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Improvement of the quality and safety of vegetable products through innovative technologies

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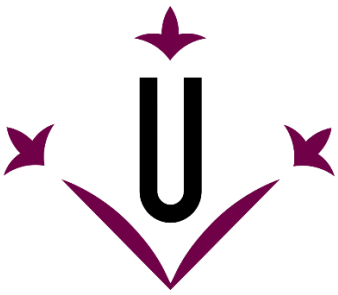

Universitat de Lleida

TESI DOCTORAL

Improvement of the quality and safety of vegetable products through innovative technologies

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Memòria presentada per optar al grau de Doctor per la Universitat de Lleida

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For you,
who have experienced
the difficulties and delights
of this quest with me.

‘Journey before destination’
Brandon Sanderson, Stormlight Archive

‘Seek, find and embrace the truths
you are fortunate enough to discover’
Disturbed, Believe

ABREVIATIONS

λ	lag time
μ	maximum growth
A	asymptotic value
AAE	ascorbic acid equivalents
AC	sodium alginate with calcium lactate
AC	antioxidant activity
AV	*Aloe vera*
AVG	*Aloe vera* gel
a_w	*Water activity*
BI	browning index
CFU	colony forming units
DPPH	2,2-diphenyl-1-picrylhydrazyl
DRBC	dichloran rose bengale chloramphenicol
DW	dry weight
EO	essential oil
FA	ferulic acid
FAE	ferulic acid equivalents
FC	flavonoid content
FNJP	fermented noni juice powder
FRAP	ferric reducing antioxidant power
FW	fresh weight
F&V	fruit and vegetable
GAE	gallic ascorbic equivalents
GE	ginseng extact
GRAS	generally recognized as safe
H°	Hue angle
IC_{50}	half inhibitory concentration
MDA	malonildialdehyde
MIC	minimal inhibitory concentration
ML	mass loss
NS	Natureseal ®
NC	non-coated
n.d.	non-determined
OCLA	Oxoid chromogenic *Listeria* agar
OD	optical density
ORP	oxido-reduction potential
PA	peracetic acid
PBS	phosphate buffer solution
PCA	plate count agar
POD	phenol peroxidase
PPO	polyphenol oxidase
RH	relative humidity
SAEW	slightly acidic electrolyzed water
SP	saline peptone
T	temperature
TA	titratable acidity
TAA	total ascorbic acid
TAC	total anthocyanin content
TAM	total aerobic mesophyll
TAP	total aerobic psicrhophylls
TCD	total color difference
TCEP	3,3',3"-Phosphanetriyltripropanoic acid
TPA	texture profile analysis
TPC	total phenolic content
TPTZ	2,4,6-tris(2-pyridyl)-s-triazine
TSA	triptone soy agar
TSB	triptone soy broth
TSS	total soluble solids
US	ultrasound
UV-C	ultra-violet C
WVR	water vapor resistance
YMA	Yeast and mould universal agar
Y&M	yeasts and molds

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Abstracts

ABSTRACT

Society evolution in developed countries has led to a consumption model based on effortless, healthy and environmentally friendly products. The attributed health benefits of fruit and vegetables (F&Vs), together with their easiness of consumption, make these products the focus of the present thesis. However, two main concerns must be highlighted. First, as such products are typically eaten raw, there are still a high number of outbreaks related to food-borne pathogens from F&Vs. Second, a third of the F&Vs produced each year become waste, making necessary to investigate and suggest strategies to maintain F&Vs at a desired commercial quality. The work in this thesis was undertaken to find solutions to the aforementioned problems, **proposing chemical or physical methods and technologies to increase safety and maintain quality of fresh and fresh-cut F&Vs**.

In front of the risk posed by several pathogens on strawberries, the first studies focus on disinfection strategies for these fruits. Considering the health risk associated with toxic vapors and by-products derived from chlorine disinfection, alternative sanitation methods were assessed to decrease pathogenic load in strawberries while maintaining their quality and nutritional parameters. Peracetic acid (PA) at 40 or 80 ppm (2 min) was effective in reducing *Listeria innocua* up to 4 log units, and fruit quality (pH, soluble solids, color, firmness) was maintained after the washing treatments (Chapter 1). Water-assisted ultraviolet C (WUV-C) light treatment alone needed 5 min to significantly reduce populations in strawberries and in washing water, so it was combined with PA at 40 ppm. This combination was successful in achieving similar reductions in artificially inoculated *L. innocua* and *Salmonella enterica* in strawberries, while keeping low (0.6 log CFU / mL) pathogenic counts in washing water (compared to 1.5 log CFU / mL after PA washing), permitting its recirculation and reducing the water expenses (Chapter 2). The optimum sanitizing combination (WUV-C + PA 40ppm) proved not to be detrimental for strawberry physicochemical (e.g. color, firmness) and biochemical (e.g. antioxidant activity, anthocyanins, vitamin C) parameters in fresh, fresh-cut (11 days) and frozen (12 months) formats (Chapter 3).

A literature review on the use of ultrasound for disinfection and preservation of F&Vs (Chapter 4) was the basis to apply this technology against *L. innocua*, *in vitro* (suspension) and *in vivo* (in artificially inoculated strawberries), alone (Chapter 5) or in combination if mild temperatures of 50 – 55 °C (Chapter 6). However, results suggested that temperature was the main factor affecting viability of *L. innocua* as well as the studied quality and nutritional parameters. Ultrasound was also applied at frequencies of 35 and 130 kHz together with a green tea extract (5 %) or Natureseal ® (7.5 %) in sliced potato, in order to decrease browning reactions during 9-day storage (Chapter 7). In this study Natureseal ® proved to be the best option to delay browning as well as increasing the antioxidant capacity of the product. Nevertheless, more studies should be performed in order to optimize the ultrasound application and the green tea effectiveness.

Finally, three compounds - two from natural sources (ginseng extract and a fermented noni juice powder) and one that could be obtained from by-products (ferulic acid, FA) - were evaluated for their antioxidant and antimicrobial properties (Chapter 8). FA outstood for its IC50 value, its polyphenol-oxidase inhibition capacity, and the low concentrations at which it had a bacteriostatic effect against several pathogenic strains of alimentary interest. For this, it was applied on fresh-cut apple and melon, proving effective in reducing artificially inoculated *L. monocytogenes* and *S. enterica* after 7 days of storage at 10 °C (Chapter 9). However, the immersion of fruit plugs in 2.5 mg / mL FA was not effective in reducing browning or preventing natural microbiota growth during storage. For this, in the next study (Chapter 10), 10 mg / mL FA were applied to apple disks, incorporated or not in edible coatings (calcium alginate casted with sodium lactate, and *Aloe vera* gel 40 %, AVG). Although no effect on *Saccharomyces cerevisiae* was observed, *L. innocua* was reduced by at least 4 log CFU / g in apple discs. Moreover, browning was delayed by all the treatments including FA and those coated with AVG alone. In fact, the mentioned antioxidant result, as well as its antimicrobial efficacy, was already reported in a literature review on AVG as an edible coating for F&Vs preservation (Chapter 11).

The present thesis makes several noteworthy contributions to the food technology field, proposing suitable strategies for specific problems that appear in the fresh and minimally processed F&V sector.

RESUMEN

La evolución de la sociedad en países desarrollados ha llevado a modelos de consumo basados en productos cómodos, saludables y respetuosos con el medio ambiente. Los beneficios saludables que se les atribuyen a las frutas y hortalizas (FyH), así como su facilidad de consumo, hacen de estos productos el tema central de la presente tesis. No obstante, se deben remarcar dos problemáticas. La primera, es que tales productos típicamente se consumen crudos, y hay aún un alto número de brotes relacionados con patógenos alimentarios. La segunda es que un tercio de las FyH que se producen anualmente se desperdician, remarcando la importancia de investigar y sugerir estrategias para mantener el nivel comercial de las FyH. El trabajo de esta tesis está enfocado a encontrar soluciones a los problemas comentados anteriormente, **proponiendo métodos químicos o físicos, así como tecnologías para incrementar la seguridad y mantener la calidad de las FyH frescas y mínimamente procesadas.**

Ante el riesgo frente a la presencia de patógenos en fresa, los primeros estudios de la tesis se enfocaron en estrategias de desinfección para este tipo de fruta. Teniendo en cuenta el riesgo asociado con los vapores tóxicos del hipoclorito, así como los subproductos derivados de su uso, se evaluaron métodos alternativos para disminuir la carga patogénica de las fresas manteniendo su calidad y valores nutricionales. El ácido peracético (AP) usado a 40 o 80 ppm (2 min) fue eficaz en reducir hasta 4 unidades logarítmicas de *Listeria innocua*, manteniendo la calidad (pH, sólidos solubles, color, firmeza) tras los lavados (Capítulo 1). En cuanto al tratamiento con luz ultravioleta-C (UV-C) asistido por agua, fueron necesarios 5 min para conseguir reducciones significativas en las fresas y en el agua de lavado, por lo que este tratamiento fue combinado con AP a 40 ppm. Esta combinación tuvo un efecto similar en la población de L. innocua y Salmonella entérica en fresa, pero mantuvo los recuentos de patógenos en el agua a un nivel bajo (0.6 logs, comparados con los 1.6 log UFC / mL tras el lavado sólo con AP). Esto permitiría la recirculación del agua reduciendo costes (Capítulo 2). La combinación seleccionada como óptima (UV-C + AP 40 ppm) demostró no ser perjudicial para las características fisicoquímicas (color, firmeza) y bioquímicas (capacidad antioxidante, antocianinas, vitamina C) de la fresa fresca, mínimamente procesada (11 días) y congelada (12 meses) (Capítulo 3).

Una revisión literaria sobre el uso de los ultrasonidos como desinfectantes y preservantes de FyH (Capítulo 4) fue la base para aplicar tal tecnología frente a *L. innocua in vivo* (suspensión) e *in vitro* (artificialmente inoculada en fresas), sola (Capítulo 5) o en combinación con temperaturas media-altas (Capítulo 6). No obstante, los resultados sugirieron que el factor que más afectaba tanto a la viabilidad de *L. innocua* como a los parámetros físico-químicos evaluados fue la temperatura. Los ultrasonidos fueron aplicados también en patata cortada a frecuencias de 35 y 130 kHz, junto con el uso de extracto de té verde (5 %) o Natureseal ® (7.5 %), con el objetivo de retrasar el pardeamiento durante un almacenamiento de 9 días (Capítulo 7). En este estudio, el Natureseal ® demostró ser la mejor opción para retrasar tal reacción, así como incrementar la capacidad antioxidante del producto. Sin embargo, deberían realizarse más estudios para optimizar el uso de ultrasonidos con este propósito, así como la eficacia del té verde.

Finalmente, tres compuestos – dos de fuentes naturales (extracto de ginseng y zumo de noni fermentado y liofilizado) y uno que podría ser obtenido de sub productos (ácido ferúlico, AF) – fueron evaluados por sus capacidades antimicrobianas y antioxidantes (Capítulo 8). AF destacó por su valor IC50, su capacidad de inhibición de la polifenol oxidasa, y por las bajas concentraciones a las cuales se observó efecto bacteriostático frente a cepas patogénicas de interés alimentario. Por ello, fue aplicado en manzana y melón mínimamente procesados, demostrando ser efectivo en reducir *L. monocytogenes* y *S. enterica* tras 7 días de almacenamiento a 10 ºC (Capítulo 9). Aun así, la inmersión de los trozos de fruta en AF 2.5 mg / mL no fue efectiva para reducir el pardeamiento ni para prevenir el crecimiento de microbiota natural durante el almacenamiento. Por ello, en el siguiente estudio (Capítulo 10), 10 mg / mL de AF se aplicaron a discos de manzana, que fueron incorporados a través de cubiertas comestibles (alginato cálcico con lactato sódico, y *Aloe vera* gel al 40 %, AVG). A pesar de que no tuvo efecto sobre *Saccharomyces cerevisiae*, se observó una reducción de *L. innocua* de hasta 4 log UFC / g en los discos de manzana. Además, el pardeamiento fue retrasado por todos los tratamientos, incluyendo el AF y aquellos cubiertos con AVG. De hecho, estos efectos antioxidantes y antimicrobianos, ya se habían incluido en una revisión literaria sobre el AVG usado como cubierta comestible en FyH (Capítulo 11).

La presente tesis aporta contribuciones sustanciales al campo de la tecnología de los alimentos, proponiendo estrategias para problemas específicos que aparecen en el sector de las FyH frescas y mínimamente procesadas.

RESUM

L'evolució de la societat en països desenvolupats ha portat a models de consum basats en productes còmodes, saludables i respectuosos amb el medi ambient. Els beneficis saludables que s'atribueixen a les fruites i hortalisses (FiH), així com la seva facilitat de consum, fan d'aquests productes el tema central de la present tesi. No obstant això, s'han de remarcar dues problemàtiques. La primera, és que aquests productes típicament es consumeixen crus, i hi ha encara un alt nombre de brots relacionats amb patògens alimentaris. La segona és que un terç de les FiH que es produeixen anualment es malgasten, remarcant la importància d'investigar i suggerir estratègies per mantenir el nivell comercial de les FiH. El treball d'aquesta tesi està enfocat **a trobar solucions als problemes comentats anteriorment, proposant mètodes químics o físics, així com tecnologies per incrementar la seguretat i mantenir la qualitat de les FiH fresques i mínimament processades.**

Davant el risc enfront de la presència de patògens en maduixa, els primers estudis de la tesi es van enfocar en estratègies de desinfecció per a aquest tipus de fruita. Tenint en compte el risc associat amb els vapors tòxics de l'hipoclorit, així com els subproductes derivats del seu ús, es van avaluar mètodes alternatius per disminuir la càrrega patogènica de les maduixes, tot mantenint la seva qualitat i valors nutricionals. L'àcid peracètic (AP) usat a 40 o 80 ppm (2 min) va ser eficaç per reduir fins a 4 unitats logarítmiques de *Listeria innocua*, mantenint la qualitat (pH, sòlids solubles, color, fermesa) després dels rentats (Capítol 1). Pel que fa a el tractament amb llum ultraviolada-C (UV-C) assistit per aigua, van ser necessaris 5 min per aconseguir reduccions significatives en les maduixes i en l'aigua de rentat, de manera que aquest tractament va ser combinat amb AP a 40 ppm. Aquesta combinació va tenir un efecte similar a la població de *L. innocua* i *Salmonella enterica* en maduixa, però va mantenir els recomptes de patògens en l'aigua a un nivell baix (0.6 logs, comparats amb els 1.6 log UFC / ml després del rentat només amb AP). Això permetria la recirculació de l'aigua reduint costos (Capítol 2). La combinació seleccionada com a òptima (UV-C + AP 40 ppm) va demostrar no ser perjudicial per a les característiques fisicoquímiques (color, fermesa) i bioquímiques (capacitat antioxidant, antocianines, vitamina C) de la maduixa fresca, mínimament processada (11 dies) i congelada (12 mesos) (Capítol 3).

Una revisió literària sobre l'ús dels ultrasons com a desinfectants i preservant de FiH (Capítol 4) va ser la base per aplicar tal tecnologia enfront de *L. innocua in vivo* (suspensió) i *in vitro* (artificialment inoculada en maduixes), sola (Capítol 5) o en combinació amb temperatures mitjana-altes (Capítol 6). No obstant això, els resultats van suggerir que el factor que més afectava tant a la viabilitat de *L. innocua* com als paràmetres fisicoquímics avaluats va ser la temperatura. Els ultrasons van ser aplicats també en patata tallada a freqüències de 35 i 130 kHz, juntament amb l'ús d'un extracte de té verd (5%) o Natureseal ® (7.5%), amb l'objectiu de retardar l'enfosquiment durant un emmagatzematge de 9 dies (Capítol 7). En aquest estudi, el Natureseal ® demostrar ser la millor opció per retardar tal reacció, així com incrementar la capacitat antioxidant del producte. No obstant això, s'haurien de realitzar més estudis per optimitzar l'ús d'ultrasons amb aquest propòsit, així com l'eficàcia de el té verd.

Finalment, tres compostos - dos de fonts naturals (extracte de ginseng i suc de noni fermentat i liofilitzat) i un que podria ser obtingut de subproductes (àcid ferúlic, AF) - van ser avaluats per les seves capacitats antimicrobianes i antioxidants (Capítol 8). L'AF va destacar pel seu valor IC50, la seva capacitat d'inhibició de la polifenol oxidasa, i per les baixes concentracions a les quals es va observar efecte bacteriostàtic enfront de soques patogèniques d'interès alimentari. Per això, va ser aplicat en poma i meló mínimament processats, demostrant ser efectiu en reduir *L. monocytogenes* i *S. enterica* després de 7 dies d'emmagatzematge a 10 ºC (Capítol 9). Així i tot, la immersió dels trossos de fruita en AF 2.5 mg / ml no va ser efectiva per reduir l'enfosquiment ni per prevenir el creixement de microbiota natural durant l'emmagatzematge. Per això, en el següent estudi (Capítol 10), 10 mg / ml d'AF van ser aplicats a discos de poma, que van ser incorporats a través de cobertes comestibles (alginat càlcic amb lactat sòdic, i Aloe vera gel al 40%, AVG). Tot i que no es va observar efecte enfront *Saccharomyces cerevisiae*, es va observar una reducció de *L. innocua* de fins a 4 log UFC / g en els discos de poma. A més, l'enfosquiment va ser retardat per tots els tractaments, incloent el AF i aquells coberts amb AVG. De fet, aquests efectes antioxidants i antimicrobians, ja s'havien inclòs en una revisió literària sobre el AVG usat com a coberta comestible en FiH (Capítol 11).

La present tesi aporta contribucions substancials al camp de la tecnologia dels aliments, proposant estratègies per a problemes específics que apareixen en el sector de les FiH fresques i mínimament processades.

Introduction

1 The importance of fruit and vegetables

The botanical definition of fruit is the seed-bearing structure in angiosperms formed from the ovary after flowering. In food science and technology, fruit are considered as the edible, usually fleshy and sweet-smelling part of a plant that may or may not contain seeds (NYBG, accessed 2020). Vegetables are commonly defined as the edible portion of a plant, and are typically grouped according to the portion of the plant that is eaten (e.g. leaves (lettuce), tubers (potato)). As other plant-derived products like nuts, fruit and vegetables (F&Vs) are a rich source of bioactive compounds. Biologically active compounds are those that produce physiological effects beyond the basic nutrition, in other words they **exert physiological benefits related to promoting health and preventing the effects of a disease** (Gnanavinthan, 2013). Together with complex carbohydrates and fiber, F&Vs provide an optimal mixture of micronutrients, such as vitamins C and E, polyphenols and carotenoids (Rico, 2007).

More and more convincing evidence suggests that the benefits of phytochemicals in F&Vs may be even greater than it is currently understood because oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases (Liu, 2003). Antioxidants have been reported to have different effects when consumed by humans; they help to control blood pressure or blood sugar influencing substances, or can act as agents with anticarcinogenic, immunity-supporting, antibacterial, antifungal, antiviral, cholesterol-lowering, antithrombotic or anti-inflammatory properties (González-Aguilar, 2008).

1.1 Production and consumption

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend a minimum of 400 g of F&Vs per day (excluding potatoes and other tubers) (WHO, 2005). These organizations claim that insufficient intake of F&Vs is estimated to cause about 14 % gastrointestinal cancer deaths, 11 % ischemic heart disease and 9 % stroke deaths globally.

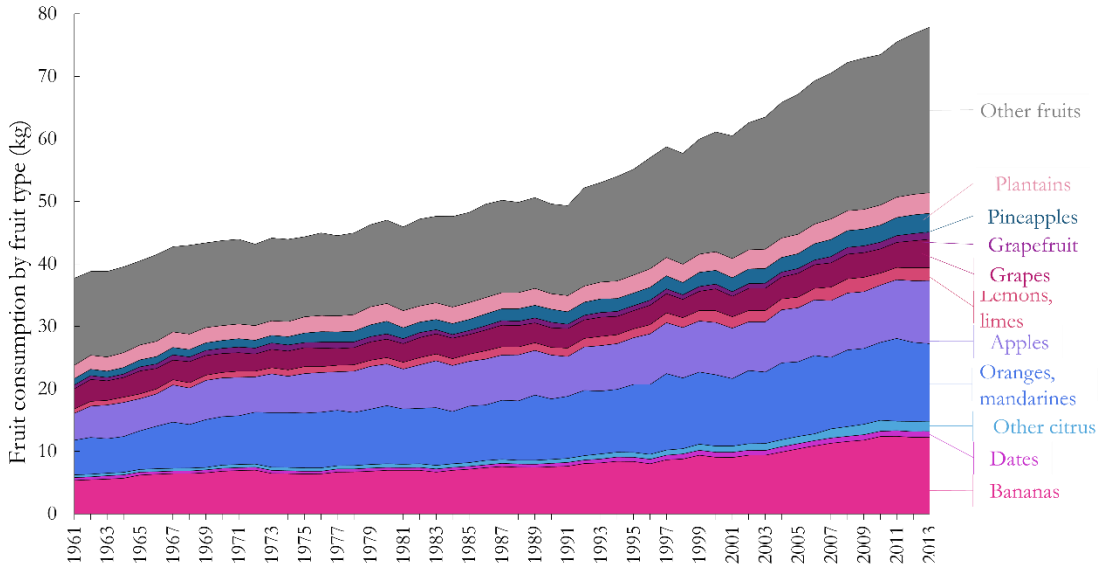


Figure 1. Time evolution of fruit consumption per capita (kg fruit / capita) by fruit type. Adapted from: https://ourworldindata.org (Accessed 2020-08).

Each year, 72,336 thousand tons of F&Vs are produced around the world, from which India and China are the major contributors, and 823,216 tons are produced in Europe. The average fruit consumption

(kg per year and capita) has increased over the past years (**Figure 1**), being the most consumed fruit bananas, oranges and mandarins, and apples. The present F&V consumption per capita is shown in Figure 2A. However, there are several countries in the world, especially those in under-developed regions that do not reach the minimum recommended (Figure 2B). For the prevention and alleviation of several micronutrients, especially in less developed countries, WHO and FAO launched a Joint Initiative to promote F&Vs for health worldwide, stablishing a minimum of 5 pieces of F&Vs each day (WHO, 2004).

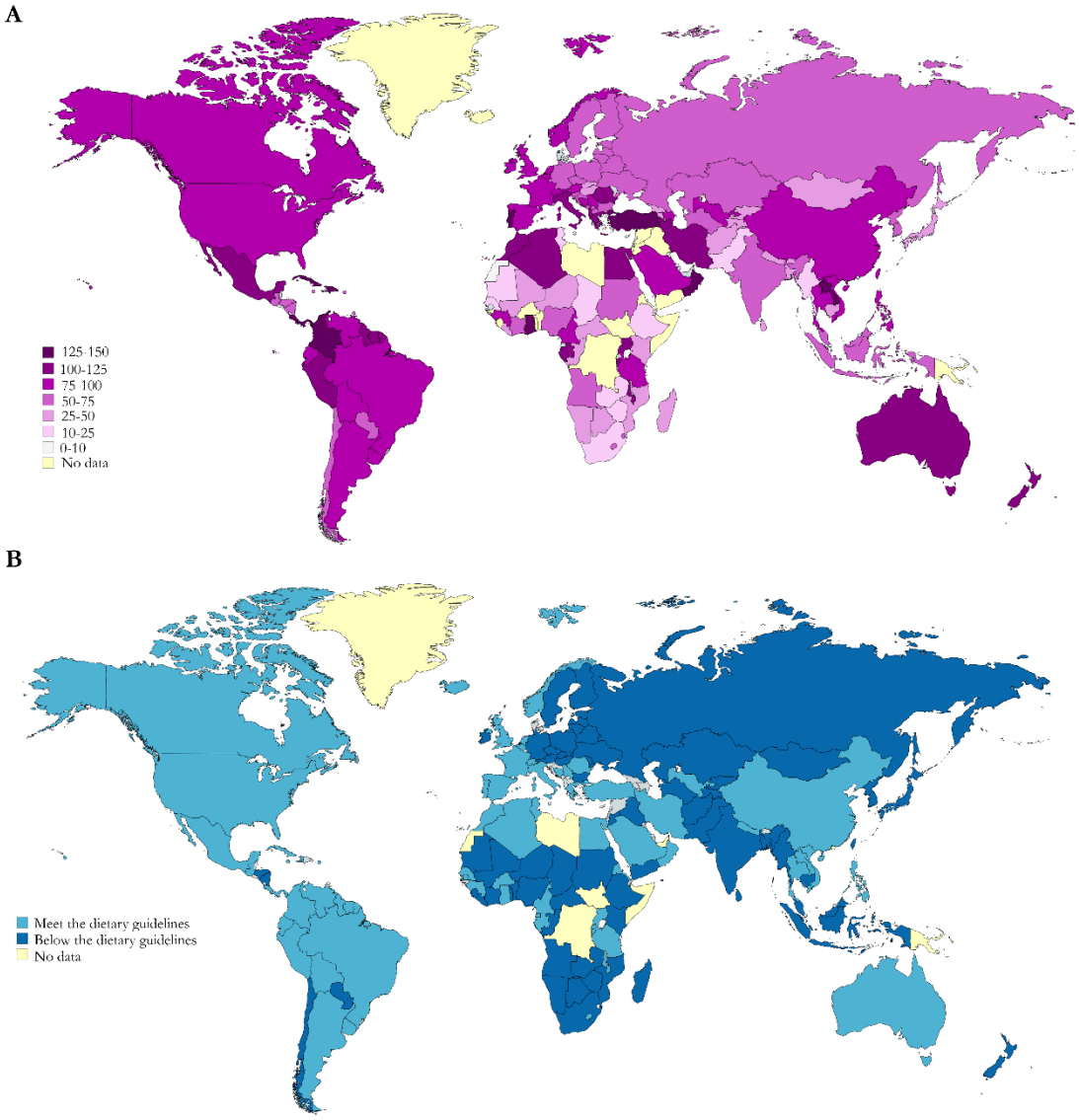


Figure 2. (**A**) Fruit consumption per capita by country (kg / year / capita) (2019), and (**B**) distribution of countries following WHO guidelines of 400 g fruit / day (B). Adapted from: https://ourworldindata.org (Accessed 2020-08)

Four vegetable products have been the main characters in the present thesis: strawberries, melon, apple and potatoes. A brief overview of the most important characteristics of each product, as well as the last years' production data is included hereunder.

Strawberries

Strawberries (*Fragaria* × *ananassa*) pertains to the *Rosaceae* family and it is described as a small, juicy, red fruit shaped like a cone with seeds on its surface. It has a relevant nutritional quality due to its high levels of micronutrients as vitamin C, folate, minerals and phenolic constituents (Battino, 2009). It is of economic and commercial relevant importance and largely consumed in both fresh (70 %) or in processed form (30 %, jams, juices, jellies, frozen), making it the most studied berry from agronomical, genomic and nutritional point of view (Alvarez-Suarez, 2014).

Data from the market analysis shows that the largest consumers are China (41 %), the USA (16 %), and Egypt (5 %). Spain is the country that produces more strawberries in Europe, with 344 thousand tons in 2018, followed by the Russian Federation and Poland (**Figure 3**A) (FAOSTAT, 2018, accessed 2020-08-03). Spain, along with the USA, Mexico and Netherlands, is responsible for 70 % of the global strawberry export, which has increased from 2012 by 18 % (IndexBox, 2018).

Melon

Melon (*Cucumis melo*) is one of the family *Cucurbitaceae* They are a type of berry known as a pepo, and they vary greatly in size, shape, surface texture, and flesh colour and flavour, depending on the variety. They generally weigh 1–4 kg and size ranges from 5 to 15 cm diameter. Most commercially important melons are sweet and eaten fresh, and have the seeds in the inner and sweetest part. Santa Claus, Christmas or 'Piel de Sapo' (toadskin) melon is one mostly cultivated in Spain, together with 'Galia', 'Amarillo' and 'Tendal' (Artes, 1992).

Melon is grown in various parts of the world due to its capacity to adapt to different kinds of soil and climates. In 2018, 4875 thousand tons of melon were cultivated in Europe, being Spain and Italy the biggest producers (**Figure 3**B). Spain is also the leading melon exporter, with around 430 thousand tons on average between 2015 and 2018 (FAOSTAT, 2018, accessed 2020-08-04). The melon market is mainly fresh, and melons are sold whole or half-cut. However, in the past decade and accompanying an increase in melon production, a variety of products have appeared using its by-products (i. e. flour, cookies, cakes and bread), minimizing waste and providing the opportunity to develop new products (Rolim, 2019).

Apples

Apple (*Malus domestica*) is a pome (fleshy) fruit pertaining to the *Rosaceae* family, in which the ripened ovary and surrounding tissue both become fleshy and edible. When harvested, apples are usually roundish, 5–10 cm in diameter, and some shade of red, green, or yellow in color; they vary in size, shape, and acidity depending on the variety. Apples represent a major proportion of the fruit supply throughout the year in most countries because of various factors: availability in the market, diversity of cultivars (Soler, 2009). These fruits are generally recognized as being healthy represented in the quite popular statement "an apple a day, keeps the doctor away". In fact, when compared to other commonly consumed fruit, they are in the top of the phenolic compound's concentration ranking, surpassing grapes, strawberries, peaches and pears (Boyer, 2004). But more important, they have the highest portion of free phenolics, which means that these compounds are not bound to others and so, are more available for eventual absorption by humans (Sun, 2002).

According to the market reports (IndexBox, 2019b), the apple market has and will grow by 2.0 % each year over the nine-year period 2016 to 2025. Overall, it seems that there was an upward trend of apple consumption, being China the biggest consumer and Venezuela the country with the highest annual growth rate. Poland leads the production of apple in Europe, with almost 4 million tons a year (FAOSTAT, 2018, accessed 2020-08-03) (**Figure 3**C). Apples are not only commercialized fresh, but

also fresh-cut as slices or as an ingredient of ready-to-eat salads, or covered by caramel or chocolate in European Northern countries.

Potatoes

The potato is the starchy tuber of the plant *Solanum tuberosum*. It is a root vegetable from the family *Solanaceae* that is available worldwide and all year long. Potato is a major staple food that is preferentially consumed mainly because of its richness in carbohydrates and essential micronutrients. Apart from starch and dietary fiber, potatoes provide other compounds and phytochemicals that are beneficial to health (i.e. potassium or vitamin C) (Jayanty, 2019).

It is a major food crop that is used for a number of purposes, beyond a fresh-vegetable for cooking at home. In fact, less than 50 % potatoes are consumed whole and fresh; the rest is processed into potato food products (i.e. sliced and fresh potatoes, pre-fried frozen chips, gnocchi) or food ingredients, such as starch for industrial uses (IndexBox, 2019a). Therefore, increased demand in the food processing industry acts as a major driving force behind the growth of the potato market. Average potato consumption per capita reached 33.5 kg in 2017 in the world (Helgilibrary, accessed 2020-08-05), but the potato market is projected to record an annual increase of 1.0 % during the period 2019-2024 (IndexBox, 2019a). In Europe, the major contributors to its production are Ukraine and the Russian Federation, with 22 million T each (FAOSTAT 2018, accessed 2020-08-03) (**Figure 3**D).

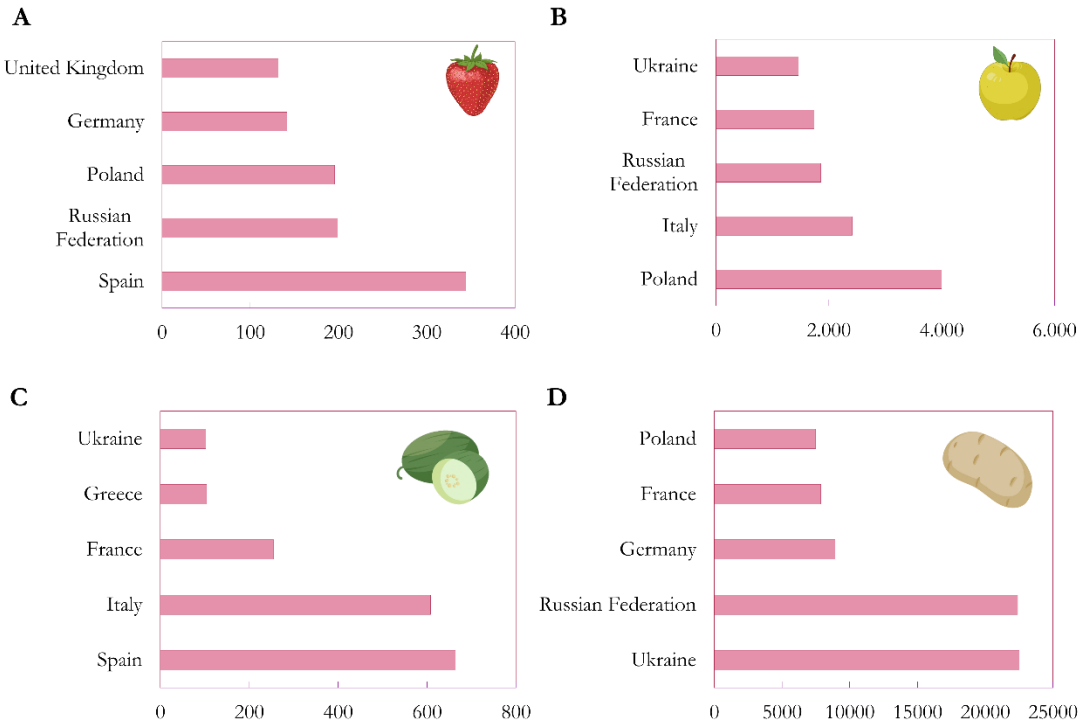


Figure 3. Million tons of (**A**) strawberry, (**B**) apple, (**C**) melon, and (**D**) potato, by top-five producer European countries. Adapted from FAOSTAT (Accessed 2020-08).

1.2 What do consumers expect from F&Vs? Present trends

Lifestyles in Europe have becoming more fast-paced and interest in easy and convenient foods is growing. Moreover, people are more aware about the impact that food can exert in their health (Güneş, 2017). Fresh F&Vs are now trending products: 28 % of the consumers refer to them as the product category in which they search more satisfaction. **Consumers are looking for faster, easier and healthier F&Vs,** demanding more emotional connection and more green conscience (**Table 1**).

It is then, the aim of industries to fulfill consumers requirements, accompanied by the knowledge science can provide in this area.

Table 1. Consumer demands to F&V attributes and expected experience of such products (Adapted from Fruit Logistica, 2019).

What are consumers asking for	Assortment	Experience
Faster, easier, healthier	Large convenience range New easier-to-cook products Ready or prepared Fortified vegetables Re-discovery of frozen	Modular convenience Suggestions: what to eat? Quick and easy shopping More information on health Encouragement to a healthier lifestyle
Emotional connection	Better tasting varieties Brands conveying trust in quality	Increased interaction: advice food tasting, cooking classes In-store gastronomy
F&Vs with good conscience	Local products Seasonal products Environmentally friendly packaging	Transparency in the supply chain Local and seasonal More information about sustainability of products and effort to achieve this

According to Fruit Logistica, the criteria for fresh food selection of 6,500 consumers from Europe and North America is based mainly in quality (33 %), assortment (21 %), and product presentation (10 %) (**Figure 4**). When deepening in what 'quality' means for consumers, the research revealed that the top-three drivers are the appearance, taste, and size and shape of the fruit (Fruit Logistica, 2019). However, taste and other attributes like odor or firmness can only be tested after consuming the products. The memory of the sensorial attributes once consumed, together with other objective (i.e. price, ecological aspects) and subjective (i.e. overall appearance, ripeness visual evaluation, size, shape) factors, and accompanied by consumer's socio-demographical situation and food health conscience will influence the intention to repeat the purchase (Ragaert, 2004) (**Figure 5**).

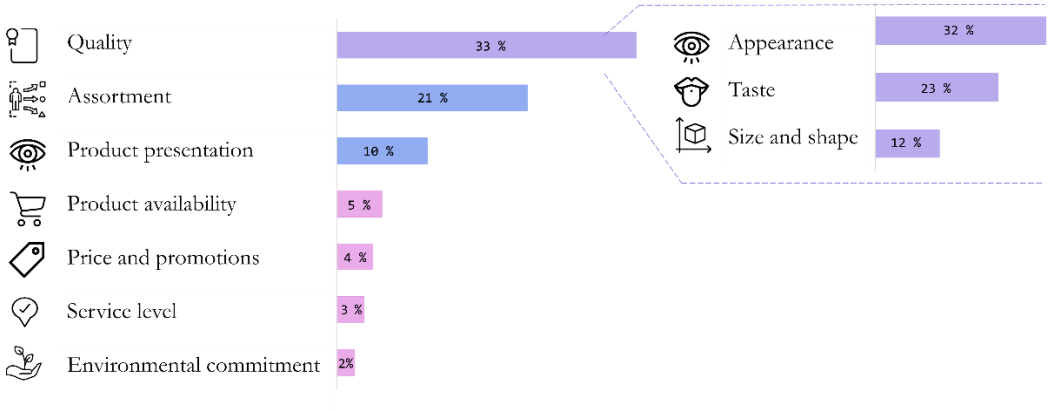


Figure 4. What do consumers demand for F&V? (Adapted from Fruit Logistica, 2020)

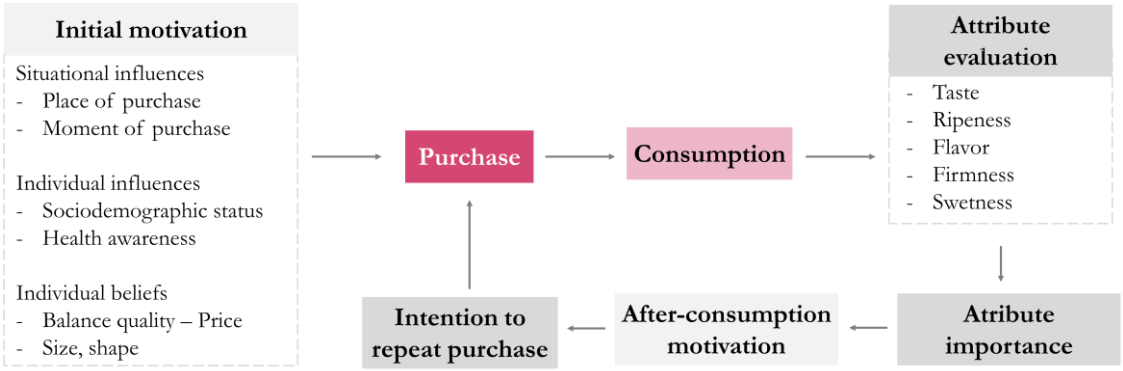


Figure 5. Attributes that affect F&V purchase intention before and after the action (Adapted from Ragaert, 2004)

1.3 The F&Vs processing

In the past two decades, **minimal processing** techniques have emerged to meet the social challenge of consuming food in a highly convenient format. This term is described as the "handling, preparation, packaging and distribution of agricultural commodities in a fresh-like state" (Shewfelt, 1987) (**Figure 6**). The original purpose of minimal processing was to minimize the quality loss caused by long- and high-temperature treatments (Brecht, 2004). This forced researchers to develop alternative processes to achieve a better balance between preservation and quality. Several typologies of products are included in this category: frozen, blanched or osmotically dehydrated (Hui, 2006)

The terms **fresh-cut**, minimally processed, or ready-to-eat F&Vs, all refer to raw produce that have been trimmed, peeled or cut into a complete usable product which has been packaged to offer consumers high nutrition and flavor while maintaining its freshness (Jideani, 2017). According to the International Fresh-Cut Produce Association (IFPA), the key criteria of a product to be included in this category is that it consists of 100 % usable material and the tissue is in a living, respiring and physiological state (IFPA, 2000). The importance of fresh-cut produce lies in its major **characteristics of freshness, convenience, nutrient retention and sensory quality** while providing extended shelf life (Smetanska, 2013).

For long-term storage, **frozen** storage is one of the most widespread used preservation methods for F&Vs, products in which the rate of most deteriorative reactions and microbial activities are significantly reduced due to reduced temperatures (Zhan, 2018). The main weakness of frozen products resides in the poor maintenance of the fresh-like properties. Even it is considered the **least destructive preservation** technology for phytochemical compounds and nutrients, and no respiration leading to physiological changes occurs, degradation of the product happens since crystal formation with volume expansion and increased osmotic pressure between cell membranes damages their microstructure (Kobayashi, 2019).

The attractiveness, convenience and health benefits related to fresh and fresh-cut fruit are factors that are promoting the consumption increase of fresh produce. However, **these benefits are offset by the rapid deterioration** (short shelf-life) of the products (section 2.1) **and the potential health hazards** (section 2.2)(Brecht, 2004). These drawbacks bring up the importance of understanding the mechanisms underlying the decline in fruit quality and safety (i.e. physiology, composition, handling, contamination ways), in order to come out with minimizing or delaying solutions.

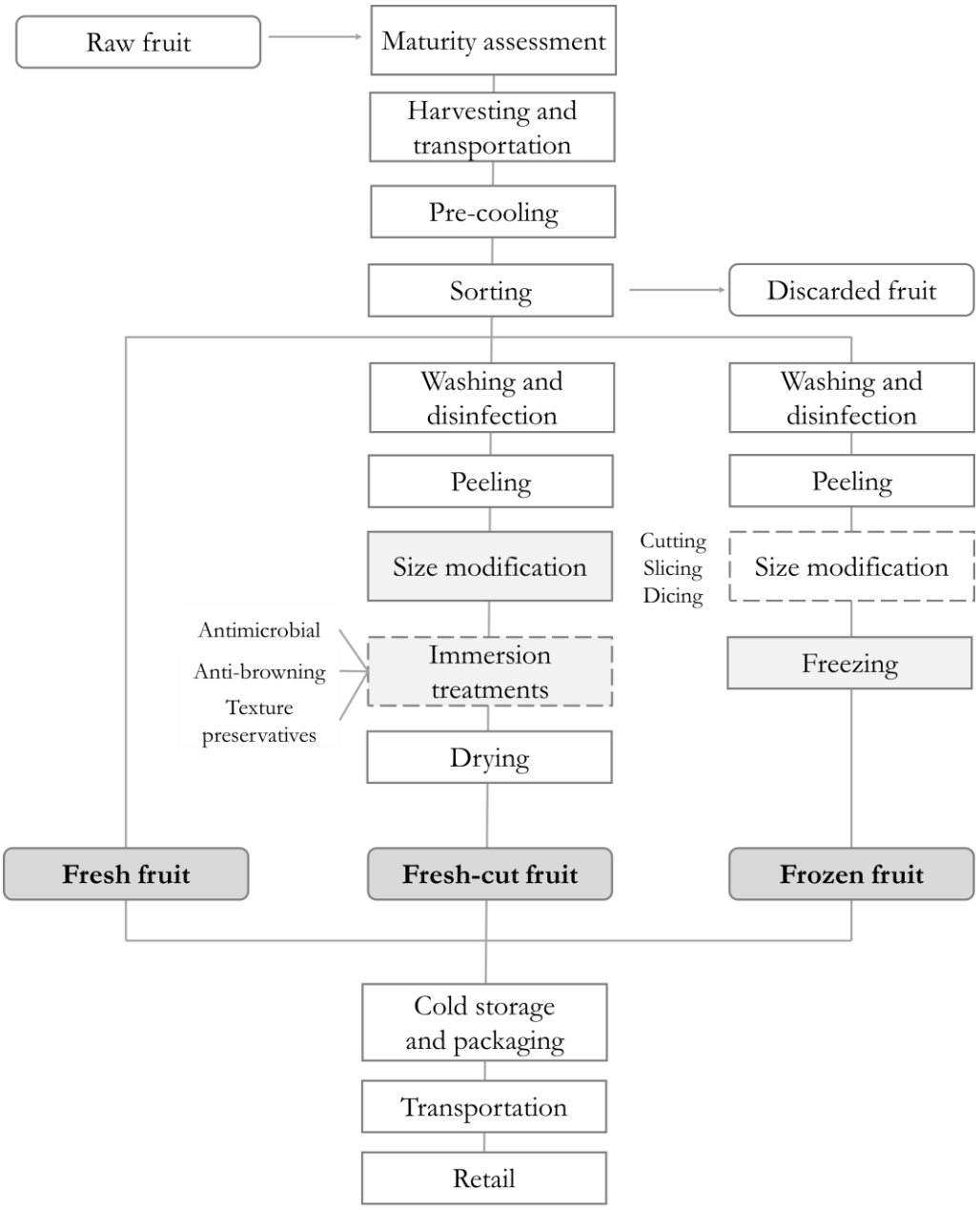


Figure 6. Flow chart of fresh, fresh-cut, and frozen fruit processing in fruit industries.

2 Main F&Vs concerns

2.1 Quality of F&Vs: losses and waste in the food chain

2.1.1 Where and how much?

Perfection does not exist, and despite their flavor, availability, nutritional values and health benefits related, fruit have, at least, one major problem: being perishable products. There are two relevant concepts, related but not equivalent, that must be explained before continuing with this problem: food loss and food waste. In particular, '**food loss**' refers to food that spills, spoils, incurs an abnormal reduction in quality such as bruising or wilting, or otherwise gets lost before it reaches the consumer. '**Food waste**' refers to food that is of good quality and fit for human consumption but that

does not get consumed because it is discarded either before or after it spoils. Food waste is the result of negligence or a conscious decision to throw food away (Lipinski, 2013).

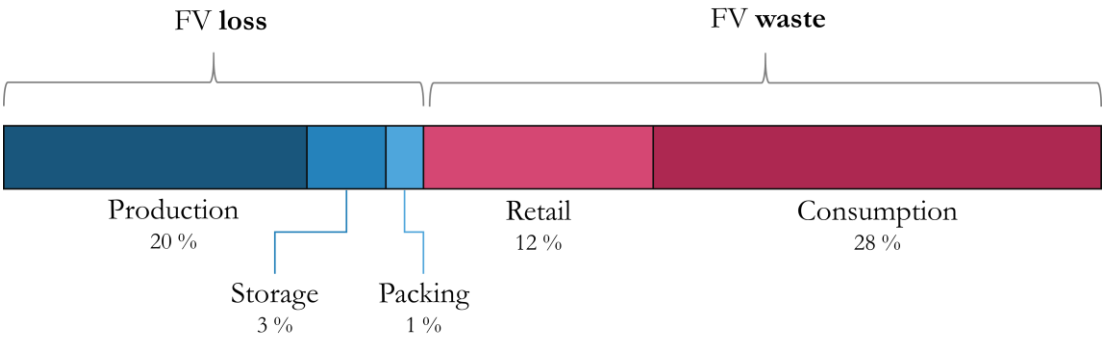


Figure 7. Percentages of F&V loss and waste in the different steps of the food chain (Adapted from Porat, 2018).

Figure 7 shows that food loss normally occurs in the production (20 % of the fruit are lost in this step), storage (3 %) and packing (1 %), while food waste occurs in the last steps, retail level (12 %) and consumption (28 % are discarded). These percentages depend on the product (Porat, 2018), but in general, more than 35 % of fresh F&V is wasted each year(Deng, 2019). **Table 2** shows where some F&Vs loss and waste occur. The table points strawberry, apple, melon and potato as some of the F&Vs subjected to losses and waste, and for which scientific knowledge can provide solutions by studying the main causes and optimizing the best approach to minimize them.

Table 2. When does food loss and food waste occur? Percentages by F&V (Adapted from Deng, 2019).

Product	Field	Grading	Storage	Packing	Retail
Strawberry	2-3	1	0.5	2-3	2-4
Raspberry	2	No data	No data	2-3	2-3
Lettuce	5-10	No data	0.5-2	1	2
Tomato	5	7	No data	3-5	2.5-3
Apple	5-25	5-25	3-4	3-8	2-3
Onion	3-5	9-20	3-10	2-3	0.5-1
Potato	1-2	3-13	3-5	20-25	1.5-3
Broccoli	10	3	0	0	1.5-3
Melon	No data	No data	No data	No data	1.6
Avocado	No data	30	5	3	2.5-5
Citrus	No data	3	No data	0.5	2-2.5
Banana	No data	3	No data	0-3	2

F&V losses at retail and consumer levels represent a significant amount of money and other resources invested in their production (e.g. land, fresh water, labor, energy, chemicals…) that will not accomplish its final purpose of feeding people. This investment loss can be prevented by finding and applying strategies and technologies along the food production, marketing and consumption chain (Buzby, 2011). **In this thesis, the focus will be placed in preventing food waste**, the causes that provoke it and how to handle it, so that F&V that fits marketability standards can last until its consumption. To be able to do so and increase the shelf-life of F&V, it is key to understand their main deterioration

causes, which lead to food waste. Knowing the aforementioned information is useful to find specific solutions for each of the steps or problems.

2.1.2 Why? The main factors that contribute to F&V deterioration

Fruit and vegetable spoilage or deterioration occurs as a consequence of strong interdependent abiotic and biotic factors happening after harvest. Harvested F&Vs continue with their respiration and metabolic processes, and various ripening changes initiated simultaneously will evolve together and interrelated. Also, when F&V age or mature, their normal defense tactics decrease, thus making it more susceptible to deterioration. When fruit is off-tree, no photosynthesis processes occur anymore, so there is no replacement of their components (Thompson, 2010). Moreover, management of fruit from the field to consumer's homes involves a series of handling steps (e.g. washing, wounding, packaging) or presentation alterations (e.g. peeling, cutting), that can increase the stress at which F&V are submitted (Hui, 2006).

A summary of the relationships between the different factors influencing F&V deterioration is shown in **Figure 8**. It must be noted **that fresh-cut F&Vs differ from their whole counterparts in terms of their physiology and form**. Associated directly to their purposely wounding (e.g. peeling, cutting, slicing, dicing…), there are physical effects, including mechanical shocks, removal of the protective epidermal layer, accumulation of moisture, cell breakage and contact of substrates, or physiological and biochemical responses to the adjacent and distant tissues, elicited by wounding (Gil, 2006). This means that a number of factors affecting viability and quality of the produce are potentiated, and these products must be handled accordingly (Brecht, 2004).

Physiological

Physiological changes continue to occur in F&V even after harvested. While climacteric fruit can ripen fully once they are harvested at completion of their growth period, non-climacteric will only fully ripe if attached to the parent plant (Paul, 2012). F&V ripening or senescence is a very well-orchestrated physiological process which is regulated by a number of factors including hormones, enzymes and environmental stimuli. It can be accelerated by external stresses such as temperature, ultraviolet light, wounding or oxidation. The metabolic processes that F&V undergo include respiration, transpiration, energy production, and plant self-defense (Palma, 2019).

Respiration is the process by which cells release energy from organic compounds to generate adenosyl tri phosphate (ATP) by a series of chemical reactions. These ATP are a source of energy for the cell, to continue with catabolic and anabolic processes (Castellanos, 2015). After harvest, it is dependent entirely upon the F&V own food reserves as no replenishment is there, and therefore, **deterioration** starts (Thompson, 2010). In general, a rapid respiration rate is well correlated with the rate of deterioration, and it is a good measure for the storage potential of the F&V. Very low respiration rate (< 10 mg CO_2 / kg · h at 10 ºC) is characteristic of nuts and dry fruit, while very high respiration rate (> 40 mg CO_2 / kg · h at 10 ºC) is characteristic of melon and strawberries (Fonseca, 2002). The effects of respiration include the loss of substrates by their utilization, such as carbohydrate metabolism or the degradation of starch to simple **sugars** (fructose, glucose and sucrose). Sugar accumulation during F&V development differs between species and changes with their growth, and peaks at maturity or ripening (Desnoues, 2014). Sugars also play a role in the **organic acid** pathway being the main ones malic, citric, galacturonic, quinic, oxalic, tartaric and shikimic (Walker, 2018). These two components are quantified by the total soluble contents (TSS), which measures mainly the sugar content, and by the titratable acidity (TA), which reflects the amount of organic acids present. In general, during postharvest, TSS increases and TA decreases, having an impact on the **taste** of F&V: sweetness increases over the

decrease in sourness. Then, a proper ratio between sugars and organic acids content is very important to provide the adequate organoleptic properties that lead to consumers' acceptance (Mikulic-Petkovsek, 2012).

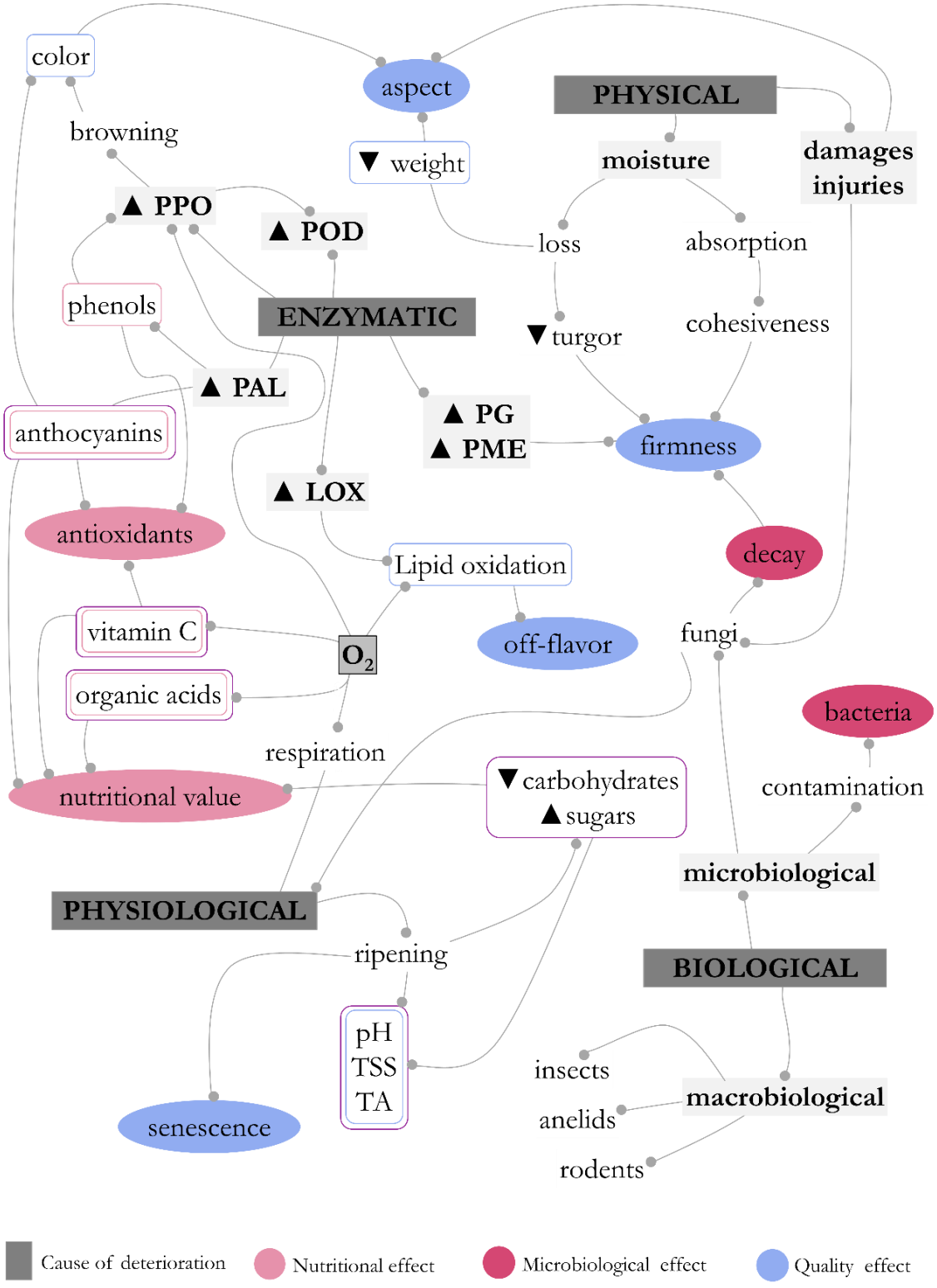


Figure 8. Complexity of the changes occurring during ripening and during storage in fruit and fresh-cut fruit. Relationship between the different causes and effects on F&V.

The anatomy of the tissue is related with **firmness**, and it is determined by interrelated factors: particular cell size and shape, cell wall thickness and strength, extent of cell wall adhesion, or turgor status. Cell wall has rigid hemicellulose fibers, typically xyloglucans, and middle lamella, that are composed by pectin with four types of galacturonic acid, are the glue holding the neighbor cells together (Thompson, 2010). After harvest, pectinolytic enzymes act in these areas, and the extent of intercellular contact decreases, the space between cells increases and softening occurs (Toivonen, 2008).

Transpiration is the loss of water from F&V that includes the transport of moisture to the skin of the commodity, its evaporation and the convective mass transport of this moisture to the surroundings (Postharvest Education Foundation, 2015). This results in **weight loss** that is patent in the visual aspect of the F&V: browning or a loss in the bright turgid appearance, which also influences mouth feel (Debeaufort, 2000), development of wrinkles in the surface or even softening of the peel, that becomes very lose and may crack (Thompson, 2010). In fresh-cut F&V, this water loss is enhanced by the higher exposed surface to the environment as a result of peeling (peel acts as a semi-permeable barrier for water) and cutting (which increases the volume-surface ratio)(Brecht, 2004). In frozen F&V, crystal formation time can influence the amount of water that the produce losses once thawed (**Figure 9**), which in turn, will affect the **firmness** of the product: water in cell exerts a pressure on the walls that contributes to turgor in the products (Toivonen, 2008) If it is slow, water crystals will form and slowly accumulate frozen water increasing in size. The bigger size will cause the break-up of cells, obstructing the water retention during thawing. If freezing process is quick, and the time within the critical zone for ice-formation is short, these crystals will form from a higher number of nuclei, but with a smaller size. These crystals are not that disrupting, so cell integrity is maintained and water is better retained after thawing (Fellows, 2000).

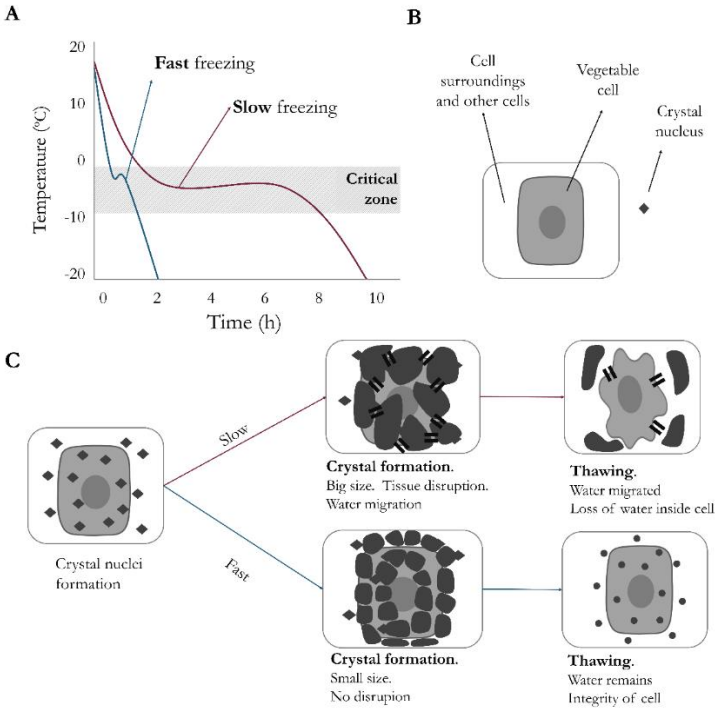


Figure 9. (A) Temperature evolution during freezing process and time in the critical zone when crystals are formed, (**B**) scheme of the different implicated parts in freezing of F&V, and (C) crystal nuclei formation during slow and fast freezing, and

Enzymatic

Changes in this category are those involving chemical reactions catalyzed by enzymes in F&V. Many of them are a continuation of the normal ripening events, mainly those involving textural changes that affect the cell wall composition. Others are triggered or potentiated by wounding or cutting, which normally will occur during handling operations or in fresh-cut F&V (Toivonen, 2008). In frozen products, the kinetics of the enzymatic activity slow down, delaying the changes that would occur in fresh products. However, with the formation of ice crystals and breakdown of cell organelles, enzymes and substrates can be released, exhibiting substantial activity after a freezing and thawing process (Zaritzky, 2010). Down below, the characteristic enzymes responsible for the main quality deterioration of F&V are explained.

Pectin methyl esterase and polygalacturonase (PME, EC 3.1.1.11 and PG, EC 3.2.1.15) are two enzymes of a heterogeneous group that hydrolyze the glycosidic bonds of pectic substances, the structural heteropolysaccharide contained in the primary and middle lamella and cells walls of plants (Kohli, 2015). In general, **softening** of F&V is related with solubilization of the pectin by these hydrolytic enzymes (Ketsa, 1999). PME removes the methyl ester groups of homogalacturonic acids (HGAs), the main component of pectin from middle lamella. The negatively charged residues associate together through ionic Ca^{2+}, forming a calcium-pectate gel that provides most of the intercellular bonding in ripe F&V (Toivonen, 2008). PG depolymerizes pectic polysaccharide chains preferably at those locations where the methyl groups have been removed (by the action of PME) (Tijskens, 1997).

Phenylalanine ammonia lyase (PAL, EC 4.3.1.24) is one of the most studied enzymes related to the secondary metabolism of plants; it is the key enzyme operative in higher plants and is mainly involved in defense mechanisms. Given a vegetable tissue, the levels of the enzyme may fluctuate significantly in a relatively short time in response to a wide variety of stimuli such as tissue wounding, pathogenic attack, light, low temperatures or hormones (Camm, 1973). PAL participates in the first step in the phenyl propanoid pathway, catalyzing the non-oxidative deamination of L-phenylalanine to ammonia and *trans*-cinnamic acid. It is therefore involved in the biosynthesis of the polyphenolic compounds such as flavonoids, phenylpropanoids and lignin (Macdonald, 2007). **Anthocyanins** are the most noticeable class of flavonoids, and have several functions in flowers and fruits, including counteraction of the negative effects of nitrogen and reactive oxygen species, maintenance of the redox homeostasis of biological fluids, or contribution to defenses against phytopathogens (Guo, 2010). Their importance for fruit quality maintenance resides in their **color**: they provide it with a wide range of pigments that go from red to blue, including purple. The type of anthocyanins, along with other factors such as their concentration or the pH of the matrix, will determine the color of the F&V (Wallace, 2015). The effect of processing on anthocyanins is, therefore, crucial to be studied in order to determine the best way to maintain these compounds and, that way, color of fruits containing them. Moreover, anthocyanins together with **polyphenols**, contribute to the **antioxidant capacity** of the fruit, permitting to neutralize the free radicals, which are very reactive and rapidly attack the molecules in nearby cells, and probably the damage caused by them is unavoidable. These repair and neutralizing processes allow to reduce the oxidative stress and thus, increase the shelf-life of F&V (Gülçin, 2012). This oxidative stress also occurs in humans: reactive oxygen species may interact with the membranes of cells or damage biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids. Free radicals attack important macromolecules leading to cell damage and homeostatic disruption (Lobo, 2010) and are involved in the etiology of a wide range of chronic diseases. For this, **nutritional value** of the F&Vs is a factor worth maintaining, as an adequate uptake of phytochemicals with antioxidant activity by diet could help in preventing such health problems (Liu, 2003).

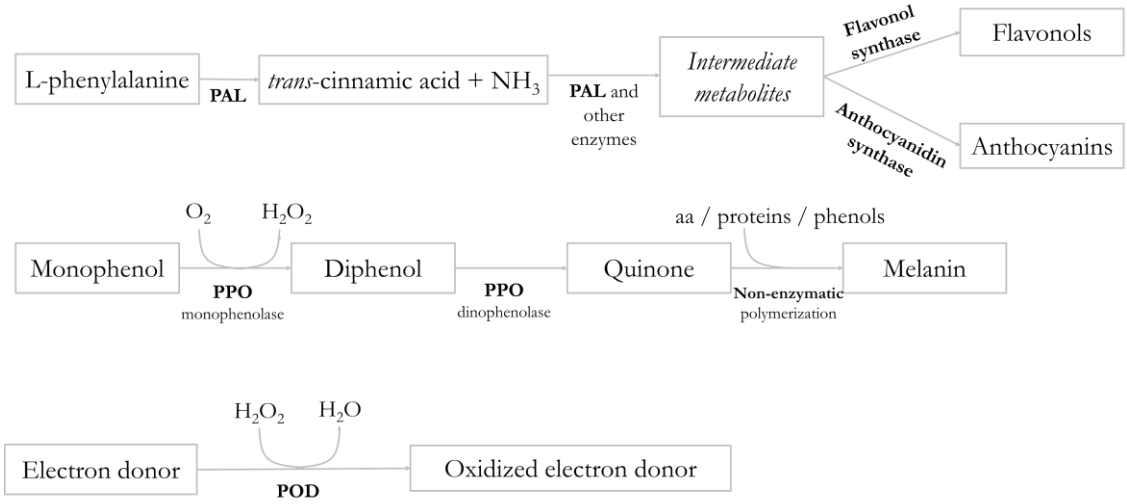


Figure 10. Main enzymatic reactions happening in fruit and vegetables in stress or after cutting operations.

Polyphenol oxidases (PPO) are a group of copper-proteins that catalyze the oxidation of phenolic compounds to quinones which will act as a defense mechanism in wounded tissue. The proposed mode of action initiates when the tissue is damaged and the rupture of plastids containing PPO leads to the enzyme getting in contact with the phenolic compounds released by the rupture of the vacuole (Pradas-Baena, 2015). This means that PPO activity is relevant mostly in fresh-cut F&Vs, which have been subjected to a cell rupture. The active site of PPO catalyzes two different reactions in the presence of molecular oxygen: (1) hydroxylation of monophenols by monophenol monooxygenase (EC 1.14.18.1) and (2) oxidation of o-diphenols to o-quinones cathecolase (EC 1.10.3.1) (**Figure 10**). The reaction is then followed by a non-enzymatic polymerization of the quinones with amino acids or proteins (mainly with the -SH and -NH_2 groups), originating melanin (Queiroz, 2008). This polymeric compounds form impermeable barriers, limiting **pathogen translocation** and protecting the fruit (Mohammadi, 2002). From the point of view of F&V industry PPO is mainly studied because it is thought to be the major cause of the **brown coloration** occurring in many post-harvest and processing steps, but essentially after cutting operations. The o-quinones generated by the reaction of PPO are themselves colored. It must be noted that reddish brown color characteristic of enzymatic browning is mostly attributed to these secondary reactions of o-quinones resulting in formation of melanin (Yoruk, 2003). The intensity of the browning depends on the amount of active PPO forms and the phenolic content of the F&V, which, are in turn dependent on numerous factors, such as variety, maturity state, or environmental factors (Soliva-Fortuny, 2003). The study of the impact that the processing steps may have on PPO becomes very important in F&Vs that are highly affected by enzymatic browning deterioration, such as potato, apple or mushroom.

Peroxidase (POD, EC 1.11.1.7) is an enzyme involved in the oxidation by hydrogen peroxide of a wide range of organic and inorganic substrates (accepts a wide range of hydrogen donors, including polyphenols such as hydroxycinnamic derivatives and flavans, the main phenolic structures implicated in enzymatic browning of fruit), with organic peroxides as terminal products (Lopes, 2015). The activity of POD is considered prejudicial to sensorial quality, as it generates **off-flavors** and **color alterations** in raw F&V (Kubo, 2017). PPO could act as a promoter for POD activity by producing hydrogen peroxide during the oxidation of phenolic compounds (Pradas-Baena, 2015). However, the role of POD in enzymatic browning is still not clear for two reasons: although in the presence of small amounts of hydrogen peroxide, POD can oxidize both mono- and diphenols PPO has a high affinity of PPO

for its natural substrate, and the very low hydroxide peroxide levels in vegetables tissues (Degl'Innocenti, 2005).

Lipoxygenase (LOX, EC 1.13.11.12) catalyzes the oxygenation of polyunsaturated fatty acids (PUFA) to form fatty acid hydroperoxides (FAHP). Some LOX isoforms generate FAHP destined for jasmonic acid, which triggers gene activation during wound response in plants(Dekker, 2002). In some plant foods (i.e. tomato and cucumber), LOX contributes to flavor formation (Thompson, 2010). Additionally, the oxidative activity of this enzyme has been linked with quality deterioration because of its involvement in **off-flavour** and odor production, **loss of pigments** such as carotenes (red-orange) and chlorophylls (green), and destruction of essential fatty acids (Baysal, 2006).

Physical

In the physical changes are included those that occur caused by external factors and can cause a stress in the plant tissue. For instance, damages caused during handling or the presence of oxygen leading to oxidation processes.

Damages or injuries take place when handling and operations are not carried out with sufficient care or attention. Cuts, scuffs, compression bruising, impact bruising and vibration bruising are included in this category (Thompson, 2010). It is one of the most common and severe defects; it has great economic repercussions, mainly due to negative changes in organoleptic attributes (skin and **flesh browning** and **off-flavors**) and internal breakdown reactions (Martinez-Romero, 2004). As these damages are not always visible, its occurrence can be detected in a later stage of the processing or marketing chain. Normally, bruises are accompanied by a number of serious quality deteriorations. Bruise damage on freshly harvested produce significantly affects physiological processes (respiration and moisture loss through the injured skin). In addition, the stress produced **accelerates or alters metabolic processes** (ethylene production, relative electrical conductivity, transpiration, senescence and nutritional composition) (Hussein, 2019). Moreover, F&V damages heighten the risk of **microbial contamination**. Rots and decay are more prevalent in mechanically damaged F&V, as they can surpass the natural barriers (peel) and have access to more nutrients that have been released with cell breakage. Decay microorganisms can also easily enter through the dead or wounded tissues and contaminate the rest of the F&V, causing its rejection and significant losses (Prusky, 2011).

Oxidation includes reactions in which a substance gains oxygen or losses electrons and hydrogen. Browning catalyzed by PPO and oxidation of lipids catalyzed by LOX have already been described in the enzyme section. This section will be focused on the oxidation of nutritional compounds, specially **vitamin C**. The ingest of this vitamin is essential because it cannot be metabolized by human body, and it is important because it acts as an antioxidant and cofactor for a family of regulatory enzymes, contributing to the immune defense, epithelial barrier function and protection against oxidative stress (Carr, 2017). Because L-ascorbic acid (AA) is easily converted (by oxidation) to L-dehydroascorbic acid (DHAA), both need to be determined in fruit to investigate the amount of vitamin C (Gil, 2006). This oxidation is reversible, and problem resides in the degradation of DHAA by oxidation or hydrolysis, which depending on the reactive oxygen species status, will result in different compounds with no vitamin C activity (Parsons, 2011).

Oxidative stress occurs when the generation of active oxygen species exceeds the capacity of the plant to maintain cellular redox homeostasis, or to scavenge them (Hodges, 2004). It has been well documented that after environmental stresses such as temperature extremes, salinity, drought, ozone exposure or UV light irradiation, oxidative stress increases, and the plant reacts by a series of mechanisms that include regulation of **antioxidant capacity** (in which many phytochemicals are included), and membrane composition (Hodges, 2004). When the plant is submitted to oxidative stress,

the plant responds in 3 steps: (1) alarm reaction, indicated by oxidative burst, increased production of superoxide anions or hydrogen peroxides, (2) resistance or acclimatization, consisting on modification of gene expression, enhancement of antioxidant enzymatic activities or non-enzymatic antioxidants, and (3) collapse or exhaustion, that derives in DNA or RNA damage, enhanced lipid peroxidation, loss of antioxidant enzyme activities, or increased membrane leakage, which leads to quicker F&V **deterioration** (Toivonen, 2004).

Biological

A biotic factor is a living organism that influences or affects its ecosystem, which in this context, is the fruit or vegetable. This category could be divided into macro biological and microbiological factors. In the latter, the epiphytic microbiota, or non-parasite microorganisms living in the surface of the product and the alterative microorganisms are included (Bailey, 2006).

Macro biological spoilage. Included here are the insects or arthropods, whose growth is typically promoted by humid environments, annelids, snails or rodents. A part from their undesirable presence in the product, their activity relays in the damages they can originate on the surface of the F&V. Insects may create an injury to lay their eggs, and the others typically damages the F&V by eating the outer or inner parts. This injuries in turn, have similar effects to those explained in the damages section, or constitute an entry way for spoilage microorganisms to colonize the fruit (Vincent, 2003).

Microbiological spoilage. Fresh F&Vs have an external toughness (i.e. water proof, wax-coated, skin) that acts as a barrier for entry of most plant pathogenic microbes. Those that attack the plant but are not pathogenic for humans are called spoilage microorganisms, as they typically alter the product (Hui, 2006). Most bacteria grow at pH values higher than 4.5, so it is difficult that they infect fruit. Bacteria that can be found in vegetables mainly includes Gram-negatives such as *Pseudomonas*, *Erwinia*, *Enterobacter* and *Lactobacillus* sp. (Siroli, 2015). Conversely, fungi, including yeasts and molds, can infect fruit as they can grow at pH values ranging from 2.5 to 6 (Thompson, 2010). Common molds involved in F&V spoilage are *Penicillium*, *Aspergillus*, *Eutorium*, *Alternaria*, *Cladosporium* sp., and *Botritys cinerea* (Lund, 2000). Typical yeasts found in fruit pertain to the genre *Saccharomyces, Zygosaccharomyces, Hanseniaspora, Candida, Debaryomyces,* and *Pichia* (Peter Ragaert, 2004). The occurrence of these microorganisms depends on the product (not only pH, but also water activity, sugar types and availability, and presence of other microorganisms - competitive microbiota) and also the processing operations (mainly cutting or peeling, which increases the exposed surface and nutrients, and the contamination caused by a poor handling or hygiene) (Francis, 2012). The spoilage microorganisms have an impact in F&V quality, affecting their flavor, texture and visual aspect. Studies that aim to increase shelf-life of F&Vs, must therefore, put attention on contamination load in order to prevent further deterioration of the products. **Flavor** is affected by the changes in volatile and non-volatile compounds following by microbial metabolism. Production of fermentative metabolites can occur when O_2 concentrations are low. Ethanol and acetaldehyde are the main compounds correlated with off-odors by a trained panel, and can be present directly after packagnig or due to a wounding response (Smyth, 1999). Other volatiles such as 2-methyl-1-butanol or 3-methyl-1-butanol have been found in spoiled F&V. Moreover, the production of different acids (lactic, acetic, malic, succinic, and pyruvic) has been described for stored FB (Ragaert, 2007). Texture is also affected, and microorganisms will typically cause a **softening** in the vegetable tissue. Several microorganisms are able to produce pectinolytic enzymes (pectin lyase, pectic lyase, polygalacturonase or pectin methyl esterases) and cellulases, that degrade the middle lamella and the primary cell wall (Laurent, 2000; Liao, 1997). According to various studies, the off-odors and softening in F&V effects are visible when bacteria and yeasts have reached populations of 8 and 5 log CFU / g, respectively (Ragaert, 2007). Other **visual**

appearance changes with the growth of microorganisms, producing a direct rejection from consumers are the growth of the hyphae of the molds, seen as filaments of various colors: green, blue, grey or white. The mold growth typically occurs in whole fruit, as other defects are determinant in fresh-cut fruit shelf-life (Mari, 2014).

2.2 Safety of F&V products

2.2.1 Pathogenic microorganisms

Although F&V are generally considered as safe and healthy products, they have been **associated with foodborne outbreaks**: when two or more people get the same illness from the same contaminated food or drink. The main binomes pathogen – food are shown in **Figure 11**.

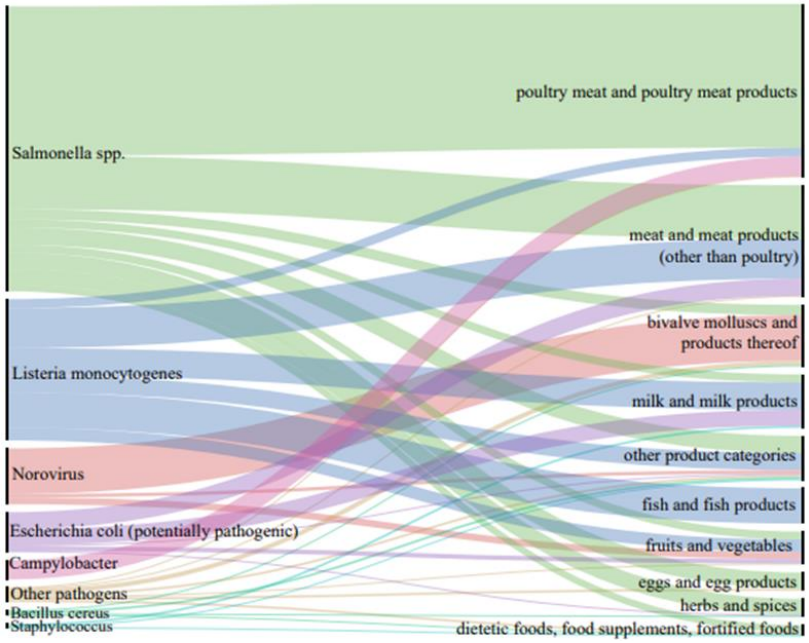


Figure 11. Binomial relationships between pathogenic agent and foods (Taken from: EFSA, 2013).

Pathogen occurrence in F&V can lead to foodborne diseases or outbreaks, depending on the number of people affected (**Table 3**). A **foodborne pathology** is a disease that is transmitted by food and caused by pathogenic microorganisms or their toxins, being food an active support for microbial growth or toxin liberation (Oromi-Durich, 2002). According to the Centre of Disease and Control Prevention (CDC), half of the foodborne outbreaks are related with fresh produce, mainly by the bacteria *Salmonella* spp., *Escherichia coli* (including the enterotoxigenic E. coli O157:H7), and *Listeria monocytogenes* (Painter, 2013). Other pathogens that can be found in fruit but in a lesser extent are the bacteria *Campilobacter* spp., *Bacillus cereus* and *Staphylococcus aureus*, and the viruses human Norovirus and Hepatitis A virus (RASFF, 2018).

The presence of pathogens in F&V is consequence of a **contamination** from different sources and in different ways in the food chain: they are produced in an environment that supports contamination, and cross-contamination can occur during post-harvesting, handling and plant-processing (Hui, 2006). In leafy greens, tubercles or fruit from creeping stems, soil, irrigation water and insects are the major causes of microbial contamination (Heaton, 2008). Although some pathogens may be naturally present in soil (*L. monocytogenes*), most of the contaminant bacteria (*Salmonella* spp., *E. coli*, and *L. monocytogenes*) can be found in the intestinal tract of animals like cows, pig or poultry, and even though they do not develop the disease, they act as vehicles, spreading them through the feces in water, or through the use

of the manure, that should be adequately handled (Emoke, 2013; Nicholson, 2005). In fruit that is collected from the tree, pathogens can be incorporated if good practices of harvesting and handling are not effectively applied: handpicking may contaminate the fruit surfaces with Staphylococcus, or with fecal-oral type microbes, including *Enterobacter*, *Shigella*, *Salmonella*, *E. coli* O157:H7 or *B. cereus*, as well as viruses like Hepatitis A virus, Rotavirus or human norovirus (Hui, 2006). In case of absence of pathogenic bacteria in F&V once in the food industry, cross-contamination could also occur due to transport or handling, or bad control of water disinfection process (i.e. dose of disinfectant, efficacy of disinfectant in presence of organic matter)(**Figure 12**) (Reij, 2004).

Table 3. Top-ten ranking of F&V and other vegetal products implicated in cases (1 person), outbreaks (2 or more people) and deaths (Adapted from: Porat, 2018)

Cases		Outbreaks		Deaths	
Strawberries	11553	Salad	202	Fenugreek	50
Radish	10126	Lettuce	84	Cantaloupe	37
Salad	9627	Raspberries	75	Lettuce	22
Raspberries	6970	Fruit	37	Coleslaw	17
Lettuce	6000	Alfalfa	33	Cucumber	7
Fenugreek	4018	Cantaloupe	24	Apples	7
Tomatoes	3231	Tomatoes	20	Celery	5
Alfalfa	3116	Bean sprouts	18	Spinach	5
Spices	2047	Spices	14	Salad	4
Fruit	2015	Onions	10	Bean sprouts	4

Some of the aforementioned **pathogenic agents** should be explained in more detail, for their importance in the recent outbreaks (**Table 3**). As it is reviewed by (Machado-moreira, 2019), a total of 571 outbreaks were identified from 1980 to 2016, accounting for 72,855 infections and 173 deaths. Contaminated leafy green vegetables were responsible for 51.7% of reported outbreaks, while contaminated soft fruit caused 27.8% of infections. Pathogenic strains of *Salmonella, E. coli*, norovirus, and hepatitis A accounted for the majority of cases. For this, one of the determinations of scientific research is to provide prevention and sanitation solutions to reduce the risk of food-borne pathogens in F&Vs. Some of them are reviewed in section 3. However, additional studies focusing on disinfection strategies for F&Vs with novel technologies are still needed.

According to the EFSA's Panel on Biological Hazard, ***Salmonella* spp**. is the most cited microorganism in the top-ranking combinations of foods and pathogens: *Salmonella* and leafy greens eaten raw, followed by *Salmonella* and bulb and stem vegetables; *Salmonella* and tomatoes; *Salmonella* and melons (EFSA Panel on Biological Hazards (BIOHAZ), 2013). This genre continues to be the most reported foodborne disease with high incidence rates per year at an estimated population of 2 million worldwide (Hui, 2006). In the last years, onions (*S. enterica* serovar Newport, 2020), cut fruit (*S.* Javiana, 2019), papayas (*S.* Uganda, 2019), pre-cut melon (*S.* Carrau, 2019 and *S.* Adelaide, 2018), salad (*S.* Typhimurium, 2018), raw sprouts (*S.* Montevideo, 2018), papayas (*S.* Urbana, *S.* Newport, *S.* Anatum, *S.* Thompson, *S.* Agona and *S.* Gaminara, 2017) or alfalfa sprouts (*S.* Reading, *S.* Abony, *S.* Muenchen and *S.* Kentucky, 2016) have been the cause of multistate outbreaks of F&V origin (CDC, 2021).

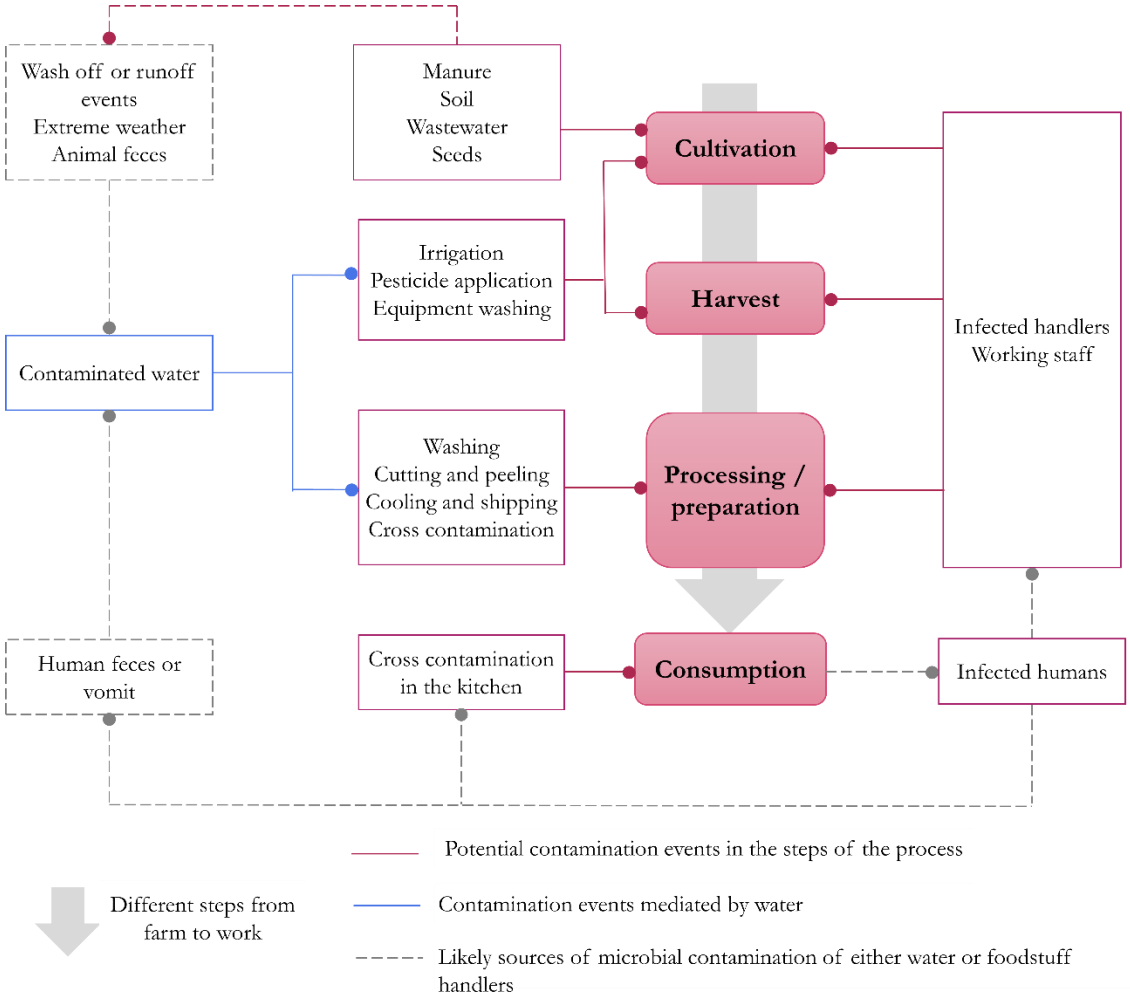


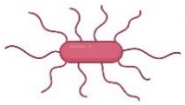
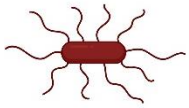
Figure 12. Microbial contamination of F&V and ready-to-eat foodstuff from farm-to-fork (Adapted from Reij, 2004).

The pathogenic ***Escherichia coli*** (shiga-toxin-producing, typically O157:H7) is also a great concern related to outbreaks, it has been highlighted by EFSA and specially linked with fresh pods, legumes or grains (EFSA Panel on Biological Hazards (BIOHAZ), 2013). It has been the main character of outbreaks related with clover sprouts (*E. coli* O103, 2020), romaine lettuce (*E. coli* O157:H7, 2019 and 2018), leafy greens (*E. coli* O157:H7, 2017) and alfalfa sprouts (*E. coli* O157:H7, 2016), and the CDC estimates that shiga-toxin-producing *E.coli* causes 3,600 hospitalizations and 30 deaths per year in U.S. (CDC website, accessed 2020-08).

Of all the pathogens found in F&V, ***Listeria monocytogenes*** has the highest mortality rate (it was responsible for the outbreak with the second highest mortality count (33 deaths out of 147 disease cases) included in the review (between 1980 to 2016) (Machado-Moreira, 2019), which resulted from the consumption of contaminated cantaloupes in the United States in 2011. Recent outbreaks related with *L. monocytogenes* have been linked with Enoki mushrooms (2020), packaged salads (2016), or bean sprouts (2014). This species represents a problem particularly in ready-to-eat food or fresh-cut fruit not only for its mortality but also because its **ubiquity and ability to grow and multiply during refrigeration,** as well as survive at relatively low water activity (Zhu, 2017). The EFSA assessed the risk to public health from *Listeria* contamination of vegetables that are blanched and frozen, and concluded that is lower than the ready-to-eat foods usually associated with this pathogen (EFSA Panel on Biological Hazards (BIOHAZ), 2020)

A review of produce-associated outbreaks in the USA from 1973–1997 found that **viruses** accounted for only 20% of outbreaks in which an agent was identified, most due to hepatitis A (Sivapalasingam, 2004). Since then, a surge in reported norovirus outbreaks occurred due to the improvements in diagnostics. This virus is now considered as the leading viral cause of foodborne illnesses (Widdowson, 2005). Most reported norovirus and hepatitis A outbreaks have been due to contamination of foods from the hands of infected workers at or close to the point of service, but other modes of transmission occur (Berger, 2010). A high number of outbreaks have been associated with the presence of norovirus in strawberries and berries, and for this, in 2014 the EFSA emitted a report on its Scientific Opinion on the risk posed by this virus in frozen berries (EFSA Panel on Biohazards (BIOHAZ), 2014).

Table 4. Main pathogenic agents implicated in F&V outbreaks (Adapted from Heaton, 2008; RASSF, 2010-2020; Predmore, 2011).

Pathogen	Characteristics	Symptoms	Contamined F&V
Salmonella spp. 	Gram-negative Rod-shaped facultatively anaerobic Enterobacteriaceae	Gastroenteritis (diarrhea, abdominal cramps, and fever) to enteric fevers (including typhoid fever)	Lettuce, sprouted seeds, melon, tomates, cauliflower, cress, endive, spinach, mushrooms, frozen strawberry
Listeria monocytogenes 	Gram-positive Rod shaped Motile Physcrotrophic Aerobic	Fiber, nausea, diarrhea, cramps, stiff neck, headache, abortion or premature delivery (in pregnant woman)	Mushrooms, vegetable mix, frozen corn, ready-to-eat salad, kale, cucumber, peppers, potato, radish, leafy vegetables, beansprout, broccoli, tomato and cabbag
Escherichia coli O157:H7 	Gram-negative Rod-shaped, Facultative anaerobic Enterobacteriaceae	Abdominal pain, watery diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome	Leafy vegetables, apples, cucumbers, sprouts, beetrot
Human norovirus 	Caliciviridae family Non enveloped Positive-stranded RNA virus	Diarrhea, vomiting, fever, chills, and extreme dehydration	Lettuce, tomatoes, melons, strawberries, raspberries, fresh cut fruit, and other vegetables
Hepatitis A virus 	Picornavirus Not enveloped Single-stranded RNA Packaged in a protein shell	Yellow skin or eyes Stomachache Fever Diarrhea / vomit Joint pain	Raspberries, strawberries, blueberries, salad

Considering that the mentioned outbreaks occurred in the last few years, there is still work to do regarding F&Vs microbiological safety, including the good handling practices, disinfection techniques and pathogenic growth control systems.

2.2.2 Other risks

Some concerns related with the chemical products aimed to reduce the number of microorganisms – pathogenic or not – have appeared and increased in the last decades. First, stricter regulatory policies have started to be imposed on the use of **pesticides**. The reasons for this are focused on human safety, the environment, and the development of pathogen strains resistant to most used products (Mari, 2014). Second, regulations on the use of **chlorine**: its reaction with organic matter present in water when used as a disinfectant for F&V origins trihalometanes, mainly trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane, that are toxic for the nervous system when inhaled, and may have long-term effects in liver, kidneys and heart (Environmental Fact Sheet, 2006). The first is not included in the scope of this thesis, and the second will be more extensively reviewed in section 3.1 Sanitation and disinfection

3 Strategies to achieve safety and maintain quality in F&Vs during shelf-life

To be sustainable in the F&V industry entails that the food chain is profitable throughout all of its stages (economic sustainability), has broad-based benefits for society (social sustainability), and has a positive or neutral impact on the natural environment (environmental sustainability). To simplify, to achieve economic and environmental sustainability it is necessary to prevent or decrease as much as possible the loss and waste occurring from the field to the fork, and to produce benefits for society, produce must be safe and possess a high-quality nutritive profile. It is of high importance, then, to prevent quality losses and increase the shelf-life of the products, so that a higher amount of fresh produce is worth consuming. Moreover, F&Vs cannot represent a risk; public health is one of the key points that must be preserved. For this, in the post-harvest working area, what can be done are techniques (Figure 13. Physical, chemical and biological strategies to increase safety and improve quality of F&V in different formats.Figure 13) for:

- reducing microbial load: in order to eliminate pathogens and reduce alterative microbiota, various disinfection methods using chemical or physical approaches must be applied,
- stablishing methods that allow to increase the shelf-life, i.e. hinder the natural processes in which F&V deteriorate, or boost some of the F&V abilities to stabilize during storage.

However, sometimes the intensity of the individual preservation techniques must be kept comparatively low, to minimize the loss of quality. For this, hurdle technology approach has been used for centuries to preserve food. **Hurdle technology** is an integrated approach of basic food preservation methods for creating safe, stable, yet nutritious foods. This concept aims to combine various mild methods that together achieve the purpose desired. The successful development of foods preserved by hurdle technology requires broad knowledge and deep insight into the impact of both, single and combined hurdles. It provides a framework for integration of individually mild preservation factors that in combination **achieve enhanced food quality while maintaining food safety and stability** (Mukhopadhyay, 2014).

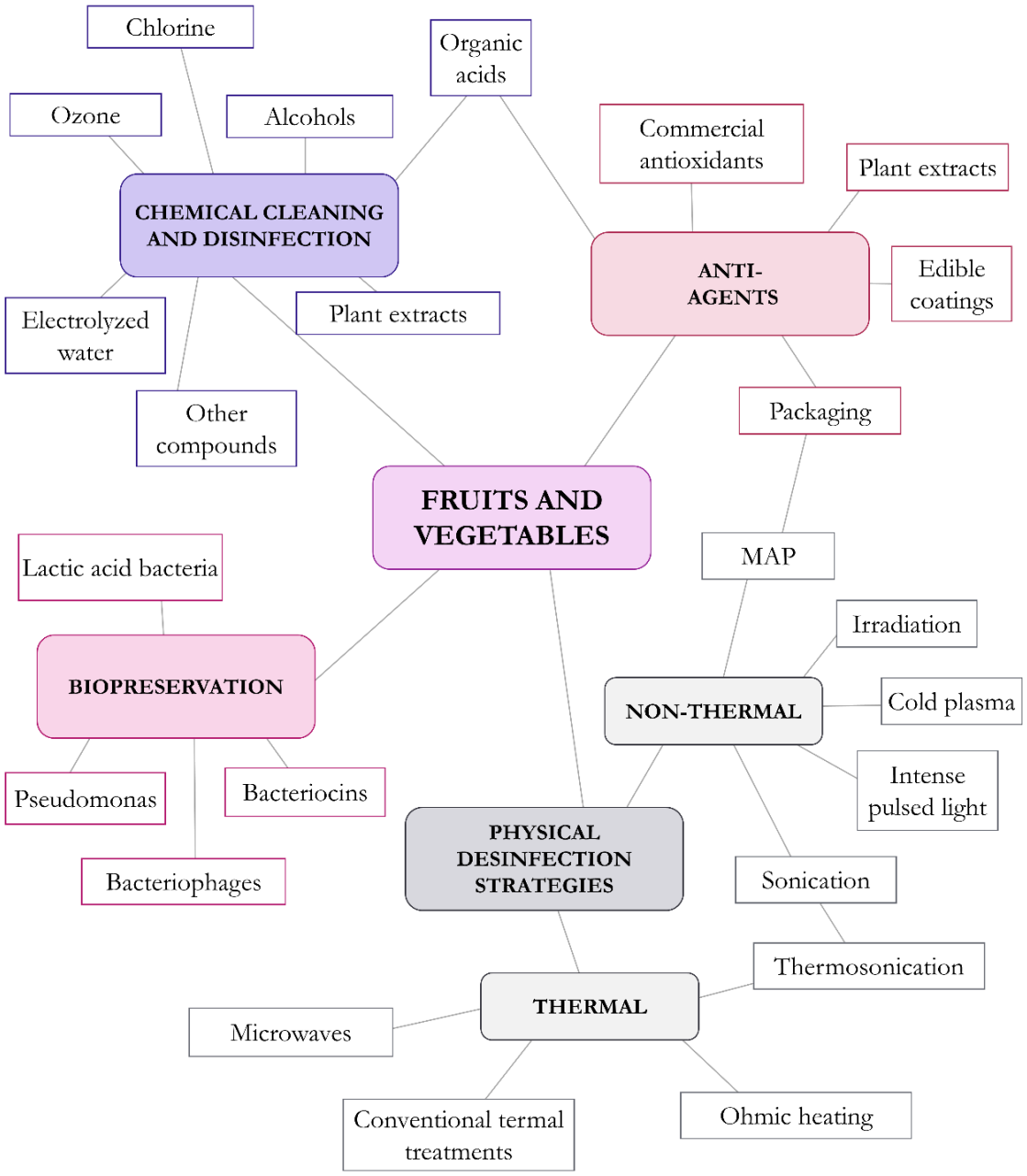


Figure 13. Physical, chemical and biological strategies to increase safety and improve quality of F&V in different formats.

3.1 Sanitation and disinfection

In the F&V industry, **sanitation or disinfection steps are essential to reduce microbial pathogens, as the produce is typically eaten raw and there is no further step in the processing that will reduce microbial populations** (e.g. cooking at home). However, pasteurization or application of high temperatures are not feasible choices, as they will affect a number of quality parameters such as firmness, color and flavor. Chlorine is the leading option, and apart from its drawbacks described below, society increasingly demands for what they call *less artificial, less chemical, less synthesized more natural, more environmentally friendly,* methods. Therefore, food industry is in the search of applications that are:

- ✓ Effective in inactivation of common and emerging pathogens
- ✓ Leading to less loss in product quality
- ✓ Adaptable to food processes
- ✓ Environmentally friendly

3.1.1 Chemical products

Chemical substances are often added to the washing water for two reasons: the disinfection of the processed F&Vs and the elimination of the pathogens that may have been dragged from the produce surfaces to water, in order to prevent cross-contamination.

Chlorine. Chlorine (Cl_2), normally applied as sodium hypoclorite (NaOCl), is the most common antimicrobial sanitizer used in the food industry. Its mode of action relays on hypochloric acid (HOCl) and hypochlorite ion (OCl^-), that are called free chlorine. The proportions in which they are present in water are variable and depend on the pH (Figure 14). As HOCl is the compound with most germicidal effect (owning to him the 80 % of the activity), optimal pH to use chlorine as a disinfectant is from 5.5 to 7.5 (Fukusaki, 2006).

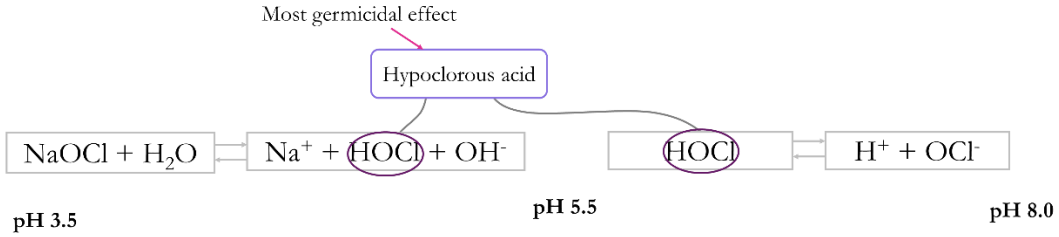


Figure 14. Forms of chlorine depending on the pH and dissociation.

HOCl can produce oxidation, hydrolysis and deamination reactions with a variety of substrates, producing physiological lesions that affect cellular processes. Cytochromes, iron-sulfur proteins and nucleotides are highly vulnerable to degradation by HOCl, suggesting that chlorine causes primarily damages to the cellular membranes (World Health Organisation, 2004). The main advantages of chlorine are that it is relatively easy to handle, the capital costs of chlorine installation are low, it is cost effective, simple to dose, measure and control (Freese, 1999). Its efficacy is yet questioned; although being useful in disinfecting water, it usually results in only 1–3 log reduction of most microbial counts on fresh produce (Brackett, 1999; Velez-Rivera, 2005). Although most disinfectants, like chlorine, may help in reducing microorganisms from washing water, they will not guarantee complete elimination of pathogens from produce. Some of the drawbacks about the use of chlorine as a disinfectant are related to its high dependence on the pH and its interaction with organic matter present in water, that leads to a loss of efficacy. In fact, this is related to one of the main concerns of the use of chlorine: its by-products. The reaction of chlorine with organic matter result in the formation of carcinogenic halogenated by-products including trihalometanes or haloacetic acids (Praeger, 2016). The safety of workers is also affected by the release of chlorine vapors, that are toxic when inhaled (Ramos, 2013). That's why its use has already been prohibited in several countries, such as the Netherlands, Switzerland, and Belgium (Deng, 2019). Since the discovering of chlorine by-products and the subsequent prohibitions, the search for equivalent alternatives that can compete with its versatility and cost has become a topic of study of many research groups (Fatica, 2017; Velez-Rivera, 2005; Yoon, 2018).

Organic acids. Organic compounds such as lactic, citric, acetic or tartaric acids, which are weak acids, are Generally Considered As Safe (GRAS) substances, by the Food and Drug Administration and the

European Comission (Lianou, 2012). Organic acids inactivate microorganisms by reducing the environmental and cellular pH of the cells, disturbing the membrane transport and permeability. The undissociated form of the acid enters the microbial cells and dissociates when encounters the near-neutral pH environment. It results in charged anions and protons resulting in acidification of the interior of the cell, that promotes inactivation intracellular enzymes and inhibition of metabolic reactions (Deng, 2019). Derived from acetic acid and needing a special mention is peracetic acid (PA). It is commercially available PA in the form of a quaternary equilibrium mixture containing acetic acid, hydrogen peroxide, PA, and water. Its disinfectant activity is based on the release of the active nitrogen, which oxidizes sulfhydryl and sulfur bonds in proteins and other metabolites. Intracellular PA may also oxidize essential enzymes, interfering in biochemical pathways, and act on the bases of the DNA of the molecule (Block, 1999). One of the main significant advantages a part of its germicidal effect is that decomposition products of PA (acetic acid, hydrogen peroxide, oxygen and water), has no to little toxic or mutagenic by-products after reaction with organic material (Kitis, 2004).

Ozone. Ozone (O_3) is formed by splitting the oxygen (O_2) molecule in the air by a high energy input. Then, single oxygen (O) molecules rapidly combine with available O_2 to form O_3, releasing heat and light (Suslow, 2004). As a disinfection method, it can be applied as a gas or in aqueous media but it decomposes quicker on the latter (Oner, 2016). Ozone destroys microorganisms by oxidizing progressively vital cellular components. The primary target of ozonation is the bacterial cell surface, and then it spreads oxidation to the internal cellular proteins, causing rapid cell death (Karaca, 2007). Ozone has also shown to eliminate off-flavors, mycotoxins and pesticide residues, while not affecting the overall quality of the produce. Despite the mentioned advantages, high ozone concentration or long exposure times (3-6 h) are needed to achieve similar reductions than those in chlorine disinfection, and excessive exposure to ozone (> 1 ppm) may cause damage to the respiratory tract, lungs and eyes of human, needing a good air-handling and an ozone destruction system (Deng, 2019).

Others. There are many other options that are being explored in the research and industry fields, including chlorine dioxide, slightly acidic electrolyzed water, high pressure carbon dioxide, washing with essential oils, etc. Until now, despite the disinfecting efficacy shown by such methods at lab- or pilot- scale, at bigger scale, where tons of product and liters of water are being processed each hour, its application is still in its early steps, as economic viability and practical feasibility must be deeper studied and optimized.

3.1.2 Technologies

In the last decades, some technologies have been proposed for they suitability to process F&Vs, as they do not reach high temperatures and have been reported to possess some antimicrobial effects:

Ultraviolet light. Ultraviolet light is the electromagnetic radiation in the wavelength range from 100 to 400 nm. The UV light spectrum is divided into four regions: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (200–280 nm) (Keklik, 2012). UV-C light is the most germicidal, as it can be absorbed by DNA, especially at 254 nm where the maximum absorption is achieved. When UV-C is absorbed by microbial DNA, it induces photochemical changes by forming mutagenic DNA lesions, being the two major classes the cyclobutane– pyrimidine dimers (CPDs) and 6–4 photoproducts (6– 4PPs), which are pyrimidine adducts. After UV irradiation the CPDs are the most abundant and probably most cytotoxic lesions but the 6–4PPs may have more serious, potentially lethal, mutagenic effects (Sinha, 2002). Traditionally, UV light irradiation has been used as a disinfectant for air, surface, and water. Recently, the food industry has shown increasing interest in the use of this technology, as it can offer multiple advantages: effective inactivation of a broad range of spoilage and pathogenic microorganisms,

minimal loss of the nutritional and sensorial quality of foods, no known toxic effects or residues from the treatment, and low energy consumption compared to other thermal and nonthermal pasteurization processes (Gayán, 2014). One of the main inconvenients of UV-C light is its low penetration, which limits its use to liquid products and to the surface of solid foods. Another is the shade effect occurring in complex surfaces of foods, which makes it more effective in smoother products (Deng, 2019). The main factors affecting UV-C efficiency are wavelength (the nearer to the maximum absorption in DNA, the better), the dose at which it is applied (which, in turn, depends on the lamps, the time, the configuration of the equipment and the optical properties of the media), and also on the target microorganism (it is suggested that Gram-positive bacteria are more resistant to UV than Gram-negative are thanks to the thicker peptidoglycan, which may hinder the penetration of UV light) (Beauchamp, 2012). For this, it is important that F&V industry, in the use of UV light in the disinfection step, should consider all these factors and have access to the scientific knowledge, in order to apply this technology at its higher potential.

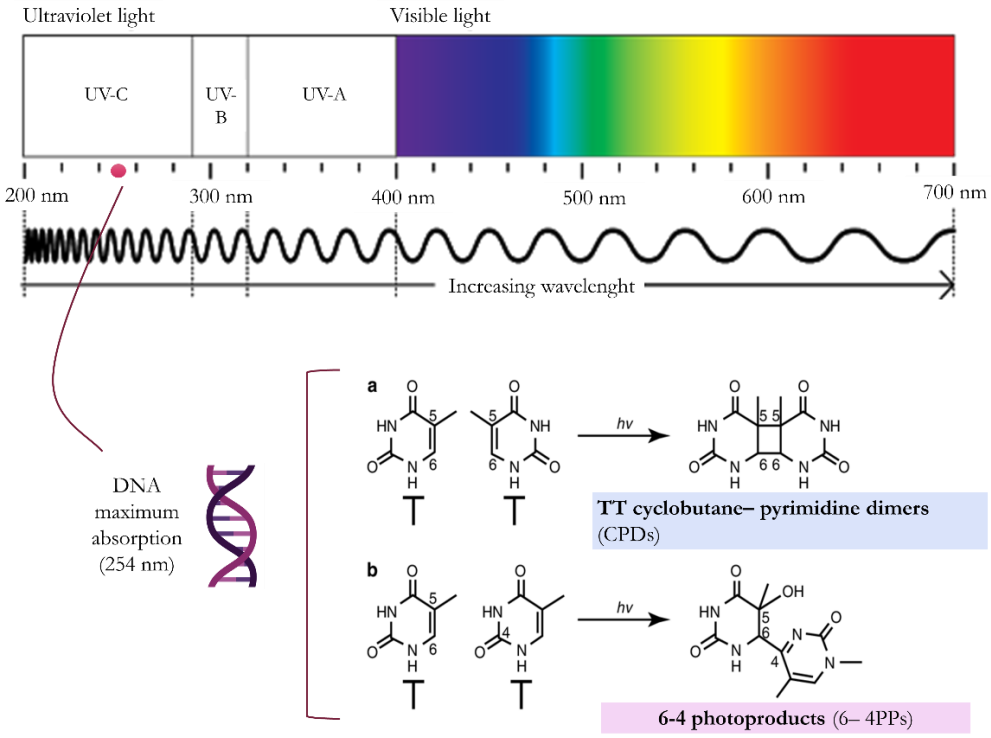


Figure 15. Ultraviolet C light (UV-C) location in the light spectrum (245 nm) and the main products when it interacts with DNA.

Ultrasounds. This technology consists on the use of ultrasonic waves at a frequency beyond 18 kHz with a specific intensity and amplitude (Bevilacqua, 2018). As it will be explained in more detail in Chapter __ (a review on the use of US in F&Vs), US may cause the loss of viability of microorganisms by destroying cellular envelopes and other microbial components. This is attributed to transient cavitation, or the formation of microscopic bubbles due to cycles of pressure caused by US. The extremely high temperature and pressure lead to the collapse of the generated bubbles, and consequently, molecules collide causing microorganism inactivation (Leong, 2017; Pérez-Andrés, 2018) (**Figure 16**).

However, the actual outcomes are not as effective as they should for F&V disinfection, and US is typically combined with other substances or technologies. As not only bacterial cells can be disrupted, and also free radical formation occurs (the high pressure and temperature reached within the bubbles

promotes the generation of primary hydroxyl radicals and the acceleration of single electron transfer), other components of the food can overcome chemical and structural changes affecting quality and nutritional values of the produce (Bilek, 2013; Leong, 2017). Despite this, US technology is still being investigated for the improved effectiveness of sanitizers when applied together with this technology, as it is reported that US helps aqueous sanitizers and substances dissolved in water to penetrate inaccessible sites (Deng, 2019). Moreover, according to Chemat (2011, 2017), it is a green food processing technology (it saves energy and water, and has a reduced carbon and water footprint when compared with other techniques), and it also provides improved processing times and enhanced quality for processes like freezing and crystallization, drying, degassing, emulsification, and demolding.

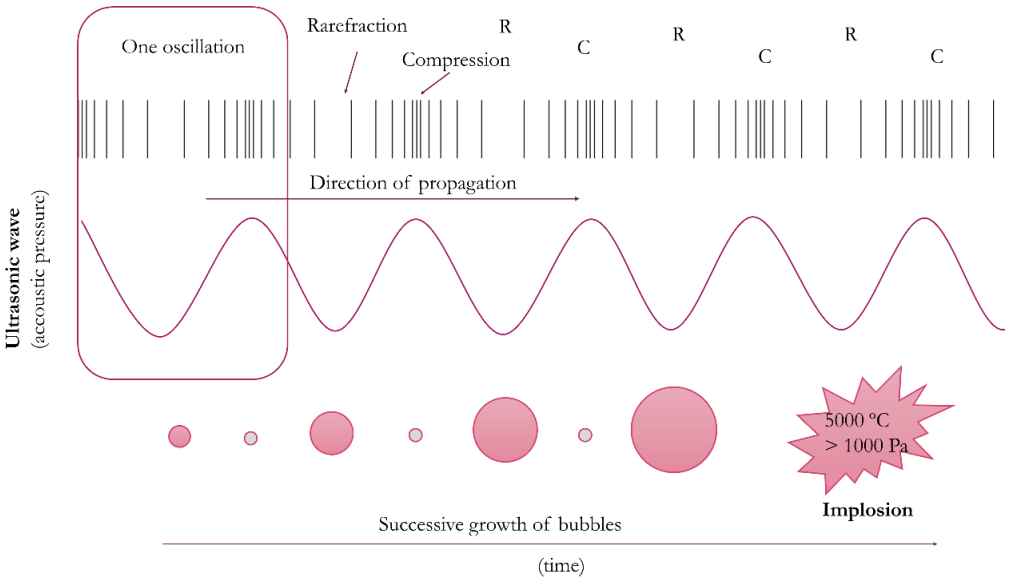


Figure 16. Acoustic pressure in ultrasonic wave, with successive cycles of rarefraction and compression, leading to growth of microbubbles until an implosion that occurs at 5,000 °C and more than 1,000 Pa (Adapted from Leong, 2011)).

Others. Other disinfection technologies are pulsed light, ionizing, radiation, high hydrostatic pressure, or cold plasma. Emerging technologies have shown improved microbial safety while maintenance of quality of the product. However, when applied alone in fresh produce, it might not meet the requirements of food safety and higher product quality. To maximize the lethality against microorganisms and minimize deterioration of produce quality, optimization of the parameters applied and combination with other techniques is often necessary.

3.2 Maintenance of F&Vs quality and safety during storage

Once disinfected, spoilage microbiota should have been reduced, so it is natural to expect a decreased alteration for this cause. Additionally, some of the aforementioned techniques (e.g. ultraviolet, ultrasound), may have an impact on the biochemical and cellular structure that may affect the evolution of the produce during storage. Despite this, it is necessary to implement further hurdles in order to completely assure safety and quality maintenance during storage. In general, each product has a related problematic (**Table 5**), depending on its intrinsic factors (climacteric or not, enzymatic activity, format: whole or fresh-cut, pH, possible contaminations…), that should be individually assessed in order to design the optimal combination of techniques for its preservation.

3.2.1 Low temperatures

In general, the lower the storage temperature, the longer the foods can be stored, and chilling and freezing continue to be important methods of processing and storing F&Vs for a longer shelf-life. Despite microorganisms and enzymes are mostly inhibited, these operations do not destroy them. Careful control is needed to maintain low temperatures, as any increase could therefore permit the growth of pathogenic bacteria or increase the rate of spoilage (Fellows, 2000).

Table 5. Main deterioration reactions in F&V occurring during storage (Adapted from Ma, 2017)

Product	Main deterioration
Apple (fresh-cut)	**Browning**
Pear (fresh-cut)	Browning
Mango(fresh-cut)	Browning, decay
Melon (fresh-cut)	**Juice leakage, softening**
Lettuce(fresh-cut)	Color changes
Potato (fresh-cut)	**Browning**
Carrot (fresh)	Loss of moisture, lignin formation
Strawberries (fresh)	**Softening, decaying**
Broccoli (fresh)	Chlorophyll degradation

Chilling. Chilling is the decrease of temperature in which F&Vs are stored, in order to decrease the rate of biochemical changes caused by either microorganisms or naturally occurring enzymes (whose action increases logarithmically with temperature (Fellows, 2000). For fruit, this temperature normally ranges between 0 and 5 ºC, except for tropical and subtropical fruit, that can suffer from chilling injury at 3-10 ºC above their freezing point (Hendley, 1985). Chilling prevents the growth of thermophilic and many mesophilic microorganisms, so the main microbiological concerns with chilled foods are a number of pathogens that can grow during extended refrigerated storage below 5ºC, such as *L. monocytogenes* (Bell, 2005)

Freezing. In frozen products, the temperature is reduced below the freezing point, leading to a change of a proportion of the water to form ice crystals. In this kind of products, preservation is achieved not only by a decrease in temperature, but also in a reduced water activity and, in some products like vegetables, by a previous blanching. In the F&V industry, freezing is used for fruits like berries and strawberries, and mainly for vegetables. In such products, there are only small changes to nutritional or sensory qualities of foods when correct freezing and storage procedures are followed (Fellows, 2000).

3.2.2 Substances with a specific functionality

Specially for fresh-cut products, compounds of different nature are added to the F&Vs surface (typically by immersion or spraying) in order to prevent deterioration during storage. What limits the use of this substances, once declared GRAS, are the requirements for their *invisibility*: consumers should not be able to detect or to be affected by their flavor or color when added to the product.

Purposes of these substances are acting as antioxidants, antimicrobials or texturizers. Substances with antioxidant properties are often incorporated to prevent reactions that involve degradation of nutrients (i.e. vitamins) or phytochemical bioactive compounds, in benefit for the F&Vs stability and human health. Also, these compounds will prevent oxidation of the product leading in browning or other changes in color, which are visual defects negatively affecting consumer's opinion. Antimicrobial compounds are essential to maintain populations low during storage, preventing the growth of epiphytic microbiota that alters the aspect and flavor of the F&Vs, and in case of being contaminated,

the increase of pathogen populations that could grow even in refrigeration (i. e. *L. monocytogenes*). Texturizers are basically used in fresh-cut products to prevent firmness losses. A product that has been used for this purpose for decades is calcium salts, which interact with the components of the cell walls and membranes, providing structural integrity (X. Deng, 2008).

A wide range of **substances** with antioxidant and antimicrobial properties have been proposed, ranging from organic acids, phytochemicals, or essential oils. Researchers and industries are in search of *non-synthetized* functional compounds, i.e. that are obtained from natural sources (plant extracts, essential oils) or from by-products (in this case, the parts that result from the processing of a food but it is not the product itself). This option is worth exploring because it adds value to a product that otherwise would be destined to feed become a residue, and also contributes to a circular economy, giving the resources a continual use. However, sometimes the extraction of functional components from by-products is not economically feasible, so by-products would then be used as they come from the industry. Some of the substances and compounds that are being used include:

- Essential oils (EOs) are defined as the volatile secondary metabolites of plants that give the plant a distinctive smell, taste, or both. They have been reported to possess antimicrobial activities attributed to their composition: EOs are rich in phenols like carvacrol, eugenol, thymol, whose hydroxyl group in the structure gives them the ability to permeabilize and depolarize the cytoplasmic membrane of microorganisms (Saad, 2013). Antioxidant activity has also been reported for most EOs; capable to stabilize free radicals thanks to phenolic compounds and terpenes with conjugated double bonds, which act as donors of hydrogen or/ and electron (Olszowy, 2016). For EOs to be a tool to be extensively applied in F&Vs, research will need to be conducted to evaluate organoleptic acceptability and applicability industry (temperature, washing times) (Winska, 2019).
- Organic acids have already been mentioned in section 3.1 as alternatives for F&V disinfection. They are also added as antioxidants in these kind of products, especially ascorbic acid. The European and national legislations, supported by numerous scientific studies, define the possible use of ascorbic acid (E-300) according to the principle of *quantum satis*, and it can be used in foods for children (Varvara, 2016). Another example is ferulic acid is a phenolic acid that can be naturally found in the cell wall of plants including bran and fruit seeds, giving structure and protection. It has proven to have a wide variety of biological activities such as antioxidant and antimicrobial, and could be a potential compound with functionality for food preservation (Kumar, 2014).
- Other plant extracts (actives with desirable properties that have been removed from the tissue of a plant, usually by treating it with a solvent, to be used for a particular purpose) are rich in phytochemicals, namely phenolic compounds, which are majorly accountable for the antioxidant and antimicrobial effects of such compounds (Kähkönen, 1999). Non-volatile secondary metabolites of plants are in this category, which include amino acids, lectins, glycoproteins, flavonoids, tannins, quinones, coumarins, terpenoids, steroids and alkaloids (Regnier, 2012). Typically, the optimal extraction mode to obtain these compounds from the plants involves the use of organic solvents, but unfortunately, these solvents have been associated with health risks (Rosenberg, 2004). Efforts must be done in this direction to obtain the maximum extraction yield from a plant from a safe extraction. In this regard, the Agrimax project, aims to extract significant amounts of valuable compounds contained agri-food wastescombining affordable and flexible processing technologies (ultrasound assisted and solvent extraction, filtration, thermal and enzymatic treatments) for the valorization of side streams from the horticultural culture and food processing industry to be used in a cooperative approach by local stakeholders. Initiatives like this are appearing to fulfill a need to replace existing preservatives (e.g. nitrites, sulphites) from those

of natural origin. Reports on the potential of several plant extracts like soy, green tea, berry, ginger (Santos-Sanchez, 2017), from extracts of tropical fruits like açai, noni or mango (Nowak, 2018), or Mediterranean plants like chicory, sowthistle or poppy (Schaffer, 2005), or even plants used in Chinese medicine, including ginger, ginkgo and ginseng (Chinaza-Godswill, 2017) demonstrate the capability of such plant extracts to be applied in food as natural preservatives. Although the exact mechanism of plant extracts is not yet fully understood, it is acknowledged that they have diverse sites of action at the cellular level. It is accepted that phenolic acids, flavonoids and other phenolic compounds act as antioxidants by chelating the ions and scavenging he free radicals particularly, superoxide (O_2^-), peroxyl and hydroxyl radicals (OH-). Antioxidant activity usually increases with an increase in the number of hydroxyl groups and a decrease in glycosylation (Fukumoto, 2000). Some plant extracts have also been reported and used for their antimicrobial activity, that results from the synergistic action and characteristics of their main components, that interact with microbial membrane or enter the cell itself, causing alterations in their metabolism and homeostasis (Bouarab-Chibane, 2019). Antimicrobial and antioxidant compounds can be added directly to the formulation of perishable food products or incorporated into food-contact materials to release them in the immediate zone of perishable foods (e.g. edible coatings or active packaging).

3.2.3 Modified atmosphere packaging

Modified atmosphere packaging (MAP) results of changing the composition of the atmosphere in the packaging headspace, resulting from the dynamic interaction between metabolic processes of the packaged product (fresh F&Vs continue with their respiration and metabolic processes after harvesting and during storage consuming O_2 and releasing CO_2 and water vapor), and the transfer of all these gases through the package. The aim is to balance these two processes to reach constant levels of these gases in the packaging headspace, and that these equilibrium levels are as favorable as possible to preserve the product (Castellanos, 2017). Benefits of using MAP are reduced respiration, ethylene production and sensitivity; retarded softening and compositional changes; palliation of the symptoms of physiological disorders; and reduced decay (Kader, 1989).

3.2.4 Edible coatings

Edible films or coatings are thin layers of edible material (typically GRAS and accepted by the FDA, the European Union standards, or the Codex Alimentarius) that cover the product surface (in addition or as a replacement for its natural protection) to provide barrier to moisture, oxygen and solute movement. They are applied directly on the fruit or vegetable by dipping, spraying or brushing (Dhall, 2013; Yousuf, 2018) .

The **function** of edible coatings is to delay the processes for which F&Vs deteriorate. Coatings with selective permeability to gases are capable of decreasing the interchange of O_2 and CO_2 between the product and the environment, slowing down the metabolism by decreasing internal O_2 concentration and increasing CO_2 concentration (Olivas, 2005) (FI). Increased CO_2 will also reduce the synthesis of ethylene, an hormone that is essential for ripening, thus delaying it (McHugh, 2000). Also, they provide a barrier to water vapor, preventing the loss of moisture and the subsequent changes in firmness and visual aspect (Yousuf, 2018). Moreover, edible coatings can serve as carriers for active ingredients, whose antioxidant, anti-browning, antimicrobial functions will be added to the already benefits of the edible coatings themselves (Rojas-Graü, 2009).

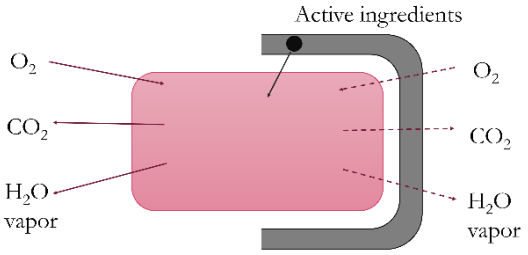


Figure 17. Schematic action mode of edible coatings (right): limitation of the gas interchange between product and environment, and vehiculation of active ingredients for the coated foodstuff.

The **composition** of edible coatings is based on in solvent such as water, alcohol, or a mixture, with polysaccharides, proteins, lipids, and composites with film forming abilities. Plasticizers and other agents can be added to improve their properties (Brecht, 2004). Polysaccharides are often obtained from marine and agricultural plants and animals (e.g. cellulose, starch, chitin and chitosan, pectin, alginate, and carrageenan), that have different barrier properties and stabilities, but in general, their hydrophilic nature makes them to not function well as physical moisture barriers. Proteins (e.g. gelatin, casein, whey protein, corn zein, soy isolates, keratin, collagen) are hydrophilic in nature that makes them absorbing humidity. In general, they need to be denatured (heat, acid, solvent) in order to extend their structures, and then they can be associated by hydrogen, ionic, hydrophobic, and covalent bonding, that makes them stronger and less permeable to gas exchange. Lipid based coatings have been used for 800 years (waxes), and nowadays, they are mainly used for their hydrophobic properties (e.g. wax, oil-based, resins, fatty acids and monoglycerides), which make them good barriers to moisture loss, but in turn, lipids form thicker and more brittle films. Composite coatings are heterogeneous in nature, consisting of a blend or a bilayer of polysaccharides, protein, and/or lipid carbohydrates and lipids, or synthetic polymers and natural polymers. The main objective of producing composite films is to improve the permeability or mechanical properties as per the need of specific application (Dhall, 2013).

In the present thesis, two edible coatings have been proposed: sodium alginate film casted with calcium lactate and *Aloe vera* gel.

- Alginate casted with calcium is a coating that has been widely studied. (The innovation in this thesis is the double casting step, which will be described Chapter 10). Alginate is naturally occurring polysaccharide with is produced and refined from various genera of brown algae (Skurtys, 2010). The molecular structure of alginates consists of unbranched, linear binary copolymers of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues linked by 1–4 glycosidic bonds. Depending on the source, the ratio M : G residues vary, giving different physical and chemical properties to the coating solution (Martinsen, 1989). Alginate gel formation relies on the cross-linking process, which is a conformational change originated by the introduction of divalent ions (usually Ca^{2+}). It causes the alignment of the G blocks forming an egg-box model due to the boundaries between the two chains by calcium ions, forming divalent salt bridges (Younes, 2017). The review by Senturk-Parreidt (2018) gives a deeper insight on the additives used to improve the coat performance of alginate casted with calcium lactate, as well as the incorporation of functional compounds when it is used as an edible coating.
    - *Aloe vera* gel is a tasteless, colorless, and odorless material that can be used to prolong the shelf-life of the coated products by possessing itself antioxidant and antimicrobial activities (Misir, 2014). These functional properties are derived from the integrated activity of its main characteristic compounds: anthraquinones aloe emodin and aloin. Due to its organic nature and

the mentioned properties, *Aloe vera* gel has created a lot of interest for exploring its potential in extending shelf-life of F&Vs. It has been applied to fresh and fresh-cut F&Vs, alone or with plasitcisers and other gel-forming agents, in order to improve its coating properties and provide better water vapor or oxygen permeability/resistance, water-solubility and mechanical strength, that are the critical factors determining the performance of edible coatings (Sarker, 2021).

As throughout the Introduction section has been reviewed, there is a wide range of physical or chemical technologies, both for disinfection and shelf-life increase, to be applied in F&Vs. However, their adequacy must be studied for each individual case: F&V intrinsic characteristics, main problematics and commercial formats must be taken into account in order to choose the meet the requirements with the solutions. In this regard, the present thesis aims to explore some technologies and chemical substances for F&Vs safety and quality.

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Objectives

The aim of this work is to find feasible **methods to increase safety and quality of fresh and fresh-cut fruits and vegetables**. This thesis is framed in the development of two projects: **FRESAFE**, *Estrategias de mitigación de problemas asociados a patógenos de transmission alimentaria para mejorar la calidad e inocuidad de fresas congeladas y listas para el consumo*, which has received funding from the Spanish Ministry *Ministerio Español de Economía, Industria y Competitivdad* (AGL2016-78086-R) and ***AGRIMAX*** which has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation program (grant agreement No. 720719). These projects stablished the main objectives of the present thesis, which have been later diversified (sub-objectives) in order to deepen in the treated topics.

Objective 1. Finding alternatives to chlorine as a sanitizer, focusing on strawberry for its safety problems.

- **1.1** Evaluating peracetic acid (PA) and water UV-C light (WUV-C) as sanitizing methods and their impact in the quality of strawberries.
- **1.2** Assessing the effect of a disinfection using PA combined with WUV-C in the shelf-life of whole, fresh-cut, and frozen strawberries.
- **1.3** Evaluating the viability of ultrasound (US) or thermosonication (TS) as a sanitizing method for strawberries and the impact of these technologies in the quality of strawberries.

Objective 2. Using plant-derived extracts and technologies in order to increase shelf-life of fresh-cut F&Vs.

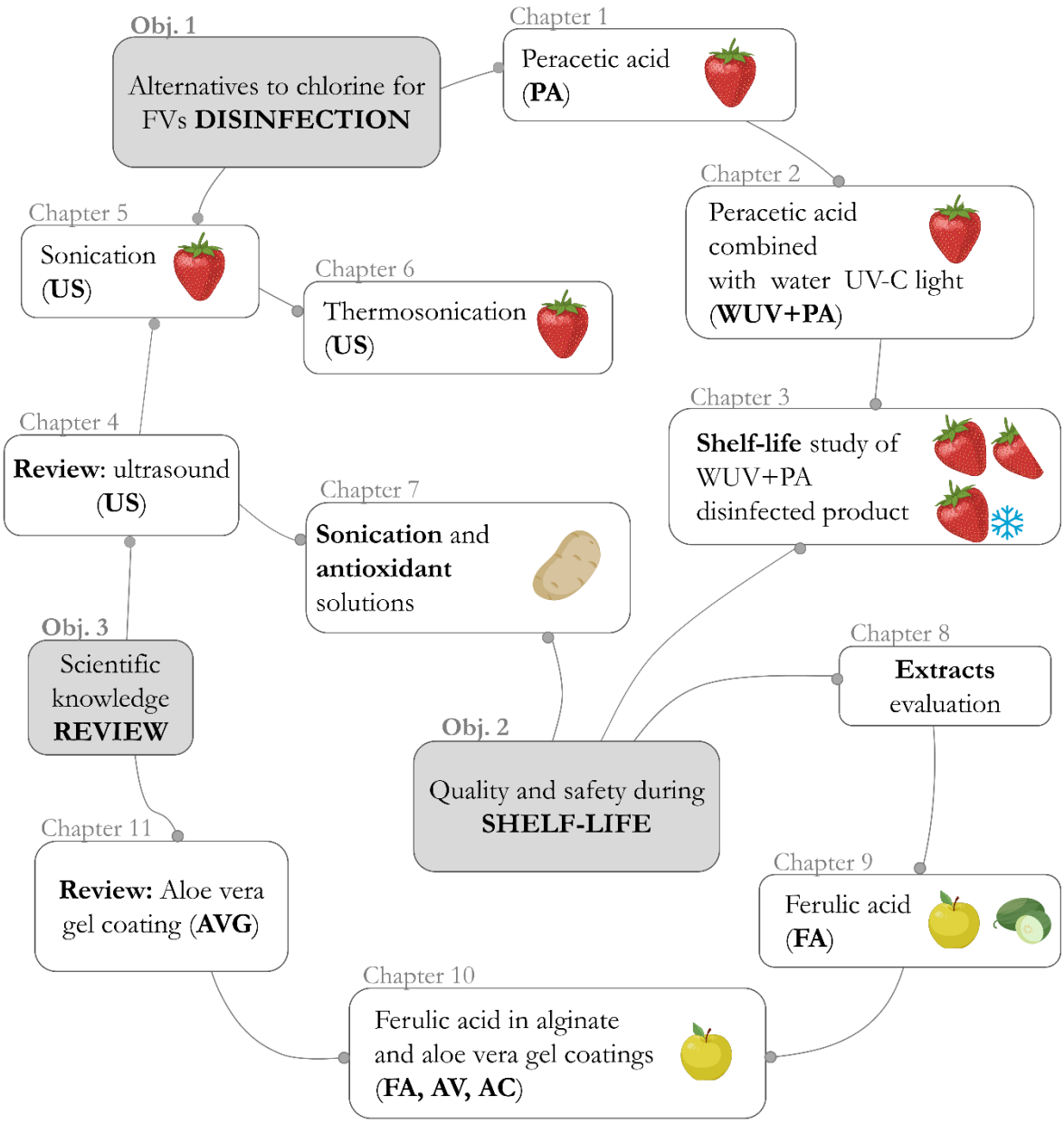
- **2.1** Evaluating solutions to prevent browning in fresh-cut potato: the use of antioxidants (green tea extract and Naturseal ®).
- **2.2** Exploring plant-derived extracts (ginseng extract, lyophilized of a fermented noni juice, and ferulic acid) to elucidate their functional activities of interest to be applied in F&Vs (antioxidant, antimicrobial).
- **2.3** Application of the most suitable extract (ferulic acid, FA) to prevent the growth of pathogenic bacteria and to decrease browning in fresh-cut apple and melon, and to combine its application with a coating (*Aloe vera gel* or alginate) to increase shelf-life of fresh-cut apple.

Objective 3. Contributing to the collection and organization of the existing knowledge about methods to improve safety and quality of F&Vs.

- **3.1** Reviewing the scientific production on ultrasound (US) treatments applied to fresh and fresh-cut F&Vs.
- **3.2** Reviewing the scientific production on the use of *Aloe vera* gel (AVG) as a coating for to fresh and fresh-cut F&Vs.

Methodology

To accomplish the stated objectives, research on each topic has been carried out, and its outcomes have resulted in scientific publications for international journals, that are presented as chapters in this thesis. Indeed, all the objectives and their related chapters are interconnected. A schematic abstract is presented below, detailing the relationship between the proposed alternatives in this thesis and the objectives they fulfill, as well as the F&V matrices on which they have been applied. The methodology used in each chapter is briefly explained hereafter, as a complete description will be found in the corresponding chapter.

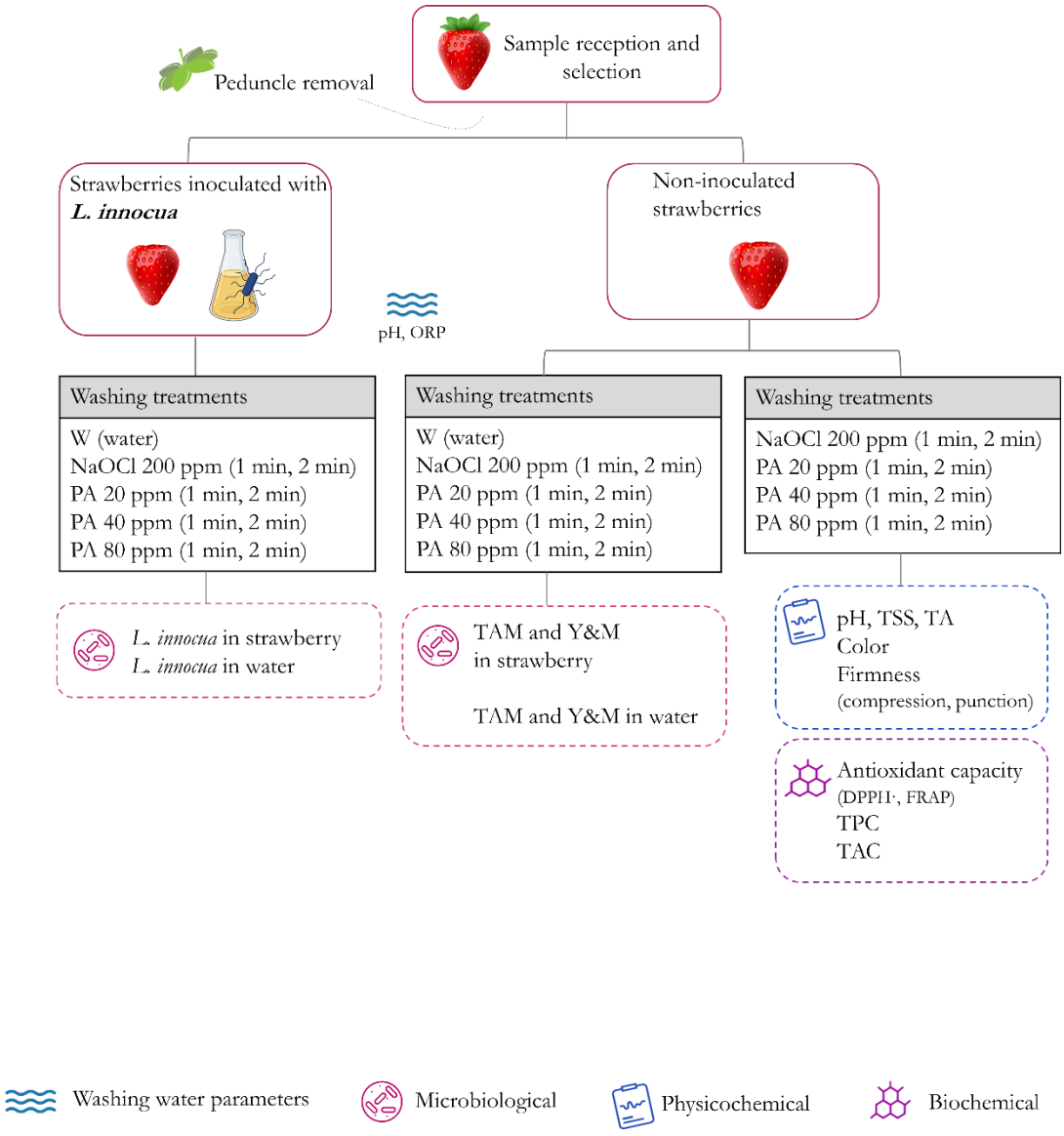


Chapter 1 Strawberry sanitization by peracetic acid washing and its effect on fruit quality

In response to the European Food Safety Agency (EFSA) publication on the risk posed by pathogenic microorganisms in frozen strawberries, the FRESAFE project aimed to find alternatives to chlorine as a sanitizer, as it has some side problems (such as the interaction with organic matter and the production of toxic vapors). A proposed sanitizer was peracetic acid (PA), whose efficacy was evaluated against artificially inoculated *Listeria innocua* (as a surrogate of *L. monocytogenes*) on strawberries. This was compared to the efficacy of chlorine, to check if the disinfection would be comparable (Objective 1.1).

In this chapter, three different concentrations of PA (20, 40, and 80 ppm) were tested for different times (1 or 2 min) for the disinfection of strawberries. Simultaneously, a chlorine solution (NaOCl, 200 ppm) was used as logarithmic reduction reference in artificially inoculated *L. innocua*, and natural occurring total aerobic mesophylls (TAM) and yeasts and molds (Y&M) populations. Moreover, one of the purposes of the use of chlorine in the F&Vs industry, is the sanitation of washing water in order to be reused preventing any further cross-contamination. To confirm the adequacy of peracetic acid for this purpose, remaining populations in washing water were also studied after the washing treatments.

In addition, the maintenance of fruit physicochemical quality after processing is of high importance to meet consumers' demands. For this, the impact of the tested sanitation condtions on pH, total soluble solids (TSS), titratable acidity (TA), color and texture were evaluated. Also, it was verified whether the PA washing affected the biochemical parameters of strawberry, including its antioxidant activity by two methods (DPPH· and FRAP), the total phenolic content (TPC), and the total anthocyanin content (TAC).

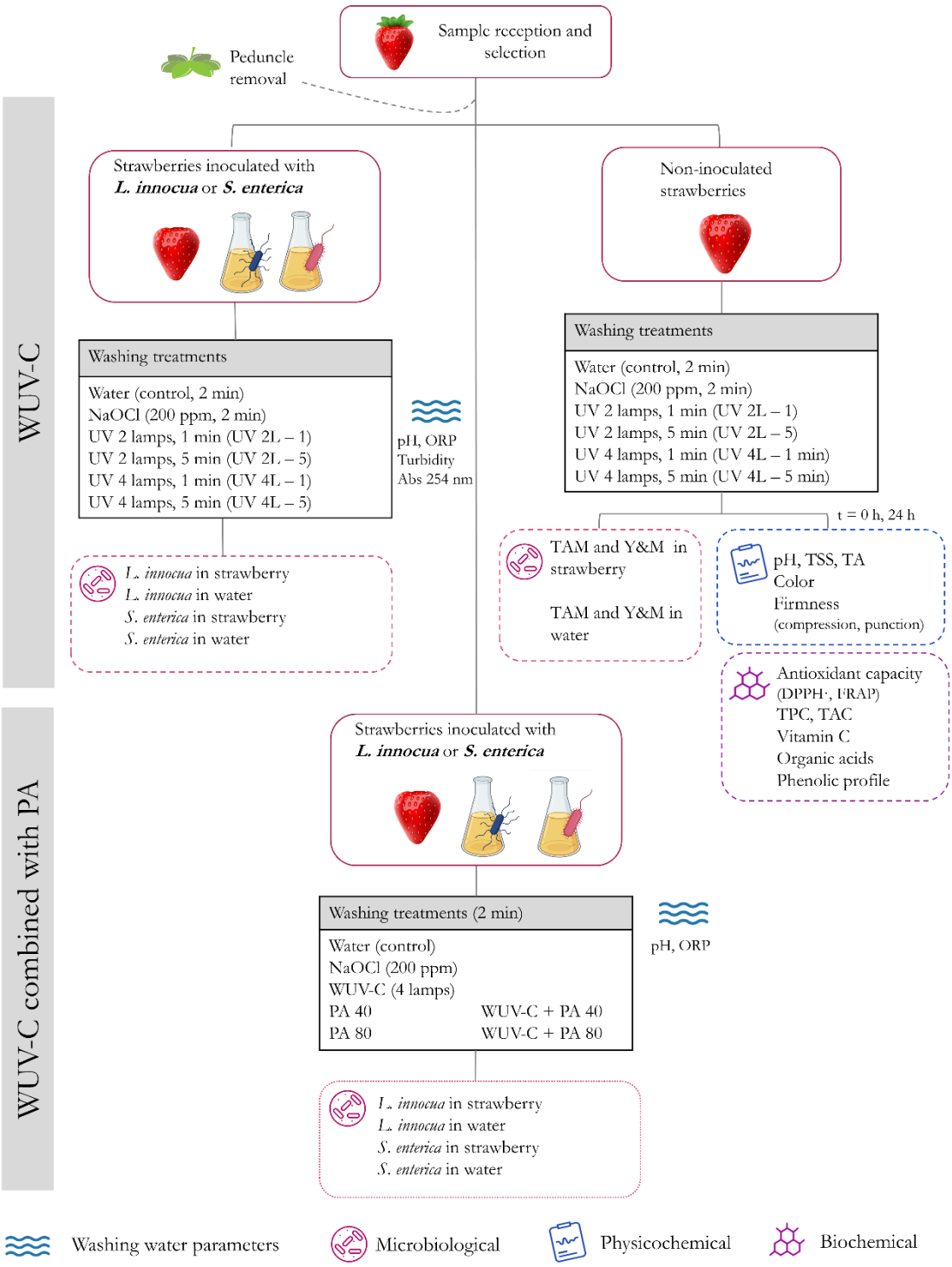

Peduncle removal
Sample reception and selection
Strawberries inoculated with *L. innocua*
Non-inoculated strawberries
pH, ORP
Washing treatments
W (water)
NaOCl 200 ppm (1 min, 2 min)
PA 20 ppm (1 min, 2 min)
PA 40 ppm (1 min, 2 min)
PA 80 ppm (1 min, 2 min)
Washing treatments
W (water)
NaOCl 200 ppm (1 min, 2 min)
PA 20 ppm (1 min, 2 min)
PA 40 ppm (1 min, 2 min)
PA 80 ppm (1 min, 2 min)
Washing treatments
NaOCl 200 ppm (1 min, 2 min)
PA 20 ppm (1 min, 2 min)
PA 40 ppm (1 min, 2 min)
PA 80 ppm (1 min, 2 min)
L. innocua in strawberry
L. innocua in water
TAM and Y&M in strawberry
TAM and Y&M in water
pH, TSS, TA
Color
Firmness
(compression, punction)
Antioxidant capacity
(DPPH·, FRAP)
TPC
TAC
Washing water parameters
Microbiological
Physicochemical
Biochemical

Chapter 2 Water UV-C treatment alone or in combination with peracetic acid: A technology to maintain safety and quality of strawberries

In this chapter, the germicide fraction of ultraviolet light (UV-C, from 100 to 280 nm), that is already being used in the disinfection of surfaces and water, is suggested as an alternative to chlorine in the sanitation of strawberries. A prototype device consisting on a tank with four UV-C lamps on and a recirculating and aerating systems was used for this purpose (Objective 1.1). This system was aimed to overcome one drawback of UV-C light: the shadow effect, as the agitation of strawberries inside the tank permitted that all sides of the fruit could be irradiated. Moreover, the fact that it is conveyed by water, could enhance the physical removal of the microorganisms from the surface to the water by dragging, being then inactivated by the UV-C light.

These possibilities were investigated by trying different lamps on (2 or 4) for different washing times (1 or 5 min). As well, a chlorine solution (NaOCl, 200 ppm) was used as a logarithmic reduction reference in artificially inoculated *L. innocua* and *Salmonella enterica*, and natural occurring total aerobic mesophylls (TAM) and yeasts and molds (Y&M) populations, both for strawberries and water. Immediately after the treatments or 24 h after, the quality and some biochemical parameters of strawberries was determined in order to assure a maintenance of the fruit properties. For this, pH, soluble solids (TSS), titratable acidity (TA), color, texture, the antioxidant activity by two methods (DPPH· and FRAP), the total phenolic content (TPC) and the phenolic profile, the total anthocyanin content (TAC) and the vitamin C were evaluated.

Once verified that the quality did not overcome major changes after the treatments, the treatment with higher sanitation efficacy (4 lamps) was selected, and combined with the application of 40 or 80 ppm of peracetic acid (PA), in order to decrease the treatment time from 5 to 2 min. In this case, only the sanitation effect against *L. innocua* and *S. enterica* in artificially inoculated strawberries and their remaining populations in water were investigated, always using 200 ppm NaOCl as a sanitizing reference.

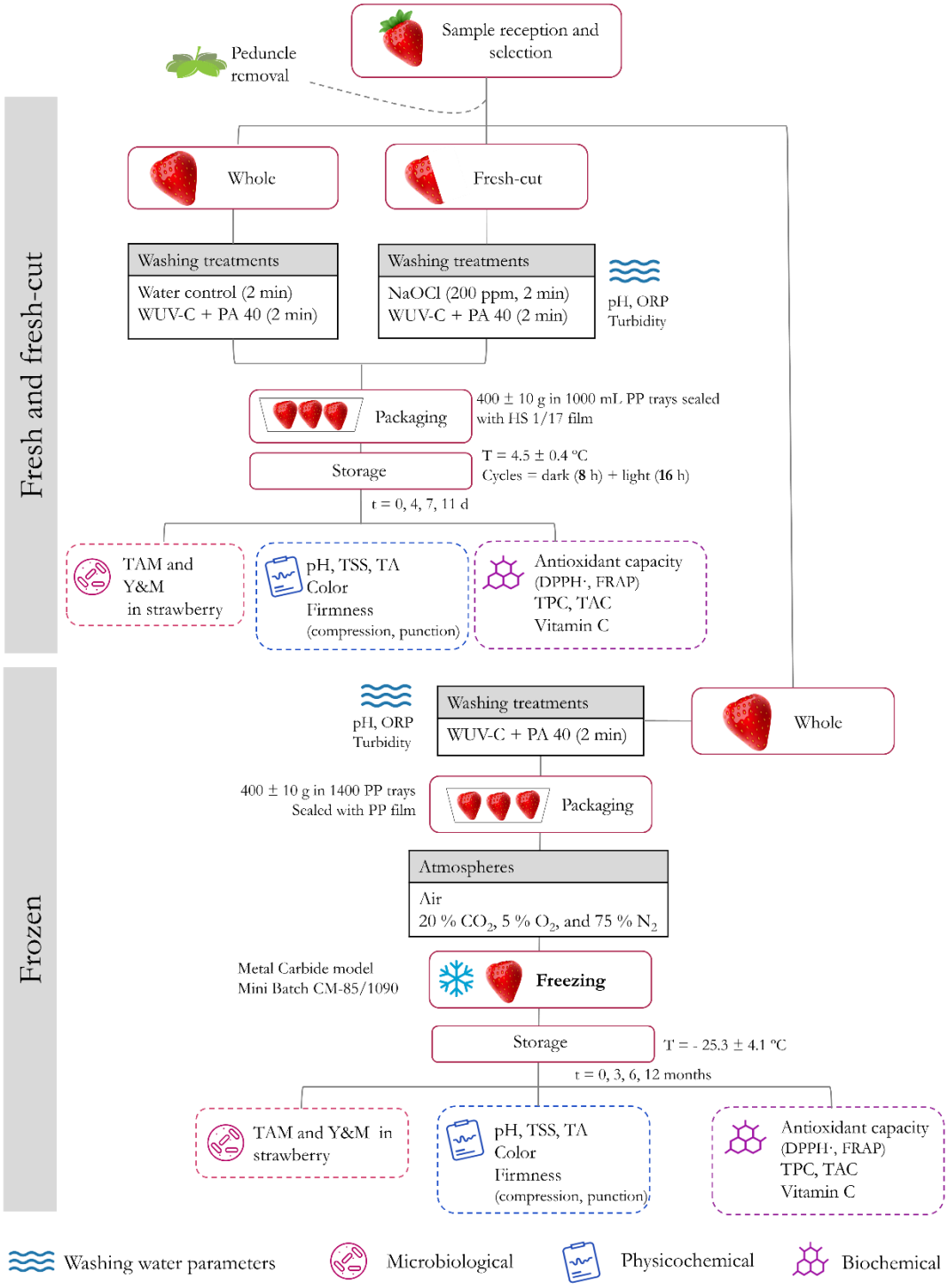

WUV-C
WUV-C combined with PA
Sample reception and selection
Peduncle removal
Strawberries inoculated with L. innocua or S. enterica
Non-inoculated strawberries
Washing treatments
Water (control, 2 min)
NaOCl (200 ppm, 2 min)
UV 2 lamps, 1 min (UV 2L – 1)
UV 2 lamps, 5 min (UV 2L – 5)
UV 4 lamps, 1 min (UV 4L – 1 min)
UV 4 lamps, 5 min (UV 4L – 5 min)
pH, ORP
Turbidity
Abs 254 nm
L. innocua in strawberry
L. innocua in water
S. enterica in strawberry
S. enterica in water
t = 0 h, 24 h
TAM and Y&M in strawberry
TAM and Y&M in water
pH, TSS, TA
Color
Firmness
(compression, punction)
Antioxidant capacity
(DPPH·, FRAP)
TPC, TAC
Vitamin C
Organic acids
Phenolic profile
Strawberries inoculated with L. innocua or S. enterica
Washing treatments (2 min)
Water (control)
NaOCl (200 ppm)
WUV-C (4 lamps)
PA 40
PA 80
WUV-C + PA 40
WUV-C + PA 80
pH, ORP
L. innocua in strawberry
L. innocua in water
S. enterica in strawberry
S. enterica in water
Washing water parameters
Microbiological
Physicochemical
Biochemical

Chapter 3 Strawberry sanitization with a combination of ultraviolet C light and peracetic acid: its impact in quality and shelf-life of fresh and frozen fruits

Once the conditions of water assisted ultraviolet light (WUV-C) combined with peracetic acid (PA) were selected (4 lamps on, emitting at 17.2 W / m^2 each, immersed in water with 40 ppm for 2 min), a shelf-life study, including fresh whole, fresh-cut, and frozen presentations, was carried out (Objective 1.2).

For this, the experimental part was divided in two blocks: fresh or frozen. In the first block, the strawberries were washed with the selected treatment (WUV-C + PA 40 / 2 min) and the controls were tap water (in case of whole fruits) and NaOCl 200 ppm (in case of fresh-cut fruits, as a sanitizing process is usually carried on in fresh-cut products). Dry product was packed in 1,000 mL PP trays that contained 400 ± 10 g strawberries, and sealed with a HS 1/17 film, and stored at 4.5 ± 0.4 in dark and light circles (8 and 16 h) to mimic store conditions. Sampling was done at 0, 4, 7, and 11 days after packing, and microbial quality including total aerobic mesophylls (TAM) and yeasts and molds (Y&M), physicochemical quality, including pH, soluble solids (TSS), titratable acidity (TA), color, texture, and biochemical parameters including the antioxidant activity by two methods (DPPH· and FRAP), the total phenolic content (TPC) and the total anthocyanin content (TAC) and vitamin C were evaluated.

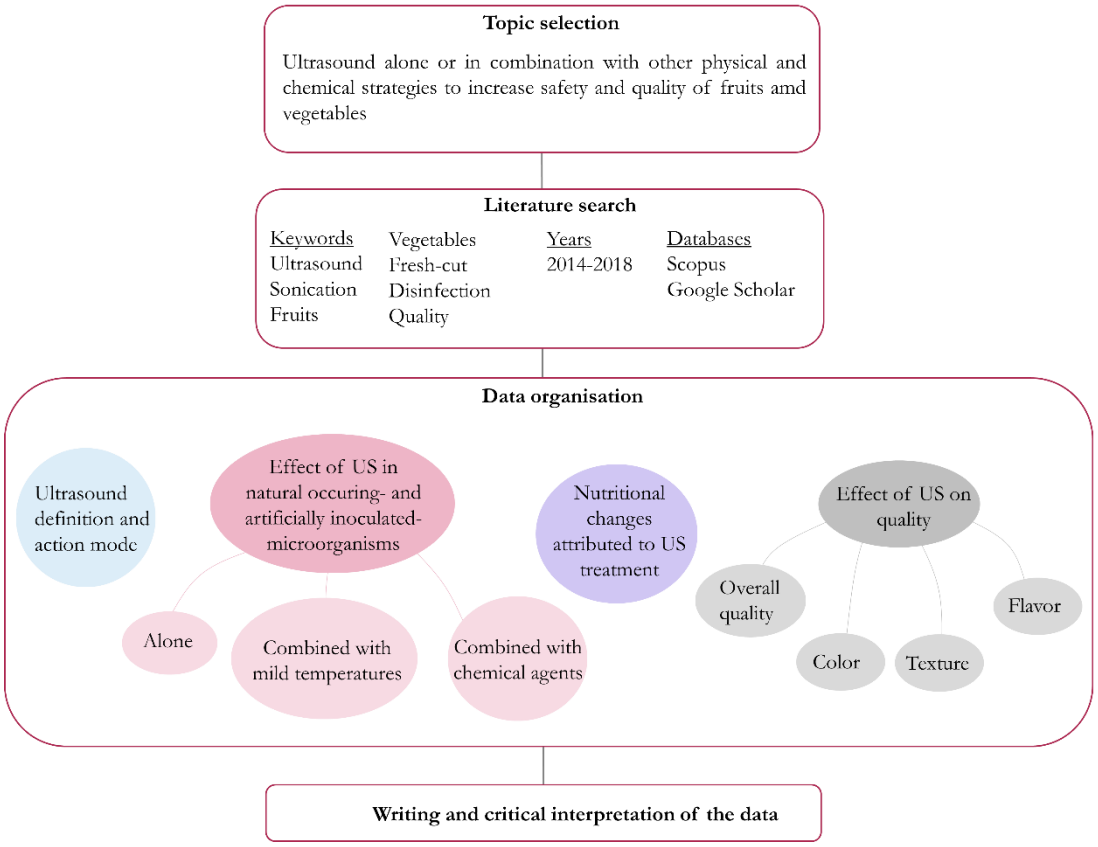
For the second block, strawberries washed with the selected treatment (WUV-C + PA 40 / 2 min) were packed under modified gas composition (20 % CO_2, 5 % O2 and 75 % N_2) or normal air. The product was frozen using a criofreezing technology (Mini Batch CM-85/1090, Carburos Metalicos), and samples were stored at -25.3 ± 0.5 °C for 12 months. Sampling was done on 24 h thawed samples, at 0, 3, 6, and 12 months after packing and criofreezing.


Fresh and fresh-cut
Sample reception and selection
Peduncle removal
Whole
Fresh-cut
Washing treatments
Water control (2 min)
WUV-C + PA 40 (2 min)
Washing treatments
NaOCl (200 ppm, 2 min)
WUV-C + PA 40 (2 min)
pH, ORP
Turbidity
Packaging
400 ± 10 g in 1000 mL PP trays sealed with HS 1/17 film
Storage
T = 4.5 ± 0.4 °C
Cycles = dark (8 h) + light (16 h)
t = 0, 4, 7, 11 d
TAM and Y&M in strawberry
pH, TSS, TA
Color
Firmness
(compression, punction)
Antioxidant capacity
(DPPH·, FRAP)
TPC, TAC
Vitamin C
Frozen
pH, ORP
Turbidity
Washing treatments
WUV-C + PA 40 (2 min)
Whole
400 ± 10 g in 1400 PP trays
Sealed with PP film
Packaging
Atmospheres
Air
20 % CO_2, 5 % O_2, and 75 % N_2
Metal Carbide model
Mini Batch CM-85/1090
Freezing
Storage
T = - 25.3 ± 4.1 °C
t = 0, 3, 6, 12 months
TAM and Y&M in strawberry
pH, TSS, TA
Color
Firmness
(compression, punction)
Antioxidant capacity
(DPPH·, FRAP)
TPC, TAC
Vitamin C
Washing water parameters
Microbiological
Physicochemical
Biochemical

Chapter 4 Ultrasound processing alone or in combination with other chemical or physical treatments as a safety and quality preservation strategy of fresh and processed fruits and vegetables: A review

Sonication is a technology with a wide range of uses in food industry, namely freezing, bleaching, degassing, extraction, drying, filtration, emulsification, sterilization, etc. Another one is the pasteurization of liquid products such as milk or F&V juices. However, its antimicrobial effect could wisely be applied to fresh and fresh-cut F&V. Prior to the experimentation with this technology, a literature review (Objective 3.1) was carried out in order to elucidate the potential of ultrasound (US) for this purpose.

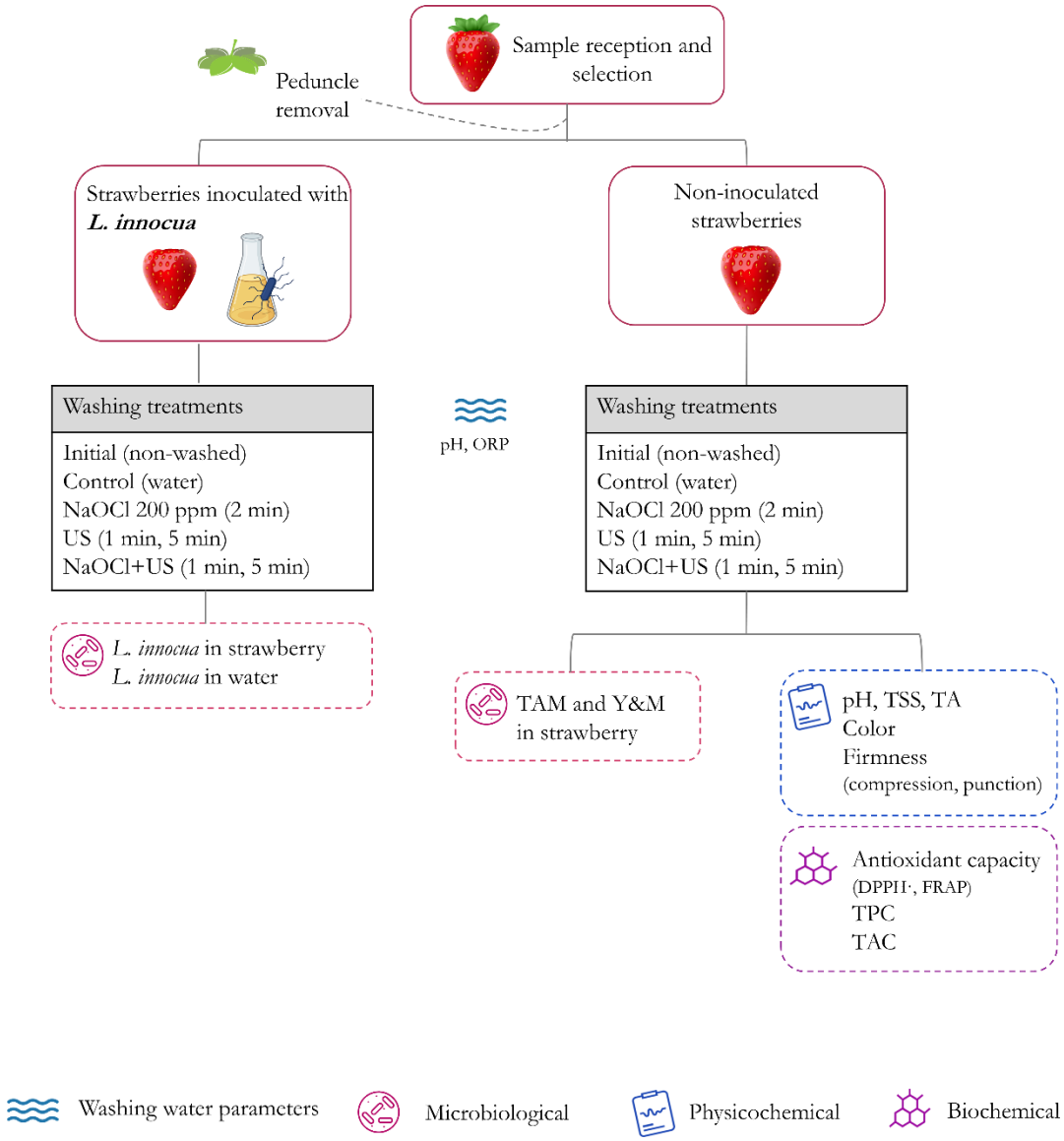
First, a screening was carried out, searching publications in databases such as Scopus and Google Scholar, using fruit, vegetable, fresh-cut, ultrasound, sonication, disinfection, quality as keywords. Papers were read through and their key points were highlighted in the review, that also considered other features of US such as its action mode or its description as 'green technology'. The review took a general view of the effects US had on microbial loads of F&V (natural occurring or artificially inoculated), on the nutritional aspects, and on the quality parameters, which included color, firmness, of flavor, amongst others.


Topic selection
Ultrasound alone or in combination with other physical and chemical strategies to increase safety and quality of fruits amd vegetables
Literature search
Keywords
Ultrasound
Sonication
Fruits
Vegetables
Fresh-cut
Disinfection
Quality
Years
2014-2018
Databases
Scopus
Google Scholar
Data organisation
Ultrasound definition and action mode
Effect of US in natural occuring- and artificially inoculated-microorganisms
Alone
Combined with mild temperatures
Combined with chemical agents
Nutritional changes attributed to US treatment
Effect of US on quality
Overall quality
Color
Texture
Flavor
Writing and critical interpretation of the data

Chapter 5 Investigating the effects of sonication as a disinfection technology for strawberries and its impact in their overall quality

This chapter presents results that were not published in any scientific journal. The purpose of this part was to evaluate US alone as a technology for strawberry disinfection, that could be used as an alternative to chlorine. In this study, US conditions were 40 kHz, 120 W (Ultrasound HD, Selecta). For this, artificially inoculated strawberries with *Listeria innocua* were washed in tap water (used as a negative control) a chlorine solution (NaOCl, 200 ppm pH 6.5) ultrasound (US) treatment for 1 or 5 min, and a combination of US with NaOCl for 1 or 5 min. The latter was carried out in order to elucidate whether the US potentiate the oxidizing power of NaOCl or its penetration through microbial membrane. In all the treatments pH and oxido-reduction potential (ORP) were evaluated before and after the washing process. Efficacy was evaluated by counting *L. innocua* populations both in strawberry and in washing water.

In a parallel assay, using non-inoculated strawberries, washing treatments were performed in the same way, and the treatments were evaluated for their effect in microbial quality including total aerobic mesophylls (TAM) and yeasts and molds (Y&M), physicochemical quality, including pH, soluble solids (TSS), titratable acidity (TA), color, texture, and biochemical parameters including the antioxidant activity by two methods (DPPH· and FRAP), the total phenolic content (TPC) and the total anthocyanin content (TAC).

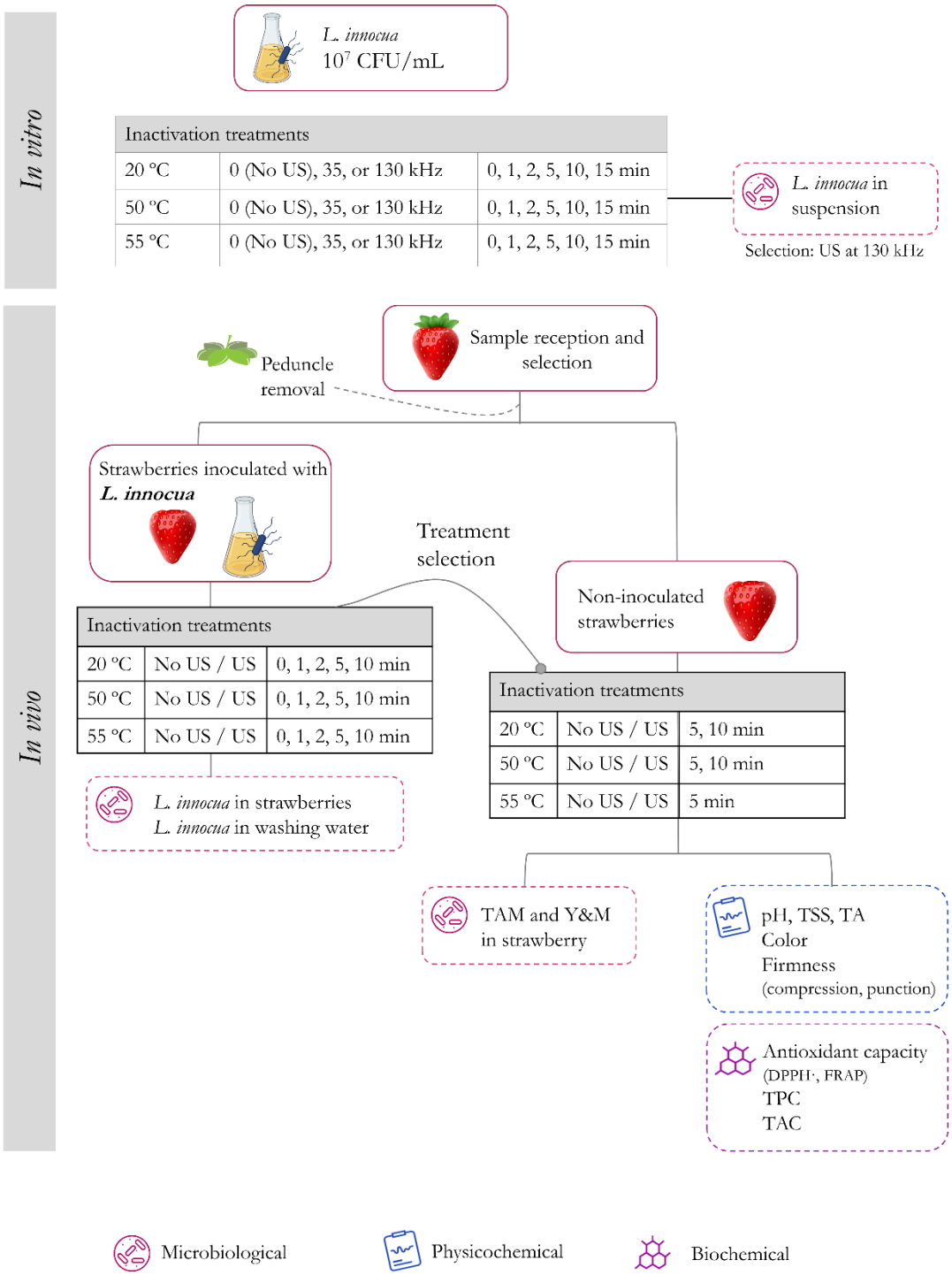

Sample reception and selection
Peduncle removal
Strawberries inoculated with *L. innocua*
Non-inoculated strawberries
Washing treatments
Initial (non-washed)
Control (water)
NaOCl 200 ppm (2 min)
US (1 min, 5 min)
NaOCl+US (1 min, 5 min)
pH, ORP
Washing treatments
Initial (non-washed)
Control (water)
NaOCl 200 ppm (2 min)
US (1 min, 5 min)
NaOCl+US (1 min, 5 min)
L. innocua in strawberry
L. innocua in water
TAM and Y&M in strawberry
pH, TSS, TA
Color
Firmness
(compression, punction)
Antioxidant capacity
(DPPH·, FRAP)
TPC
TAC
Washing water parameters
Microbiological
Physicochemical
Biochemical

Chapter 6 Exploring thermosonication as non-chemical disinfection technology for strawberries

Often, as a standalone treatment, sonication is not useful for disinfection purposes unless times longer than 60 min are applied. However, such long treatments are not feasible for the F&V industry. Alternatively, sonication can be combined with other technologies or chemical agents, in order to improve their performance. In the present study, thermosonication, or the combination of the ultrasound technology (US) (TRANSSONIC TI-H-20 bath, Elma Schmidbauer GmbH, Singen, Germany) with mild temperatures (50 and 55 °C), was evaluated as a non-chemical method for sanitizing strawberries.

First, an *in vitro* trial was carried out, in order to select the most suitable US frequency. Here, nine treatments, consisting on three temperatures (20 °C – used as a control –, 50 or 55 °C) with three US conditions each (0 – no US –, 35 or 130 kHz) were performed on a *Listeria innocua* suspension (10^7 CFU / mL). Survival curves were plotted against time (15 min) and, according to the results, 130 kHz were selected for further experiments.

Next, *in vivo* trials were performed using strawberries as the fruit matrix, which was artificially inoculated with *L. innocua*. Strawberries were immersed in the ultrasonic bath, and the sonication at the three selected temperatures was carried out, for 1, 2, 5, and 10 min. The efficacy of thermosonication was assessed in *L. innocua* populations, both in strawberries and in washing water. According to the sanitation results obtained in this trial, and excluding the treatments that provided the strawberry a cooked appearance (55 °C, 10 min), the following treatments were selected: 5 and 10 min at 50 °C, and 5 min at 55 °C (sonicated or not). As a control, 5 and 10 min at 20 °C were applied (sonicated or not). The effects on natural microbiota (total aerobic mesophylls, TAM, and yeasts and molds, Y&M), were assessed, as well as the impact on physicochemical quality, including pH, soluble solids (TSS), titratable acidity (TA), color, texture, and biochemical parameters including the antioxidant activity by two methods (DPPH· and FRAP), the total phenolic content (TPC) and the total anthocyanin content (TAC).

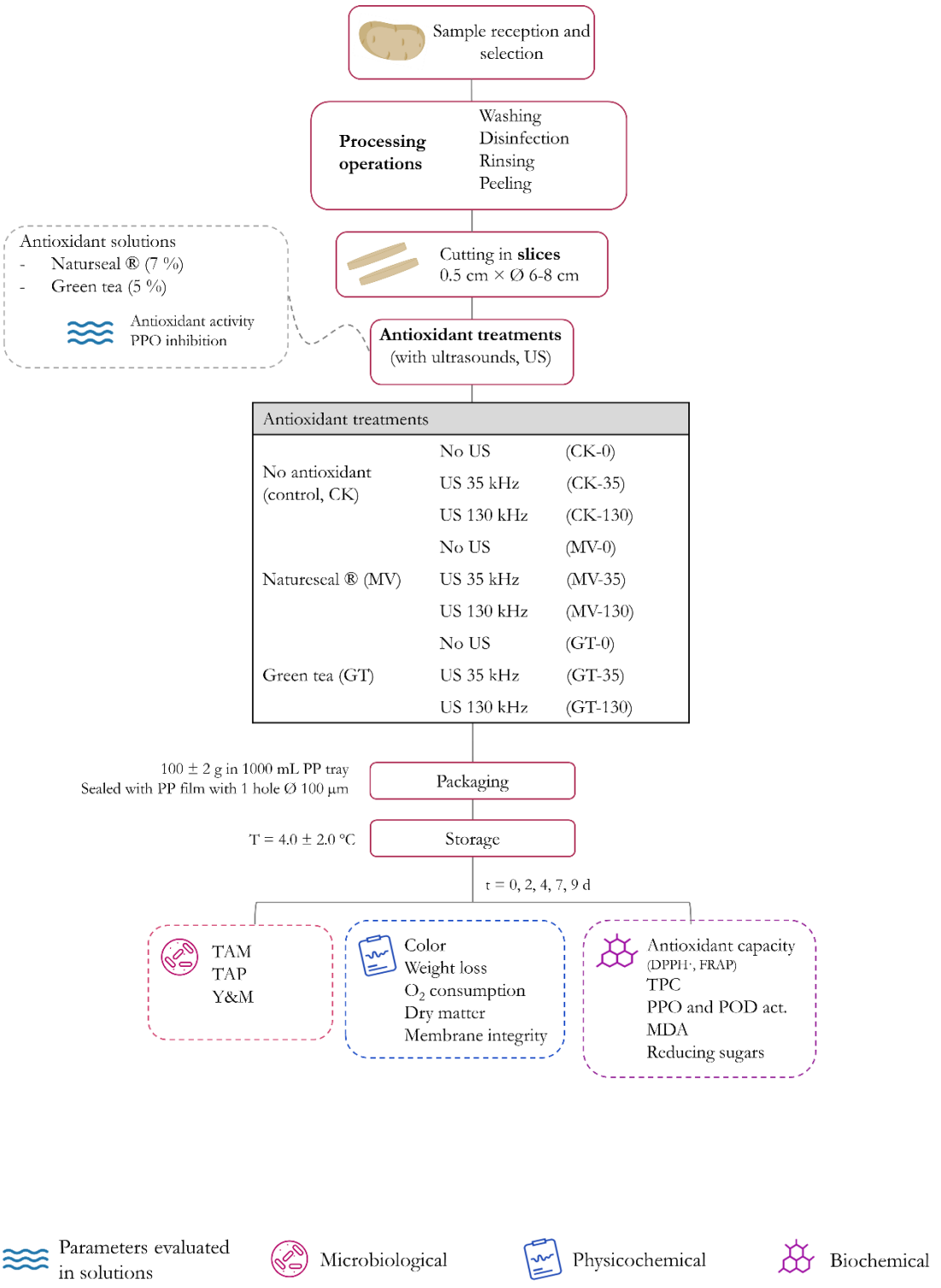

In vitro
L. innocua
10⁷ CFU/mL
Inactivation treatments
20 °C | 0 (No US), 35, or 130 kHz | 0, 1, 2, 5, 10, 15 min
50 °C | 0 (No US), 35, or 130 kHz | 0, 1, 2, 5, 10, 15 min
55 °C | 0 (No US), 35, or 130 kHz | 0, 1, 2, 5, 10, 15 min
L. innocua in suspension
Selection: US at 130 kHz
In vivo
Peduncle removal
Sample reception and selection
Strawberries inoculated with L. innocua
Treatment selection
Non-inoculated strawberries
Inactivation treatments
20 °C | No US / US | 0, 1, 2, 5, 10 min
50 °C | No US / US | 0, 1, 2, 5, 10 min
55 °C | No US / US | 0, 1, 2, 5, 10 min
L. innocua in strawberries
L. innocua in washing water
Inactivation treatments
20 °C | No US / US | 5, 10 min
50 °C | No US / US | 5, 10 min
55 °C | No US / US | 5 min
TAM and Y&M in strawberry
pH, TSS, TA
Color
Firmness
(compression, punction)
Antioxidant capacity
(DPPH·, FRAP)
TPC
TAC
Microbiological
Physicochemical
Biochemical

Chapter 7 Combination of sonication with anti-browning treatments as a strategy to increase the shelf-life of fresh-cut potato (cv. Monalisa)

One of the main problems in sliced potato is the browning of its surface due to enzymatic reactions occurring after cutting operations that put into contact the enzymes with their substrates. In these cases, the solution is whether to reduce the available oxygen that participates in such reactions or use substances capable to inhibit the activity of the enzymes, preventing browning (Objective 2.4).

In this chapter, two solutions reported as antioxidants, Natureseal ® (NS) and green tea (GT), were evaluated for their Half Inhibitory Concentration (IC_{50}) and polyphenol oxidase (PPO) inhibition capacity. After processing operations (washing, peeling, slicing) the potatoes, the slices were immersed in a 7.5 % Natureseal ® or a 5 % green tea solution or in water (control). Each treatment was divided in three conditions: their combination with the application of ultrasound at two different frequencies: 35 and 130 kHz or not. This was meant to increase the penetration of the antioxidant solutions into the potato tissue. After the drying, potatoes were packed (100 ± 2 g in 1000 mL in a polypropylene tray) and stored at 4.0 °C during 9 days.

Each sampling day, potatoes were evaluated for their microbial quality (total aerobic mesophylls (TAM), total aerobic psychrophiles (TAP), and yeasts and molds (Y&M)), for their commercial quality, being CIE L*a*b* coordinates of color the most important parameter as its values are related to browning in the potato. Also, weight loss, O_2 consumption, dry matter and membrane integrity (related to malondialdehyde (MDA) content and its relationship with the potato oxidative stress) were measured. Moreover, the biochemical characterization studied the evolution in the antioxidant activity by DPPH· and FRAP methods, the total phenolic content (TPC), the polyphenol oxidase (PPO) and peroxidase (POD) enzymatic activities, as well as the reducing sugars.

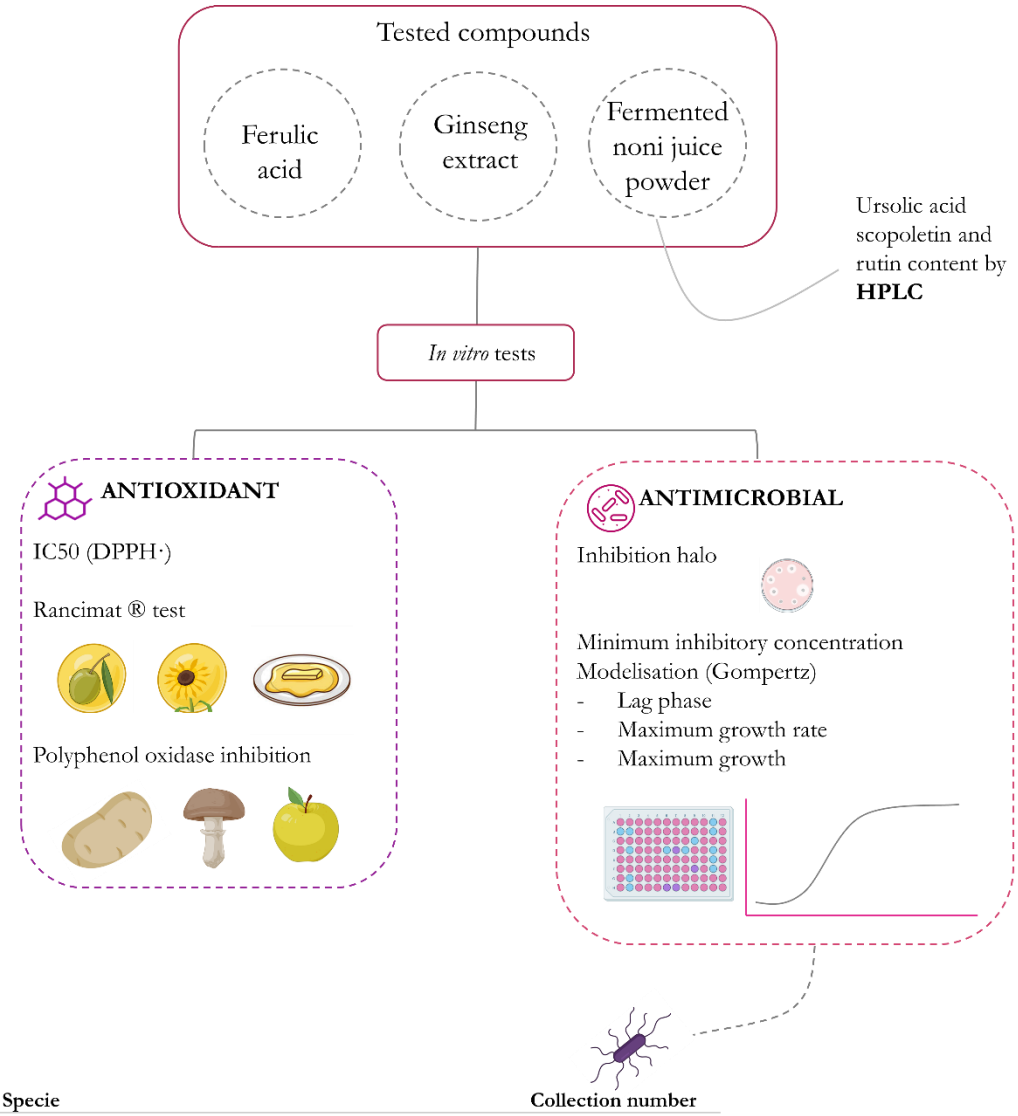

Sample reception and selection
Processing operations
Washing
Disinfection
Rinsing
Peeling
Antioxidant solutions
- Naturseal ® (7 %)
- Green tea (5 %)
Antioxidant activity
PPO inhibition
Cutting in slices
0.5 cm × Ø 6-8 cm
Antioxidant treatments
(with ultrasounds, US)
Antioxidant treatments
No antioxidant (control, CK)
No US (CK-0)
US 35 kHz (CK-35)
US 130 kHz (CK-130)
Naturesal ® (MV)
No US (MV-0)
US 35 kHz (MV-35)
US 130 kHz (MV-130)
Green tea (GT)
No US (GT-0)
US 35 kHz (GT-35)
US 130 kHz (GT-130)
100 ± 2 g in 1000 mL PP tray
Sealed with PP film with 1 hole Ø 100 µm
Packaging
T = 4.0 ± 2.0 °C
Storage
t = 0, 2, 4, 7, 9 d
TAM
TAP
Y&M
Color
Weight loss
O2 consumption
Dry matter
Membrane integrity
Antioxidant capacity
(DPPH·, FRAP)
TPC
PPO and POD act.
MDA
Reducing sugars
Parameters evaluated in solutions
Microbiological
Physicochemical
Biochemical

Chapter 8 Antioxidant and antimicrobial activities of ginseng extract, ferulic acid and fermented noni juice powder in the evaluation of their potential to be incorporated in food

In order to elucidate the potential of synthetic or natural compounds or extracts to be incorporated in food, *in vitro* tests must be carried out. Their antioxidant activities will be related to the compounds' ability to prevent or delay oxidative stress degradation, as well as browning inhibition (together with inhibition of polyphenol oxidase, PPO). Their antimicrobial activity is also important to predict their potential use in food to control and assure microbial safety.

In this study, 3 compounds were evaluated for these properties: ferulic acid, ginseng extract and a powder obtained by freeze-drying a fermented noni juice. Antioxidant activity was evaluated by IC_{50} (Concentration to achieve 50 % of DPPH· radical inhibition), which is an index that allows the antioxidant comparison between compounds. Also, a Rancimat ® test was used to elucidate the compounds ability to delay lipid peroxidation, using three model matrices: olive oil, sunflower oil, and butter. Moreover PPO is an enzyme related to browning, and its inhibition could prevent this reaction. For this, the inhibition of PPO obtained from potato, mushroom and apple, matrices in which it is present and active, was also evaluated.

The antimicrobial activity was investigated by two methods. One is the inhibition halo, or the observation of the area in which the plated and spread microorganism in a plate does not grow. The other is the microdilution method, that is carried out in a 96-well plate in which microorganisms grow in a liquid media with increasing concentrations of the studied compounds. The Minimum Inhibitory Concentration (MIC) is determined by the lowest concentration that prevents growth in the wells. Moreover, the absorbance at 620 nm, which is related to the concentration of the microorganisms in the well, was recorded. With this, modelisation of the growth, using mathematical complements of Excel (DMFit), permitted to determine the lag phase, maximum growth rate and maximum growth of each microorganism submitted to each concentration.



Specie		Collection number
Listeria monocylogenes	4b	CECT[1] -935
Listeria monocytogenes	1/2 a	Isoleated in Lab
Listeria monocylogenes	1/2	CECT-4031
Salmonella enterica subsp. Enterica	Typhimurium	CECT-4594
Salmonella enterica subsp. Enterica	Agona	ATCC[2] BAA-707
Salmonella entérica subsp. Enterica	Montevidco	ATCC BAA-710
Salmonella enterica subsp. Enterica	Gaminara	ATCC BAA-711
Escherichia. coli (virulent factor deleted)	O157:H7	NCTC[3]-12900
Escherichia. coli		CECT-516
Staphylococcus aureus		CECT-435
Bacillus cereus		CECT-131
Enterococcus faecalis		CECT-795
Enterobacter aerogenes		CECT-684

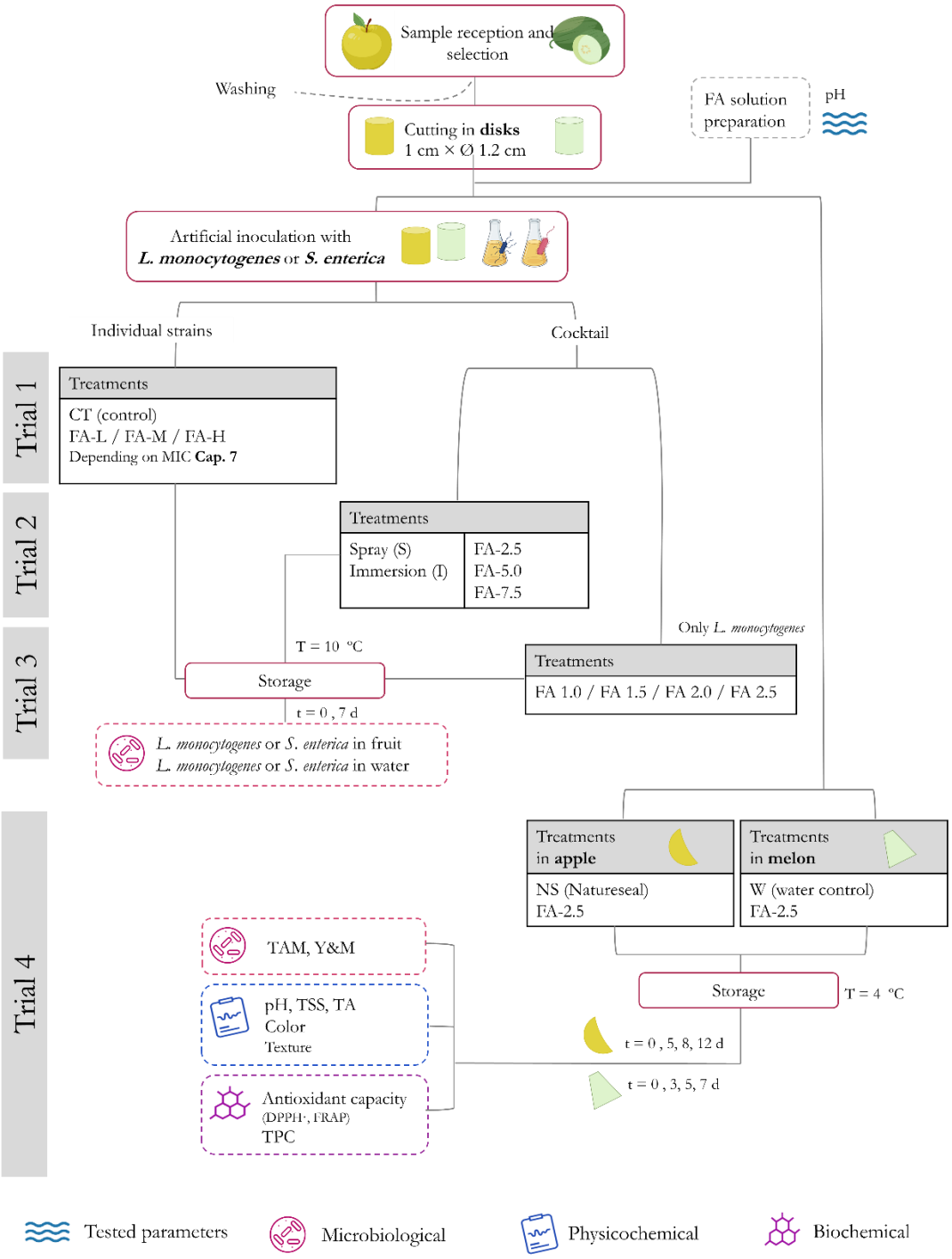
[1] Colección Española de Cultivos Tipo
[2] American Type Culture Collection
[3] National Collection of Type Cultures

Microbiological Biochemical

Chapter 9 Ferulic acid application to control growth of *Listeria monocytogenes* and *Salmonella enterica* on fresh-cut apples and melon, and its effect in quality parameters

The evaluation of the antioxidant and antimicrobial properties of ferulic acid (FA) carried out in Chapter 7 revealed that this substance has potential to be used in food with a specific objective. In the present chapter, the addition of FA to fresh-cut fruit (apple and melon) was investigated, primarily for its antimicrobial properties, and then, its effect on overall quality was assessed. The study was divided in 4 trials, each one with one specific objective.

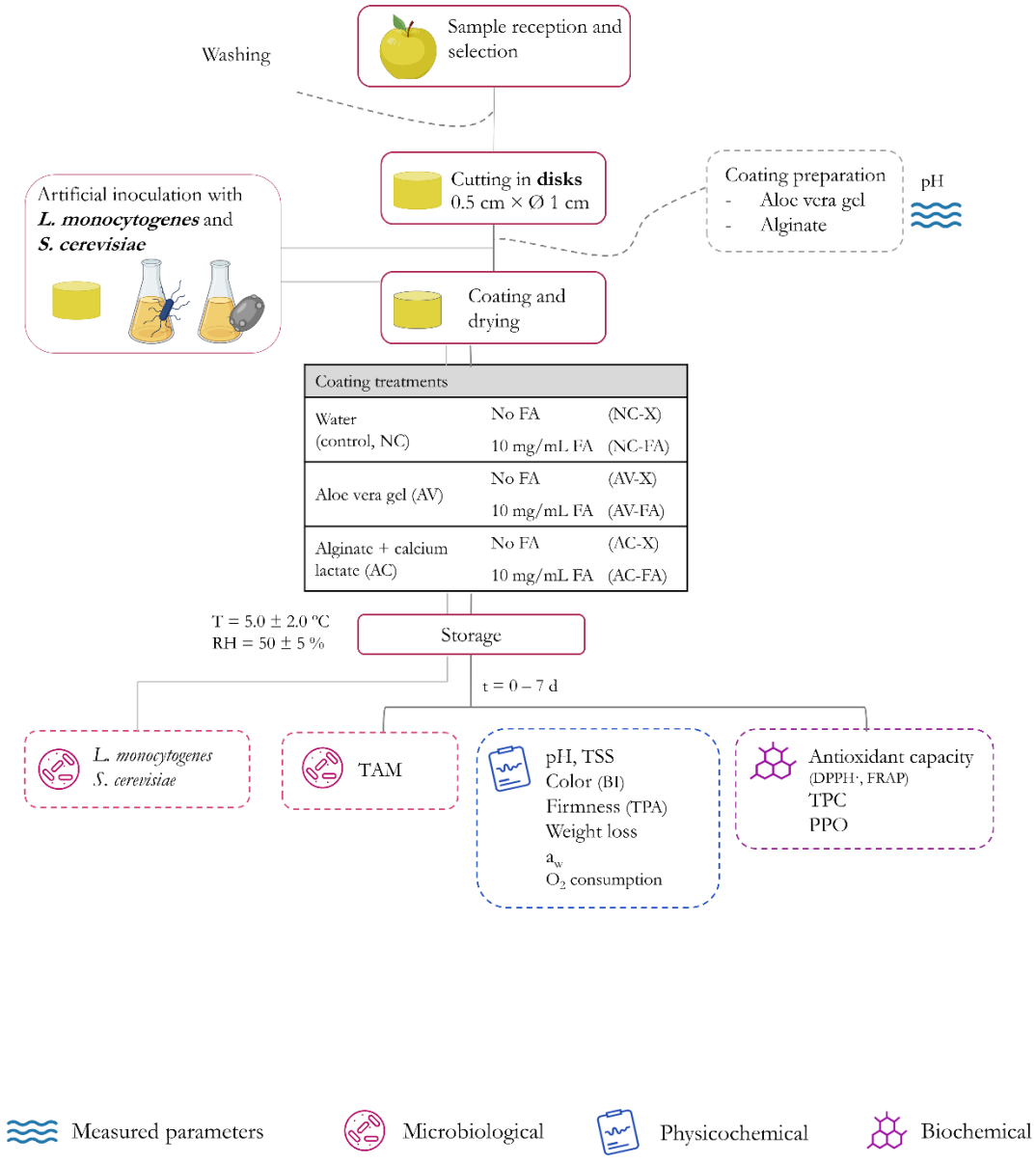
In the three first trials, apple and melon were cut like disks, and in the fourth trial, the typical shape of fresh-cut products was mimicked. The selection of the tested FA concentrations was done according to the minimum inhibitory concentration (MIC) determined in Chapter 7 for each strain of *Listeria monocytogenes* (serovars 1/2, 4b and 1/2a) and *Salmonella enterica* (serovars Agona Michigan, Montevideo and Typhimurium). Following this, three different concentrations were selected: a low (FA-L), a medium (FA-M) and a high concentration (FA-H). The FA sanitizing effect on artificially inoculated fruit disks was evaluated immediately after the immersion of the disks in the solution (both in fruit and in washing water), and the microbial growth control was evaluated 7 days after. Once the effect that each concentration had on each individual strain was evaluated, a cocktail containing all *L. monocytogenes* or all *S. enterica* strains was used for further experiments. In the second trial, the application mode of FA to the samples was investigated (immersion (I) or spray (S)), at three concentrations (2.5, 5.0, and 7.5 mg / mL). Again, the sanitizing effect and the microbial growth control were evaluated. In the third trial, the tested concentrations of FA against *L. monocytogenes* were decreased, as the growth control efficacy of FA against this pathogen was similar for all the previously tested concentrations. Here, the aim was to achieve the same result with lower concentrations of FA, so immersion of fruit disks in 1.0, 1.5, 2.0, and 2.5 mg / mL FA was evaluated, and the sanitizing effect and the microbial growth control were also evaluated. Finally, a concentration of 2.5 mg / mL FA was selected, as it covered the control of both microorganisms. In the fourth trial, fresh-cut apple was treated both with FA-2.5 and with Naturseal ® as a control for its antioxidant effect (that would prevent browning in apple). Similarly, fresh-cut melon was treated with FA-2.5 and with water (CK), as it doesn't show this browning problematic. Both products were stored at 4 °C for 12 days (apple) or 7 days (melon). During this time, their microbial quality (total aerobic mesophylls (TAM) and yeasts and molds (Y&M), their physicochemical quality (pH, total soluble solids (TSS), titratable acidity (TA), color, and firmness) and some biochemical parameters (antioxidant capacity by DPPH· and FRAP methods, and the total phenolic content (TPC)) were assessed.


Sample reception and selection
Washing
Cutting in disks
1 cm × Ø 1.2 cm
FA solution preparation
pH
Artificial inoculation with
L. monocytogenes or S. enterica
Individual strains
Cocktail
Trial 1
Treatments
CT (control)
FA-L / FA-M / FA-H
Depending on MIC Cap. 7
Trial 2
Treatments
Spray (S)
Immersion (I)
FA-2.5
FA-5.0
FA-7.5
Trial 3
T = 10 °C
Storage
t = 0 , 7 d
L. monocytogenes or S. enterica in fruit
L. monocytogenes or S. enterica in water
Only L. monocytogenes
Treatments
FA 1.0 / FA 1.5 / FA 2.0 / FA 2.5
Trial 4
Treatments in apple
NS (Natureseal)
FA-2.5
Treatments in melon
W (water control)
FA-2.5
Storage
T = 4 °C
t = 0 , 5, 8, 12 d
t = 0 , 3, 5, 7 d
TAM, Y&M
pH, TSS, TA
Color
Texture
Antioxidant capacity
(DPPH·, FRAP)
TPC
Tested parameters
Microbiological
Physicochemical
Biochemical

Chapter 10 Combination of ferulic acid with *Aloe vera* gel or alginate coatings for shelf-life prolongation of fresh-cut apples

The edible coatings are aimed to protect the fresh or fresh-cut fruit by creating a surrounding layer that decreases gas exchange and reduces stresses at which they are submitted, reducing in turn their metabolism and deterioration. Sometimes, substances and extracts can be added in order to add more functionality to edible coatings. Ferulic acid (FA) was tested in Chapter 7 for its antioxidant and antimicrobial properties, and applied by immersion (or spray) in fresh-cut apple and melon in Chapter 8. So, in this chapter, the effect of FA in fresh-cut apple was studied in combination of the effect two edible coatings: a sodium alginate coating casted with two steps of calcium lactate, and *Aloe vera* gel. These coatings had been previously formulated in previous studies found in the literature.

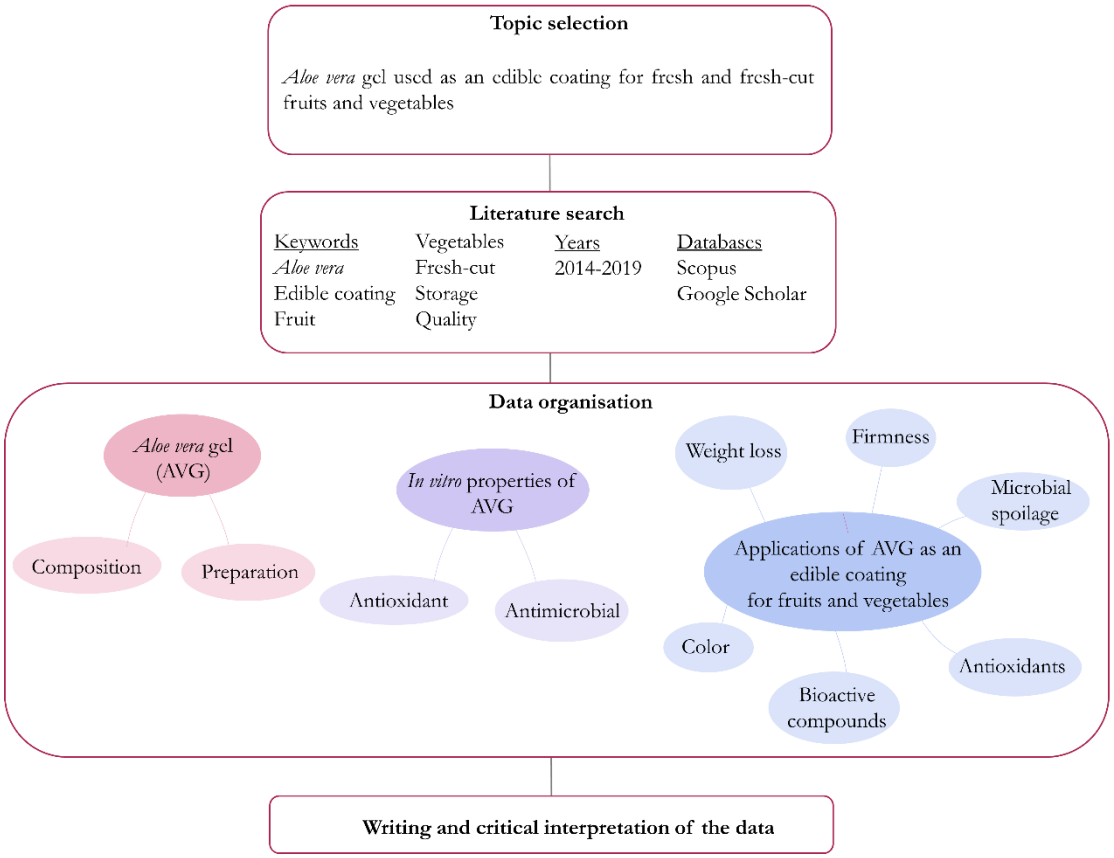
A factorial design was applied, combining three coating types (non-coated (NC), coated with *Aloe vera* gel (AV) or with alginate and calcium (AC)) with two antioxidant types (no antioxidant (0) and ferulic acid (FA)). Once the apples were washed, cut in disks, coated and dried, they were stored at 5 °C for 7 days. The main parameters evaluated were browning index, as it is a limiting factor in the shelf-life of these products, the weight loss, as has a great impact in the visual appearance of the product, and the control of the growth of artificially inoculated *L. monocytogenes*, a microorganism that can grow at refrigeration temperatures. *Saccharomyces cerevisiae* and total aerobic mesophylls (TAM) populations during storage were counted for microbial quality. For physicochemical quality, pH, total soluble solids (TSS), a texture profile analysis, weight loss, water activity, and O_2 consumption were evaluated. The biochemical parameters studied were the antioxidant capacity by DPPH· and FRAP methods, the total phenolic content (TPC) and the polyphenol oxidase (PPO) enzymatic activity.


Washing
Sample reception and selection
Cutting in **disks** 0.5 cm × Ø 1 cm
Coating preparation
- Aloe vera gel
- Alginate
pH
Artificial inoculation with ***L. monocytogenes*** and ***S. cerevisiae***
Coating and drying
Coating treatments
Water (control, NC)
No FA (NC-X)
10 mg/mL FA (NC-FA)
Aloe vera gel (AV)
No FA (AV-X)
10 mg/mL FA (AV-FA)
Alginate + calcium lactate (AC)
No FA (AC-X)
10 mg/mL FA (AC-FA)
T = 5.0 ± 2.0 °C
RH = 50 ± 5 %
Storage
t = 0 – 7 d
L. monocytogenes
S. cerevisiae
TAM
pH, TSS
Color (BI)
Firmness (TPA)
Weight loss
a_w
O_2 consumption
Antioxidant capacity
(DPPH·, FRAP)
TPC
PPO
Measured parameters
Microbiological
Physicochemical
Biochemical

Chapter 11 Aloe vera gel: An update on its use as a functional edible coating to preserve fruits and vegetables

Aloe vera is a succulent plant that has caught the attention of the scientists for its properties related to health and skin-care. Besides, its potential to be used in food has been highlighted in some studies and reviews, for its antioxidant and antimicrobial properties. Much work has been done in the last five-year period, but there was no publication in the literature that reviewed the last discovering regarding the use of Aloe vera as an edible coating for whole or fresh-cut F&V. This chapter makes an overview (Objective 3.2) on the properties of AVG, as well as its use as an edible coating towards the safety and quality of the studied fruits during their storage period.

First, a screening was carried out, searching publications in databases such as Scopus and Google Scholar, using fruit, vegetable, fresh-cut, *Aloe vera*, edible coating, quality, storage as keywords. Selected papers were organized and classified, and the main outcomes were sum up in the review. The review not only included studies in F&V matrices, but also the *in vitro* antioxidant and antimicrobial properties that *Aloe vera* had shown and could be useful for its further use on food. Regarding its use as an edible coating, alone or with other chemical and functional compounds, its effect on parameters such as respiration rate, firmness, color, antioxidant activity, bioactive compounds, or flavor, was widely reviewed.


Topic selection
Aloe vera gel used as an edible coating for fresh and fresh-cut fruits and vegetables
Literature search
Keywords
Aloe vera
Edible coating
Fruit
Vegetables
Fresh-cut
Storage
Quality
Years
2014-2019
Databases
Scopus
Google Scholar
Data organisation
Aloe vera gel (AVG)
Composition
Preparation
In vitro properties of AVG
Antioxidant
Antimicrobial
Weight loss
Firmness
Microbial spoilage
Applications of AVG as an edible coating for fruits and vegetables
Color
Antioxidants
Bioactive compounds
Writing and critical interpretation of the data

Scientific studies

Strawberry sanitization by peracetic acid washing and its effect on fruit quality

Chapter 1

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The risk posed by outbreaks associated with strawberries together with the safety issues of by-products from chlorine disinfection in the fruit industry has led to a search for alternative sanitizers. The disinfection capacity of peracetic acid (PA) at three concentrations (20, 40 and 80 ppm) and washing times (1 and 2 min) was compared to sodium hypochlorite (200 ppm) (NaClO) treatments and a water control, and its influence on the physico-chemical, biochemical and nutritional quality of strawberries was also studied. Counts on total aerobic mesophilic microorganisms were comparable between NaClO and PA. For yeasts and molds, only NaClO and 80 ppm PA reduced contamination in washing water, but no differences were observed in strawberries. Artificially inoculated *L. innocua* was reduced by at least 4 log CFU/g in strawberry by all the PA treatments, except at 20 ppm PA for 1 min. Total soluble solids, pH, titratable acidity, antioxidant activity and total phenolic content values were maintained after all treatments. Only anthocyanin content was affected. Treatments of 20 and 40 ppm PA did not significantly affect fruit color, and there were no losses on strawberry firmness. PA, as a GRAS substance that has shown potential to reduce microorganisms present in strawberries without any major physicochemical or sensorial alteration, could be a suitable alternative to chlorine disinfection.

Listeria innocua, native microbiota, nutritional, biochemical, disinfection

1 Introduction

Strawberries are rich in vitamins (i.e. ascorbic acid) and other antioxidants (i.e. phenolic acids, anthocyanins), and other bioactive molecules. There is increasing evidence to suggest that these active phytochemicals have anti-inflammatory, antimicrobial, anti-carcinogenic, anti-mutagenic and neuroprotective effects. Thus, berry consumption seems to be beneficial for human health (Mortas, 2017).

Strawberry production exceeds 740,000 tones in Europe, and it is widely consumed in both fresh and frozen forms (Fruit Logistica, 2018). Fresh strawberries have a short life of 13 days on average if correctly stored at 5 °C (Leithner, 2017) and losses due to shelf-life issues can range up to 53%, as reported in Meyer (2017). Even though no bacterial pathogenic microorganisms have been found on strawberries (Delbeke, 2015), the EFSA (European Food Safety Authority, 2014) emitted a scientific opinion on the risk posed by *Salmonella* spp. and norovirus in berries. Hadjilouka (2014) reported presence of *Listeria monocytogenes* in 3.8% of strawberry samples.

Strawberry contamination can occur at the pre-harvest or post-harvest stage by numerous sources including insects, soil, water, equipment or human handling (Zhu, 2017). Disinfection is a critical step in the inactivation of pathogenic and spoilage microorganisms. In fruits, a first approach for this purpose consists of a washing step in which fruits are immersed in a sanitizer solution. Among available sanitizers, chlorine is the first choice due to its low price, simplicity of use and effectiveness against vegetative bacteria. But since its action is highly pH dependent and it reacts with organic matter, producing unhealthy by-products including carcinogenic and mutagenic chlorinated compounds, it has already been banned in some European countries (Fallik, 2014; Meireles, 2016). It has also been included in the indicative list of the Directive on Industrial Emissions (IPPC, 2007/0286(COD), to reduce harmful industrial emissions across the EU (European Commission, 2007).

Subsequently, effective disinfection alternatives to chlorine have been studied, including other sanitizers like organic acids or essential oils, or physical methods such as ultrasound or ultraviolet processing (Ramos, 2013). As the washing water may also increase the bacterial counts by cross-contamination, it is important that the washing step not only removes bacteria from the strawberry surface but also maintains water quality (Pablos, 2018). Peracetic acid (PA) is an unspecific, persistent oxidizer of C-C double bonds and reduced atoms. This mode of action would imply a poor chance for the development of resistance in microorganisms, as borne out by the absence of such reports in the literature (Wessels, 2013). It has revealed to be effective on decontamination procedures, making it a good choice as a sanitizing agent (Singh, 2018). Its use up to 80 ppm is permitted in USA for the washing of fruits and vegetables (FDA CFR 173.315).

Alternative disinfection methods to chlorine must be found in order to provide consumers with safe fresh-cut fruits and vegetables. Hence, the objectives of this study were to assess the adequacy of peracetic acid as a sanitizer in strawberry washing processes to decrease native microbiota and artificially inoculated *L. innocua* and to study its effect on the nutritional and commercial quality of the fruits.

2 Materials and methods

2.1 Materials

Strawberries (*Fragaria* x *ananassa*) were purchased from local distributors. Calix and leaves were carefully removed before the treatment.

Peracetic acid 15% was purchased from PanReac AppliChem (Barcelona, Spain). Triptone soy broth (TSB), triptone soy agar (TSA), Palcam base agar, yeast extract, plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), potassium bisulfate, sodium chloride and peptone were purchased from Biokar Diagnostics (Allonne, France).

Ascorbic acid, gallic acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol, acetone, chlorhidric acid (37%), sodium acetate, sodium hydroxide, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were purchased from Panreac (Llinars del Vallès, Spain).

2.2 Bacterial strains and culture conditions

L. innocua strain CECT-940 (*Colección Española de Cultivos Tipo*, Burjassot, Spain) was used in this study. It was grown for 24 h in 50 mL of TSB supplemented with 6 g / L of yeast extract, 2.5 g / L glucose and 2.5 g/L K_2HPO_4 (TSBYE) at 37 ± 1°C in a rotatory shaker set at 150 rpm. Afterwards, the culture was centrifuged at 9,800 × *g*, at 10°C, for 10 min, and the pellet was suspended in an adequate volume of saline peptone (8.5 g NaCl and 1 g peptone) to obtain a concentrated suspension, which was approximately 10^{10} CFU / mL. Concentration in the suspension was checked by plating in TSAYE and Palcam followed by incubation at 37 ± 1 ºC for 48 h.

2.3 Strawberry inoculation with *Listeria innocua*

The day before the experiment, strawberries were inoculated with 50 µL of the prepared suspension of *L. innocua* at 10^{10} CFU/mL, to reach a theoretical initial concentration of 2×10^7 CFU / g. Inoculation was done by pipetting small droplets on the surface of each strawberry and allowing them to dry for approximately 3 h at room temperature (22 ºC). Inoculated strawberries were stored at 4 ± 1°C for 20 h until the assay. Prior to the experiments, the initial concentration of *L. innocua* was checked as explained below.

2.4 Experimental design

Two types of experiments were carried out. On one hand, an experiment was conducted in artificially inoculated strawberries to determine *L. innocua* populations after the treatments (**Figure 18**).

This experiment was done once, with 3 determinations (repetitions). On the other hand, the experiment in non-inoculated strawberries was replicated three times, two to ascertain the effect of washing treatments on epiphytic microbiota and one to perform the quality and nutritional determinations. Treatment solutions were prepared: tap water with sodium hypochlorite at 200 ppm pH 6.6 (NaClO) adjusted using 3 M citric acid, and tap water with peracetic acid at concentrations of 20 ppm (PA20), 40 ppm (PA40) or 80 ppm (PA80). In microbiological assays, tap water (W) was added as a control in order to verify whether reductions could be due to the physical removal of water itself or if further reductions could be achieved by the use of a germicidal effect of PA. For washing treatments, 20 fruits were submerged for 1 or 2 min in 2 L of each solution. After the hypochlorite treatment, fruits were rinsed in 2 L of tap water. Fruits were kept to dry at room temperature. Free chlorine concentration

was checked with an ion specific meter Hanna Instruments HI 95734-11 (Rhode Island, USA) and peracetic acid concentration was determined by titration.

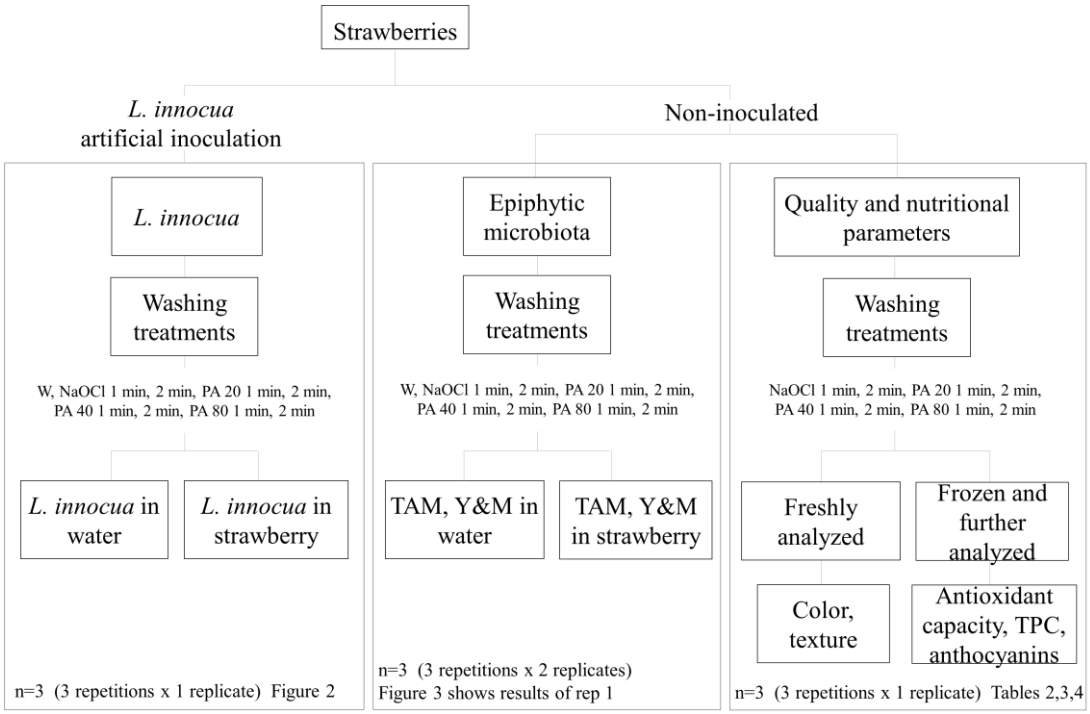

Figure 18. Experimental design.

Moreover, in the experiments with non-inoculated strawberries, microbiological and quality analysis were performed. For biochemical determinations, an aliquot of each replication was frozen with liquid nitrogen, milled using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain) and stored at -80°C until analysis.

2.5 Microbiological analysis

In the artificially inoculated experiments, one strawberry per repetition was weighted, placed in a sterile filter bag (80 mL BagPage®, Interscience BagSystem, Saint Nom, France) and diluted with buffered peptone water 1:4 (w:v). It was mashed in a paddle blender (MiniMix, Interscience, France) for 2 min at 9 strokes/s. Aliquots of the mixture were serially diluted in saline peptone (SP), plated in duplicate on Palcam agar and plates were incubated at 37 ± 1°C for 48 h.

In experiments with epiphytic microbiota, two strawberries per repetition were weighed, placed in a sterile filter bag, diluted and homogenized as explained above. A 10-fold serial dilutions were made in SP and plated in duplicate on PCA for total aerobic mesophilic counts (TAM) and in DRBC for yeasts and molds (Y&M). Plates were incubated at 30 ± 1 ºC for 3 days for TAM and at 25 ± 1 ºC for 3 to 5 days for Y&M. Results were expressed as log CFU/g and the detection limit was 20 CFU / g. This experiment was repeated twice.

Moreover, after each washing treatment, the population of *L. innocua* and TAM and Y&M was determined in the wash water. One milliliter of water was added to neutralizing Dey-Engley medium and plated as described before. Results were expressed as log CFU / mL, and the detection limit was 50 CFU / mL. When quantification was below the detection limit, its presence was confirmed by Dey-Engley change in color followed by streaking onto PCA, DRBC or Palcam.

2.6 Quality analysis

Quality analyses were only determined in non-inoculated strawberries

2.6.1 pH, titratable acidity and total soluble solids

For pH, titratable acidity (TA) and total soluble solids (TSS) determination, strawberries were smashed in a blender to obtain their juice. For each replication, 25 mL of strawberry juice were prepared, and determined twice. pH was determined using an electrode in a pH-meter model GLP22 (Crison Instruments SA, Barcelona, Spain). TA was measured by diluting 10 mL of strawberry juice with 10 mL of distilled water and titrated with 0.1 M NaOH until pH 8.2 was reached. Results were expressed as mg of citric acid per L. TSS was measured at 20 °C with a refractometer (Atago Co. Ltd., Tokyo, Japan), and the results expressed as °Brix.

2.6.2 Color

Color of 20 strawberries was measured on 3 sides of each sample by using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Color was expressed as CIE L* a* b* coordinates, using a D65 illuminant and 10° observer angle. These values were used to calculate the total color difference (TCD) (Eq. 1),

$$TCD = [(L^*_f - L^*_i)^2 + (a^*_f - a^*_i)^2 + (b^*_f - b^*_i)^2]^{1/2} \quad \text{Eq. 1}$$

where f = final (strawberries after each treatment) and i = initial (strawberries before any treatment).

2.6.3 Texture

To assess changes in texture, compression and firmness measured by the maximum penetration force were determined using the TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England).

Compression force readings were taken by recording the maximum force required to compress a strawberry half 6 mm using 2 horizontal parallel plates. The compression pre-test and test were both run at 5 mm/s speed with a trigger force of 0.1 N.

The firmness test was performed using a cylindrical probe (4 mm). Pre-test and test were both run at 5 mm/s speed and using a trigger force of 0.1 N, allowing the probe to enter 8.0 mm deep into the tissue, measuring the maximum force encountered.

2.7 Biochemical analysis

2.7.1 Antioxidant activity

Antioxidant activity was assessed in the frozen strawberries using two methodologies: ferric reducing antioxidant power (FRAP) and DPPH scavenging activity assays. For the extraction, 6.0 ± 0.1 g were mixed with 20 mL of methanol 70 % (v/v) and homogenized in a vortex for 20 s. Samples were immediately placed in a stirrer at 4 °C working at 195 rpm for 5 min and centrifuged using a Sigma-3-18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 13,500 × *g* for 20 min at 4 °C. Supernatant was then filtered and marked to 25 mL with methanol 70%. Extracts were stored at -80 °C for further determinations.

The **FRAP** reagent was prepared with a mixture of acetate buffer 0.3 M pH 2.6, TPTZ 40 mM in HCl and $FeCl_3 \cdot 6H_2O$ 20 mM in distilled water in 10:1:1 (v:v:v) proportion. The determination was performed by adding 0.1 mL of the extract to 1.4 mL of FRAP reagent and incubating in a thermostatic

bath at 37 °C for 20 min in the dark. Absorbance was read at 593 nm using GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA).

DPPH· radical was prepared daily by diluting a stock solution of DPPH· 1mM in methanol 100 %, until an absorbance at 515 nm of 0.750 ± 0.50 was reached. Then, the determination was performed by adding 0.1 mL of the extract to 1.4 mL of DPPH· reagent and incubating at RT for 1 h in the dark. Absorbance was read at 515 nm using GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA).

Standard curves with ascorbic acid for both methods were prepared daily by using the same procedure as with the samples. Results were expressed as mg of ascorbic acid equivalents (AAE)/ 100 g of fresh weight (FW).

2.7.2 Anthocyanin content

Anthocyanin extraction for further determination was performed as following. Briefly, 5.0 ± 0.1 g of frozen sample were mixed with 10 mL of methanol 80% (v/v) and vortexed for 20 s. After stirring at 200 rpm for 10 min at 4 °C, the mixture was centrifuged using a Sigma-3-18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 12,000 rpm for 15 min at 4 °C. Supernatant was then filtered and stored at -80°C until needed.

Determination was accomplished by adding a 0.5 mL aliquot of the extract to potassium chloride buffer 0.025 M, pH 1.0 and also to sodium acetate buffer 0.400 M, pH 4.5 to a final volume of 5 mL. Absorbance of both solutions was read at 510 and 700 nm. For quantification, Eq. 2 was used:

$$\Delta A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}} \qquad \text{Eq. 2}$$

where A is absorbance at a certain wavelength. Anthocyanin content was expressed as mg of cianidine-3-glucosyde / 100 g FW following the calculations described by (Meyers, 2003).

2.7.3 Total phenolic content (TPC)

The TPC was determined by the Folin-Ciocalteau method. The test was performed on the same extract used for antioxidant activity determination.

The assay was performed by adding 4.3 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent to 0.7 mL of extract. After shaking and incubation for 5 min at RT in the dark, 2 mL of saturated sodium carbonate were added. The mixture was again shaken and incubated for 1 h in the dark. Absorbance was read at 760 nm using GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). Standard curve with gallic acid was prepared daily using the same procedure as with the samples. Results were expressed as mg of gallic acid equivalents (GAE) / 100 g FW.

2.8 Statistical analysis

Results are expressed by mean ± standard deviation (SD) of 3 repetitions. All data were checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analysis was carried out using JMP 13 (SAS Institute Inc., Cary, USA).

3 Results and discussion

3.1 Effect of PA on microorganisms

Concentrations of sanitizers, pH and ORP values are detailed in **Table 6.** Water parameters: pH, ORP, concentration of sanitizer. Values are the mean of the 3 reps ± standard deviation. In the PA washing solutions, pH and POR values were lower than those observed in NaClO treatment, which ranged from 6.5 to 6.65 and 881 to 894 mV, respectively.

Table 6. Water parameters: pH, ORP, concentration of sanitizer. Values are the mean of the 3 reps ± standard deviation.

	Water for native microbiota experiment			**Water for *L. innocua* experiment**		
Treatment	pH	ORP (mV)	Concentration of free chlorine or PA (mg / L)	pH	ORP (mV)	Concentration of free chlorine or PA (mg / L)
Water	7.84 ± 0.15	279 ± 5	< 0.01	7.84 ±0.15	279 ± 5	< 0.0.1
NaClO	6.55 ± 0.04	894 ± 14	138.2 ± 67.6	6.65 ± 0.07	881 ± 3	173.0 ± 4.2
PA20	5.53 ± 0.04	460 ± 3	26.53 ± 2.19	6.54 ± 0.09	464 ± 5	23.1 ± 1.2
PA40	4.54 ± 0.02	493 ± 5	46.36 ± 2.63	4.83 ± 0.01	506 ± 7	46.9 ± 6.8
PA80	4.11 ± 0.02	515 ± 3	76.96 ± 1.23	4.17 ± 0.03	523 ± 6	87.1 ± 8.2

3.1.1 *L. innocua* experiments

The initial population of *L. innocua* on strawberries was 5.7 ± 0.5 log CFU/g (**Figure 19**). After all washing treatments, *L. innocua* populations were statistically lower than the initial population. When washing with 200 ppm hypochlorite (NaClO) for 2 min, *L. innocua* population in strawberries was 0.50 ± 0.50 log CFU/g fruit. This 5.5 log CFU/g reduction was higher than those reported in other studies on fresh-cut produce such as avocados disinfected with hypochlorite 75 ppm for 15 s (Rodríguez-García, 2011), or romaine lettuce and cantaloupe, immersed in a 200 ppm NaClO solution for 10 min (Guzel, 2017). *L. innocua* populations achieved after PA treatments in all combinations were equivalent to those observed after hypochlorite washing, ranging from 1.7 ± 0.7 to 0.4 ± 0.5 log CFU / g, when washing with PA40 for 2 min or PA80 for 2 min, respectively. Reductions of about 4 log units observed in this study were in accordance with other authors, who also found no statistical differences between different concentrations of 45 or 85 ppm PA washings for 5 min on lettuce, cantaloupe, tomato, lemon, and blueberry (Singh, 2018). Contrarily, other authors found lower reductions at similar PA concentrations (25, 50 and 75 ppm) on sprouts (Neo, 2013). These differences could be attributed to variations in the inoculation step (method or pathogen concentration), the strain used or on the characteristics of the fruit and vegetable surface, as this parameter affects the adherence of the microorganism (São José, 2014). As *L. monocytogenes* is a pathogen that can grow in the conditions in which strawberries are stored, other studies have used different sanitizers to reduce its populations. For instance, Zhou (2017) used 0.5 % levulinic acid plus 0.5 % sodium dodecyl sulphate, achieving 2 log CFU / g reductions. In strawberries, other pathogenic microorganisms have been reported to pose a health concern, namely *Salmonella* spp., *E. coli* O157:H7 and norovirus (EFSA, 2014). Guo (2018) have studied the effect of PA at 90 ppm for 2 min and found a reduction of *Salmonella* and *E. coli* O157:H7 of 1.2 log CFU / g after the washing treatments. In other vegetable products, Silveira (2018) found a decrease of *S. enterica* Typhimurium of 2.4 log CFU/g when using PA 50 ppm for 5 min. Wang (2014) also found that the decrease of *Salmonella* Typhimurium after washing tomatoes with PA 40 ppm for 2 min was 2.5 log CFU / g.

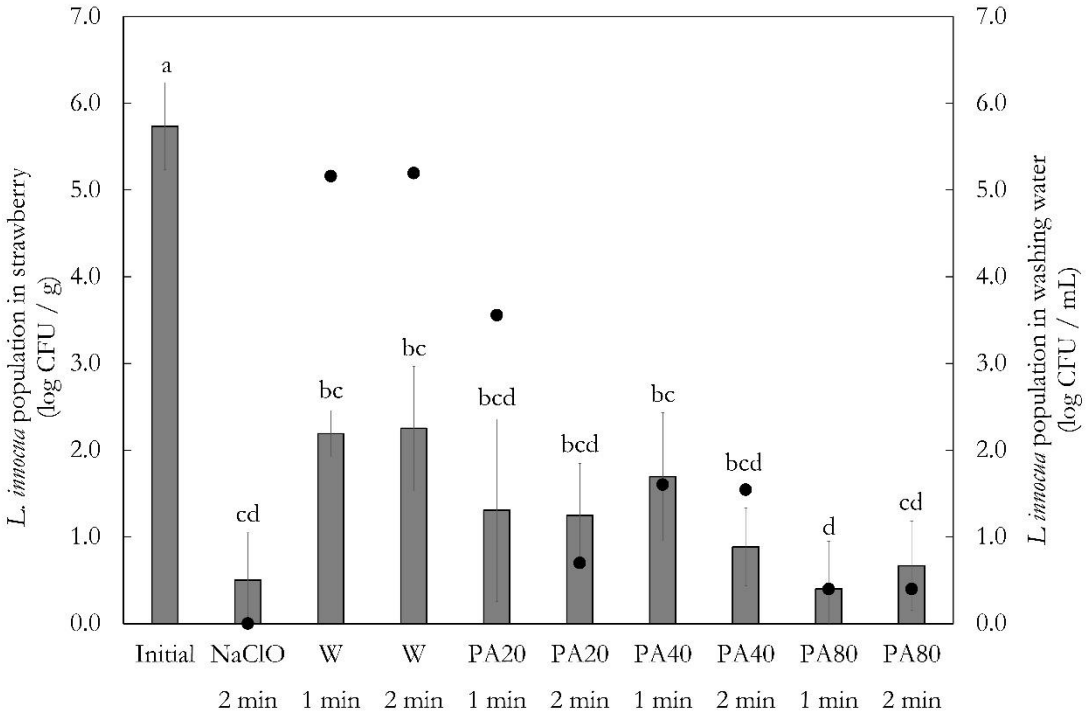

Figure 19. Population of *L. innocua* in strawberries (bars, log CFU / g) and in water (●, log CFU / mL). *L. innocua* values in strawberries are the mean of 3 reps ± standard deviation. *L. innocua* values in water were obtained from one sample.

L. innocua has demonstrated to be a good surrogate for *L. monocytogenes* (Francis, 1997). However, the lower reductions of other pathogens compared to ones found in our study with *L. innocua* should be considered. Further investigations should be done targeting common pathogenic microorganisms of strawberries, so as to confirm the effect of PA on them. Removal of microorganisms from the produce surface as a result of washing is critical, as it is the quality of water used. In this study, *L. innocua* on strawberries after W washing was not statistically different from other treatments, demonstrating that there was a physical removal of microorganism during washing. However, the remaining population in wash water after treatments was higher (more than 5.0 log CFU / mL) than it was when a sanitizer was used. Except for PA20 for 1 min, other PA and NaClO treatments achieved a final population of less than 1.5 log CFU / mL in water, thus preventing subsequent cross contamination of *L. innocua*. However, as can be seen below, the population of natural microbiota found in washing solutions was higher than it was for the pathogenic strain. The 2-4 log CFU/mL of TAM and Y&M found after treatments in washing solutions could be a drawback when recommending PA for water reprocessing. On the other hand, the reported ability of PA to reduce biofilm formation would make this product a suitable sanitizer to add in the washing step (Barbosa, 2016). Furthermore, compared to other wash water disinfectants, PA has less potential of producing degradation by-products, which are easily dissolved in water and non-toxic, thus making this sanitizer a good alternative to chlorine (Banach, 2015a).

3.1.2 Native microbiota

Regarding epiphytic microbiota, remaining TAM population after NaClO washing was 3.32 ± 0.68 log CFU / g (**Figure 20**). The PA and NaClO effect were comparable, as there were no significant

differences between populations. Washing time, 1 or 2 min, did not significantly affect the results. These counts were significantly lower than those observed after the washing with water for 2 min (W, control) with populations of 4.74 ± 0.58 log CFU / g, thus implying a sanitizing effect attributed to PA. Nevertheless, no significant differences were found on Y&M populations between the treatments and the control, so the cell decrease could be attributed to a physical removal due to water forces on the surface (Castro-Ibáñez, 2017). Microbial contamination of washing solutions after washing was between 2.5 and 4.2 log CFU / mL, except for sodium hypochlorite, in which both TAM and Y&M were reduced below 2 log CFU / mL. The experiment was repeated using a different batch of strawberries. Results showed that even if the initial population on strawberries was similar (3.96 ± 0.14 and 3.88 ± 0.14 log CFU / g strawberry), the effectiveness of some of the treatments was statistically different. Overall, reductions observed were lower in the second repetition than they were in the first assay. However, PA80 results were comparable to those obtained with NaClO being final populations of TAM after NaClO and PA80 for 1 min treatments 3.32 ± 0.68 and 3.51 ± 0.14 log ± CFU / g strawberry, respectively. These differences could be partially explained by the fact that native microbiota of fruits and vegetables is a complex and heterogenic community. Bacteria belonging to *Serratia*, *Pseudomonas*, *Enterobacter* and *Rahnella* genera, yeasts like *Candida*, *Cryptococcus* and *Rhodotorula* and molds such as *Cladosporium*, *Penicillium* and *Botrytis cinerea* are most likely to be found in strawberries (Baugher, 2016). However, dissimilar proportions of each genre and different loads can be found between cultivars, batches or years and even among fruits (Baugher, 2016; Jensen, 2013). Hereto, a higher sensitivity to washing procedures depending on the main genres existing in the population may occur, as it has been proved that there are inter-specific differences on how microorganisms are inhibited by this product (Banach, 2015a,b). It is suggested that PA disrupts the chemiosmotic function of the lipoprotein cytoplasmic membrane and transport by dislocation or rupture of cell walls and promotes catalase inactivation. Variances in membrane composition could be a reason for comparative sensitivity (Banach, 2015c).

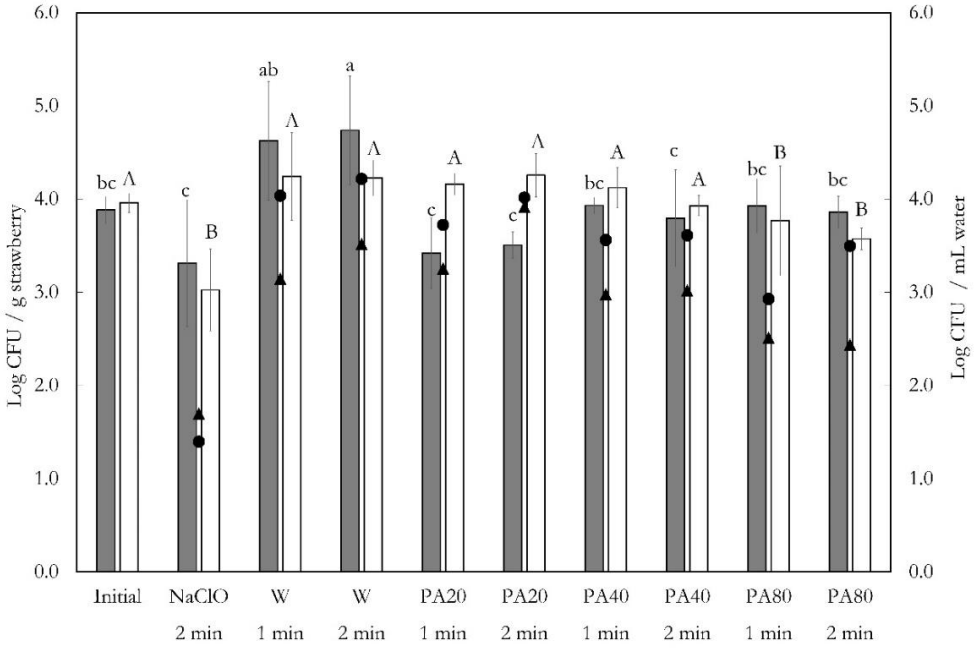

Figure 20. Population (log CFU / g strawberry) of total aerobic mesophylls (grey), or molds and yeasts (white) on strawberries. Values are the mean of 3 reps ± standard deviation. Different letters indicate significant statistically differences ($p < 0.05$) between treatments. Counts (log CFU / mL) of total aerobic mesophylls (●), or molds and yeasts (▲) in washing solutions. Values were obtained from one sample.

Other sanitizers have been used in order to reduce natural microbiota of strawberries. For instance, organic acids such as citric acid (20 g / L, pH 2.1), lactic acid (20 mL / L, pH 2.1), and malic acid (20 g / L, pH 3.3) were used for strawberry washing by Wei (2017). They reported maximum TAM reductions of 1.5 log CFU / g when using citric or malic acid, whereas Y&M reductions below 1 log CFU / g were achieved. This was attributed to the observed results in non-washed strawberries regarding the TAM, Y&M counts being less than those obtained after the different treatments.

3.2 Quality changes

3.2.1 pH, TSS, TA

Physicochemical changes in strawberries, pH, TSS contents and TA are shown in **Table 7**. Values of these parameters of non-washed strawberries were 3.39 ± 0.01, 5.9 ± 0.1 and 6.37 ± 0.30 mg citric acid / L juice, respectively, which were in concordance with the literature (Ayala-Zavala, 2004). Values of pH and TSS contents indicated barely detectable statistically significant differences among treatments. Although existing differences between treatments, there was not a general tendency that explains changes in pH and TSS contents. TA values were higher when strawberries were washed with PA80, achieving a maximum of 8.54 ± 0.17 mg citric acid / L juice when treatment time was 2 min.

Table 7. Values of pH, TSS and TA of strawberries for each washing treatment. Values are expressed as the mean of 3 reps ± standard deviation. Different letters indicate statistically significant differences ($p < 0.05$) between treatments.

Treatment	**Treatment time**	**pH**	**TSS (°B)**	**TA** (g citric acid/ L juice)
Initial	-	3.39 ± 0.01 ^{bc}	5.9 ± 0.1 ^{a}	6.37 ± 0.30 ^{cd}
NaClO	2 min	3.36 ± 0.02 ^{bcd}	5.8 ± 0.1 ^{ab}	6.56 ± 0.22 ^{c}
PA20	1 min	3.40 ± 0.01 ^{b}	5.5 ± 0.1 ^{bc}	5.96 ± 0.02 ^{de}
PA20	2 min	3.47 ± 0.03 ^{a}	5.2 ± 0.1 ^{d}	6.19 ± 0.07 ^{e}
PA40	1 min	3.33 ± 0.01 ^{d}	5.1 ± 0.1 ^{d}	6.32 ± 0.06 ^{cd}
PA40	2 min	3.45 ± 0.03 ^{a}	5.5 ± 0.1 ^{bc}	5.5 ± 0.16 ^{e}
PA80	1 min	3.34 ± 0.01 ^{cd}	5.5 ± 0.0 ^{c}	7.06 ± 0.15 ^{b}
PA80	2 min	3.26 ± 0.01 ^{e}	5.2 ± 0.1 ^{d}	8.54 ± 0.17 ^{a}

3.2.2 Color

Strawberry color before any sanitization washing, expressed as CIE-Lab coordinates, was L* 40.04 ± 3.20, a* 32.69 ± 2.57 and b* 26.14 ± 5.40 (**Table 8**). These values were comparable to those found in the literature (Van de Velde, 2014). Statistical differences were observed among treatments regarding each CIE-Lab coordinate, and PA-washed samples seem to have more luminosity and to be less yellowish and reddish, as L* values are higher and a* and b* lower in these samples. However, TCD was not statistically influenced by treatments. It has been stablished that when TCD is higher than 3.5, a clear difference in color is noticed by the inexperienced viewer (Mokrzycki, 2011). A general trend was found in TCD, markedly observed when using PA at 80 ppm, with values of 4.76 ± 1.69 and 4.85 ± 3.88 for 1 and 2 min, respectively. When washed with hypochlorite, TCD was 0.84 ± 1.13, indicating that there was no visible alteration in color. Color is one of the sensory parameters that may affect consumers' acceptance and buying intention (Barrett, 2010).

3.2.3 Texture

Texture was evaluated by compression and firmness tests (**Table 8**). The obtained results for firmness showed no statistical differences among treatments and initial value. Firmness values were in the range of those reported by other authors (Duvetter, 2005). However, compression values showed a statistical difference between non-washed and PA80 2 min washed strawberries. After washing with PA 80 ppm for 2 min, maximum force at compression was 48.57 ± 12.28 N, higher than the 30.67 ± 7.30 N obtained in non-washed strawberries (initial). This increase in texture may be considered to be an undesirable impact of this washing treatment on strawberry quality, as consumers search for 'moderate hardness' against firm or smooth strawberries (Bhat, 2015).

Table 8. Values of CIE Lab coordinates, total color difference (TCD) and firmness measured by compression and pricking tests. Values are the mean of 20 samples by 3 reps ± standard deviation. Different letters indicate statistically significant differences ($p < 0.05$)

Treatment	Treatment time	Color			
		L*	a*	b*	TCD
Initial	-	40.04 ± 3.20 ^{ab}	32.69 ± 2.57 ^{ab}	26.14 ± 5.40 ^{abc}	-
NaClO	2 min	39.28 ± 3.66 ^{ab}	32.66 ± 1.56 ^{ab}	26.48 ± 5.62 ^{abc}	0.84 ± 1.13 ^{a}
PA20	1 min	39.83 ± 3.10 ^{ab}	33.18 ± 1.80 ^{a}	28.49 ± 4.74 ^{ab}	2.41 ± 1.02 ^{a}
PA20	2 min	41.61 ± 3.63 ^{ab}	32.84 ± 1.78 ^{ab}	28.27 ± 5.34 ^{ab}	2.65 ± 0.90 ^{a}
PA40	1 min	41.17 ± 3.05 ^{ab}	32.66 ± 1.88 ^{abc}	27.31 ± 5.03 ^{abc}	1.61 ± 0.80 ^{a}
PA40	2 min	38.82 ± 2.80 ^{b}	31.73 ± 1.81 ^{abc}	22.86 ± 5.12 ^{bc}	3.63 ± 0.90 ^{a}
PA80	1 min	38.70 ± 3.83 ^{b}	30.55 ± 3.28 ^{bc}	22.11 ± 6.81 ^{c}	4.76 ± 1.70 ^{a}
PA80	2 min	42.92 ± 6.66 ^{a}	29.74 ± 4.17 ^{c}	28.09 ± 6.13 ^{a}	4.85 ± 3.90 ^{a}

Treatment	Treatment time	Firmness	
		Compression (N)	Firmness (N)
Initial	-	30.67 ± 7.30 ^{a}	2.67 ± 1.06 ^{a}
NaClO	2 min	36.51 ± 12.01 ^{ab}	2.23 ± 0.88 ^{a}
PA20	1 min	34.06 ± 10.02 ^{a}	2.46 ± 1.22 ^{a}
PA20	2 min	31.37 ± 8.90 ^{a}	2.30 ± 0.65 ^{a}
PA40	1 min	34.32 ± 9.92 ^{a}	2.82 ± 0.90 ^{a}
PA40	2 min	32.23 ± 6.90 ^{a}	2.31 ± 0.63 ^{a}
PA80	1 min	38.09 ± 12.50 ^{ab}	2.98 ± 0.91 ^{a}
PA80	2 min	48.57 ± 12.28 ^{b}	3.59 ± 1.67 ^{a}

3.3 Biochemical characterization

3.3.1 Antioxidant activity

Antioxidant activity of samples washed with NaClO or PA was assessed by FRAP and DPPH· free radical scavenging ability assays (**Table 9**).

FRAP results indicated that control strawberries had an antioxidant capacity equivalent to 145.93 ± 8.09 mg ascorbic acid / 100 g FW. DPPH· results showed values of 138.04 ± 12.21 mg AAE / 100g FW. Nevertheless, antioxidant activity was maintained in strawberries washed with hypochlorite or PA at different concentration and time combinations, as no statistical differences were observed between samples.

Table 9. Anthocyanin content, total phenolic content (TPC), and antioxidant activity (FRAP and DPPH· methods) of strawberries for each washing treatment. Values as expressed as a mean of 3 reps ± standard deviation. Different letters indicate significant statistically differences ($p < 0.05$) between treatments.

Treatment	Treatment time	Anthocyanin content (mg / 100 g FW)	TPC (mg GAE/ 100 g FW)	FRAP (mg AAE / 100 g FW)	DPPH· (mg AAE / 100 g FW)
Initial	-	1.90 ± 0.18 ^{cd}	83.01 ± 1.58 ^{a}	145.92 ± 8.09 ^{a}	138.04 ± 12.21 ^{a}
NaClO	2 min	1.91 ± 0.13 ^{cd}	83.56 ± 5.01 ^{a}	136.05 ± 21.75 ^{a}	133.35 ± 6.51 ^{a}
PA20	1 min	1.96 ± 0.06 ^{cd}	75.78 ± 2.79 ^{a}	123.90 ± 5.44 ^{a}	119.77 ± 1.16 ^{a}
PA20	2 min	2.42 ± 0.05 ^{ab}	75.84 ± 4.26 ^{a}	216.35 ± 6.63 ^{a}	118.26 ± 5.95 ^{a}
PA40	1 min	1.82 ± 0.11 ^{cd}	72.09 ± 0.25 ^{a}	133.81 ± 4.32 ^{a}	124.98 ± 8.99 ^{a}
PA40	2 min	2.08 ± 0.02 ^{bc}	77.89 ± 8.47 ^{a}	125.80 ± 6.70 ^{a}	112.86 ± 4.83 ^{a}
PA80	1 min	2.56 ± 0.01 ^{a}	82.89 ± 3.5 ^{a}	140.94 ± 1.75 ^{a}	129.6 ±1.58 ^{a}
PA80	2 min	1.66 ± 0.05 ^{d}	77.77 ± 2.92 ^{a}	59.07 ± 6.19 ^{a}	116.88 ± 11.3 ^{a}

3.3.2 Anthocyanin content

Initial anthocyanin content of strawberries was 1.90 ± 0.18 mg / 100 g FW (**Table 9**). Significant increases of anthocyanin values were found after the treatments PA20 2 min and PA 80 1 min, but a general tendency was not observed. To date, no studies have been found on how PA can affect anthocyanin content of strawberries. Anthocyanin values obtained with strawberries used in this study were lower than those found by Nowicka (2019) and Van de Velde (2014). This could be attributed to the use of different strawberry varieties or maturity stage, or by differences in the anthocyanin extraction method, as ultrasound was used to assist extraction in those studies, which makes anthocyanins more accessible as it helps to break cell walls and remove boundaries (Meyers, 2003).

3.3.3 Total phenolic content

Values of TPC are shown in **Table 9**. Initial phenolic content of strawberries was 83.01 ± 1.58 mg GAE/100 g FW, which was in similar amounts to those reported in the literature (Perin, 2019; Yeoh, 2017). Even so, Avalos-Llano (2018) found greater values of TPC in strawberry (550 mg GAE/100 g FW). These dissimilarities could be attributed to fruit differences in maturity stage (Ban, 2018) or cultivar (Šamec, 2016), for instance. Also, different extraction methods and interferences by other compounds could mark a difference on the values obtained (Azmir, 2013). TPC in washed strawberries did not statistically change either with NaClO solution (83.56 ± 5.01 mg GAE / 100 g FW) or PA solutions at different concentrations or times. Similarly, no significant differences were observed by Vandekinderen (2017), in carrots washed with 80 or 250 ppm PA. Contrarily, Ling (2018) found a significant increase in TPC when washing loquat fruit with a higher dose of PA (4,000) ppm for a longer time (6 min) with respect to the control.

4. Conclusions

The results of this study demonstrated the effectivity of peracetic acid treatments in reducing artificially inoculated *L. innocua* in both, strawberries and wash water, which would reduce cross-contamination in washing steps. Concerning native microbiota, mesophilic bacteria and molds and yeasts reduction values were lower than those observed with *L. innocua* but similar to those obtained with a standard treatment using sodium hypochlorite. PA in general did not affect the physicochemical and nutritional quality of strawberries.

Future experiments should be carried on in order to validate the efficacy of PA against the strawberry pathogens of concern, namely *Salmonella*, STEC, norovirus, or hepatitis A virus. Further investigations should be focused on the effect of PA during shelf life and subsequent processing of strawberries.

In this paper, the effect of PA has been studied against a pathogen surrogate and epiphytic microbiota, and the results have shown that PA washing seems to be a good alternative to chlorine disinfection for pathogens. However, results demonstrated that its efficacy against natural microbiota was lower than hypochlorite treatment, especially for the number of these microorganisms that remained in the wash water. To overcome this weakness, more studies should be carried on, including combination of PA with other physical technologies, such as ultrasounds or ultraviolet light, in order to promote a synergistic effect and increase shelf-life of strawberries washed with these procedures.

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Conflict of interests

The authors declare no conflict of interests.

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Chapter 2

Water UV-C treatment alone or in combination with peracetic acid: A technology to maintain quality and safety of strawberries

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Disinfection of fruits is one of the most important steps since they are going to be eaten fresh-or minimally-processed. This step affects quality, safety, and shelf-life of the product. Despite being a common sanitizer in the fruit industry, chlorine may react with organic matter leading to the formation of toxic by-products. Alternative sustainable disinfection strategies to chlorine are under study to minimize environmental and human health impact. Water-assisted UV-C light (WUV-C) is proposed here as an alternative sanitizing method for strawberries. In this study, strawberries were washed for 1 or 5 min in a tank with 2 or 4 lamps on, each emitting UV-C light at 17.2 W/cm^2, or in a chlorine solution (200 ppm, pH 6.5). Moreover, trials with 4 lamps on, together with a washing solution consisting on peracetic acid at 40 or 80 ppm, were carried out. Overall, quality and nutritional parameters of strawberries after treatments were maintained. Changes in color were not noticeable and fruits did not lose firmness. No major changes were observed in antioxidant activity, organic acid, anthocyanin, vitamin C, and total phenolic content. Yeasts and molds were not affected by the WUV-C treatment, and 5 min were needed to significantly reduce total aerobic mesophylls population. However, reductions of artificially inoculated *Listeria innocua* and *Salmonella* Typhimurium after WUV-C treatments were comparable to those obtained with chlorine-wash, which were 3.0 log CFU / g. Moreover, WUV-C light was effective to minimize microorganisms remaining in washing water, avoiding cross-contamination and thus, allowing water recirculation. This effect was improved when combining the action of UV-C light with peracetic acid, showing the suitability of this combined treatment, understood as an alternative to chlorine sanitation, for sanitizing strawberries and keeping the populations of pathogenic bacteria in washing water lower than 0.6 ± 0.1 log CFU/mL.

Sanitization, organic acids, *Listeria innocua*, *Salmonella* Typhimurium, UV-C light, fruit

1 Introduction

Strawberry (*Fragaria* × *ananassa*) production and consumption has practically doubled over the past 15 years (Indexbox, 2017), being. Strawberries are generally considered a safe product, and some authors reported the absence of pathogenic microorganisms in the sampled fruits (Ortiz-Solà, 2020). However, mold growth and loss of firmness of strawberries may cause losses of 10 % to 35 % at retail and at consumer level, respectively, making the control of alterative microbiota a challenge for fruit industry (Kelly, 2019). Moreover, berries including strawberries have been linked to safety issues associated with foodborne pathogens, such as *Salmonella* spp. and Norovirus (EFSA, 2014) and *Listeria monocytogenes* (Hadjilouka, 2014). The reported problem is mostly related to frozen strawberries, that normally are washed and disinfected with chlorine before freezing.

Chlorine is a widespread sanitizer used as a water disinfectant to reduce pathogenic and other microbiota loads in fruits and vegetables. However, its dependence on a number of factors, including pH, concentration, and presence of organic matter (Chen, 2017) along with the health concerns associated with its toxic by-products, such as chloroform and other trihalomethanes, chloramines and haloacetic acids (Meireles, 2016), have led to a search for safer alternatives. Moreover, a recent regulation from the European Comission (Regulation Comission (EU) 2020/685 stablishes the limits of perchlorates, which are by-products of chlorine, in some foods specially in fruits and vegetables. A chemical alternative that does not leave any residue on the food and has been proposed in the literature is peracetic acid (PA), which is effective in decreasing the native microbiota and the pathogenic contamination of produce such as strawberries, without decreasing the quality of the fruits (Méndez-Galarraga, 2019; Nicolau-Lapeña, 2019; Van de Velde, 2014). Ultraviolet (UV) light has also been proposed due to its inexpensiveness, efficacy on pathogen inactivation and reduced unwanted physicochemical changes (Usaga, 2017). UV light is the portion of the electromagnetic spectrum with wavelengths ranging between 100 to 400 nm. Within this, the fraction that has the most germicidal effect is comprised between 100 and 280 nm, and it is known as UV-C light (Pigeot-Rémy, 2012). Its mode of action consists of UV absorption by DNA and RNA, which in turn, origins the formation of cyclobutene-pyrimidine and pyrimidine-pyrimidine dimers, blocking the elongation of nucleic acid transcripts (Seltsam, 2011). This blocks and compromises cellular functions and replication, causing the eventual cell death (Barba, 2017).

This study evaluates the role of an emerging technology consisting in UV-C light transmitted by lamps immersed in stirring water (WUV-C), as an alternative to chlorine disinfection for strawberries. This approach could overcome some of the drawbacks of air-transmitted UV-C light. Shallow penetration ability, sample heating and shadowing effect of UV treatment limit its application in decontamination of fresh produce (Liu, 2015). Agitation of fruits conveyed by water could serve to prevent shadowing effect, which happens with static fruits. Otherwise, if the strawberries were agitated in a dry surface, an increase of mechanical damages would occur. Moreover, water may enhance the removal of microorganisms from rough surfaces or hidden in trichomes. There are studies in which UV-C irradiation assisted by water has already been used to disinfect fresh produce (Guo, 2017). The same WUV-C device used in this study was tested in vegetables in other investigations (Collazo, 2018), but to the best of authors' knowledge, this is the first approach to disinfect strawberries in a system where lamps and fruits are immersed in water.

The objective of this study was to evaluate the efficacy of this WUV-C system as a sanitizer treatment for strawberries, for both epiphytic microbiota and artificially inoculated *Listeria innocua* and *Salmonella* Typhimurium. Its efficacy was compared to that of chlorine, in order to evaluate WUV-C as a potential alternative to this well stablished sanitation method. Moreover, microorganisms that could remain in water were investigated in order to see the efficacy of UV-C lamps in sanitizing washing water, in order to prevent cross-contamination. The effect on the quality and nutritional parameters was also investigated, so as to provide a product that meets consumers' needs. Once verified the UV-C sanitizing effect and its

impact on strawberry quality, this method was combined with the use of peracetic acid in solution with the washing water, so as to improve the results obtained with only UV-C irradiation while being able to diminish the washing time.

2 Materials and methods

2.1 Materials

Strawberries (*Fragaria x ananassa*), had been harvested in Huelva between March and May in the 2019 campaign and transported and kept in a cold store. They were bought in a local supermarket the same day of the beginning of the experiment. Before the treatment, peduncle and leaves were carefully removed.

Triptone soy broth (TSB), triptone soy agar (TSA), Palcam base agar and Palcam selective supplement for *Listeria*, xilose lysine deoxycholate agar (XLD), yeast extract, plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), and peptone were obtained from Biokar Diagnostics (Allonne, France). Dey-Engley broth was obtained from Honeywell Fluka (Madrid, Spain). Peracetic acid (PA) 15 % was purchased from Panreac AppliChem (Barcelona, Spain).

Ascorbic, gallic, quinic, malic, citric, tartaric and fumaric acids, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, metaphosphoric acid, acetic acid, 3,3',3"-phosphanetriyltripropanoic acid (TCEP), were acquired from Sigma-Aldrich (Steinheim, Germany). Catechin, cinnamic acid, coumaric acid, quercetin and kaempferol standards were obtained from Merck (Darmstadt, Germany). Pelargonidin standard was purchased from Extrasynthese (Genay, France). Methanol, acetone, chlorhidric acid (37 %), sodium acetate, sodium hydroxide, sodium chloride, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were procured by Panreac (Llinars del Vallès, Spain).

2.2 UV-C water-assisted equipment

Treatments were conducted in the UV-C water-assisted (WUV-C) equipment LAB-UVC-Gama (UVC-Consulting Peschl España, Castellón, Spain) (**Figure 21** A and **Figure 21** B). This apparatus consists of a tank where 4 UV-C lamps (GPH303T5L/4, 254 nm) are installed, emitting a power of 17.2 W each. The lamps are enclosed by a quartz tube (25 mm of outer diameter) to prevent the lamp's contact with the wash water and product. The equipment has a recirculating system connected to a water pump and an aeration system that provides bubbling, which improves accessibility to UV-C light from all sides of the fruit. Radiation, according to the simulation and calculations given by the manufacturer, is distributed inside the empty tank as shown in **Figure 21** C.

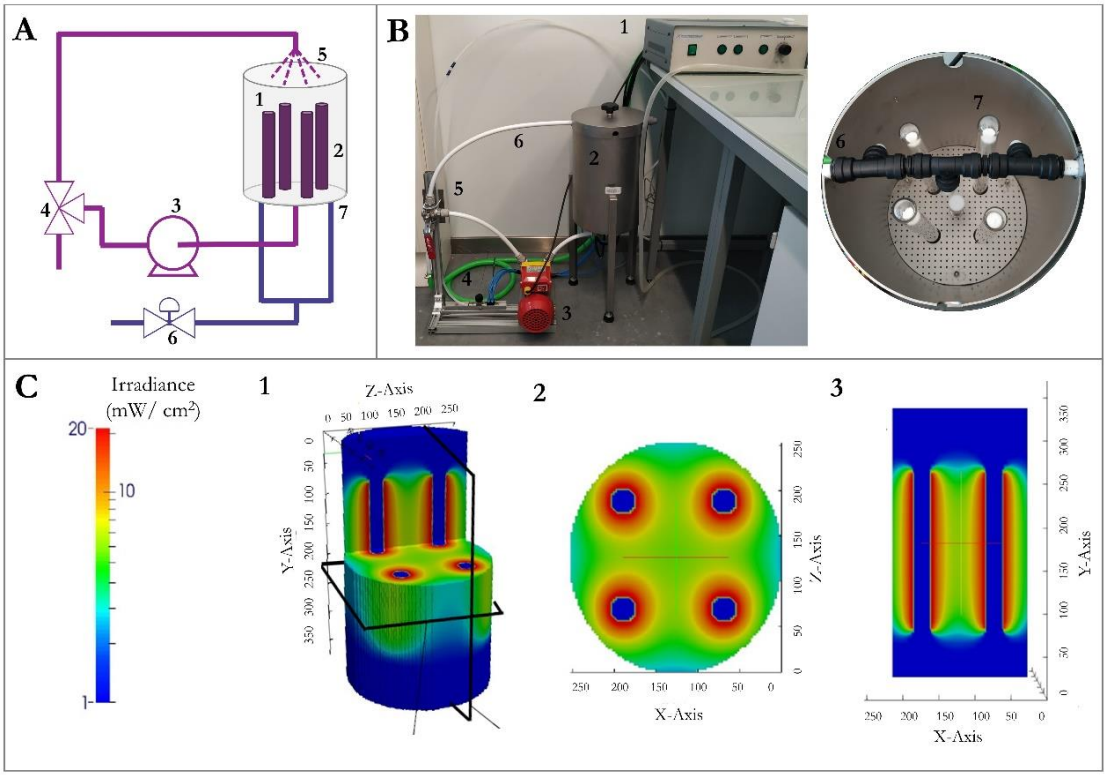


Figure 21. (**A**) Scheme of the equipment: tank (1), UV-C lamps (2), water pump (3), water circuit valve (4), water recirculation (5), air regulator valve (6), air inlet (7); (**B**) equipment: controller (1), tank (2), water pump (3), air regulator valve (4), water circuit valve (5), water recirculator (6), UV-C lamps (7); (**C**) irradiance distribution inside the tank: 3-D view (1) elevation view (2), section view (3).

Before the experiment, lamps were preheated for 10 min, to reach the maximum irradiance at the start point of washing treatments. After this time, irradiance values in the empty tank were 5.8 and 10.5 W/cm² with 2 and 4 lamps on, respectively, measured with a UV-sensor Easy H1 (Peschl Ultraviolet, Mainz, Germany) through an orifice located on the lid of the tank. Afterwards, the WUV-C tank was filled with 12 L of cold (6 ± 2 °C) water and the UV-C lights were switched on for 12-15 min. Absorbance at 254 nm of the water inside the tank was measured spectrofotometrically using a GENESYS™ 10S UV–Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). Turbidity was measured using a portable turbidimeter (TN-100, Eutech, Singapore) measuring in Nephelometric Turbidity Units (NTU). These measures were taken before and after each treatment, to check the ability of UV-C light to be transmitted in this media.

2.3 Washing treatments

For each treatment, 20 strawberries – average weight 25 ± 5 g – were immersed in 12 L of cold (6 ± 2 °C) tap water in agitation. Experiments, with 3 repetitions each, were performed separately for non-inoculated (one replication, n=3) and inoculated (two replications, n=6) fruits. First, effect of WUV-C irradiation alone was studied. Four WUV-C treatments were proposed, combining 2 or 4 lamps on with different contact times: 1 or 5 min: 2 lamps on for 1 min (2L-1 min), 2 lamps on for 5 min (2L-5 min), 4 lamps on for 1 min (4L-1 min), and 4 on lamps for 5 min (4L-5 min). Then, the best WUV-C approach was selected for its combination with PA. For this second trial, five treatments were proposed: WUV-C alone (WUV), PA at 40 ppm (PA 40) or 80 ppm (PA 80) alone, and WUV-C with PA 40 ppm or 80 ppm (WUV + PA40 or WUV + PA 80). A 200 mg / L of free chlorine solution, adjusted to pH 6.5 using citric acid 2 M (NaOCl) was used as a reference to compare the efficacy of WUV-C treatments with that of chlorine, to check if the proposed UV-C method can be a good alternative to it. After NaOCl disinfection,

strawberries were rinsed in tap water. Moreover, a tap water control was added when performing the microbial trials to determine the removal of the bacteria due to a physical effect. Water parameters including pH and oxidation-reduction potential (ORP) were measured before and after each treatment. ORP and pH were measured in a pH-meter (GLP22, Crison, Alella, Barcelona, Spain) equipped with a pH probe (ref. 52-03, Crison) or ORP probe (ref.62-51 Hach, Vésenaz, Geneva), respectively.

After the washing, fruits were let at room temperature to drain the excess of water.

2.4 Effect of WUV-C treatment on the quality of strawberries

The trial to study quality and nutritional parameters was performed once, with 3 replications (n=3) in non-inoculated strawberries. Determinations on fresh-product were carried out just after the draining period, and aliquots of each treatment were frozen with liquid nitrogen, milled using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain) and stored at -80 °C for further biochemical analysis. Finally, the remaining strawberries were stored at 4 ± 1 °C for 24 h, and they were and analyzed or frozen after this time.

2.4.1 Quality analysis

For determining **pH, total soluble solids** and **titratable acidity**, strawberries were smashed in a blender to prepare 25 mL of juice. Each parameter was evaluated twice for each repetition (three repetitions) according to Nicolau-Lapeña (2019).

Color of 10 strawberries was measured on 3 sides, using a CR-200 Minolta Chrome Meter (Minolta, INC., Tokyo, Japan) with a D65 illuminant and 10° observer angle. The instrument was calibrated using a standard white reflector plate. Color was expressed as CIE L* a* b* coordinates. Total color difference (TCD) (Eq. 1), and Hue angle (H°) (Eq.2) were calculated.

$$TCD = [(L^*_f - L^*_i)^2 + (a^*_f - a^*_i)^2 + (b^*_f - b^*_i)^2]^{1/2} \quad \text{Eq. 1}$$

Where f = final (strawberries after each treatment) and i = initial (strawberries before any treatment)

$$H° = \tan^{-1}(b^* / a^*) \quad \text{Eq. 2}$$

Texture changes were evaluated on 10 strawberries halves for treatment, that were cut immediately before the determination. Two textural tests using the TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England) were performed. In the compression test, the maximum force required by 2 parallel plates to compress 6.0 mm a strawberry half was recorded. Puncture test was performed with a 4 mm cylindrical probe, measuring the maximum force encountered when the probe enters 8.0 mm deep into the tissue. Both tests were run at 5 mm/s speed with a trigger force of 0.1 N.

2.4.2 Biochemical analysis

Antioxidant activity of strawberries was assessed by ferric reducing antioxidant power (FRAP) and DPPH scavenging activity assays, as described in Nicolau-Lapeña (2019). Results are expressed as μmol ascorbic acid equivalents (AAE) / 100 g FW of 3 repetitions (n=3).

Content of **organic acids**, including tartaric, malic, fumaric, citric and quinic acid was determined by high-performance liquid chromatography (HPLC) in a Waters 717 plus Autosampler HPLC system (Waters Corp., NJ, USA) coupled to a UV detector, following the method described by Scherer et al. (2012) with minor changes. Duplicate injections were performed, and average peak areas were used for quantification (n=2). Concentrations of organic acids in samples were calculated by the area interpolation on the adequate calibration curve.

Vitamin C contents expressed as the sum of ascorbic acid and dehydroascorbic acid (TAA), was determined by high-performance liquid chromatography (HPLC) in a Waters 717 plus Autosampler HPLC system (Waters Corp., NJ, USA) coupled to a UV detector, following the method described by

Lafarga (2018). Average peak areas of duplicate injections were used for quantification (n=2). Concentration of vitamin C, expressed as mg TAA / 100 g FW, was calculated by the area interpolation on the adequate calibration curve.

Anthocyanin extracts and quantification were carried out in triplicate (n=3) according to the method described by Meyers (2003). Anthocyanin content was expressed as mg of cianidine-3-glucosyde / 100 g of strawberry.

The **total phenolic content** (TPC) was assessed by Folin Ciocalteau method on the same extract used for antioxidant activity determination, following the procedure described by Nicolau-Lapeña (2019). Results were expressed as mg gallic acid equivalents (GAE) / 100 g FW of 3 repetitions (n=3)

For a **phenolic profile**, extracts of phenolic were analyzed according to Aaby (2012), and da Silva (2007) with minor modifications, on an Acquity UPLC system equipped with a diode array detector (DAD) (Waters, Milford, MA, USA). The peaks were tentatively identified according to chromatographic data from literature Aaby (2012) and da Silva (2007) and quantified by DAD detection and external calibration curves with pure standards.

2.4.3 Microbiological quality

The effect of the washing treatments on total aerobic mesophylls (TAM) and yeasts and molds (Y&M) was evaluated. For this, 25 g per repetition (n=3), taken from pieces of 2 strawberries to ensure representativity, were diluted 1:4 in peptone buffered solution. The count process followed the method described in Nicolau-Lapeña (2019). Results were expressed as log CFU / g, and the detection limit was 20 CFU / g.

Remaining populations of, TAM and Y&M were also determined in wash water. Results were expressed as log CFU / mL. When counts were below the limit of detection (5 CFU / mL), and presence was confirmed by Dey-Engley color change, an arbitrary value of ½ limit of detection was assigned.

2.5 Effect of WUV-C system in the survival of *Listeria innocua* and *Salmonella* Typhimurium artificially inoculated on strawberries.

2.5.1 Strains and strawberries inoculation

Listeria innocua strain CECT-940 (*Colección Española de Cultivos Tipo*, Burjassot, Spain) was used as a surrogate of *L. monocytogenes* in this study (Francis, 1997). *Salmonella enterica* subs. *enterica* serovar Typhimurium CECT-4594 was also used to inoculate strawberries. Cultures were prepared as described in Nicolau-Lapeña (2019).

The day before the experiment, strawberries designated for this purpose were inoculated with a suspension containing 10^{10} CFU / mL of *L. innocua* or *S.* Typhimurium at stationary phase, by pipetting 50 µL in small droplets on the surface. Once dried, strawberries were stored at 4 ± 1 ºC overnight. Concentration immediately after the inoculation and drying, and also after storage was checked by plating in duplicate in selective Palcam or XLD media for *L. innocua* and *S. Typhimurium*, respectively.

Washing treatments were performed as indicated in section 2.3. The experiment was repeated twice.

2.5.2 Determination of *L. innocua* and *S.* Typhimurium populations

One strawberry per repetition was used for microbiological analysis (n=6). Populations were determined by plate count on selective Palcam medium for *L. innocua* or XLD for *S.* Typhimurium in duplicate, as it has been previously described in Nicolau-Lapeña (2019). Results were expressed as log CFU / fruit, and detection limit was 20 CFU / fruit.

Logarithmic reductions of the pathogens were calculating by the following equation (Eq. 1)

$$\text{Log reductions (Log cfu/fruit)} = \text{Log}_{10}(\overline{N}_0) - \text{Log}_{10}(N_t) \qquad \text{Eq. 1}$$

where $\overline{N}_0$ is the mean of the initial population (CFU / fruit), and N_t is the population after the washing treatment (CFU / fruit).

Remaining populations of *L. innocua* and *S.* Typhimurium were determined in wash water. Duplicate 1-mL samples of wash water after treatment were neutralized in 9 mL Dey-Engley medium. Results were expressed as log CFU / mL. When counts were below the limit of detection (50 CFU / mL), and presence was confirmed by Dey-Engley color change, an arbitrary value of ½ limit of detection was assigned.

2.6 Statistical analysis

All data were checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. Principal components analysis (PCA) was carried out to obtain correlations among phenolic profile of strawberries. All statistical analyses were carried on using JMP 13 (SAS Institute Inc., Cary, USA).

3 Results and discussion

3.1 Properties of washing water

Water used to wash strawberries was controlled during each treatment. For those treatments with no chemical solution (control or WUV-C), pH values were 7.95 ± 0.22, while for those with PA 40, PA 80 ppm or NaOCl, pH values were, 5.79 ± 0.21, 4.64 ± 0.08, or 6.56 ± 0.11, respectively. For the same conditions, ORP values were 256 ± 22 (control or WUV-C), 444 ± 16 (PA 40 ppm), 504 ± 3 (PA 80 ppm), and 891 ± 4 (NaOCl). Washing treatments were carried out at 7.5 ± 0.5 ºC. To check that dispersion of radiation through water was not reduced by any turbidity of the media, turbidity and absorbance at 254 nm were measured. For all treatments, turbidity values were 0.9 ± 0.2 NTU, and absorbance was 0.073 ± 0.035, indicating no interference of irradiation caused by presence of particles or dirt in water.

3.2 Quality changes in strawberries

3.2.1 Physicochemical quality

Physicochemical quality of non-washed strawberries indicated that samples had a pH of 3.57 ± 0.07, TSS values of 6.60 ± 0.01 °Brix and TA of 6.12 ± 1.87 mg citric acid / L juice (Data not shown). When strawberries were washed, pH statistically decreased by approximately 0.2 points, reaching 3.21 ± 0.04 when NaOCl was used in water (**Table 10**). This treatment also showed the highest TA, reaching a concentration of 8.07 ± 0.70 mg citric acid / L juice. This was not attributed to residual chlorine on the surface of strawberries, because an additional washing step with water was added to remove any residue after this treatment. Although statistically significant differences were observed on pH, TSS and TA values, a general tendency was not detected, and changes could not be attributed to WUV-C doses or times.

Table 10. Quality assessment of strawberries after washing treatments. Values are the mean of 3 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments.

	pH	**TSS** (°B)	**TA** (mg citric acid / L)
NaOCl	3.21 ± 0.04 ^{d}	5.67 ± 0.42 ^{c}	8.07 ± 0.70 ^{a}
UV-2L-1 min	3.36 ± 0.02 ^{bc}	6.17 ± 0.82 ^{a}	6.63 ± 0.89 ^{b}
UV-2L-5 min	3.35 ± 0.07 ^{c}	5.93 ± 0.68 ^{b}	5.26 ± 0.55 ^{c}
UV-4L-1 min	3.32 ± 0.05 ^{c}	5.90 ± 0.35 ^{d}	5.70 ± 0.43 ^{bc}
UV-4L-5 min	3.43 ± 0.00 ^{a}	5.50 ± 0.26 ^{b}	6.21 ± 0.48 ^{bc}

3.2.2 Color and texture

Color of strawberries, expressed as CIE L*a*b* coordinates, was L* of 43.5 ± 4.8, a* of 32.9 ± 0.3 and b* 29.1 ± 8.5 (see Supplementary material). The higher L* and b* values than those reported by other authors (Kelly, 2019) mean a greater luminosity and yellowish color of the strawberries of this study. There were no statistical differences in L*a*b* coordinates nor in H°, which was on average 40.92 ± 0.01, between the treatments. In strawberries, color is highly correlated with the anthocyanin content. When washed with NaOCl or 4L-1 min, TCD reached the highest values of 3.1 and 3.0, but in all cases, TCD was lower than 3.5 which, according to Mokrzycki (2011), would not be noticed by the inexperienced viewer. No changes in color were also reported by Liu (2014), after applying 4,1 kJ / m² UV-C irradiation (in air) on strawberries.

Firmness of strawberries before and after the washing treatments was assessed by compression and pricking tests. There were no significant statistical differences in firmness immediately after the WUV-C

washings, neither between the treatments or when compared to the NaOCl treatment. Average compression force was 44.6 ± 3.1 N and pricking test results were 3.6 ± 0.3 N (see Supplementary material). The preservation of firmness is important in strawberries to maintain quality through all the supply chain steps, as soft fruits are more likely to mechanical damage and waste at consumer level (Kelly, 2019). Liu (2014) also reported no changes in strawberry firmness after WUV-C irradiation.

3.3 Biochemical characterization

3.3.1 Antioxidant activities

Antioxidant activity of strawberries, washed or not with WUV-C, was assessed by FRAP and DPPH· free radical scavenging ability assays.

Initial antioxidant values were 797 ± 46 and 608 ± 8 μmol AAE / 100 g FW for FRAP and DPPH· assays, respectively (Data not shown). These values were in accordance with those reported in literature for 90 different strawberry cultivars (Nowicka, 2019). Antioxidant activity in fruits was maintained whether WUV-C was applied or not with no significant differences observed among treatment conditions (Data not shown).

3.3.2 Acid organic contents

Content of organic acids was determined in both WUV-C light treated and non-treated strawberries, immediately after the treatment and 24 h later (**Figure 22**). Initial amounts of quinic, malic, citric, tartaric and fumaric acids in strawberries were 126.6 ± 13.5, 18.5 ± 1.8, 820.5 ± 45.9, 155.0 ± 15.7 and 2.4 g / 100 g FW, respectively. No significant differences were detected in quinic and tartaric acids values between WUV-C treatments. For the other acids, changes did not show a clear tendency related with treatment times or WUV-C light dose. This independent variation has also been reported in cucumber treated with UV-C light at 8.2 W / m^2 for 1, 5 or 10 min (Erkan, 2001). In general, all the treatments showed the same trend in organic acid content after 24 h. The only exception was observed after applying 2L-5 min treatment to strawberries, which showed an inverse progression when compared to the other treatments of the same acid. As far as we know, there is no study in the literature reporting the evolution of organic acids in fruit matrices depending on WUV-C treatment.

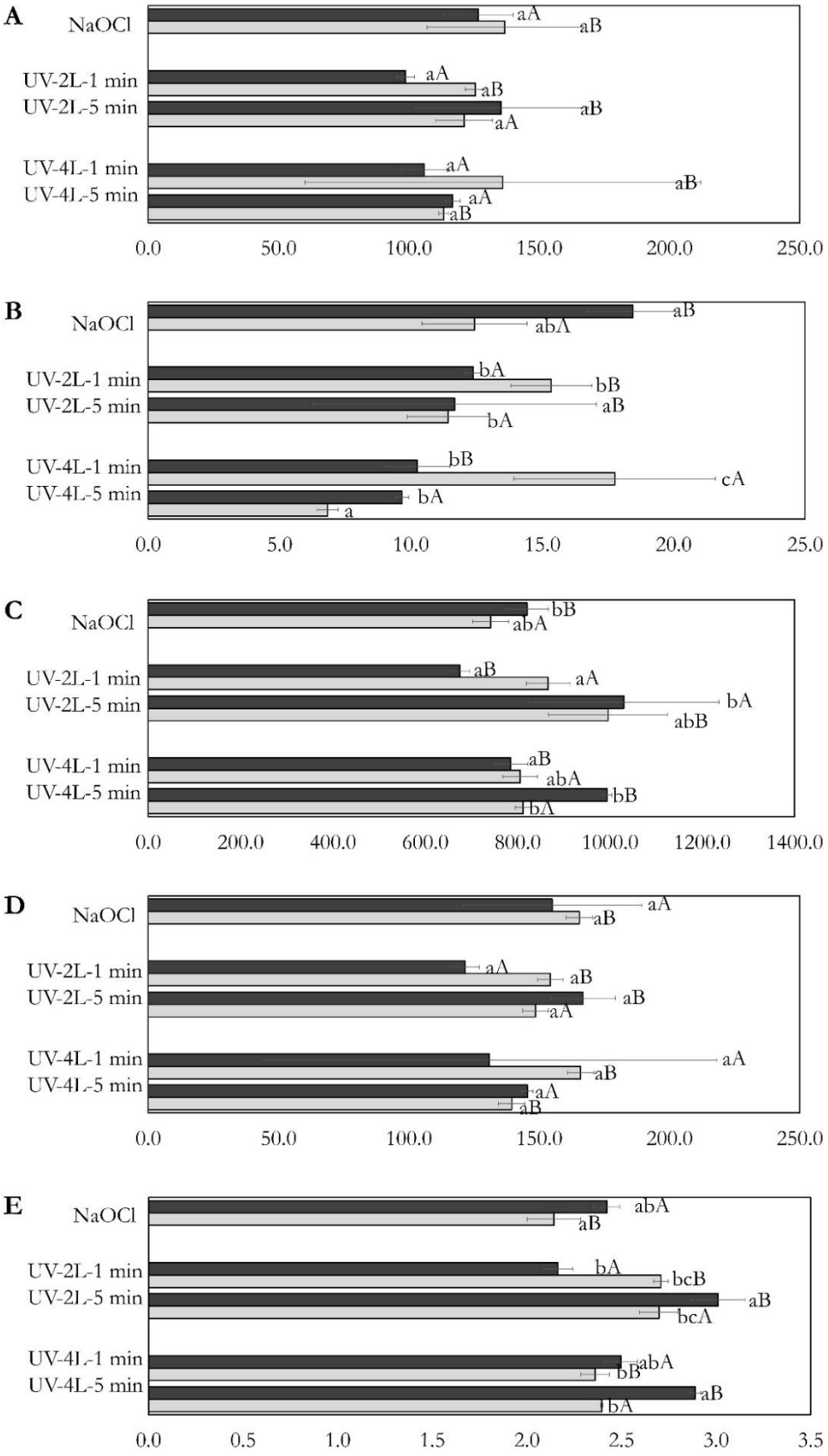


Figure 22. Organic acid content of strawberries immediately after the washing treatments (■) and 24 after storage at 4°C (□). Contents expressed as mg quinnic acid / 100 g FW (**A**), mg mallic acid / 100 g FW (**B**), mg citric acid / 100 g FW (**C**), mg tartaric acid / 100 g FW (**D**), and mg fumaric acid / 100 g FW (**E**). Results are the mean of 2 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments and days.

3.3.3 Vitamin C content

Vitamin C, expressed as the sum of ascorbic acid and dehydroascorbic acid (TAA), was determined in fresh strawberries. Initial values were of 29.8 ± 1.0 mg TAA / 100 g FW (Data not shown), and immediately after the WUV-C treatments (**Table 11**), no statistical differences were observed when compared to NaOCl washing. A decrease in AA has been accounted (Gopisetty, 2018), explained by induced molecular excitation and subsequent photochemical reactions. In contrast, no changes were reported by Allende (2007) when irradiating strawberries at doses of 0.28 kJ / m². In this study, after 24 h, TAA slightly increased in all the treatments and in the control. A similar increase was also reported by Jagadeesh (2011), who applied UV-C doses of 3.7 kJ / m² to tomatoes, and AA increased throughout storage time.

Table 11. Total ascorbic acid (TAA), anthocyanins and total phenolic contents of strawberries just after the treatments (0 h) or after 1 day storage at 4 ºC (24 h). Values are the mean of 3 repetitions ± standard deviation. Different lowercase letters mean statistically significant differences between treatments within the same day, and different capital letters mean statistically significant differences between days for one treatment ($p < 0.05$).

	TAA (mg ascorbic acid/100g FW)		**Anthocyanins** (mg/kg)		**TPC** (mg GAE /100 g FW)	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	26.4 ± 0.1 ^{abA}	30.9 ± 1.6 ^{aB}	19.1 ± 0.2 ^{aA}	20.7 ± 0.4 ^{abB}	106.5 ± 2.3 ^{aA}	126.7 ± 10.0 ^{aB}
UV-2L-1 min	25.6 ± 1.4 ^{bA}	32.5 ± 2.0 ^{aB}	15.0 ± 1.3 ^{abA}	19.2 ± 0.7 ^{bcB}	122.7 ± 4.8 ^{aA}	120.7 ± 13.6 ^{aA}
UV-2L-5 min	29.5 ± 2.2 ^{abB}	25.2 ± 0.7 ^{bA}	16.5 ± 0.9 ^{abA}	17.4 ± 0.1 ^{cB}	115.6 ± 8.0 ^{aA}	114.8 ± 8.9 ^{aA}
UV-4L-1 min	28.. ±1.9 ^{abA}	32.4 ± 0.8 ^{aB}	14.3 ± 0.4 ^{bA}	19.5 ± 0.8 ^{bcB}	110.3 ± 1.3 ^{aA}	111.7 ± 0.4 ^{aA}
UV-4L-5 min	31.3 ±1.6 ^{aB}	30.2 ± 0.1 ^{aA}	17.7 ± 1.6 ^{abA}	22.2 ± 1.0 ^{aB}	115.9 ± 2.1 ^{aA}	118.9 ± 11.5 ^{aA}

3.3.4 Anthocyanin contents

Initial anthocyanin content of strawberries was 12.5 ± 0.4 mg / kg FW (Data not shown). Maximum values of 22.2 ± 1.0 mg / kg FW were achieved after 2L-5 min WUV-C washing (**Table 11**). As has been reported by other authors (Sheng, 2018), phenylalanine ammonia lyase (PAL) expression and activity is enhanced when hormetic doses of UV-C light are applied, suggesting a possible further increase in anthocyanins after irradiation. Nevertheless, no significant differences were found in this study, neither immediately after washing nor after 24 h of treatment. UV-C light, did not increase anthocyanin content after 1 day, comparably to Li (2014) results, who irradiated strawberries with 4.1 kJ/m² UV-C and did not found any change on anthocyanin content.

3.3.5 TPC and phenolic profile

Strawberries processed in this study had an initial TPC value of 113.2 ± 11.8 mg GAE / 100 g FW (Data not shown), which are in agreement with the literature (Tarola, 2013) and no significant differences were observed in TPC values among the treatments or within days (**Table 11**). WUV-C light, could induce an accumulation of phenolics during storage, as it may trigger the accumulation of WUV-C light absorbing flavonoids and other phenolic compounds (Mditshwa, 2017).

Xu (2017) used different UV-C light doses and found an increment of 25 to 75 % of the TPC, namely cyanidin 3- glucoside, pelargonidin 3-glucoside or ellagic acid. Predominant compounds were pelargonidin derivatives, which give color to strawberries, followed by kaempferol derivatives (Table 3 and Supplementary material), which was in accordance to Aaby (2012). No direct relationship has been found between WUV-C doses and changes in phenolic profile in the present study (Table 12). To a better understanding of the variations in phenolic profile, a study of the effect of UV-C doses on enzymes related to the flavonoids and the shikimate pathway would be worth to be carried out (Tomás-Barberán, 2001).

Table 12. Content of phenolic compounds on strawberries just after the treatments (0 h) or after 1 day of storage at 4 ºC (24 h). Values are the mean of 2 repetitions ± standard deviation. Different lowercase letters mean statistically significant differences between treatments within the same day, and different capital letters mean statistically significant differences between days for one treatment ($p < 0.05$).

	Galloyl-diHHDP-glucose (mg/100 g DW)		**(+)-Catechin** (mg/100 g DW)		**Cinnamoyl glucose** (mg/100 g DW)	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	31.8 ± 2.9 ^{aA}	29.2 ± 1.7 ^{aA}	18.3 ± 0.7 ^{aA}	20.1 ± 0.3 ^{aA}	42.2 ± 1.1 ^{aA}	49.5 ± 3.9 ^{aA}
UV-2L-1 min	25.4 ± 2.3 ^{aA}	33.8 ±4.4 ^{aA}	14.9 ± 2.5 ^{aA}	16.5 ± 0.8 ^{aA}	41.6 ± 1.4 ^{aB}	37.5 ± 3.6 ^{aA}
UV-2L-5 min	28.5 ± 2.2 ^{aA}	27.6 ±0.6 ^{aA}	18.4 ± 0.8 ^{aA}	19.6 ± 1.9 ^{aA}	45.0 ± 4.9 ^{aA}	46.1 ± 1.5 ^{aA}
UV-4L-1 min	29.3 ± 5.8 ^{aA}	25.7 ± 3.1 ^{aA}	17.3 ± 0.7 ^{aA}	16.9 ± 1.5 ^{aA}	55.1 ± 4.7 ^{aA}	53.7 ± 6.2 ^{aA}
UV-4L-5 min	27.6 ± 3.0 ^{aA}	26.7 ± 2.0 ^{aA}	19.7 ± 0.5 ^{aA}	19.2 ± 0.4 ^{aA}	50.8 ± 3.7 ^{aA}	47.9 ± 3.3 ^{aA}

	Coumaroyl hexose I (mg/100 g DW)		**Coumaroyl hexose II** (mg/100 g DW)		**Quercetin glucuronide** (mg/100 g DW)	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	14.7 ± 0.1 ^{aA}	14.7 ± 0.6 ^{aA}	17.8 ± 0.8 ^{aA}	18.5 ± 1.9 ^{aA}	14.0 ± 0.4 ^{aB}	8.8 ± 0.2 ^{cA}
UV-2L-1 min	11.4 ± 0.2 ^{bA}	9.9 ± 0.5 ^{bA}	13.4 ± 0.8 ^{aA}	11.8 ± 1.7 ^{aA}	8.2 ± 0.1 ^{cdA}	12.1 ± 0.0 ^{aB}
UV-2L-5 min	12.1 ± 0.9 ^{bA}	15.0 ± 0.6 ^{aA}	14.3 ± 1.9 ^{aA}	17.7 ± 1.3 ^{aA}	12.1 ± 0.6 ^{bA}	11.0 ± 0.1 ^{abA}
UV-4L-1 min	14.7 ± 0.8 ^{aA}	15.0 ± 0.7 ^{aA}	17.1 ± 1.6 ^{aA}	17.7 ± 2.7 ^{aA}	9.6 ± 0.3 ^{cA}	8.9 ± 0.5 ^{cA}
UV-4L-5 min	13.5 ± 0.2 ^{abA}	14.9 ± 0.2 ^{aA}	16.1 ± 1.5 ^{aA}	18.2 ± 1.5 ^{aB}	7.5 ± 0.1 ^{dA}	9.9 ± 0.5 ^{bcB}

	Kaempferol glucuronide (mg/100 g DW)		**Kaempferol malonylglucoside** (mg/100 g DW)		**Kaempferol coumaroylglucoside** (mg/100 g DW)	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	60.2 ± 1.1 ^{aB}	47.8 ± 1.4 ^{cA}	22.8 ± 1.7 ^{abA}	25.8 ± 0.3 ^{abA}	40.1 ± 3.8 ^{aA}	32.1 ± 13.9 ^{aA}
UV-2L-1 min	45.4 ± 1.9 ^{bA}	64.7 ± 0.4 ^{aB}	20.0 ± 0.1 ^{bA}	28.1 ± 1.5 ^{aB}	36.3 ± 1.0 ^{aA}	73.6 ± 7.9 ^{aB}
UV-2L-5 min	52.0 ± 2.4 ^{abA}	61.4 ± 2.3 ^{abA}	24.4 ± 0.3 ^{aA}	27.5 ± 1.0 ^{aA}	26.1 ± 8.4 ^{aA}	64.1 ± 10.2 ^{aB}
UV-4L-1 min	48.2 ± 3.7 ^{aA}	45.5 ± 2.6 ^{cA}	23.6 ± 0.0 ^{aA}	23.2 ± 0.5 ^{bA}	28.1 ± 3.3 ^{aA}	36.5 ± 8.4 ^{aA}
UV-4L-5 min	46.6 ± 2.5 ^{aA}	53.8 ± 2.9 ^{bcA}	22.2 ± 0.3 ^{abA}	25.7 ± 1.5 ^{abA}	26.6 ± 4.3 ^{aA}	53.7 ± 12.8 ^{aB}

	Pelargonidin galactoside (mg/100 g DW)		**Pelargonidin glucoside (mg/100 g DW)**		**Pelargonidin acetylglucoside (mg/100 g DW)**	
	0 h	24 h	0 h	24 h	0 h	24 h
NaOCl	205.7 ± 0.4 ^{aB}	161.1 ± 1.8 ^{bA}	418.2 ± 27.1 ^{aA}	407.2 ± 19.7 ^{aA}	109.9 ± 4.9 ^{aA}	95.4 ± 1.9 ^{bcA}
UV-2L-1 min	132.9 ± 6.9 ^{cA}	182.8 ± 2.2 ^{abB}	334.1 ± 0.6 ^{bA}	374.2 ± 31.2 ^{aA}	80.5 ± 1.3 ^{aA}	108.7 ± 2.4 ^{abB}
UV-2L-5 min	167.6 ± 1.6 ^{bA}	195.3 ± 1.3 ^{aA}	408.0 ± 36.3 ^{abA}	455.5 ± 1.9 ^{aA}	105.2 ± 7.1 ^{aA}	117.1 ± 2.6 ^{aA}
UV-4L-1 min	151.4 ± 0.3 ^{bcB}	108.6 ± 8.5 ^{cA}	418.7 ± 44.7 ^{abA}	417.3 ± 19.3 ^{aA}	97.2 ± 6.2 ^{aA}	82.7 ± 6.1 ^{cA}
UV-4L-5 min	138.7 ± 0.3 ^{bcA}	177.4 ± 13.0 ^{abA}	381.8 ± 24.7 ^{bA}	412.6 ± 1.6 ^{aA}	85.7 ± 2.2 ^{aA}	102.5 ± 2.8 ^{bB}

3.4 Effect of WUV-C on microbial load of strawberries and wash water

Total aerobic mesophylls (TAM) and yeasts and molds (Y&M) initial population in **strawberries** were 4.3 ± 0.3 and 4.0 ± 0.3 log CFU / g, respectively (**Figure 23** A). Washing processes with 2 or 4 lamps for 5 min were needed to significantly reduce these populations in strawberry. TAM counts after 2L-5 min and 4L-5 min UV-C doses were reduced by 1.8 ± 0.4 and 1.5 ± 0.6 log CFU / g, respectively, which were equivalent to the NaOCl counts. Y&M population was maintained after all treatments except for NaOCl and 4L-5 min, in which the decrease was 1.8 ± 0.8 and 1.2 ± 0.5 log CFU / g, respectively. These results are in dissonance with those published by Collazo (2018), where the highest reduction of spoilage

microorganisms in broccoli did not correlate to higher WUV-C dose, applied with the same equipment. Variability in fruit or vegetable surface may be a factor that influences bacterial attachment and further removal, due to surface roughness, hydrophobicity, and presence of trichomes (Adhikari, 2015). Moreover, complexity and predominance of certain genres and species above others may lead to a diverse susceptibility mechanisms to UV-C light (Kim, 2018).

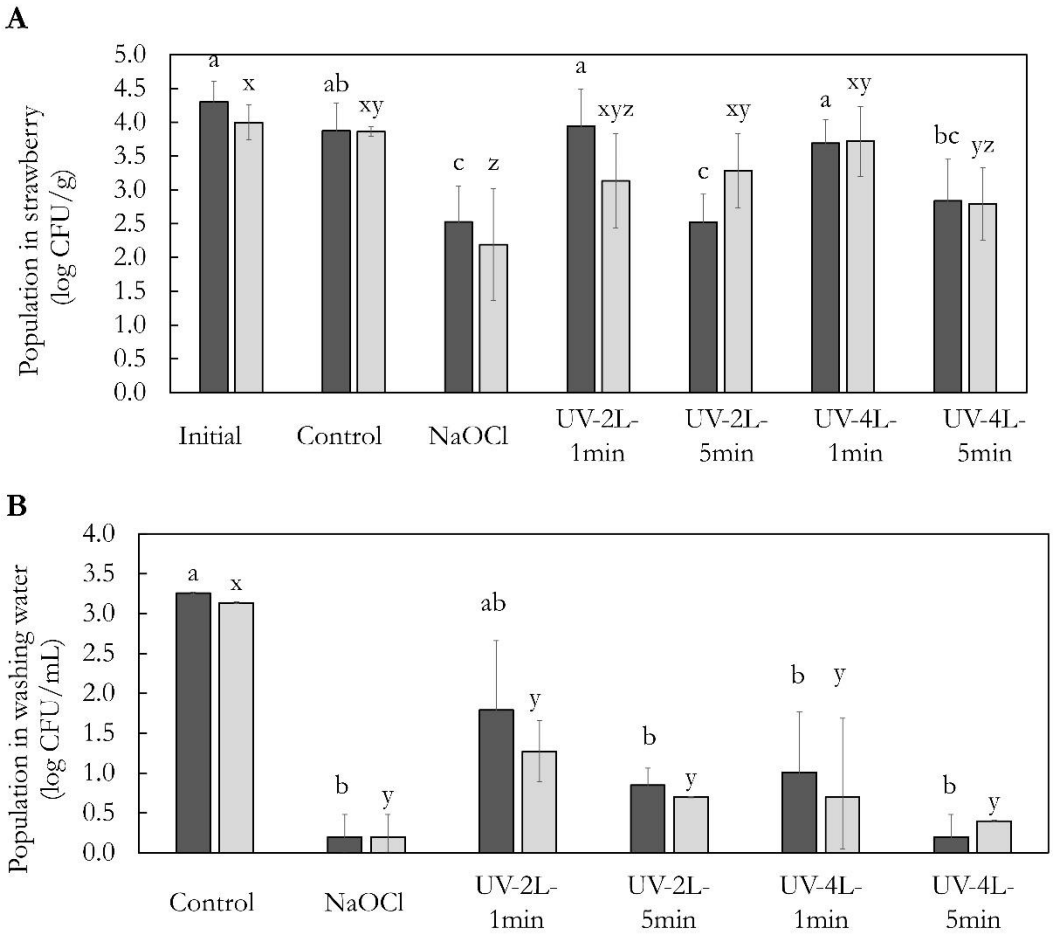


Figure 23. (**A**) Counts of total aerobic mesophylls (■) and yeasts and molds (□) in strawberries before (initial) and after washing treatments. Detection limit was 1.70 log CFU / g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments. (**B**) Remaining population of total aerobic mesophylls (■) and yeasts and molds (□) in washing water. Detection limit was 0.7 log CFU/g. Results are the mean of 3 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments

In **washing water**, population of TAM and Y&M was 3.6 ± 0.1 and 3.1 ± 0.1, log CFU / mL, respectively, in control water without NaOCl nor WUV-C light (**Figure 23** B). This load could be attributed to the transference of the microorganisms from fruit surface to water due to physical action of water pressure, agitation and aeration (bubbles), explaining the reduction of microbial load in strawberries in water control, as detailed above. The most effective treatment – whose reductions comparable to the NaOCl washing – against TAM and Y&M in water was 4L-5min, which in the end, remained in water 0.2 ± 0.1 and 0.4 ± 0.1 log CFU / mL, respectively.

3.5 Effect of WUV-C on *L. innocua* and *S.* Typhimurium in strawberries and wash water

Regarding pathogenic bacteria, initial *L. innocua* and *S.* Typhimurium populations in artificially inoculated **strawberries** were 6.4×10^6 and 1.7×10^7 CFU / strawberry, respectively (Data not shown). After storage at 4 ºC for 22 ± 2 h, *L. innocua* populations were maintained, while *S.* Typhimurium populations in strawberry decreased 1 log unit. WUV-C washing procedures did not differ statistically in reductions, having counts decreased 4.5 ± 0.3 and 3.7 ± 0.5 log CFU / strawberry for *L. innocua* and *S.* Typhimurium, respectively (**Figure 24** A). Moreover, reductions after washing treatments with UV-C light were similar to those after NaOCl washing, which were 3.0 ± 1.2 and 4.9 ± 0.6 log CFU / strawberry of *L. innocua* and *S.* Typhimurium, respectively. Only when 4 lamps were on, reductions of *L. innocua* were over 2-log higher from those obtained with the water control, which were 2.4 ± 0.9 log CFU / fruit. Regarding *S.* Typhimurium, no treatment, except for NaOCl, achieved statistically more reductions than control. In our study, water-assisted UV-C light acted as an effective disinfectant method whose effects could be comparable with those obtained with NaOCl at the same doses used in the food industry. In fact, its effect has been demonstrated in a number of foodborne and spoilage microorganisms, including *E. coli*, *Salmonella* Typhi, *Shigella sonnei*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Bacillus subtilis* (Chang, 1985), *L. monocytogenes* and *Clostridium sakazakii* (Cebrián, 2016), in different extents. In contrast, Collazo (2019) did not achieve effective inactivation for either of the pathogens studied, *L. monocytogenes* and *S. enterica*, in baby spinach leaves, when applied a 0.5 kJ/m² dose. Butot (2018) reported no more than 1 log reduction on artificially inoculated blueberries, raspberries or strawberries with *L. monocytogenes*, *E. coli* O157:H7 and *S. enterica*. This trial was conducted with a UV-C device using intensities ranging from 2.12 to 13.31 kJ / m², but it was not water-assisted. In fact, water-assisted UV-C light has been more successful in reducing pathogenic loads on fruits, and this could be attributed to the higher efficacy of the water-assisted procedure, which may have overcome the limitations of UV-C light transmitted by air, such as shadowing effect (Liu, 2015). Water agitation can also be helpful in removing bacteria that would otherwise be lodged in trichomes or cracks (Butot, 2018).

In **washing water**, counts of *L. innocua* and *S.* Typhimurium in control water without NaOCl and WUV-C light reached 4.2 ± 0.2 and 4.1 ± 0.2 log CFU / mL, respectively (**Figure 24** B). *L. innocua* population in wash water after WUV-C treatments and NaOCl sanitization were not statistically different, and this microorganism persisted in concentrations of 0.2 ± 0.2 log CFU / mL in all cases except for 2L-1 min, which was 1.00 ± 0.9 log CFU / mL. Counts of *S.* Typhimurium in water did not differ between WUV-C treatments or NaOCl, averaging 0.2 ± 0.2 log CFU / mL. Indeed, UV-C irradiation has been widely used as a non-thermal method of disinfecting drinking, waste and recreational water (Beck, 2015) and its effectiveness has been already demonstrated in disinfecting liquid matrices of different natures (Gunter-Ward, 2018; Jeon, 2018). Minimizing microbial load in washing water is crucial in the fruit industry, to prevent cross-contamination when it is reused in the process. The amount of wastewater generated per mass unit of product depends on the disinfection technique employed, so being UV-C irradiation capable of disinfecting efficiently both the process water and the product, a higher ratio of recycling can be achieved, with a lower impact on the environment (Kretzschmar, 2009).

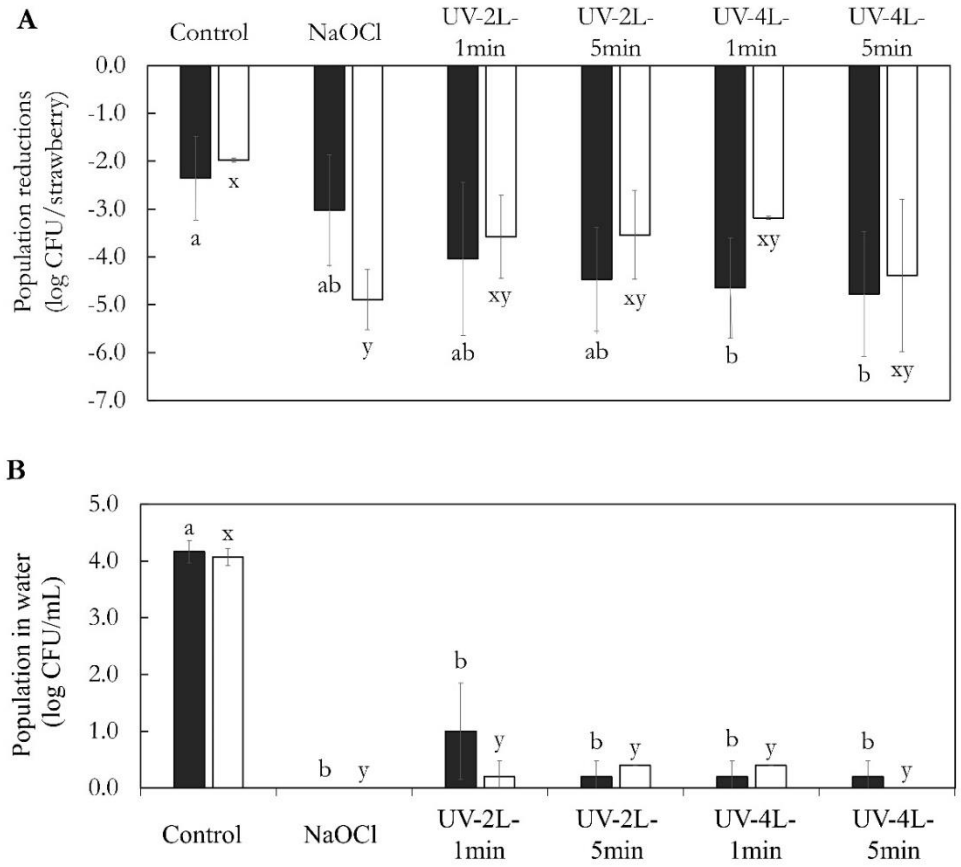


Figure 24. **(A)** Reductions of *L. innocua* (■) and *S. enterica* (□) populations in strawberries after washing treatments with WUV-C irradiation alone. Detection limit was 1.70 log CFU / g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments. (**B**) Remaining population of *L. innocua* (■) and *S. enterica* (□) in washing water. Detection limit was 0.7 log CFU / g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments.

3.6 Efficacy of WUV-C combined with PA on the reduction of pathogens artificially inoculated on strawberries

To combine UV-C irradiation with the use of PA, treatments with 4 lamps were chosen. One of the reasons was because in some cases, higher reductions of microorganisms in strawberry and in water were observed with this dose. The other, is that in the trials with PA there was the intention to maximize the UV-C irradiation, to reduce treatment time in order to avoid mechanical damages that could affect strawberries during commercialization. According to previous studies of the research group (Nicolau-Lapeña, 2019), 2 min washing with PA at concentrations of 40 and 80 ppm were needed to exert any significant change in disinfection of strawberries, so these concentrations and time were selected to this optimization trial. It was assumed that if 2 min washing with PA was effective, the time could be decreased from 5 min to 2 min. Moreover, an additive effect was expected when using PA in combination with the UV-C lamps; so that the concentration of PA at 40 ppm with UV-C irradiation could equal the effect of 80 ppm PA without UV-C application. As Regarding strawberry quality, nutritional parameters and biochemical characterization immediately after the washing treatments, no substantial changes attributed to WUV-C irradiation or PA were observed in this or previous studies (Nicolau-Lapeña, 2019). Considering this, determinations of parameters other than the two pathogens counts were not carried out in the combination of WUV-C and PA trials.

In **strawberries**, the initial load of artificially inoculated *L. innocua* and *S.* Typhimurium was 6.3×10^7 and 1.7×10^7 CFU / strawberry (Data not shown). *L. innocua* was reduced 2.4 ± 0.8 and 4.3 ± 1.0 log CFU / strawberry, and *S.* Typhimurium 2.4 ± 0.1 and 4.7 ± 0.1 log CFU / strawberry, by control and by NaOCl washing, respectively (**Figure 25** A). Reductions caused by WUV, PA 40 or PA 80 alone, even though similar to NaOCl values, were no statistically different from control. However, reductions after treatments with the combinations, WUV + PA 80 for *L. innocua*, and WUV + PA 40 for both pathogens, were statistically higher than control and comparable to NaClO. These results contrast with those obtained by Collazo (2019) in lettuce or spinach leaves inoculated with *L. monocytogenes* or *S.* Typhimurium. They explained that the lack of synergistic effect could be related to the ability pathogens of interacting with the plant-associated microbiota, or to their internalization and attachment to the plant tissue during overnight incubation, which could have reduced the accessibility of UV-C and PAA or led to induced resistance of bacteria against antimicrobial mechanisms. In this paper, combination of both mechanisms, physical and chemical, showed improved results than their separate applications. One possible reason could be that the attachment of the pathogens to the fruit surface is different than in the leaves surface, or that the distribution in the tank of both products may differ due their structural differences.

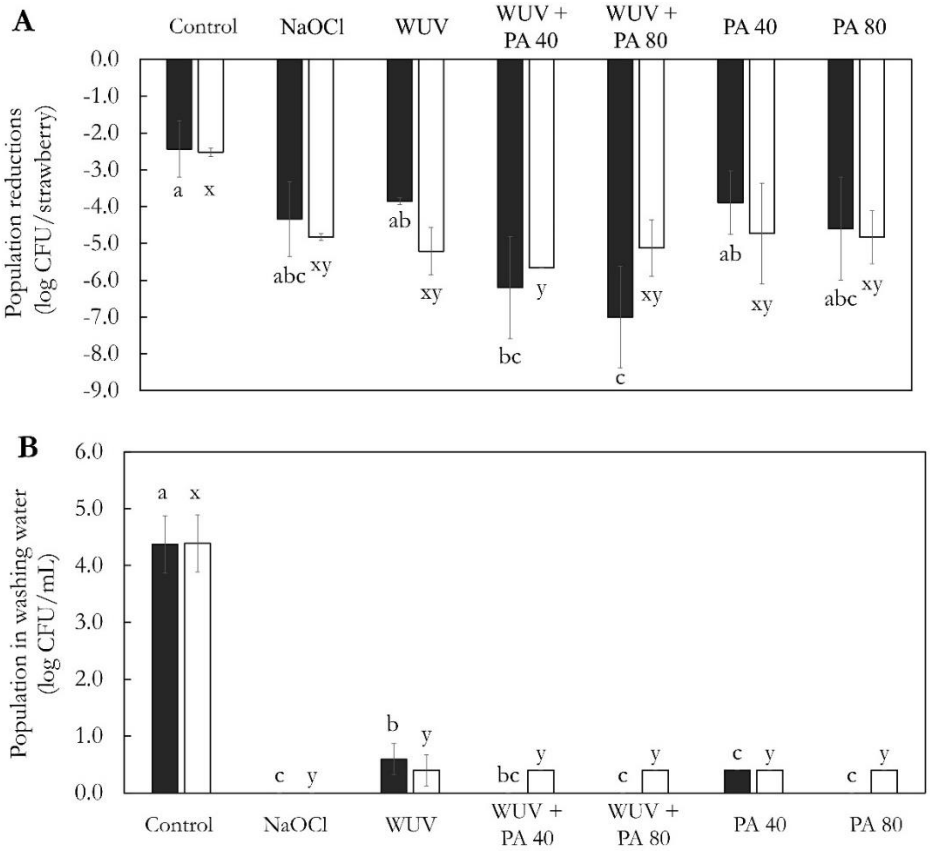
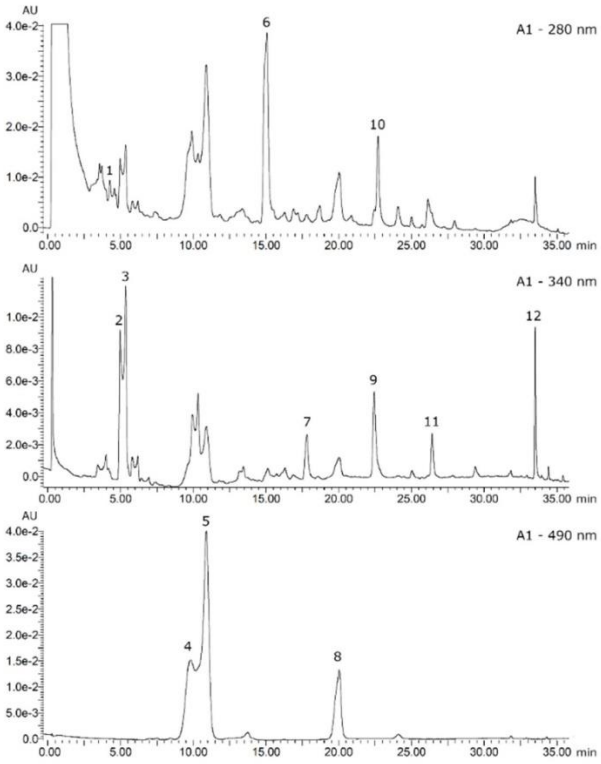


Figure 25. (**A**) Reductions of *L. innocua* (■) and *S. enterica* (□) populations in strawberries after washing treatments with WUV-C combined with PA at different doses for 2 min. Detection limit was 1.70 log CFU / g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments. (**B**) Remaining population of *L. innocua* (■) and *S. enterica* (□) in washing water. Detection limit was 0.7 log CFU / g. Results are the mean of 6 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments.

The efficacy of the combination of WUV-C with PA at two different concentrations was also studied in the **wash water after treatments**, focusing on the counts of the viable pathogens. After washing with water alone (control) 4.4 ± 0.5 and 4.4 ± 0.3 log CFU / mL of *L. innocua* and *S.* Typhimurium remained in water (**Figure 25** B). Therefore, even the water wash was as effective as the other treatments evaluated, which could be attributed to the mechanical effect caused by water agitation that drags the microorganisms

from the surface of the fruit to the water, pathogenic microorganisms remained in the water, allowing cross-contamination. As expected, NaOCl achieved absence of pathogens in washing water. *S.* Typhimurium remained at concentrations of 0.4 ± 0.1 log CFU / mL in water after all the other treatments. Meanwhile, *L. innocua* was not detected in WUV + PA 40, WUV + PA 80 and PA 80, in contrast with 0.6 ± 0.3 and 0.4 ± 0.1 log CFU / mL found in WUV and in PA 40, thus meaning and improvement in the efficacy of the combination of WUV + PA compared to those treatments applied separately. In fact, this combination has been studied for the disinfection of *E. coli*, *Enterococcus* spp., somatic coliphage, and *Cryptosporidium parvum* in waste water, and a pilot plant to escalate this application has been set up recently (Hassaballah, 2019; Hassaballah, 2020), showing promising outcomes. But it should be pointed out that, the fact that some bacteria may remain viable in the washing water must be controlled. Recirculation of this water could constitute a problem if incoming strawberries were contaminated. The potential of the lamps permanently irradiating the recirculated water should also be assessed, in order to verify if that could be reused safely after more exposure time to UV-C light.



Supplementary figure. HPLC chromatogram of strawberry phenolic compounds, obtained at 280, 360 and 490 nm, showing (+)-catechin (1), coumaroyl hexose I (2), coumaroyl hexose II (3), pelargonidin galactoside (4), pelargonidin glucoside (5), cinnamoyl glucose (6), quercetin-3-*O*-glucuronide (7), pelargonidin acetylglucoside (8), kaempferol-3-*O*-glucuronide (9), galloyl-diHHDP-glucose (10), kaempferol-3-*O*-malonylglucoside (11), and kaempferol-3-*O*-coumaroylglucoside (12).

4 Conclusions

The results of this study indicate that water-assisted UV-C light disinfection has been useful in reducing artificially inoculated *L. innocua* and *S.* Typhimurium in strawberries. Reductions could be comparable to those obtained when using a standard sodium hypochlorite treatment. WUV-C light helped minimizing remaining population of both, pathogenic and spoilage microorganisms in washing water. It would make this technique suitable for its use in the washing step in fruit industry to allow water recirculation and reduce the risk of cross-contamination. In general, the innovative WUV-C treatment evaluated did not affect physicochemical and nutritional quality of strawberries.

In order to improve the effectiveness of the WUV-C light in strawberry sanitization, this water-assisted system can be combined with other substances that can be solved in water, such as organic acids or essential oils, or with other technologies not involving high temperatures, such as ultrasound application. In this study, PA was combined with the use of WUV-C light. In this case, WUV-C light combined with PA at 40 ppm for 2 min proved to be effective in the disinfection strawberries and their washing water, with results comparable to those obtained with chlorine. This treatment allowed the reduction of treatment time from 5 to 2 min. It is important to note that, even though this study has shown the potential of UV-C light assisted by water, and the suitability of combining this treatment with PA, it was carried out at lab scale (using a low proportion strawberries:water). More studies should be carried out in order to determine the feasibility to scale up this disinfection procedure and its suitability for the actual fruit industry conditions. Normally, strawberries that will be sold in the fresh market are not washed to prevent further softening and mold growth (because these fruits are a delicate product). In this paper, only immediate effect of the proposed UV-C technique has been determined. For this, further investigations will be focused on the shelf-life of strawberries, both fresh and frozen. Also, more studies will be needed to study possible synergistic effects of combined methodologies.

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Conflicts of interest

The authors declare no conflict of interests.

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Chapter 3

Strawberry sanitization with a combination of ultraviolet C light and peracetic acid: its impact in quality and shelf life of fresh and frozen fruits

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A process consisting on the immersion in a 40 ppm peracetic acid (PA) solution while irradiating with ultraviolet-C lamps for 2 min (WUVPA) was proposed as a sanitizing method for strawberries prior to their commercialization. The shelf-life of the washed strawberries was evaluated in fresh product (whole or half-cut) for 11 days at 4 ºC, or in frozen product for 12 months at –20 ºC. Fresh and fresh-cut fruit maintained firmness (2.3 ± 0.5 N at day 11, D11), and lightness (expressed by L*, averaging 31.0 ± 0.1 at D11). Although antioxidant activity expressed by DPPH· values decreased during storage in fresh-cut samples, that expressed by ferric reducing antioxidant power (FRAP), total phenolic content and total anthocyanin content were maintained for all the formats during storage (averaging 133.2 ± 4.1 mg ascorbic acid equivalents/100 g fresh weight (FW), 21.1 ± 0.7 gallic acid equivalents / 100 g FW, and 2.8 ± 0.1 mg pelargonidine-3-glucoside/100 g FW). Modified atmosphere did not influence the changes that strawberries overcame in the frozen product. An increase in red color (from 32.1 to 39.3 a* values) and a loss of the firmness up to 46.8 % was observed at the first month of storage after freezing and thawing process with no changes in the nutritional quality. Considering the sanitizing effect of WUVPA and the results obtained in the present study, the addition of this process in the production chain of strawberries could be an effective method to maintain shelf-life of strawberries.

Irradiation, fresh-cut, antioxidant, vitamin C, storage

1 Introduction

Strawberries (*Fragaria × ananassa*) are widely consumed, both because their excellent organoleptic properties and their high nutritional quality, characterised by a high content in antioxidants (Gani, 2016). However, their postharvest life is short due to the relatively high metabolic activity, sensitivity to fungal decay, and susceptibility to water loss. Also, they are susceptible to mechanical injuries owing their soft texture and lack of a protective rind (Pinzon, 2019). For this, storage and selling of these fruits is very challenging in domestic and international markets, and sometimes, spoilage or deterioration occurs even before reaching the consumers (Hazarika, 2019). If not solved, the actual product loss – 10 % to 35 % at retail and at consumer level, respectively – can have a great impact, causing economic losses in the sector (Kelly, 2019).

The need to develop new methods to control postharvest diseases appears with the rise in consumer's awareness about pesticide residues on foods, pathogen resistance, and nutritional losses (Yang, 2011). Postharvest handling operations may be detrimental for strawberries, mainly for their susceptibility to mechanical damages. Refrigeration at 4-5 ºC, combined or not with modified atmosphere, has proved to slow down the metabolic processes and increase shelf-life of strawberries (Bovi, 2018).

Fresh strawberries are often packaged directly in the field and commercialized without any sanitation process. Fresh-cut and frozen strawberries are typically commercialized after washing and/or disinfection steps. However, in any of the cases, fruits are eaten raw (Janowicz et al., 2007; Velickova et al., 2018). For this, the need to offer innocuous products to consumers is a key factor to developing new sanitizing strategies. Chlorine is one of the most used disinfectants in fruit industry due to its low cost, easiness of use and effectiveness against some vegetative bacteria and enteric viruses (Luo, 2018). However, its action is highly pH-dependent and it reacts with organic matter, producing undesirable by-products, such as trihalomethanes. For this reason, other products or technologies are being investigated in order to find effective alternatives to chlorine.

Frozen strawberries have also been linked to safety issues associated with foodborne pathogens, such as *Salmonella* spp. and human norovirus (European Food Safety Authority Panel on Biohazards, 2014). The importance to maintain their safety resides in the fact that these products are not only consumed at homes, but they are also used as ingredient in a variety of groceries, including yoghourts, smoothies and ice-creams (Šamec, 2016).

Different alternatives have been studied to reduce microbial growth and to extend the shelf-life of strawberries. In this regard, the combination of water assisted ultraviolet C light (WUV-C) and peracetic acid (PA) proved effective in reducing *L. monocytogenes* and *S. enterica* populations in whole strawberries, and it did not negatively affect the quality and nutritional parameters of the fruit (Nicolau-Lapeña, 2020). Moreover, in previous studies, the combination of WUV-C and PA at 40 ppm reduced artificially inoculated Norovirus in strawberry up to 2 log $TCID_{50}$ / g and artificially inoculated *L. monocytogenes* and *S. enterica* were also reduced up to 4.5 and 2.8 log CFU / g after washing treatments (Ortiz-Solà, under peer revision).

Therefore, in this study, the combination of WUV-C combined with 40 ppm of PA for 2 min was suggested as a disinfectant step in the processing chain of strawberries, to increase their shelf-life of this product presented in three different formats: fresh, fresh-cut, and frozen. For this, quality parameters (including pH, TSS, and TA, color and firmness evolution), biochemical characteristics (including antioxidant capacity, total phenolic, total anthocyanin or total ascorbic acid retention), and the evolution of the epiphytic microbiota in strawberries were evaluated.

2 Materials and methods

2.1 Materials

Strawberries (*Fragaria* × *ananassa*) cv. San Andreas were purchased from local providers (Spain) during the campaign of 2019 and stored at 4 ºC.

Yeast extract, plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), and peptone were obtained from Biokar Diagnostics (Allonne, France). Peracetic acid (PA) 15 % was purchased from Panreac AppliChem (Barcelona, Spain).

Ascorbic and gallic acids, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, metaphosphoric acid, acetic acid, 3,3',3"- phosphanetriyltripropanoic acid (TCEP), were acquired from Sigma-Aldrich (Steinheim, Germany). Methanol, acetone, chlorhidric acid (37 %), sodium acetate, sodium hydroxide, sodium chloride, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were procured by Panreac AppliChem (Barcelona, Spain).

2.2 Treatments

The effect of water-assisted UV-C (WUV-C) combined with 40 ppm peracetic acid (PA) on shelf-life of strawberries was studied in two formats: fresh and frozen. Treatments were conducted in the UV-C water-assisted (WUV-C) equipment LAB-UVC-Gama (UVC-Consulting Peschl España, Castellón, Spain) already described in Nicolau-Lapeña et al., (2020) and Ortiz-Solà et al. (2020). Before the experiment, the 4 UV-C lamps were preheated for 10 min, to reach the maximum irradiance at the start point of washing treatments. After this time, irradiance value in the empty tank, which was measured with a UV-sensor Easy H1 (Peschl Ultraviolet, Mainz, Germany) through an orifice located on the lid of the tank, was 10.5 ± 0.5 W/m^2. Afterwards, the WUV-C tank was filled with 12 L of cold (6 ± 2 °C) water and the UV-C lights were switched on for 5 min. When needed, PA or chlorine were added in the tank. Water parameters including pH, oxidation-reduction potential (ORP) and turbidity were measured before and after each treatment. ORP and pH were measured in a pH-meter GLP22 Crison, Alella (Barcelona, Spain) equipped with a pH probe (ref. 52-03, Crison) or ORP probe 62-51 Hach (Geneva, Switzerland), respectively. Turbidity was measured using a portable turbidimeter (TN-100, Eutech, Singapore) measuring in Nephelometric Turbidity Units (NTU).

Before the treatment, peduncle and leaves were carefully removed. For each treatment, the strawberries were immersed in the water tank in agitation in a ratio 3:10 (strawberries:water, w:v). For **fresh presentation**, whole strawberries were washed using WUV-C 4 lamps with 40 ppm PA for 2 min (WUVPA, W-TREAT). Tap water during 2 min was used as a control (W-CON), as normally disinfection is not a common step in whole strawberry industry. A lot of strawberries washed in WUV-C 4 lamps with 40 ppm PA for 2 min were half-cut afterwards (C-TREAT). For half-cut strawberries, sodium hypochlorite 200 ppm (NaOCl), adjusted to pH 6.5 using citric acid 2 M was used as a control (C-CON), because fresh-cut products need to meet the safety standards as they are eaten without further processing. After NaOCl disinfection, strawberries were rinsed in tap water during 2 min. After washing, fruits were let at room temperature to drain the excess of water. Afterwards, 400 ± 10 g of strawberries were weighed in 1000 mL polypropylene (PP) trays (CL1000TPE, Alphacel) and sealed with a propylene HS 1/17 film (ACSA, Valencia, Spain). Packaged product was stored in a display case (INFRICO Z017ERC110, Córdoba, Spain) at 4.5 ± 0.4 ºC for 11 days. Light and dark cycles were programed each 18 and 8 h, respectively, simulating opening and stocking hours in a supermarket display.

For **frozen presentation**, whole strawberries were washed using WUV-C 4 lamps with 40 ppm PA for 2 min (WUVPA). After the washing, fruits were let at room temperature to drain the excess of water.

Strawberries (400 ± 10 g) were weighed in 1400 mL (MW1-50, Wiezoplast) trays and sealed with a HS 1/17 film. Half the samples were packed under modified atmosphere packaging (MAP) (20 % CO_2, 5 % O_2, and 75 % N_2). Strawberries were frozen at -80 ˚C in a freezing cabinet with liquid nitrogen (N_2) (model Mini Batch CM-85/1090, Metal Carbide, Carburos Metalicos, Spain). Transition time was 40 min and internal temperature of strawberries reached -15 °C after 60 min (**Figure 26**). Strawberries were stored at -25.0 ± 0.5 ºC for 12 months.

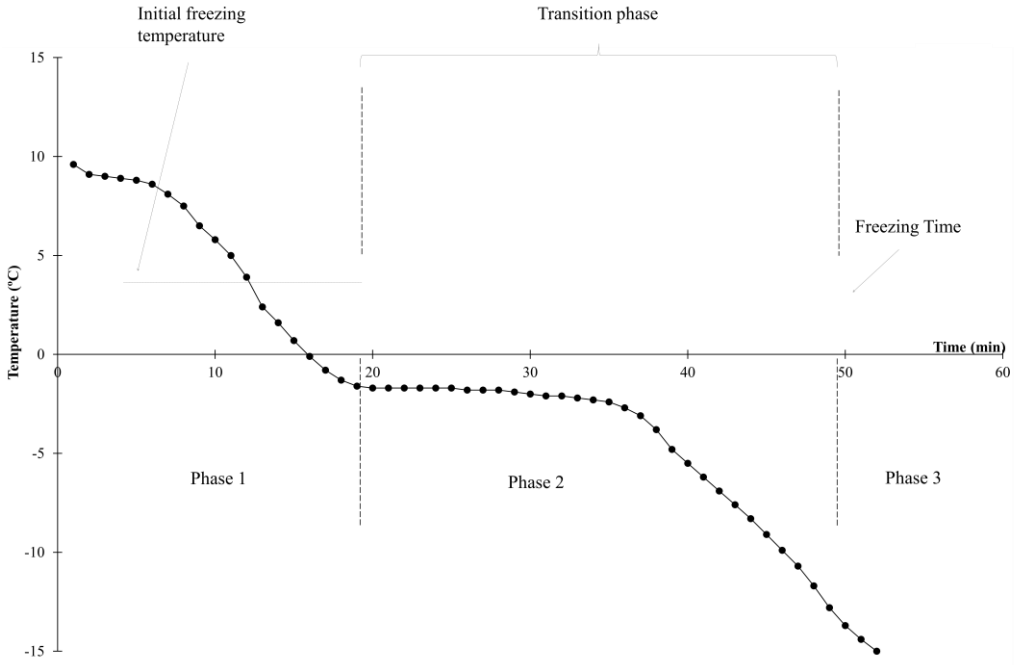

Figure 26. Freezing parameters and curves of strawberry samples during supercooling in a freezing cabinet functioning with liquid nitrogen (N2), from the brand Metal Carbide model Mini Batch CM-85/1090.

2.3 Effect of WUV-C treatment on the quality of strawberries

Fresh strawberries, whole or half-cut, were sampled at days 0, 2, 4, 7, and 11 (D0, D2, D4, D7, D11). Quality parameters were determined each sampling day. Then, aliquots of each treatment were frozen with liquid nitrogen, milled using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain) and stored at -80 °C for further biochemical analysis.

Frozen strawberries were sampled at months 0, 3, 6, and 12 (M0, M3, M6, and M12) and defrosted at 4 ºC overnight. Quality determinations were performed on thawed product, and aliquots were prepared and stored at -80 °C for further biochemical analyses.

2.3.1 Quality parameters. Physiochemical evaluation

For determining **pH, total soluble solids** and **titratable acidity**, strawberries were smashed in a blender to prepare 25 mL of juice. Each parameter was evaluated twice for each repetition (n=3) according to Nicolau-Lapeña (2019).

Respiration rate (RR) of fresh and fresh-cut strawberries was determined immediately after the processing. For this, 100 ± 5 g strawberries were put inside a hermetic plastic pot and stored at 4 ºC. After 24 h, O_2 and CO_2 concentrations were measured using headspace gas analyser CheckMate 3 (Dansensor, Spain). RR was calculated following Equation 1:

$$\text{RR (mL gas / kg · h)} = \frac{[CO2]f-[CO2]i \cdot (Vt - V0) \cdot 0.01}{W \cdot (tf - t0)} \qquad \text{Eq. 1}$$

where $[CO_2]_f - [CO_2]_i$ is the change in concentration between measurements (% in volume), V_t is the total volume of the container (600 mL), V_0 is the volume of the fruits (mL), $t_f - t_i$ is the time difference between measurements (h), and W is the weight of the fruits in the container (kg).

Evolution of **internal gas composition** in sample trays was followed by measuring O_2 and CO_2 concentration at each storage day (D) and expressed in % (n=3).

Colour of 10 strawberries was measured on 3 sides, using a CR-200 Minolta Chrome Meter (Minolta, INC., Tokyo, Japan) with a D65 illuminant and 10° observer angle. The instrument was calibrated using a standard white reflector plate. Color was expressed as CIE L* a* b* coordinates (n=30).

Texture changes were evaluated on 10 strawberries for treatment with two textural tests by using the TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England). Puncture test was performed with a 4 mm cylindrical probe, measuring the maximum force encountered when the probe enters 8.0 mm deep into the tissue. Both tests were run at 5 mm / s speed with a trigger force of 0.1 N

Drip losses were determined by weighing the exudates immediately after the freezing– thawing process. It was calculated by difference in weights, as expressed in Equation 2:

$$\text{DL (\%)} = \frac{We}{Wf} \times 100 \qquad \text{Eq. 2}$$

where *We* is the weight of the exudate and *Wf* is the weight of the frozen product.

2.3.2 Biochemical parameters

Antioxidant activity of strawberries was assessed by ferric reducing antioxidant power (FRAP) and DPPH scavenging activity assays, as described by Nicolau-Lapeña (2019). Results are expressed as μmol ascorbic acid equivalents (AAE) / 100 g FW of 3 repetitions (n=3).

The **total phenolic content** (TPC) was assessed by Folin Ciocalteau method on the same extract used for antioxidant activity determination, following the procedure described by Nicolau-Lapeña (2019). Results were expressed as mg gallic acid equivalents (GAE) / 100 g FW of 3 repetitions (n=3).

For the **total anthocyanin content** (TAC), extracts and quantification were carried out in triplicate (n=3) according to the method described by Meyers (2003). Anthocyanin content was expressed as mg of cianidine-3-glucosyde / 100 g of strawberry.

Total ascorbic acid contents expressed as the sum of ascorbic acid and dehydroascorbic acid (TAA), was determined by high-performance liquid chromatography (HPLC) in a Waters 717 plus Autosampler HPLC system (Waters Corp., NJ, USA) coupled to a UV detector, following the method described by Lafarga (2018). Average peak areas of duplicate injections were used for quantification (n=2). Concentration of vitamin C, expressed as mg TAA / 100 g FW, was calculated by the area interpolation on the adequate calibration curve.

2.3.3 Microbiological quality

The effect of the washing treatments on total aerobic mesophylls (TAM) and yeasts and molds (Y&M) was evaluated. For this, 25 g per repetition (n=3), taken from pieces of 2 strawberries to ensure representativity, were diluted 1:4 in peptone buffered solution. The count process followed the method described in Nicolau-Lapeña (2019). Results were expressed as log colony-forming unit (CFU) / g, and the detection limit was 1.30 log CFU / g.

2.4 Statistical analysis

For fresh-product, data was checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, a contrast analysis was applied, comparing formats with the same treatment (W-TREAT vs. C-TREAT), and treatments within the same format (W-CON vs. W-TREAT and C-CON vs. C-TREAT). For frozen product, a T-student test was run between the two gas composition conditions, and a Tukey HSD (Honest significant difference) test to evaluate differences between times within the same treatment. The criterion for statistical significance was $p < 0.05$. All statistical analyses were carried on using JMP 13 (SAS Institute Inc., Cary, USA).

3 Results and discussion

3.1 Water characteristics during treatments

The pH and ORP values of washing water solutions were measured before and after the treatments. For water containing PAA at 40 ppm, values were 5.3 ± 0.2 and 447.2 ± 13.4 mV, respectively. For water with chlorine at 200 ppm, values were 6.7 ± 0.2 and 901.8 ± 11.3 mV, respectively. Turbidity of the water (2.0 ± 0.1 NTU) was in agreement to those observed in previous studies of the group, indicating that absorbance of water at 254 nm (corresponding to that of the UV light) was low and no interference of irradiation was caused by the presence of particles or dirt in water (Nicolau-Lapeña, 2020)

3.2 Fresh and fresh-cut strawberries

3.2.1 Commercial quality of the fruits

The washing treatments using WUVPA and the respective controls for whole and fresh-cut presentation formats were evaluated for different quality aspects during the storage. One important parameter is respiration rate, as its increase often relates with an increased stress of the fruit (Montanez, 2010). No differences were found in **respiration rate** (RR) of whole and half-cut samples, and neither between the treatments. RR was moderate, averaging 7.6 ± 0.4 mL CO_2 / kg · h. **Gas composition** in the package showed the same evolution for all 4 presentations and treatments, achieving final values of 11.1 ± 0.7 % O_2 and 12.3 ± 0.9 % CO_2 (Data not shown).

Overall quality of fresh strawberries was evaluated by means of **pH, TSS and TA** values (**Table 13**). At D0, there were no significant differences between treatments and presentations, and the values found were in accordance to those in the literature (Costa, 2011). The pH values of samples after 11 days of storage ranged between 3.3 and 3.4. However, some variations during the storage period were observed. Contrarily, TSS decreased in W-CON treatments (6.3 ± 0.3 to 5.3 ± 0.2 º Brix) after 11 days of storage, trend that was also observed by (Mehmet Seçkin Aday, 2013) in strawberries during storage after sonication treatment (30 – 90 W, 5 – 10 min). Some authors explain this trend by a decrease fruit metabolism, leading to a lower decomposition of these constituents, and a slow hydrolysis of sugars (Aday, 2014). However, in the present study, no differences were observed between the RR of the different treatments and formats that could explain why the TSS changes only happened in one treatment. The results on TA values of strawberries (**Table 13**) show that there was a decrease in this parameter during storage. According to Aday (2014), the use of organic acids might be related to this decrease, which was faster in samples treated with WUVPA than it was in control treatments.

Table 13. Quality assessment of fresh and fresh-cut strawberries: pH, total soluble solids (TSS) titratable acidity (TA) and firmness. Values are the mean of 3 repetitions ± standard deviation (n=10 in the case of firmness). Different lowercase letters show statistically significant differences ($p < 0.05$) between treatments within the same presentation (whole or half-cut), analyzed with a contrast test, asterisks show statistically significant differences ($p < 0.05$) between presentation formats (whole vs. half-cut) for water assisted assisted ultraviolet-C with peracetic 40 ppm treated samples, and capital letters show statistically significant differences ($p < 0.05$) during time within the same treatment analyzed with Tukkey's test.

	Format	Treatment	D0	D4	D7	D11
pH	**Whole**	**W-CON**	3.5 ± 0.1 ^{aB}	3.5 ± 0.1 ^{aB}	3.6 ± 0.1 ^{aA}	3.3 ± 0.1 ^{aB}
		W-TREAT	3.4 ± 0.1 ^{aAB}	3.7 ± 0.1 ^{bA}	3.6 ± 0.2 ^{aAB}	3.3 ± 0.1 ^{aB}
	Half-cut	**C-CON**	3.3 ± 0.1 ^{xB}	3.8 ± 0.2 ^{xA}	3.8 ± 0.1 ^{xB}	3.4 ± 0.1 ^{xA}
		C-TREAT	3.4 ± 0.1 ^{xB}	3.6 ± 0.1 ^{xB}	4.1 ± 0.3 ^{xA}	3.4 ± 0.1 ^{xB}
Total soluble solids (TSS, °Brix)	**Whole**	**W-CON**	6.3 ± 0.3 ^{aA}	5.7 ± 0.1 ^{aB}	5.7 ± 0.1 ^{aB}	5.3 ± 0.2 ^{aB}
		W-TREAT	6.3 ± 0.1 ^{aA}	5.2 ± 0.3 ^{aB}	5.8 ± 0.3 ^{aAB}	6.4 ± 0.1 ^{b*A}
	Half-cut	**C-CON**	5.9 ± 0.2 ^{xAB}	6.5 ± 0.3 ^{xA}	5.9 ± 0.3 ^{xAB}	5.6 ± 0.1 ^{xB}
		C-TREAT	6.3 ± 0.3 ^{xA}	5.9 ± 0.8 ^{xA}	6.0 ± 0.8 ^{xA}	5.8 ± 0.1 ^{x*A}

Titratable acidity (TA, mg citric acid / L)	**Whole**	**W-CON**	8.7 ± 0.3 ^{aA}	8.9 ± 0.8 ^{aA}	6.6 ± 0.4 ^{aB}	6.7 ± 0.4 ^{aB}
		W-TREAT	8.6 ± 0.2 ^{aA}	7.3 ± 0.2 ^{bB}	7.0 ± 0.6 ^{aBC}	6.2 ± 0.3 ^{aC}
	Half-cut	**C-CON**	9.5 ± 0.4 ^{xA}	6.8 ± 0.2 ^{xB}	6.4 ± 0.1 ^{xB}	6.2 ± 0.4 ^{xB}
		C-TREAT	9.0 ± 0.4 ^{xA}	7.0 ± 0.8 ^{xB}	6.6 ± 0.2 ^{xB}	6.1 ± 0.2 ^{xB}
Firmness (N)	**Whole**	**W-CON**	1.7 ± 0.2 ^{aB}	2.2 ± 0.3 ^{aB}	1.9 ± 0.2 ^{aB}	2.9 ± 0.2 ^{aA}
		W-TREAT	1.9 ± 0.2 ^{aA}	1.6 ± 0.3 ^{b*A}	1.9 ± 0.4 ^{aA}	1.8 ± 0.2 ^{aA}
	Half-cut	**C-CON**	2.4 ± 0.3 ^{xA}	1.7 ± 0.2 ^{yA}	2.1 ± 0.6 ^{xA}	2.5 ± 1.1 ^{xA}
		C-TREAT	2.0 ± 0.1 ^{yA}	2.3 ± 0.3 ^{x*A}	2.3 ± 0.8 ^{xA}	2.1 ± 0.2 ^{xA}

To understand **colour** of strawberries, the study focuses on L* and a* coordinatesfrom the CIELab space, which are indicators of luminosity and redness, respectively. Initially, these parameters did not differ between treatments, and averaged 38.7 ± 2.2 and 32.5 ± 0.5, for L* and a*, respectively, indicating a characteristic red light colour of strawberries as reported in the literature (de São José, 2015). At the end of the storage, luminosity was maintained and redness decreased regardless the treatment (**Figure 27**). The decrease in a* values was sharper for whole fruits than for cut samples. The decrease in a* could be attributed to the higher stress induced to the fresh-cut strawberries, which could accelerate browning reactions and progressive loss of red colour in the plant tissue. This trend was also reported by Aday (2014), who also found a decrease in redness in fresh-cut strawberries treated ultrasound.

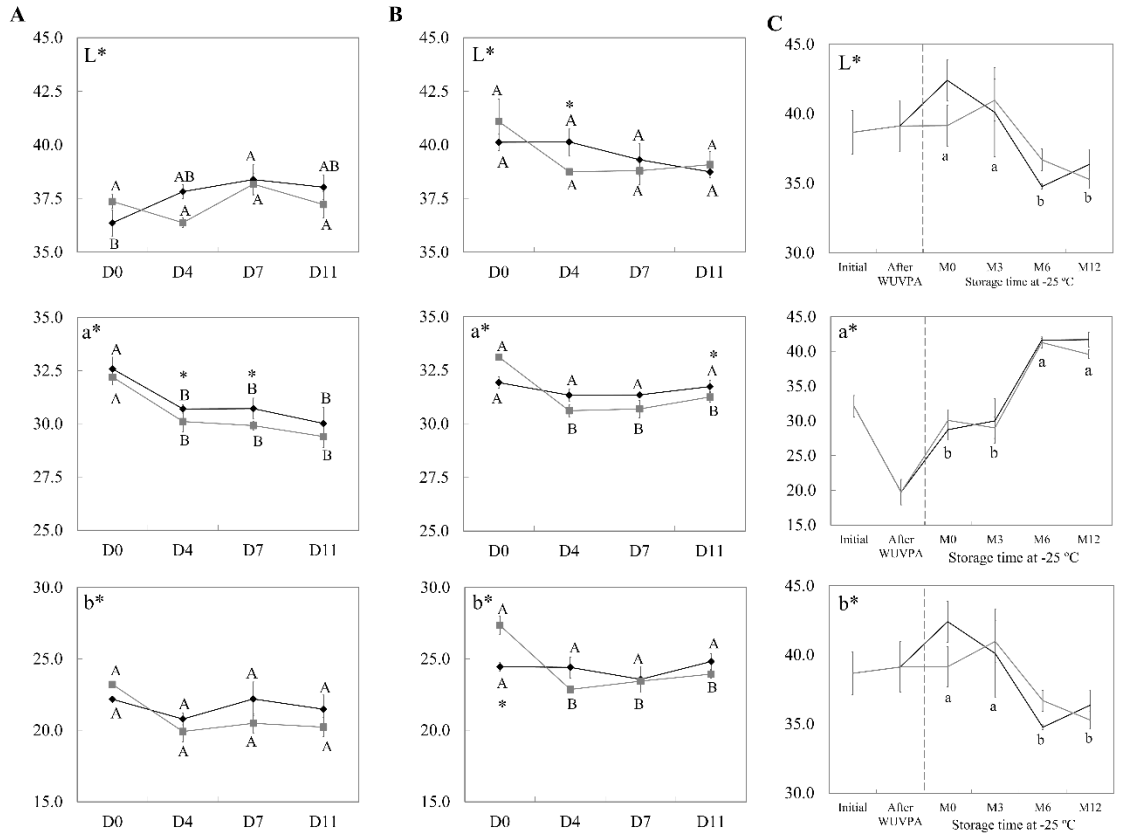

Figure 27. Color, expressed as L*a*b* coordinates, in fresh and whole (**A**) or fresh and half-cut (**B**), showing control (**black lines**) or WUVPA treated (**grey lines**). In (C), color, expressed as L*a*b* coordinates in frozen strawberries (-25 °C), showing modified gas composition (**black lines**) or air composition (**grey lines**). Values are the mean of 3 repetitions ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) among storage time (4 °C) within the same treatment (Tukkey's HSD test), and asterisks show statistically significant differences ($p < 0.05$) between treatments in the same day.

In this study, initial **firmness** values for whole fruits ranged from 2.4 to 1.7 N. In half-cut strawberries, statistical differences were observed between C-CON and C-TREAT. Firmness values of C-CON strawberries (2.4 ± 0.3 N) were significantly higher than C-TREAT were (1.9 ± 0.1 N) (**Table 13**)). The present study shows that firmness was maintained through 11-day storage at 4 ºC. In contrast, other

studies reported a decrease in strawberries firmness during storage after PA at 40 or 90 ppm treatment (de São José, 2015; Guo, 2018). Differences between studies could be attributed to the maturity stage of strawberries can influence the firmness maintenance, as described by Nunes (2002), who concluded that strawberries being mature enough but not ripe or overripe maintained their firmness during cold storage.

Regarding **weight loss**, all strawberries lost 2.9 ± 0.2 % of their weight in form of water (data not shown), which was accumulated as drops in the internal surface of the package at the end of the storage. This value was reached by W-CON at D4, while other samples reached it at D11. Other studies reported weight losses in whole strawberries packaged with an ozone atmosphere of 1.5 % after 3-day storage at 2 °C (Nadas, 2003) or in whole strawberries with ascorbic acid of 8 to 15 % after 12-day storage at 1 °C (Sogvar, 2016).

3.2.2 Biochemical changes

Antioxidant capacity of the samples was measured by DPPH· and FRAP methods (**Table 14**). Antioxidant capacity of the strawberries prior to any treatment was 546.2 ± 39.4 and 134.2 ± 3.3 mg AA / 100 g FW determined by DPPH· or FRAP methods, respectively. Antioxidant values obtained by FRAP method were in accordance with those obtained in our previous studies of strawberries washed with 40 ppm PA (Nicolau-Lapeña, 2019), but those obtained by DPPH· method were 4 times higher. This could be attributed to the different profile of phenolic compounds, including anthocyanins, which are reported to be the main antioxidant molecules of strawberries along with tannins (Fierascu, 2020), and the different antioxidant mechanism they use, hydrogen atom or single electron transfer (Schlesier, 2002). Antioxidant values by FRAP were maintained at 133.2 ± 4.6 mg AAE / 100 g FW at the end of storage, whereas by DPPH· method decreased up to 473.5 ± 16.6 mg AAE / 100 g at the end of the storage in half-cut samples, process that was also reported for fresh-cut strawberries in (Odriozola-Serrano, 2009).

Table 14. Changes in antioxidant activity, expressed by DPPH· and FRAP values (mg AAE / 100 g FW), in total phenolic content (TPC, mg GAE / 100 g FW), total anthocyanin content (TAC, mg pelargonidin-3-glucosyde / 100 g FW), and total ascorbic acid (mg AA / 100 g FW) in fresh strawberries. Values are the mean of 3 repetitions ± standard deviation. Different lowercase letters show statistically significant differences ($p < 0.05$) between treatments within the same presentation (whole or half-cut), analyzed with a contrast test, asterisks show statistically significant differences ($p < 0.05$) between presentation formats (whole vs. half-cut) for WUVPA treated samples, and capital letters show statistically significant differences ($p < 0.05$) during time within the same treatment analyzed with Tukkey's test.

	Format	Treatment	D0	D4	D7	D11
DPPH· (mg AAE / 100 g FW)	**Whole**	**W-CON**	490.7 ± 61.0 ^{aA}	566.4 ± 25.1 ^{aA}	547.1 ± 22.4 ^{aA}	542.1 ± 73.6 ^{aA}
		W-TREAT	548.2 ± 24.7 ^{aA}	561.3 ± 32.1 ^{a*A}	556.6 ± 28.7 ^{a*A}	524.4 ± 23.9 ^{aA}
	Half-cut	**C-CON**	564.2 ± 38.2 ^{xAB}	575.7 ± 43.1 ^{yA}	486.4 ± 48.7 ^{xAB}	461.8 ± 45.1 ^{xB}
		C-TREAT	581.6 ± 19.4 ^{xA}	470.3 ± 27.0 ^{y*B}	400.6 ± 36.9 ^{y*C}	485.3 ± 11.8 ^{xB}
FRAP (mg AAE / 100 g FW)	**Whole**	**W-CON**	136.6 ± 10.6 ^{aA}	145.5 ± 6.2 ^{aA}	143.4 ± 2.7 ^{aA}	139.1 ± 8.8 ^{aA}
		W-TREAT	132.3 ± 1.8 ^{aA}	143.6 ± 3.4 ^{aA}	148.0 ± 4.4 ^{aA}	127.7 ± 21.1 ^{aA}
	Half-cut	**C-CON**	130.5 ± 2.0 ^{xB}	149.2 ± 5.7 ^{xA}	128.8 ± 9.1 ^{xB}	132.6 ± 5.8 ^{xB}
		C-TREAT	137.3 ± 4.9 ^{xA}	126.9 ± 1.1 ^{y*B}	117.9 ± 3.3 ^{y*C}	133.3 ± 2.2 ^{xAB}
TPC (mg GAE / 100 g FW)	**Whole**	**W-CON**	22.9 ±1.3 ^{aA}	23.3 ±0.9 ^{aA}	23.4 ± 1.3 ^{aA}	22.1 ± 0.4 ^{aA}
		W-TREAT	22.6 ± 0.6 ^{aA}	23.0 ± 0.7 ^{a*A}	21.0 ± 2.8 ^{aA}	20.5 ± 2.5 ^{aA}
	Half-cut	**C-CON**	21.0 ± 0.4 ^{xA}	22.7 ± 1.6 ^{xA}	20.6 ± 1.0 ^{xA}	20.8 ± 0.5 ^{xAB}
		C-TREAT	22.0 ± 1.0 ^{xA}	20.2 ± 0.2 ^{y*B}	18.6 ± 0.4 ^{xC}	21.0 ± 0.3 ^{xA}
TAC (mg pelargonidin-3-glucoside / 100 g FW)	**Whole**	**W-CON**	2.8 ± 0.1 ^{aA}	2.8 ± 0.1 ^{bA}	2.9 ± 0.1 ^{bA}	2.9 ± 0.1 ^{aA}
		W-TREAT	2.7 ± 0.1 ^{aA}	3.1 ± 0.1 ^{a*B}	3.2 ± 0.1 ^{a*B}	2.9 ± 0.2 ^{aAB}
	Half-cut	**C-CON**	2.2 ± 0.1 ^{xA}	2.7 ± 0.1 ^{xB}	2.8 ± 0.1 ^{xB}	2.7 ± 0.1 ^{xB}
		C-TREAT	2.7 ± 0.1 ^{yAB}	2.8 ± 0.1 ^{x*AB}	2.9 ± 0.1 ^{x*A}	2.7 ± 0.1 ^{xB}

TAA (mg AA / 100 g FW)	**Whole**	**W-CON**	26.7 ± 0.1 ^{aB}	28.2 ± 0.1 ^{aA}	27.1 ± 0.1 ^{aAB}	21.9 ± 0.1 ^{aC}
		W-TREAT	27.8 ± 0.2 ^{aAB}	28.8 ± 0.3 ^{aA}	24.9 ± 0.2 ^{aAB}	22.7 ± 0.6 ^{aB}
	Half-cut	**C-CON**	28.0 ± 0.1 ^{xA}	25.8 ± 0.0 ^{xB}	22.8 ± 0.1 ^{xC}	19.5 ± 0.2 ^{xD}
		C-TREAT	27.6 ± 0.1 ^{xA}	25.6 ± 0.1 ^{xA}	21.9 ± 0.1 ^{xB}	23.3 ± 0.2 ^{yB}

In this study, **TPC and TAC values** (**Table 14**) did not differ between treatments of formats immediately after the treatments and during storage, despite the fact that the biosynthetic pathway for anthocyanins and phenolic compounds is still operative after harvest and storage at low temperatures is not supposed to inhibit this process The anthocyanin content was lower than those reported in the literature (Li, 2019; Van De Velde, 2016). Differences could be attributed to fruit differences in maturity stage (Ban, 2018) or cultivar (Šamec, 2016) or to different extraction methods (Azmir, 2013).

Initial content of **total ascorbic acid** (TAA) in strawberries ranged from 26.7 to 28.0 mg AA / 100 g FW. Results showing the evolution in TAA during storage (**Table 14**) indicated that the application of WUVPA treatment was not detrimental for strawberry vitamin C: the evolution of this parameter in WUVPA samples was similar to that of their respective controls. Despite this, a sharper decrease in TAA was observed for the control fresh-cut strawberries (C-CON) when compared to whole fruits (W-CON, W-TREAT) and fresh-cut treated with WUVPA (C-TREAT). In general, fruits show a gradual degradation of vitamin C content as the storage temperature or duration increases due to the augmented oxidation, that lead to compounds that do not possess vitamin function (Avina, 1986), which can be delayed by low temperatures (Lee, 2000). At the end of the storage, TAA of whole (W-CON and W-TREAT) and fresh-cut strawberries (C-TREAT) had decreased 17.7 %, while TAA content decreased 29.1 %, in control fresh-cut strawberries (C-CON). Other authors have reported higher decreases (up to 50 %) in strawberry vitamin C after 6 days of storage at 6 °C (Cordenunsi, 2003)

3.2.3 Effect in native microbiota

Initial populations in strawberries are those obtained in W-CON treatment at D0, as it represented initial non-treated fruits. Initial TAM and Y&M counts were 2.3 ± 0.1 log CFU / g for both parameters (**Table 15**). This, together with the fact that the exact same evolution of TAM and Y&M during storage, make evident that native microbiota in strawberries is mainly composed of yeasts and moulds. This composition is in accordance to what Ortiz-Solà (2020) described after a survey of strawberries of Spanish origin. After treatments, Y&M counts were slightly reduced in W-TREAT, C-CON, and C-TREAT. This can be explained by the germicidal effect of UV-C light, which basically causes damages on the DNA structure (Barba, 2017). Besides, PA is a highly effective biocide, being a powerful oxidizer of C-C double bonds and reacting with sulfhydryl and sulfur bonds in proteins (Rossi, 2007). Reductions of bacterial pathogens have been demonstrated by Nicolau-Lapeña (2020) in strawberries by using this combination (WUVPA). However, the decrease in the counts of natural occurring microbiota of strawberry in that study was lower compared to that of artificially inoculated *L. monocytogenes* and *S. enterica* with the same sanitizing treatment. São José (2015) reported a 3 log CFU / g decrease in Y&M population after washing strawberries with 40 ppm PA. The higher reductions could be attributed to the treatment time (10 min), but that could be deleterious for strawberry firmness. Overall, during shelf-life, populations were maintained under 3.1 log CFU / g. Even so, the EU regulation on microbial criteria for foodstuffs (EC 2073/2005 and subsequent modifications) does not include maximum levels of TAM in fresh and pre-cut fruit.

Table 15. Total aerobic mesophylls (TAM) and yeasts and moulds (Y&M) counts (log CFU / g) in fresh strawberries. Values are the mean of 3 repetitions ± standard deviation. Different lowercase letters show statistically significant differences ($p < 0.05$) between treatments within the same presentation (whole or half-cut), analyzed with a contrast test, asterisks show statistically significant differences ($p < 0.05$) between presentation formats (whole vs. half-cut) for WUVPA

treated samples, and capital letters show statistically significant differences ($p < 0.05$) during time within the same treatment analyzed with Tukkey's test.

	Format	Treatment	D0	D4	D7	D11
TAM (log CFU / g)	**Whole**	**W-CON**	2.3 ± 0.1 ^{aB}	2.7 ± 0.2 ^{aAB}	2.9 ± 0.4 ^{aAB}	3.1 ± 0.2 ^{aA}
		W-TREAT	1.8 ± 0.3 ^{aA}	1.9 ± 0.4 ^{aA}	1.0 ± 0.4 ^{bA}	1.0 ± 0.1 ^{b*A}
	Half-cut	**C-CON**	1.2 ± 0.2 ^{xA}	1.0 ± 0.1 ^{xA}	1.0 ± 0.1 ^{xA}	1.0 ± 0.1 ^{xA}
		C-TREAT	1.8 ± 0.6 ^{xA}	1.7 ± 0.4 ^{xA}	1.0 ± 0.1 ^{xA}	1.6 ± 0.1 ^{y*A}
Y&M (log CFU / g)	**Whole**	**W-CON**	2.3 ± 0.4 ^{aB}	2.5 ± 0.1 ^{aAB}	2.9 ± 0.4 ^{aAB}	3.0 ± 0.2 ^{aA}
		W-TREAT	1.8 ± 0.5 ^{aA}	2.1 ± 0.8 ^{aA}	1.3 ± 0.1 ^{bA}	1.9 ± 0.5 ^{bA}
	Half-cut	**C-CON**	1.4 ± 0.2 ^{xA}	1.4 ± 0.2 ^{xA}	1.5 ± 0.3 ^{xA}	1.7 ± 0.1 ^{xA}
		C-TREAT	1.8 ± 0.6 ^{xA}	1.8 ± 0.4 ^{xA}	1.3 ± 0.1 ^{xA}	1.6 ± 0.2 ^{xA}

3.3 Frozen strawberries

3.3.1 Overall quality

After the washing of strawberries in WUVPA treatment, they were packed and frozen with air or under modified gas atmosphere (MAP) (20 % CO_2, 5 % O_2, and 75 % N_2). The **gas composition** inside the package was measured on each sampling time, and results indicated that it was maintained during all the storage, as expected in frozen products (data not shown). One of the purposes of the MAP application was to protect strawberries from detrimental changes during thawing. However, no significant effect in quality and biochemical parameters was observed in thawed strawberries that could be attributed to air conditions inside the package.

The quality parameters **pH, TSS and TA** were evaluated on thawed strawberries (**Table 16**). Although statistical analysis revealed that differences between pH values before and after the washing treatments were statistically significant, pH values measured moved within the reported range for strawberries, so they could be neglected (Costa, 2011). The TSS content did not vary, neither when strawberries were washed in WUV-C and PA 40 ppm nor after the chilling process, and values were maintained at 6.2 ± 0.4 ºB. In contrast, TA showed a significant decrease in strawberries that were frozen (from 7.8 ± 0.3 up to 6.6 ± 0.2 mg citric acid / L), but increased afterwards during the 12-months chilled storage.

Table 16. Quality assessment of strawberries: pH, total soluble solids (TSS), titratable acidity (TA), and firmness in frozen presentation. Values are the mean of 3 repetitions ± standard deviation. Different lowercase letters show statistically significant differences ($p < 0.05$) between gas compositions in the same day, and capital letters show statistically significant differences ($p < 0.05$) during time within the same treatment analyzed with Tukkey's test. Underlined values show statistically significant differences ($p < 0.05$) between the three steps (initial, after WUVPA[1], and frozen-thawed at M0), analyzed with Tukkey's test.

	Packing	Before WUVPA	After WUVPA	M0	M3	M6	M12
pH	**MAP**	3.7 ± 0.1	<u>3.5 ± 0.1</u>	3.8 ± 0.1 ^{aA}	3.6 ± 0.1 ^{aB}	3.7 ± 0.1 ^{aA}	3.8 ± 0.1 ^{aA}
	Air			3.6 ± 0.1 ^{aA}	3.5 ± 0.1 ^{aB}	3.7 ± 0.1 ^{aA}	3.8 ± 0.1 ^{aA}
Total soluble solids (TSS, °Brix)	**MAP**	5.8 ± 0.3	6.4 ± 0.3	6.3 ± 0.3 ^{aA}	5.9 ± 0.5 ^{aA}	6.3 ± 0.3 ^{aA}	6.2 ± 0.4 ^{aA}
	Air			5.9 ± 0.5 ^{aA}	6.0 ± 0.5 ^{aA}	6.4 ± 0.2 ^{aA}	7.0 ± 0.4 ^{aA}
Titratable acidity (TA, mg citric acid / L)	**MAP**	7.8 ± 0.3	7.8 ± 0.4	<u>6.7 ± 1.1</u> ^{aB}	6.5 ± 1.3 ^{aB}	7.5 ± 1.7 ^{aAB}	8.7 ± 1.4 ^{aA}
	Air			<u>6.5 ± 0.8</u> ^{aB}	6.6 ± 1.0 ^{aB}	8.2 ± 0.4 ^{aAB}	7.9 ± 0.3 ^{aA}
Firmness (N)	**MAP**	1.6 ± 0.1	1.6 ± 0.1	<u>0.7 ± 0.1</u> ^{aA}	1.2 ± 0.8 ^{aA}	1.1 ± 0.3 ^{aA}	1.3 ± 0.2 ^{aA}
	Air			<u>0.8 ± 0.1</u> ^{aA}	1.1 ± 0.5 ^{aA}	1.1 ± 0.3 ^{aA}	1.3 ± 0.1 ^{aA}

Firmness of strawberries evaluated before and after the washing treatment did not vary, and was maintained at 1.6 ± 0.1 N (**Table 16**). However, firmness decreased after chilling and thawing, regardless the storage time. Frozen fruits are especially susceptible to damage during freezing process, resulting in softening of texture, loss of water holding capacity and deterioration of colours after thawing. It is accepted that this occurs with the formation of ice crystals accompanied by an increase in volume, which damages cells and the middle lamellae of fruits, but that a quick freezing process may reduce it (Kobayashi, 2019). The measurement of **drip loss** revealed that after thawing, strawberries had lost between 4.7 ± 2.6 and 10.4 ± 0.5 % weight in form of water, resulting in a turgor loss in the cell tissue (data not shown). Drip loss is typically attributed to three main factors: high internal pressure in the product, formation of ice crystals in the product and the irreversibility of water removal from cells (Jul, 1984). And these, in turn, depend on the matrix and on the freezing thawing conditions (Sirijariyawat, 2012). For instance, (Holzwarth, 2012) evaluated different thawing conditions, including temperatures ranging between 4 and 37 ºC, times ranging from 8 to 48 h, or microwaving the samples. In that study, drip losses of strawberries that had been frozen with liquid N_2 and stored at – 20 ºC ranged between 9.1 ± 0.0 and 19.2 ± 0.4 %.

Color of frozen strawberries did not vary after the washing with WUVPA **(Figure 27**C). After freezing and thawing, no changes were observed at 0- and 3-month storage, while at 6- and 12-month storage, L* and b* values decreased, and a* values increased. The color parameters at M12 were 35.8 ± 0.8, 40.6 ± 1.4, and 19.2 ± 1.4, for L*, a*, and b*, respectively, meaning that color was darker and more purplish. Other authors also reported a darkening in the typical red color, regardless the application of a pretreatment of pulsed electric field coupled with vacuum infusion of cryoprotectants was applied to frozen-thawed strawberries (Velickova, 2018). In contrast, Holzwarth (2012) observed that when strawberries were thawed at low temperatures and low times (4 ºC and 8 h), color parameters were better retained than when thawing was performed at higher temperatures (20 or 37 ºC). It is highly important to implement freezing and thawing methods that enable the color retention, that will be unnoticeable by the consumer.

3.3.2 Biochemical changes

The evolution in the **antioxidant capacity** in strawberries was evaluated by two methods: DPPH· and FRAP (**Table 17**). Initial values were 694.8 ± 56.5 and 759.4 ± 61.7 mg AAE / 100 g FW, respectively. These values were maintained immediately after the washing with WUVPA. Although other authors have reported a decrease in antioxidant capacity of strawberries when frozen (Marques, 2010), in the present study, it was not after 6 months of storage when the antioxidant capacity decreased significantly. Antioxidant formation in strawberries is a delicate system that can be easily affected, and according to what has been reviewed in the literature, it can be attributed to **phenolic compounds anthocyanins and vitamin C** (Alvarez-Suarez, 2014; Fierascu, 2020) TPC initial value was 15.6 ± 1.2 mg GAE / 100 g FW (**Table 17**) and after freezing, the value increased significantly to 42.4 – 44.9 mg GAE / 100 g FW. This increase in the TPC value could be explained by the collapse of the cell wall caused by the formation of frozen crystals, and its subsequentially liberation of some phenolic compounds that were not previously accessible for their determination (Kobayashi, 2019). However, the TPC value decreased gradually with storage time, and at after of 12 months at – 20 ºC, it was 7.2 – 7.5 mg GAE / 100 g FW. Initial TAC values were 2.5 and 2.6 mg pelargonidin-3-glucoside / 100 g FW, before and after WUVPA treatment. The content of anthocyanins was maintained throughout the 12-months storage, similar to what other authors have reported (Oliveira, 2015).

Table 17. Changes in antioxidant activity, expressed by DPPH· and FRAP values (mg AAE / 100 g FW), in total phenolic content (TPC, mg GAE / 100 g FW), total anthocyanin content (TAC, mg pelargonidin-3-glucosyde / 100 g FW), and total ascorbic acid (mg AA / 100 g FW) in frozen strawberries. Values are the mean of 3 repetitions ± standard deviation. Different lowercase letters show statistically significant differences ($p < 0.05$) between gas compositions in the same day, and capital letters show statistically significant differences ($p < 0.05$) during time within the same treatment analyzed with

Tukkey's test. Underlined values show statistically significant differences ($p < 0.05$) between the three steps (initial, after WUVPA, and frozen-thawed at M0), analyzed with Tukkey's test.

	Packing	Before WUVPA	After WUVPA	M0	M3	M6	M12
DPPH· (mg AAE / 100 g FW)	**MAP**	694.8 ± 56.5	784.8 ± 61.0	691.9 ± 74.2 ^{aA}	618.3 ± 31.6 ^{aAB}	479.9 ± 37.8 ^{aC}	616.9 ± 76.0 ^{aB}
	Air			759.4 ± 7.8 ^{aA}	679.8 ± 68.0 ^{aAB}	484.5 ± 64.1 ^{aC}	625.5 ± 39.4 ^{aB}
FRAP (mg AAE / 100 g FW)	**MAP**	759.4 ± 61.7	774.4 ± 54.4	740.0 ± 54.3 ^{aA}	692.9 ± 52.8 ^{aAB}	600.1 ± 33.7 ^{aC}	667.7 ± 69.3 ^{aBC}
	Air			785.9 ± 64.6 ^{aA}	804.8 ± 28.8 ^{aAB}	625.7 ± 76.3 ^{aC}	668.7 ± 52.2 ^{aBC}
TPC (mg GAE / 100 g FW)	**MAP**	15.6 ± 1.2	17.7 ± 1.3	42.4 ± 2.9 ^{aA}	30.7 ± 2.3 ^{aB}	18.5 ± 1.2 ^{aC}	7.5 ± 1.6 ^{aD}
	Air			44.9 ± 3.5 ^{aA}	36.8 ± 0.7 ^{bB}	21.4 ± 3.8 ^{aC}	7.2 ± 1.5 ^{aD}
TAC (mg pelargonidin-3-glucoside / 100 g FW)	**MA**	2.5 ± 0.3	2.6 ± 0.2	2.9 ± 0.1 ^{aA}	2.2 ± 0.2 ^{aA}	2.6 ± 0.1 ^{aA}	2.7 ± 0.1 ^{aA}
	Air			2.8 ± 0.3 ^{aA}	2.1 ± 0.2 ^{aA}	2.6 ± 1.1 ^{aA}	2.4 ± 0.3 ^{aA}
TAA (mg AA / 100 g FW)	**MAP**	30.6 ± 2.6	31.5 ± 2.0	30.4 ± 1.0 ^{aA}	31.2 ± 0.5 ^{aA}	24.6 ± 0.6 ^{aB}	29.4 ± 4.3 ^{aA}
	Air			29.5 ± 0.6 ^{aA}	32.4 ± 1.0 ^{aA}	26.1 ± 1.8 ^{aA}	28.6 ± 1.5 ^{aA}

Additionally, one of the aspects of the major nutritional relevance of strawberry is its extremely high content of **vitamin C** (Alvarez-Suarez, 2014). Vitamin C is very labile, so if a given process keeps its levels relatively remained unchanged, it is likely that most other nutrients have survived the process as well (Bouzari, 2015). In the present study, the TAA content ranged from 30.6 ± 2.6 to 29.4 ± 4.3 mg AA / 100 g FW for strawberries before any treatment and for strawberries after the 12-months storage period, respectively (**Table 17**). Similarly, other authors reported a maintenance in ascorbic acid values of frozen strawberries with time (Bouzari, 2015; Li, 2020).

3.3.3 Effect in native microbiota

Initial TAM and Y&M counts were 2.5 ± 0.1 log CFU/g. After washing treatment, counts decreased between 0.5 to 0.8 log units, for TAM and Y&M, respectively. Regardless of the storage time, from 0 to 12 months, the TAM and Y&M counts after freezing remained at 1.4 ± 0.1 and 1.3 ± 0.1 log CFU / g, respectively (**Table 18**). Microbial populations on frozen strawberries probably decreased due to the cell damage that occurred during freezing. Although the formation of intracellular ice should be lower in quick freezing than in conventional freezing, the formation of intracellular ice affecting microbial viability could also occur (Jeremiah, 1996).

Table 18. Total aerobic mesophylls (TAM) and yeasts and moulds (Y&M) counts (log CFU / g) in frozen strawberries. Values are the mean of 3 repetitions ± standard deviation. Different lowercase letters show statistically significant differences ($p < 0.05$) between gas compositions in the same day, and capital letters show statistically significant differences ($p < 0.05$) during time within the same treatment analyzed with Tukkey's test. Underlined values show statistically significant differences ($p < 0.05$) between the three steps (initial, after WUVPA, and frozen-thawed at M0), analyzed with Tukkey's test.

	Packing	Initial	After WUVPA	M0	M3	M6	M12
TAM (log CFU / g)	**MAP**	2.5 ± 0.1	2.1 ± 0.3	1.2 ± 0.3 ^{aA}	1.2 ± 0.2 ^{aA}	1.2 ± 0.3 ^{aA}	1.4 ± 0.4 ^{aA}
	Air			1.5 ± 0.3 ^{aA}	1.4 ± 0.2 ^{aA}	1.2 ± 0.4 ^{aA}	1.1 ± 0.2 ^{aA}
Y&M (log CFU / g)	**MAP**	2.5 ± 0.2	1.8 ± 0.2	1.4 ± 0.2 ^{aA}	1.4 ± 0.1 ^{aA}	1.4 ± 0.2 ^{aA}	1.3 ± 0.1 ^{aA}
	Air			1.6 ± 0.3 ^{aA}	1.4 ± 0.1 ^{aA}	1.5 ± 0.3 ^{aA}	1.3 ± 0.1 ^{aA}

4 Conclusions

The present study evaluated the shelf-life of fresh and fresh-cut strawberries after a washing step consisting on the combination of peracetic acid 40 ppm with ultraviolet -C light, which proved to be effective as a sanitation method for strawberries in previous studies. Here, it was demonstrated that this sanitation method was not detrimental for strawberry quality during storage at 4 °C during 11 days. It did not to be deleterious to fresh strawberries in comparison to non-treated samples. Moreover, the overall effect, including colour, firmness, or antioxidant activity, could be comparable to that of the chlorine sanitation, a common procedure for this type of fruit presentation.

Strawberries washed with the combination of PA 40 ppm and UV-C light were also frozen using a supercooling technology. Even though firmness decreased after freezing and thawing cycle in both normal packaging or modified air composition (20 % CO_2, 5 % O_2, and 75 % N_2), and a slight darkening of the strawberries was observed after 6 months of storage, the nutritional quality, including antioxidant activity, total anthocyanin content and total ascorbic acid, values were maintained.

The results of the study highlight the potential use of WUV+PA as an alternative to produce safer strawberries, without any major decrease in their quality during storage. Scaling-up to industrial process can be challenging, so future studies on the current topic are therefore recommended.

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Conflict of interests

The authors declare no conflict of interests.

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Chapter

4

Ultrasound processing alone or in combination with other chemical and physical treatments as a safety and quality preservation for fresh and processed fruits and vegetables: a review

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Ultrasound (US) processing has emerged as a novel food preservation technology. This strategy has proved antimicrobial effects due to cavitation, which is the formation, growth, and collapse of bubbles that generate a localized mechanical and chemical energy. This technology can be applied by water so introducing it in the washing step to obtain safe fresh or fresh-cut products could be promising. The current review provides an overview of the current knowledge and recent findings on the use of US, alone or in combination with other mild physical technologies or chemical agents, to reduce microbial loads, and to better retain their quality attributes including color and texture, as well as the content of bioactive compounds such as antioxidant, phenolic compounds, or vitamins of minimally processed fruits and vegetables. As the effects of US depends on several factors related with treatment parameters, target microorganism, and matrix characteristics, further research efforts should be directed on optimizing US processes in accordance with their further application.

Sonication, microorganisms, fresh-cut, non-thermal technologies, antimicrobial

1 Introduction

Minimally processed or fresh-cut produce has been defined by the International Fresh-cut Produce Association as "any fruit, vegetable or their combination subjected to a physical alteration from its original form, remaining in a fresh state" (Grau-Rojas, 2010). These products are completely edible, packaged, and should be stored under refrigerated conditions thus providing convenience to consumers (Grau-Rojas, 2010). The fruit and vegetable processing industry is experiencing an expanding period as the global demand for healthy, fresh, and sustainable products is increasing (Qadri, 2015). However, consumption of minimally processed fruits and vegetables (F&V) has been associated to concerns on their safety due to the emergence of several outbreaks of foodborne pathogens linked to their consumption (Pinela, 2015).

Main causes of foodborne diseases are due to bacteria 53.0%, viruses 42.5%, and parasites 4.5% (Ramos, 2013). *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* were the main contaminants involved in outbreaks related to F&V over the past years (Birmpa, 2013; Park, 2012; Silva, 2017; Tango, 2017). According to the European Food Safety Authority (EFSA), the top ranking food / pathogen combinations are *Salmonella* spp. and leafy greens, bulb, stem vegetables, tomatoes, or melons, and *E. coli* and fresh pods, legumes, or grains (Andreoletti, 2013). Another major problem of the F&V processing industry is related to alterative microbiota, which do not suppose any health risk to humans, but can lead to quality deteriorations, shortening the products' shelf-life, and causing significant economic losses (Rico, 2007). For example, strawberry spoilage losses can be as high as 40 % (Luksiene, 2013) leading to strawberry producers to look for strategies to extend their shelf life. In addition, fresh-cut operations such as peeling or cutting result in increased nutrient availability and senescence rates as a consequence of the natural epidermal breach leading to the growth of microorganisms (Qadri, 2015; Ramos, 2013). For these reasons, disinfection procedures are crucial to maintain safety and quality of fresh-cut F&V.

Among available sanitizers, chlorine is the most widely used due to its low cost, ease of use, and effectiveness against vegetative bacteria and some enteric viruses (Luo, 2018). However, chlorine has been associated with negative health outcomes and it has already been banned in some European countries including Belgium, Denmark, Germany and the Netherlands (Meireles, 2016). Therefore, the need to find effective alternatives to chlorine led to a number of novel chemical and non-thermal strategies. Proposed methods include the use of electrolyzed water, high pressure processing, ozone, Generally Recognized As Safe (GRAS) substances such as organic acids or essential oils, pulsed electric fields, ultraviolet irradiation, ultrasounds (US), or combinations of chemical and physical strategies (Barba, 2017; Cebrián, 2016).

US has been reported as a green food processing technology, as it implies a saving of energy and water use, and it is environmentally friendly, with a reduced carbon and water footprint when compared with traditional techniques (Chemat, 2017). It offers an advantage in terms of productivity and yield, with improved processing times and enhanced quality, and it has been reported to improve processes such as freezing and crystallization, drying, degassing, emulsification and demolding (Chemat, 2011).

US consists on the use of ultrasonic waves at a frequency beyond 18 kHz with a specific intensity and amplitude (Bevilacqua, 2018). It has been acknowledged and reviewed that microorganism lethality caused by sonication is due to a phenomenon known as transient cavitation (Pérez-Andrés, 2018). Generated bubbles collapse and, consequently, molecules collide, spots of extremely high temperature and pressure occur (5,000 ºC and 50 MPa), and cellular envelopes and other microbial components are destroyed (Leong, 2017), thus reducing the viable microorganisms (Van Impe, 2018). Moreover, US can induce other chemical and structural changes, affecting quality and nutritional values of the processed products (Leong, 2017).

Most of the review papers published to date discussed the potential utilisation of sonication in liquid matrices such as milk or juices (Anaya-Esparza, 2017; Ortega-Rivas, 2014; Potoroko, 2018; Van Impe, 2018). The current manuscript summarises the most recent findings on the effect of sonication on the physicochemical and nutritional attributes as well on the safety of fresh and minimally processed fruit and vegetables. To the best of the authors' knowledge, this is the first paper that reviews the effect of sonication of fresh and

minimally processed vegetables. Furthermore, this paper also highlights the possible use of sonication combined with other physical or chemical aids and discusses the potential large-scale utilisation of this technology if a correct optimisation and scaling-up is conducted.

2 Antimicrobial effects of US processing

2.1 Effect of sonication on natural-occurring and inoculated microorganisms

Antimicrobial effects of US have been attributed to two main causes: cavitation and free radical formation. The former can shear and break cell wall and membrane structures, thus increasing permeability and losing selectivity (Bilek, 2013). The micro-mechanical shocks of the collapsing bubbles, can cause disruption of cell components and DNA injuries, breakages, and fragmentation (Birmpa, 2013). The latter is caused by the high pressure and temperature reached within the bubbles, which promotes the generation of primary hydroxyl radicals and the acceleration of single electron transfer. This originates a series of reactions that form, among others, hydrogen peroxide with bactericidal properties (Bilek, 2013). Hydroxyl radicals are also able to react with the sugar-phosphate backbone of DNA, causing the withdrawal of phosphate-ester bonds and breaking the double strand microbial DNA, leading to cell unviability, dysfunction, and further death of microorganisms (Mañas, 2005).

Fresh and minimally processed F&V must be microbiologically safe as they are generally consumed raw. As US processing has been reported to be a potential alternative to chlorine in disinfection steps, studies have been and must be carried out in order to better understand the outcomes of US processing of F&V. The most commonly used conditions when applying US to F&V are shown in Table 1 and Table 2. Briefly, the most commonly used frequencies ranged between 20 and 40 kHz, obtained by applying sonication powers between 10 and 200 W and temperatures ranging from 20 to 40 °C. Treatment times ranged from 1 to 60 min. In addition, several food matrices have been evaluated, and these included vegetables such as lettuce, kale, or carrots, and fruits such as strawberries, plums, or kiwis. So far, most studies reported the effects of US processing on either alterative microbiota, typically mesophilic bacteria, yeasts and molds (see **Table 19**) or pathogenic microorganisms, namely *E. coli*, *Salmonella* spp., and *L. monocytogenes* (see **Table 20**).

Antimicrobial effects of US depend on treatment parameters. Indeed, Mansur (2016) recently suggested that sonication power is a factor which has a key impact on the antimicrobial efficacy of US. Moreover, the higher was the intensity used when treating kale (ranging between 100 to 400 W / L), the higher were the reductions observed for all the microorganisms studied (ranging between 3.2 to 3.9 log cycles). Overall, it seems that US application mode is not a factor that has a significant effect on microorganisms, as there seems to be no differences between continuous – constant sonication – or pulsed – intermittent sonication – modes (Hashemi, 2018a). Still, the use of continuous or pulsed US affected differently the content of certain compounds in fruits and vegetables previously. For example, Pan (2012), reported different values of total phenolics content and antioxidant activity on pomegranate after application of continuous US when compared to pulsed US. We would like to highlight that this does not mean that US processing can alter the content of polyphenols in fruits. It is likely that continuous or pulsed ultrasonic waves can lead to different extraction yields and therefore higher phenolic contents and antioxidant activities of the water and organic extracts. Another parameter that affects cavitation activity is frequency, as bubble size is inversely proportional to it. Application of lower frequencies results in larger bubbles, liberating higher energy (São José, 2014a).

Effects of US on microorganisms also depend on the target specie and on matrix properties. In this sense, São José (2014b) obtained different antimicrobial effects depending on the studied matrix. They reported a reduction of *E. coli* populations of 2.3 or 1.6 log cycles when processing green pepper or melon at 40 kHz for 2 min, respectively. When using these same conditions, reductions of *S. enterica* Enteritidis on green pepper or melon were lower, 1.8 and 1.9 log cycles, respectively. These differences were attributed to the behavior of each microorganism on different surfaces. According to Tan (2017), sonication alone

significantly affects the flagella and fimbriae of bacteria, decreasing the cell adhesion of artificially inoculated *S. enterica* Typhimurium by 0.5 to 1.0 log cycles, a relevant reduction if taken into account that *Salmonella* contamination in real production lines typically contains <1 log CFU / mL of this bacteria. US capacity to remove bacterial cells from the surface is recognized, as it influences the attachment ability of microorganisms before and after biofilm formation. Biofilms, or aggregates of microorganisms whose cells are frequently embedded within a self-produced matrix of extracellular polymeric substances, may be another source of resistance to sanitizers and surfactants (Brilhante de São José, 2012). In fact, several studies have evidenced its effects on *L. monocytogenes* biofilms alone (Hamman, 2018) or combined with surfactants (Torlak, 2004). US are widely used on machinery surfaces and food pipelines, as a physical method to eliminate biofilms, since there is no residue left over in the removal process (Zhao, 2017).

Yeasts and molds can also be inhibited by US. Some authors have reported 0.5 log cycles reductions in strawberry processed at 33 kHz and 60 W for 10 min (Gani, 2016). Other studies observed reductions of 2.3 log cycles in kiwi, when processed at 30 kHz and 368 W / cm^2 during 8 min (Vivek, 2016). Overall, in the majority of the studies published to date, decay incidence, or percentage of fruits with visible mold growth, was significantly reduced when comparing US processed with a non-treated control (Muzaffar, 2016; Vivek, 2016). Even though reported reductions of pathogenic bacteria seem to be higher than those of epiphytic microbiota, pathogenic microorganisms are normally artificially inoculated in the food matrix before the assay, so internalization and attachment are typically lower than what occurs regarding natural microbiota. Moreover, total bacteria count includes a wide range of microorganisms within which some strains could be more resistant to specific US conditions. In this regard, more assays should be carried out in order to elucidate whether US could be capable of reducing microbial loads that occur in the stomata, vasculature, cut edges or intercellular tissues, where other strategies have proven to be ineffective (Meireles, 2016).

Another factor that affects the effectiveness of US is processing or dipping time. It seems that longer processing times result in higher microbial inactivation (Birmpa, 2013). It is important to highlight that for each target microorganism, matrix, and US conditions, a minimum application time is necessary to report significant changes on the microbiota (Hashemi, 2018a). Temperature of the matrix and the media can increase by the application of US for a period of time, due to acoustic energy produced (Marques-Silva, 2017). The temperature achieved could affect the results, leading to a possible increase in microorganism inactivation but also to an alteration or degradation of biochemical and nutritional compounds. In order to implement this technology at large scale production of minimally processed fruits or vegetables, processing times should be minimized and should not exceed a few minutes. Although US processing alone can exert antimicrobial effects (do Rosário, 2017), to reduce treatment time and to achieve a sufficient microbial inactivation, US can be combined with other chemical or physical strategies, because synergistic or additive effects may take place when it is combined (Barba, 2017; Park, 2018).

2.2 US combined with mild temperatures

So far, there are no publications that use a combination of mild temperatures and US (thermosonication) to disinfect F&V for fresh-cut produce. It has been widely studied in F&V juices, with good results on pathogenic microorganism reductions (Sánchez-Rubio, 2018), alterative microbiota growth and bioactive compounds maintenance (Lafarga, 2018; Hashemi, 2018b), and enzyme inactivation (Illera, 2018). Nonetheless, application of thermosonication on F&V for fresh-cut industry could lead to changes in texture that may not be a shortcoming in juices but could have detrimental effects on fresh-cut F&V. As previously mentioned, long processing times are not feasible in industry, as they can have detrimental effect on firmness (Terefe, 2011). However, further studies are needed in order to assess the real potential of this technology in the fresh and minimally processed fruit and vegetable industry.

2.3 US processing combined with chemical agents

Because of the limitations of US processing alone and the limited applications of the combinations of US with mild temperature for fresh produce, chemical agents used as sanitizers may become effective alternatives to chlorine. Among others, organic acids and ozone have proved to be able to reduce microbial load in F&V (Meireles, 2016). Many of these compounds have GRAS status, and have already demonstrated to exert antimicrobial activity. For example, carvacrol, vainillin, or peracetic acid were used against *E. coli* O157:H7, *Listeria* spp., and *Salmonella* spp. and reductions between 1.0 and 3.0 log cycles were observed (Abadias, 2011). This, together with the possibility of combining them with US, makes them good choices for the fresh-cut industry.

2.3.1 Organic acids

Organic acids seem to have two distinct antimicrobial action modes. The first involves pH depression, as a release of protons to the surrounding media creates unfavorable conditions for bacterial growth. The second is based on the diffusion of the non-dissociated form of the organic acid across the semi-permeable membrane of the microorganisms. Once within the cell, the acid may undergo a dissociation process, as the pH of the cytoplasm, which is approximately 7, may be different to the pH outside the cell. Once the organic acid is dissociated, the pH drop can suppress cell enzymes and nutrient transport systems, causing the death of the pathogen (Calmont, 2010).

The most widely studied organic acids are lactic, citric, acetic, and peracetic acid, at concentrations ranging between 0.04 and 2 %. Reductions of 3.2 or 3.0 log cycles have been achieved against *Salmonella* Typhimurium when combining US with citric (2 %) or peracetic acid (5 %) respectively (Sagong, 2011; Silveira, 2018), which were higher than those of non-treated product. Lower reductions were observed when using lactic acid 1 % against *Salmonella* Enteritidis, which reduced by 1.9 to 2.8 log units (Sâo José, 2014a; São José, 2015). *L. monocytogenes* and *E. coli* have also been studied, and reductions of approximately 2.5 log cycles have been reported when processing lettuce leaves at 40 kHz and 90 W for 5 min combined with lactic, citric, or malic acid at 2 % (Sagong, 2011).

Except for Silveira (2018), who reported no significant differences between US alone (40 kHz, 500 W, 5 min) or in combination with peracetic acid 50 mg / L, studies published to date show a significant synergistic or additive effect on the combination of both mechanisms (**Table 19** and **Table 20**). The intense pressure gradients caused by US seem to enhance the penetration of the organic acids through the cell membrane of the microorganisms, and along with cavitation, it assists the disaggregation of the microorganisms, leading to an increased efficiency of the sanitization treatment (Sâo José, 2015).

2.3.2 Essential oils

Sonication can also be combined with essential oils (EOs). EOs are effective antimicrobials (Ribeiro-Santos 2018). Their action mechanism includes membrane rupture, ATP-ase inhibition, leakage of essential biomolecules, proton motive force disruption, and enzyme inactivation (Pisoschi, 2018). According to Salvia-Trujillo (2015), the key features for the effectiveness of EOs are their composition, concentration, and droplet size, that promotes faster inactivation of microorganisms. Millan-Sango (2016) suggested that EOs' droplet size is not as important. However, when EOs and US processing are combined, US frequency and processing time are directly related with antimicrobial effects.

In fact, cinnamon, oregano, and thyme EOs have been studied against several pathogens. When using cinnamon EO (2 %), reductions of *L. monocytogenes* ranging from 0.8 to 1.6 log cycles have been reported. Cinnamon EO in combination with 140W, 5 min US processing, also stopped the growth of the microorganism during 9 days of storage (Park, 2018). Oregano (10-18 mg / L) and thyme (14-18 mg / L) EOs in combination with US processing at 26 kHz and 200 W for 5 min, were used against *Salmonella* spp. increasing the effect achieved when using US alone (Millan-Sango, 2015). In addition, a 4- to 5-fold higher

decrease in the *E. coli* O157:H7 populations was observed when compared with disinfection with EOs only (Millan-Sango, 2016).

This synergism, or the greater effect observed when combining US and EOs compared to the sum of their individual effects, and the ease to apply both methods together, make the tandem a promising alternative for disinfection processes in F&V industry.

2.3.3 Ozone

Briefly, the antimicrobial action mode of ozone consists of two mechanisms. On the one hand, the oxidation of sulfhydryl groups and amino acids of enzymes and proteins generating small peptides. On the other hand, oxidation of polyunsaturated fatty acids to acid peroxides by ozone induces cell envelope damage or disintegration, leakage of cell content, and lysis (Brodowska, 2017; Horvitz, 2014). Ozone is one of the most potent oxidizing agents, and it is more soluble in water than it is in air, making it suitable to be combined with US (Aguayo, 2014). Moreover, as ozone is unstable in aqueous phases, it decomposes to form oxygen and therefore, food products treated with ozone are free from chemical residues (Souza, 2018). To the best of the authors' knowledge, there is only one study published so far evaluating the combined effect of ozone and US. Aday (2014) combined US (20 kHz, 30 W, 5 min) and ozonation (0.075 mg / L) on strawberries. The authors reported a 21 and 35 % incidence of *Botrytis cinerea* in non-treated fruits at the 3rd and 4th weeks respectively, whereas a complete inhibition of mold growth was observed after the treatment with ultrasound and ozone during the whole storage (See **Table 20**). In order to determine whether the combination of ozonation and sonication could be an effective option for F&V disinfection, more studies should be carried out using both methodologies and applying them to a range of matrices, target microorganisms and at different conditions.

2.3.4 Slightly acidic electrolyzed water

Slightly acidic electrolyzed water (SAEW) is produced by means of an electrolytic cell without a separating membrane, producing the electrolysis of dilute sodium chloride and hydrochloric acid solutions. Its bactericidal effect is attributed to the available chlorine compounds including ClO^-, HClO, and Cl_2 (Ye, 2017). SAEW is commonly used at pH values ranging from 5.0 to 5.5 and oxide-reduction potential values of 930-980 mW.

Despite the potential of SAEW for disinfecting fresh foods, it seems that this technique alone might not be able to completely disinfect all F&V, especially those that might have hidden places where adherent microorganisms are difficult to remove by aqueous sanitizers (Luo, 2016b). Indeed, Koide (2009) found that SAEW was effective to remove bacteria from the surface of fresh-cut cabbage but residual contamination could be caused by microorganisms embedded inside the cellular tissues, namely stomata. The combined effect of SAEW and US has been proved to be more efficient in reducing microbial loads when compared to their individual application. For instance, SAEW has been applied in lettuce or tomato in combination with US at 20 kHz, 130 / 210 W, for 5 to 15 min against *L. monocytogenes* and *E. coli*, achieving reductions of 4.0 log cycles (Afari, 2016). The combination did not only reduce the population but also allowed the control of the remaining microorganisms in F&V. Indeed, for *Bacillus cereus* in potato processed with US at 40 ºC 40W / L for 3 min, the lag time increased by 0.2-10.5 h, and the specific growth rate decreased 0.01-0.23 log CFU / h in comparison to the 0.46 log CFU / h of the non-treated control. The authors indicated that the cells stressed by the treatment had lower metabolic activity compared to those untreated (Luo, 2016a). In addition, SAEW and US combination was also effective reduce spoilage microbiota, as it was reported by Wu (2018), who applied pH 5.5 and ORP 514 mV water combined with 40 kHz, 200 W, 3 min US treatment to mushrooms and found significant differences in spoilage microbiota in comparison to the water-treated control. The combination is worthy as well to inactivate the pathogens that could remain in water (Afari, 2016).

2.3.5 High pressure CO_2

Supercritical CO_2 is being increasingly studied as an antimicrobial agent, due to its advantageous characteristics. These include being a GRAS substance, and that its critical temperature (31.1 ºC) and pressure (7.3 MPa) are compatible with the thermal stability of most food matrices, facilitating its application in industrial processes (Hossain, 2013; Hossain, 2016; Tamburini, 2014).

So far, most commonly used pressures and temperatures were 6 to 12 MPa and 22 to 35 °C, respectively (**Table 19** and **Table 20**). Studies published to date have reported an 8.0 log cycle reduction of *E. coli* when combining supercritical CO_2 10 MPa, 22ºC with US at 40 kHz, 10 W, after processing for 5 min, while 15 min were needed to achieve the same levels using CO_2 alone (Ferrentino, 2015b). Ferrentino (2015a) detailed that mesophilic microorganisms, coliforms, yeasts and molds were also reduced by 3.0 log cycles when combining CO_2 at 12 MPa, with US at 40 kHz, 10 W for 30 min, at a mean temperature of 39.7 ºC. Also, a 7.0 log cycle reduction of *S. typhimurium* was achieved with the same treatment. Effect on *S. tyhphimurium* was not observed when applying US alone.

Combination of supercritical CO_2 with US demonstrated to have an improved effect than it had when US was applied alone (Ferrentino, 2015a; Ferrentino, 2015b). As one of the main drawbacks of US is that the transmitting media seems to partially absorb the acoustic energy, preventing its transfer to the solids to be treated, the use of CO_2 could potentially overcome this issue as it is a dense fluid, and acoustic waves would not be reflected but absorbed by the solid (García-Pérez, 2006). Moreover, with an increase of temperature from 22 to 40 ºC, higher diffusivity of CO_2 and increased fluidity of cell membrane allows a faster penetration of CO_2 into it. US enhances this effect, as it induces a better contact between CO_2 and the membrane, accelerating the diffusion through the membrane, thus causing a drastic drop in intracellular pH and extraction of vital constituents (Ferrentino, 2015).

3. Nutritional changes

The effect of US processing on F&V nutritional components has been widely studied. Results listed in **Table 21** suggest a higher content of phytochemicals in extracts obtained from sonicated fruits and vegetables. US is commonly used to promote the extraction of compounds from food sources including phenols (Soquetta, 2018), carbohydrates (Vilkhu, 2008), or proteins (Lafarga, 2018). This does not mean that US processing promotes the generation of these valuable compounds. Higher yields reported in the literature could be attributed to the enhanced extraction efficacy when US have been applied. US causes cell disruption, allowing permeation of intracellular compounds and therefore, a higher liberation of molecules to the extracting media (Hidalgo, 2017). In order to obtain improved extraction yields, processing times in the range 20-60 min are generally required (Annegowda, 2012; Lafarga, 2019). However, it has been suggested that sonication can increase the degradation of natural products (Pingret, 2013). Two chemical reactions have been proposed as probable mechanisms responsible for the degradation connected with sonication. One is related with pyrolysis within cavitation bubbles or gas pockets trapped in the crevices of the solid boundaries, which cause the degradation of polar compounds, and the other is the generation hydrogen ions (H+), free radicals (O− , OH− ,) and hydrogen peroxide (H_2O_2) that are produced by cavitation effect (Rawson, 2011). For instance, isomerization of carotenoids can also occur, as there are extreme physical conditions of temperature and pressure during processing (Kumcuoglu, 2014). Also, antioxidant capacity of cyaniding 3-glucoside was evaluated after US treatment (20 kHz) and showed a 20 % of its original antioxidant capacity. The authors suggested that hydroxylation occurred during sonication, causing such decrease (Ashokkumar, 2008). Degradation or oxidation of biochemical compounds has been related with increased treatment times (Gani et al., 2016; Jahouach-Rabai, 2008)

There are scarce studies focusing on the effects of US on the macromolecules of F&V. In fact, from the recent past years, there is only one paper reporting values of fat content, and the results showed that there was no statistical effect on this parameter when combining US and high pressure CO_2 on coconut

(Ferrentino, 2015a). Regarding proteins, US could induce changes in native form: conformational changes, damage to secondary structure, re-structuration of disulfide bond or generation of other intra / inter molecular interactions (Chizoba-Ekezie, 2018). Studies on proteins in F&V after US have focused mostly on its extraction yield and allergenicity (Nayak, 2017). Only one study carried on by Li (2017) evaluated the effect of US (40 kHz, 350 W) on total soluble proteins of straw mushrooms. They reported that this parameter – an indicative of tissue destruction – was negatively affected by over-time treatments (30 min), but 1 to 10 min served to prevent soluble protein utilization, allowing metabolic activity prolongation.

The effect of US processing on the total phenolic content (TPC), of F&V has been largely studied. However, only few studies evaluated whole pieces and most of them focused on processed products such as juices or purees. Bal (2017) processed grapes with US (32 kHz, 60 W / L, 10 min) and observed an increase on the yield TPC of the sonicated product at the end of a 60-day storage period when comparing to the untreated control. Related to flavonoids, Bal (2017) suggested that even though there were no statistical differences between samples, total anthocyanin content of grapes processed with US tended to increase during storage. Other authors observed an increment of 7.9 % in TPC values on strawberries processed with US (33 kHz, 60 W, 10-40 min, US bath maintained at 25 ºC) from day 1 (Gani, 2016). Increase of TPC was partially explained by a better extraction of polyphenols attributed to an increase in temperature that occurs in US treatment as a consequence of cavitation phenomena, and it was also attributed to hydroxylation of flavanols, which has a positive effect on antioxidant activity (Soria, 2010). Increases in yield of TPC were reported by Yu (2016), who found that the TPC values of US-treated romaine lettuce (25 kHz, 26 W / L, 1-3 min) were up to 22 % higher than those quantified in the untreated product. As an abiotic stress, US could enhance the biosynthesis of secondary metabolites in plant cells, through stimulating their physiological activities. That could partially explain why TPC increases during storage when compared with the non-sonicated products (Wang, 2015). In addition, US could promote the liberation of phenols, as these compounds can be bound to other compounds present in cell walls (polysaccharides, proteins, etc.) and be disrupted by US cavitation (Khan, 2018). On the contrary, Ferrentino (2015b) found that applying US (30 kHz, 40 W, 30 min) and high pressure CO_2 (12 MPa, 35ºC), the TPC decreased when compared with the untreated product. Still, these results could not be attributed directly to the ultrasonic effect, because no control of both individual treatments was used in that study.

Ascorbic acid (AA) forms part of Vitamin C, and its content can be affected by US processing. Alexandre (2013) reported that sonication reduced the loss of AA during the freezing of red bell pepper when processing at 35 kHz, 120 W and 15 ºC compared to water-washed ones. In terms of the US mode application, Hashemi (2018a) did not observe significant differences between the use of pulsed or continuous mode in the AA content of plums. Treatment time was a significant factor to take into account in US processing. The same authors reported an increase in the AA content when longer US treatment times (1, 15, 30, 45 and 60 min) were applied to plums at 30 kHz and 100 W. The increase of this compound was attributed to the elimination of entrapped oxygen due to cavitation, which is essential for AA degradation (Bhat, 2011; Cheng, 2007).

As summarized above, effects of US on nutritional values of F&V may differ between studies, conditions, and matrices, and they can partially be attributed to different extraction yields when applying US (Chemat, 2017). These differences may also occur when scaling up from lab or pilot plant scales to industry. With this purpose, several papers reviewing the potential of US in food industry have been published to date (Bilek, 2013; Kentish, 2014; Prakash, 2003).

4. Effect of US processing on F&V quality

As highlighted in previous sections, US processing combined with chemical sanitizers shows potential for being used for the large scale disinfection of fresh and minimally processed fruit and vegetables. However, US processing can result not only in antimicrobial or increased extraction yields but also in a detriment in quality attributes. The quality of F&V is based on several properties: physical parameters, such as texture or color, organoleptic attributes like aroma or flavor, and nutritional and bioactive properties

including TPC or antioxidant capacity. Therefore, in order to obtain high-quality products, it is important to assess the effects of processing on these key parameters.

4.1 Overall quality changes

Physical properties of F&V processed with US generally remain unchanged after treatment. As it is shown in **Table 21**, pH and titratable acidity tend to maintain the values of the control samples after the US treatment. In some cases, F&V processed with US have higher total soluble sugars values than those from the control. This has been attributed to the fact that US might accelerate the depolymerization process of the starch gel (Amaral, 2015; Bal, 2017) in the outer parts (< 1 mm) and at deeper tissues, changes are attributed to water removal (Schössler, 2012). These structure alterations can be related with the increment of exposure time to US, increasing the temperature and the further destruction of cellular structure (Jurek, 2012).

4.2 Color

Color is an important sensory attribute of a fruit or vegetable that provides an indication of freshness and flavor quality. It could affect the consumer buying decision to acquire a certain product or to prefer one to another. Not appropriate color will suggest loss of freshness or lack of ripeness that will repel the potential buyer (Barrett, 2010), thus the importance of monitoring the effect of US on this attribute.

Several studies, including those listed in **Table 22**, evaluated the effect of US processing on the color parameters of F&V. Overall, no changes in color were observed, processing with US alone or in combination with chlorine or high pressure CO_2 (Ferrentino, 2015b; Salgado, 2014). However, some studies reported significant differences between US-processed and untreated samples in the a* and b* values, either once treated or after storage (Ferrentino, 2015a) or in L* values in different matrices such as coconut, mango, or strawberries (Aday, 2014; Amaral, 2015; Santos, 2015).

The observed changes in color can be attributed to the possible inactivation of enzymes such as poly-phenol oxidase (PPO) and phenol peroxidase (POD). These enzymes are proposed to cause off-colors in raw and frozen vegetables and browning reactions (São José, 2014a; Toivonen, 2008). US treatments have demonstrated to be able to inactivate such enzymes in certain conditions, occurring at higher rates when combining US technology with heat (40-60 °C) (Illera, 2018). Enzyme inactivation also depends on treatment time (Cao, 2018; Zhu, 2017) and US intensity (Liu, 2017). Causes of enzyme inactivation involve shear stress and pressure, which cause oligomeric enzymes dissociation, free radicals affecting the structure, and creation of a large interfacial area by US that disturbs the hydrophobic interaction and hydrogen bonding, thus destabilizing proteins (Terefe, 2015). For instance, Li (2017) observed an inhibition in PPO activity when processing straw mushrooms with US (40 kHz, 350 W, 1-30 min). A decrease in POD and PPO activities of fresh-cut pineapple was reported by Yeoh (2017) after processing pineapple slices with 25-29 W, 37 kHz, for 10 to 15 min US . These enzymes had significantly lower activity in sonicated fruit than they had in water-washed fruit. Besides, after a 5-day storage period, POD and PPO activities were 3.8 and 4.5-fold lower than they were in the water-dipped control. Moreover, US was suggested to facilitate the penetration of ascorbic acid to vegetable cells, as cell wall disruption occurred, thus enhancing antioxidant processes. On the contrary, Wang (2015) found an increase on POD activity of cherry tomatoes and Ferrentino (2015a) of coconut. It has been proposed that low US power level could promote enzyme production, whereas high power US could induce the contrary effect, but it could affect the quality parameters of the product. Also, the effectiveness of US depends on the differences in the resistance of each enzyme to the treatment (Kentish, 2014).

In red fruit, changes in color may be attributed to the degradation of anthocyanins when cavitation occurs for long period times (Gani, 2016). For mushrooms, it has been suggested that US exerts a protective effect on surface color changes as hydrogen peroxide is formed in distilled water when cavitation occurs, and this compound helps to maintain their whiteness (Lagnika, 2013). Factors affecting other F&V are pigments,

such as carotenoids and chlorophyll, or other compounds like ascorbic acid (Bermúdez-Aguirre, 2013), that may be altered by US treatments. Carotenoids, lycopene, and other liposoluble pigments undergo isomerization processes that can lead to color alterations (Adekunte, 2010). Indeed, Sun (2010) observed that the appliance of US at 21-25 kHz, 950 W, for 10 min to β-carotene resulted in several carotene degradation products, including 15-*cis*-β-carotene and di-*cis*-β-carotene. Eh (2012) also found that processing tomatoes with US with 37 kHz, at 140 W for 45 min resulted into changes in lycopene forms *cis* and *trans*.

For what has been reviewed, changes in F&V color after US treatment can occur as a consequence of a number of reasons, namely activity reduction or inactivation of browning enzymes, penetration of antioxidant agents to vegetable cells, and alteration or degradation of pigments. As so, more studies should be carried out in order that US conditions be optimized for each purpose in order to maintain overall color quality of F&V.

4.3 Texture

There are two main factors influencing the consumer's mouth feel of a fruit or vegetable: firmness and juiciness. Firmness is determined by the physical anatomy of the plant tissue, cell size and shape, wall thickness and strength, and cell-to-cell adhesion. In turn, juiciness is related to the cell sap content and the ease to be split (Toivonen, 2008). Consumers have clear expectations for the texture of fresh-cut F&V, and panel testing indicates that they are more sensitive to small differences in texture than in flavor, being textural defects and the interaction of flavor and texture the features that cause most reject (Barrett, 2010).

A review of the data found to date is summarized on **Table 22**. Some studies report no significant differences in textural parameters after US processing. However, most of the accounts suggest that US processing can affect the firmness of fresh F&V depending on the intensity of the treatment. The effect of US processing on texture also depends on several parameters, which include food matrix, variety, maturity stage, intensity, or processing duration of US treatment. Results obtained so far seem to be contradictory and matrix-dependant, texture changes should be assessed independently for each fruit or vegetable. Softening of fruits has been attributed to inner changes of cell wall constituents, mostly pectin, which can be de-esterified by the activity of pectin methyl esterase, followed by a depolymerization of methoxy pectin or pectic acids due to endo-polygalacturonase activity (Wang, 2018). For instance, Saeeduddin (2015) found that 20 kHz, 0.30 W / mL US applied for 10 min at 45 or 25 ºC could inactivate pear pectin methyl esterase by 60 or 7%, respectively. Thus, US, combined or not with mild temperatures or high pressures, can cause partial or total inactivation of enzyme activity, thus leading to changes on the textural quality of the F&V (Marques-Silva, 2017).

From above, one can gather that texture and color change or maintenance depends on a number of factors including matrix, treatment conditions, enzymes and plant components. Therefore, the effect of US processing on F&V quality parameters should be assessed for each product independently.

4.4 Antioxidant capacity

Fruit and, to a less extent, vegetables, are along with beverages the main sources of the daily intake of phenolic antioxidants (Shahidi, 2015). Apart from preventing browning and deterioration of different constituents of F&V, antioxidant compounds are now on the focus for health reasons, as they are presumed to prevent the deleterious effects of free radicals in the human body (Pisoschi, 2012).

According to recent studies, antioxidant activity values obtained by *in vitro* methods of F&V processed with US increases in comparison with the control samples. As an example, Wang (2015) reported that applying US (22 kHz, 100 W) to tomatoes led to an increase DPPH· inhibition by 8.22 to 17.56%, depending on the power intensity used (66.64 and 106 W / L respectively) and an increase in FRAP values from 6.03 to 13.18 % respectively. Yu (2016) observed similar results in romaine lettuce treated with US (25 kHz, 26 W, 1 or 3 min). Gani (2016) also stated that antioxidant activity of US treated samples increased with processing and

was higher proportionally to the treatment time. However, a slight decrease was observed at 60 min, attributed to the excessive damage to cell structure which could lead to greater chances of oxidation as well as degradation of polyphenolic compounds. It has been suggested that due to the generation of hydroxyl radicals, hydroxylation of food materials could be increased during US, leading to an increased antioxidant activity (Ashokkumar, 2008). Increased antioxidant capacity can also be attributed to an increased phenolic content in F&V, as this two values are positive correlated (Gani, 2016). Nonetheless, and as it has been previously described, antioxidant compounds may be maintained in amount in F&V but better extracted due to tissue disruption, leading to higher antioxidant capacity values regarding sonication time does not exceed. More studies should be done concerning antioxidant capacity of sonicated fruits, in order to find a relationship between the higher yields observed and a higher bioavailability once ingested.

4.5 Flavor

In relation to flavor, data that can be found in the literature is not extensive. Feng (2018) reported no differences in astringency, aftertaste, bitterness, umami, richness, and saltiness between US processed cucumber (20 kHz, 200 W, 10 min) and the non-sonicated control. The concentration of the main volatile compounds of cucumber increased with treatment. Yu (2016) found that 1 min sonicated romaine lettuce had a good punctuation on overall sensory evaluation, and it was higher than it was for the control and samples processed for 2 or 3 min.

Effects of US on flavor has not been thoroughly studied, so more investigation in this line could be done in order to elucidate the effects of US on aromatic and sapid molecules.

Table 19. Effect of US processing alone or in combination with other strategies on F&V natural microbiota.

Fruit / vegetable	US conditions	Target microorganisms	Effect	Source
Kale	40 kHz, 100 – 400 W / L, 1 min, 40°C	TBC, yeasts and molds, and Enterobacteriaceae	Reductions: 1.0, 0.9 and 1.0 log CFU / g (100 W / L) and 1.8, 1.5 and 1.7 log CFU / g (400 W / L). Reductions at 20°C were lower than they were at 40°C.	(Mansur, 2015)
Cherry tomatoes	20 kHz, 100 W, 8 min	TBC, and yeasts and molds	Reductions: 0.8 and 0.7 log CFU / g. Microorganism populations were reduced by US treatments compared with the control group. The higher the power density was, the lower the counts.	(Wang, 2015)
Kiwis	30 kHz, 368 W / cm2, 8 min	TBC and yeasts and molds	Reductions: 2.3 and 3.5 log CFU / cm2. Not better compared with treatment using NaOCl, that achieved 5.83 and 3.68 log CFU / cm2 respectively.	(Vivek, 2016)
Strawberries	33 kHz, 60 W, 10 – 60 min	TBC and yeasts and molds	Reductions: 3.6 ± 0.1 and 2.0 log CFU / mL. After 15 days storage, best conditions to preserve were 40 min, and reduced 3.9 and 3.3 log CFU / mL respectively.	(Gani, 2016)
Grapes	32 kHz, 60 W / L, 10 min	Decay incidence	Decay incidence was lower when compared with the control.	(Bal, 2017)

Fruit / vegetable	US conditions	Target microorganisms	Effect	Source
Mirabelle plums	30 kHz, 100 W, 0 – 60 min, pulsed / continuous	TBC and decay incidence	Reductions: 0.4 – 1.5 log CFU / g. Decay incidence was reduced when compared with the control. No differences between pulsed and continuous mode. Highest decrease was observed at 60 min.	(Hashemi, 2018a)
Strawberries	20 kHz, 30 W, 5 min combined with 0.075 mg / L ozone or 6 mg / L chlorine dioxide	Decay incidence	US combined with ozone or chlorine dioxide prevented mold growth, while in control group, mold presence was of 21 and 35% at the 3rd and 4th week.	(Aday, 2014)
Carrots	40 kHz, 10 W, 30 min combined with high pressure CO2 (12 MPa, 22°C)	Mesophyll microorganisms, acid lactic bacteria, total coliforms and yeasts and molds	Reductions: 3.7, 2.5, >6, and 3 log CFU / g for mesophyll microorganisms, acid lactic bacteria, total coliforms, and yeasts and molds.	(Ferrentino, 2015b)
Strawberries	40 kHz, 100 W, 5 min combined with acetic acid (800 mg / L), SDS (1,200 mg / L) or PAA (40 mg / L)	TBC and yeasts and molds	Reductions: 1.0 ± 0.2 log CFU / g and 1.2 ± 0.2 log CFU / g higher when compared with the control. The most effective treatment was US combined with PAA, which achieved 2.0 ± 0.8 log CFU / g reductions more than the control.	(do Rosário, 2017)
Calçot (Allium cepa L.)	40 kHz, 250 W, 1 to 45 min	TBC	Reductions: 1.0 log CFU / g after 45 min of ultrasonication. Populations did not exceed 106 CFU / g in any case.	(Zudaire, 2018)
Melons	40 kHz, 500 W, 5 min, combined or not with NaOCl (100 mg / L)	TBC	Reductions: 0.4 log CFU / g after combination US+NaOCl. Statistically different from the application of NaOCl or US individually, where reductions were of 0.1 and 0.2 log CFU / g, respectively.	(do Rosário, 2018)

CFU, colony forming units; ORP, oxide-reduction potential; PAA, peracetic acid; SDS, sodium dodecylbenzenesulfonate; TBC, total bacteria counts; US, ultrasounds. Decay incidence, % of fruits with visible mold growth

Table 20. Effect of US processing alone or in combination with other strategies on pathogenic microorganisms in F&V.

Fruit / vegetable	US conditions	Combined with	Target microorganisms	Reductions (log CFU / g)	Source
Lettuce leaves	37 kHz, 90 W, 10 - 60 min	-	*E. coli* *S. aureus* *Salmonella* Enteritidis *L. innocua*	2.3 ± 0.3 1.7 ± 0.2 5.7 ± 0.1 1.9 ± 0.6	(Birmpa, 2013)
Strawberries	37 kHz, 90 W, 10 - 60 min	-	*E. coli* *S. aureus* *Salmonella* Enteritidis *L. innocua*	3.0 ± 0.7 2.1 ± 0.6 5.5 ± 0.1 6.1 ± 0.0	(Birmpa, 2013)
Kale	40 kHz, 100 W / L, 1 min	-	*E. coli* O157:H7 *L. monocytogenes*	2.5 ± 0.2 2.6 ± 0.1	(Mansur, 2015)
Lettuce leaves	40 kHz, 90 W, 5 min	Malic acid (2%)	*S.* Typhimurium *L. monocytogenes* *E. coli* O157:H7	2.7 ± 0.5 2.8 ± 0.3 2.5 ± 0.6	(Sagong, 2011)
		Lactic acid (2%)	*S.* Typhimurium *L. monocytogenes* *E. coli* O157:H7	2.7 ± 0.4 2.5 ± 0.8 2.8 ± 0.7	
		Citric acid (2%)	*S.* Typhimurium *L. monocytogenes* *E. coli* O157:H7	3.2 ± 0.2 2.3 ± 0.3 2.4 ± 0.1	
Lettuce leaves	40 kHz, 500 W, 5 min	PAA (50 mg / L)	*S.* Typhimurium	3.0	(Silveira 2018)
Pears	40 kHz, N / A	-	*S.* Enteritidis *E. coli*	0.9 ± 0.6 [1] 1.5 ± 0.4 1	(São José, 2015)
		Lactic acid (1%)	*S.* Enteritidis *E. coli*	1.9 ± 0.4 1.9 ± 0.4	
		Acetid acid (1%)	*S.* Enteritidis	1.6 ± 0.3	

[1] Log CFU / cm^2

Fruit / vegetable	US conditions	Combined with	Target microorganisms	Reductions (log CFU / g)	Source
			E. coli	1.4 ± 0.6	
181Strawberries	40 kHz, 500 W, 5 min	-	*S.* Enterica	1.2 ± 0.3	(do Rosário, 2017)
		Acetic acid (800 mg / L)	*S.* Enterica	1.0 ± 0.3	
		SDS (1200 mg / L)	*S.* Enterica	1.0 ± 0.4	
		PAA (40 mg / L)	*S.* Enterica	2.0 ± 0.4	
Green Peppers	40 kHz, 2 min		*S.* Enteritidis ATCC 13076 *E. coli* ATCC 11229	1.8 ± 0.2 2.3 ± 0.3	(Sâo José, 2015) (São José, 2014b)
		Lactic acid (1%)	*S.* Enteritidis ATCC 13076 *E. coli* ATCC 11229	2.8 ± 0.6 2.9 ± 0.5	
		Acetic acid (1%)	*S.* Enteritidis ATCC 13076 *E. coli* ATCC 11229	2.4 ± 0.3 2.6 ± 0.3	
Melons	40 kHz, 2 min		*S.* Enteritidis ATCC 13076 *E. coli* ATCC 11229	1.9 ± 0.3 1.6 ± 0.5	(Sâo José, 2015) (São José, 2014b)
		Lactic acid (1%)	*S. Enteritidis* ATCC 13076 *E. coli* ATCC 11229	3.1 ± 0.7 2.5 ± 0.3	
		Acetic acid (1%)	*S. Enteritidis* ATCC 13076 *E. coli* ATCC 11229	2.4 ± 0.2 2.1 ± 0.2	
Carrots	40 kHz, 10 W, 30 min	-	*E. coli* ATCC 25922	No effect	(Ferrentino, 2015b)
		High pressure CO2 6-12 MPa, 22 / 35°C	*E. coli* ATCC 25922	8.0	
Coconuts	30 kHz, 40 W, 30 min	-	*S.* Typhimurium	No effect	(Ferrentino, 2015a)
		High pressure CO2 12 MPa, 35°C	*S.* Typhimurium	7.0	
Endives	N / A, 140 W, 5 min, 20ºC	-	*L. monocytogenes* (KCTC 13064, ATCC 15313) *E. coli O157:H7* (ATCC 43889, NCTC 12079)	0.4 0.5	(Park, 2018)

Fruit / vegetable	US conditions	Combined with	Target microorganisms	Reductions (log CFU / g)	Source
		Cinnamon leaf oil + surfactants CPC or BC	*L. monocytogenes* (KCTC 13064, ATCC 15313) *E. coli* O157:H7 (ATCC 43889, NCTC 12079)	1.6 (CPC), 1.5 (BC) 1.6 (CPC), 1.5 (BC)	
Lettuce leaves	26 kHz, 200 W, 5 - 25 min	Oregano EO (10 mg / L)	*E. coli* 0157:H7 NCTC 12900	4.0 ± 0.1 [2]	(Millan-Sango, 2015)
		Oregano EO (14 mg / L)	*E. coli* 0157:H7 NCTC 12900	> 5.0 *2	
Lettuce leaves	26 kHz, 200 W, 6 min	Oregano EO (18 mg / L)	*Salmonella* spp.	3.1 ± 0.3 1	(Millan-Sango, 2016)
		Thyme EO (18 mg / L)	*Salmonella* spp.	2.9 ± 0.31	
Parsley, lettuce and dill mix	20 kHz, 500 W, 5 min	Cinnamon EO	*L. monocytogenes*	0.8 ± 0.1	(Özcan, 2015)
Tomatoes	-	Calcium oxide, fumaric acid, SAEW	*L. monocytogenes* (ATCC 19111, 19118, Scott A) *E. coli* O157:H7 (ATCC 23150, 43894, 43895)	4.5 ± 0.1 4.3 ± 0.6	(Tango, 2017)
	40 kHz, 400 W, 3 min	Calcium oxide, fumaric acid, SAEW	*L. monocytogenes* (ATCC 19111, 19118, Scott A) *E. coli* O157:H7 (ATCC 23150, 43894, 43895)	> 5 > 5	
Potatoes	40 kHz, 400 W / L, 40°C, 3 min	-	*B. cereus*	2.9 ± 0.2	(Luo, 2016a)
		SAEW (pH 5.3-5.5, ORP 958-981 mV)	*B. cereus*	3.0 ± 0.2	
Lettuce leaves	20 kHz, 130 - 210 W, 5 – 10 - 15 min	Near neutral electrolyzed water (pH 6.5)	*E. coli* O157:H7 *S. enterica* Typhimurium	4.7 ± 0.5 4.3 ± 0.5	(Afari, 2016)
Tomatoes			*E. coli* O157:H7 *S. enterica* Typhimurium	8.4 ± 0.5 8.5 ± 0.5	

[2] Log CFU / mL

Fruit / vegetable	US conditions	Combined with	Target microorganisms	Reductions (log CFU / g)	Source
Bell peppers	40 kHz, 400 W / L, 10 min, 60 °C	SAEW (pH 5.0-5.2, ORP 930-950 mV)	*L. monocytogenes* *S. enterica* Typhimurium	3.0 ± 0.1 3.0 ± 0.1	(Luo, 2015)

CFU, colony forming units; EO, essential oil; ORP, oxide-reduction potential; PAA, peracetic acid; SAEW, slightly acidic electrolyzed water; SDS, sodium dodecylbenzenesulfonate; US, ultrasounds.

Table 21. Changes in quality parameters of F&V after US processing.

Fruit / vegetable	US conditions	Parameter	Obtained results	Source
Strawberries	20 kHz, 30 W, 5 min, combined with 0.075 mg / L ozone or 6 mg / L chlorine dioxide	pH	The greatest increase in pH during the storage was observed in untreated samples in comparison to the individual or combined treatments.	(Aday, 2014)
		TSS	Untreated samples had lower TSS content than other treatments. No significant difference between the treatments.	
		Respiration rate	Samples treated with US + ClO2 and US + O3 had a lower respiration rate than the individual treatments.	
Potatoes	24 kHz, 400 W, 1 / 5 / 10 min	pH	pH of sonicated potato was reduced after 5 and 10 min of treatment. Longer time the sonication, the greatest decrease in pH	(Amaral, 2015)
		TSS	TSS was higher on samples treated for 10 min.	
		Dry matter	No significant differences ($p>0.05$).	
		Cell structure	Differences in microstructure of potato after 10 min US. Disruption of the vacuole and the polygonal cell wall.	
Coconut	40 kHz, 10 W, 30 min, combined with high pressure CO2 12 MPa, 35ºC	pH, TA	pH and TA of processed samples remained unchanged during storage. Contrarily, in control samples, pH values decreased and TA increased after 21 d storage	(Ferrentino, 2015b)
		POD	Treatment was not able to induce POD inactivation. Its activity slightly increased by the end of storage period.	
		Fat content	No significant differences ($p>0.05$).	
		TPC	Processed samples showed lower TPC values than controls did.	
		Antioxidant activity	A slight decrease was observed after the combined treatment compared with the untreated samples.	

Fruit / vegetable	US conditions	Parameter	Obtained results	Source
Strawberries	33 kHz, 60 W, 10 / 20 / 30 / 40 / 60 min	TPC	TPC increased when strawberries were processed with US. The longer the time was, the higher the TPC.	(Gani, 2016)
		Antioxidant activity	Antioxidant activity of US treated samples increased with the increase in treatment time.	
Mirabelle plums	30 kHz, 100 W, 0 / 15 / 30 / 45 / 60 min, pulsed / continuous	TA	No significant differences ($p>0.05$) between the control and 15 min US processed samples. 30, 45 and 60 min sonication significantly inhibited the decrease of TA content.	(Hashemi, 2018a)
		TSS	Only 60 min treatment showed significant differences in TSS compared with the control. Higher amounts were observed.	
		AA	Significant increase in all sonicated samples when compared with the control	
Cucumber	20 kHz, 100 / 200 W, 10 min	TSS	100 and 200 W better retained SSC in samples. 300 W had a negative effect on TSS value	(Feng, 2018)
		Flavor	No significant difference in astringency, umami, richness or saltiness between processed samples and fresh ones.	
		Volatile compounds	Characteristic aromatic compounds, although decreased with time, were better retained if samples had been sonicated.	
Straw mushroom	40 kHz, 300 W, 3, 10, 30 min	Respiration rate	US significantly inhibited the respiration od straw mushroom. 10 min US treatment resulted in the minimum CO2 production rate.	(Li, 2017)
		Weight loss	Us treatment delayed the weight loss. 10 min treatment had the greatest effect.	
		TSS	In all tested groups, TSS increased after the first 12 h period	
		Total soluble proteins	Over-time US treatment (30 min) had a negative effect on total soluble proteins, indicating tissue destruction.	

Fruit / vegetable	US conditions	Parameter	Obtained results	Source
		PPO	US processing inhibited PPO.	
Romaine lettuce	25 kHz, 70 W, 1 / 2 / 3 min	TPC	Samples processed with US had higher TPC than control had. Only 1 min treatment was statistically significant ($p<0.05$)	(Yu, 2016)
		Antioxidant activity	During the first 30 h of storage, DPPH· inhibition was higher on sonicated samples, and they were followed by a significant increase	
		PAL	Samples processed during 2 and 3 min expressed higher PAL activity than the control did.	
		Sensory evaluation	Samples treated with US 1 min were rated higher than the control and maintained an acceptable score after 150 h. No significant differences ($p>0.05$) between samples treated with US 2 and 3 min and the control.	
Kiwi	400 W, 8 min	pH, TSS, TA	No significant differences ($p>0.05$).	(Vivek, 2016)
Cherry tomatoes	20 kHz, 100 W	Ethylene production	Ethylene production of treated samples was lower than it was for the control after 12 days storage. Climacteric peak was delayed by 4 d.	(Wang, 2015)
		TSS, TA	No significant differences ($p>0.05$).	
		POD	US processed fruits had higher POD activity than control group after 0 to 8 days.	
		TPC	At the end of the 16 d storage, US processed fruits showed higher TPC than the control did.	
		AA	At the end of the 16 d storage, US processed fruits had higher ascorbic acid content than the control had.	
		Antioxidant activity	At the end of the 16 d storage, US processed fruits had DPPH·, FRAP and ORAC values than the control had.	
Red bell pepper	35 kHz, 120 W, 15ºC	pH	No significant differences ($p>0.05$)	(Alexandre, 2013)

Fruit / vegetable	US conditions	Parameter	Obtained results	Source
		AA	US treated samples retained more ascorbic acid than water washed ones did.	
Grapes	32 kHz, 600 W, 10 min	TSS	No significant differences ($p>0.05$) immediately after the treatment. US processed samples had the highest TSS compared with the control.	(Bal, 2017)
		TA	No significant differences ($p>0.05$)	
		Anthocyanin content	No significant differences ($p>0.05$)	
		TPC	US processed samples had the highest TPC values, and control samples had the lowest TPC values	
Pear	42 kHz, 200 W, 5-15 min	AA	No changes were observed in ascorbic acid content after US treatment.	(Plaza, 2015)
		TPC	Total phenolic content was significantly higher in US treated pears for 5 min than it was in non-treated samples. No differences in TPC were observed at 10 or 15 min treatments.	
Melon	40 kHz, 500 W, 5 min	pH	No significant differences ($p>0.05$)	(do Rosário, 2018)
		TA	No significant differences ($p>0.05$)	

AA, ascorbic acid; DPPH·, 2,2-Diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ORAC, ozygen radical absorbance capacity; POD, phenol peroxidase; PPO, polyphenol oxidase; TA, titratable acidity; TPC, total phenolic content; TSS, total soluble solids; US, ultrasound.

Table 22. Changes in color and texture of F&V after US processing .

Fruit / vegetable	US conditions	Color	Texture	Source
Lettuce leaves	40 kHz, 90 W, 5 min combined with organic acids (malic, citric, and lactic) 0.3, 0.5, 0.7, 1.0 and 2.0%	Processing did not affect color parameters immediately after the treatment nor at 7 days of storage	No significant differences immediately after processing or after 7 days of storage.	(Sagong, 2011)
Lettuce leaves	37 kHz, 90 W, 10 / 20 / 30 / 45 / 60 min	Decrease in L* when treated with US. TCD was higher and positively correlated with treatment time (significantly different after 30 min)	Not significantly affected	(Birmpa, 2013)
Strawberries		Significant differences in L*, a*, and b* values when treatment time was higher than 30 min	Not significantly affected	
Strawberries	20 kHz, 30 W, 5 min, combined with 0.075 mg / L ozone or 6 mg / L chlorine dioxide	Ozone caused an increase in L* due to its bleaching effect. a* values of untreated strawberries were lower than treated ones. Strawberries treated with ultrasound plus ClO2 preserved their a* values significantly better than other treatments.	All treated strawberries had higher firmness values than the controls. No difference was noticed between strawberries treated with ultrasound or ozone	(Aday, 2014)
Romain and iceberg lettuce leaves	25 kHz, 2 000 W, 1 min, combined with chlorine, surfactantants and Sodium dodecylbenzenesulfonate (1200 mg / L)	No significant effect on color. TCD between samples not significant. TCD<4 Chlorine helped to retain color.	No difference between samples immediately after processing or after storage for 14 days. Firmness evolved equally for all treatments.	(Salgado, 2014)
Coconuts	40 kHz, 10 W, 30 min, combined with high pressure CO2 12 MPa, 35ºC	L* values were not statistically different after the treatment or during 4 weeks of storage. a* and b* parameters decreased. TCD of treated samples was higher than 4 after 3 weeks of storage.	No differences in hardness were observed between treated and non-treated samples. Hardness significantly increased after 2 weeks of storage in treated samples.	(Ferrentino, 2015a)

Fruit / vegetable	US conditions	Color	Texture	Source
Mangoes	25 kHz, 50 W, 30 min	TCD was higher for US processed samples. º Hue was the most affected by US. Significant differences were observed immediately after the process, and a greater decrease occurred after 7 days of storage.	Firmness decreased when products were US processed. Firmness had more decay after 7 days of storage in treated samples.	(Santos, 2015)
Potatoes	24 kHz, 400 W, 1 / 5 / 10 min	L* was affected by US for all treatment times. After frying, color was correct (L* > 60) for all the treatments. L* and chroma decreased with time when US for 1 min. Hue values were not affected.	Losses of texture were observed but there were no statistical differences with the control.	(Amaral, 2015)
Carrots	40 kHz, 10 W, 30 min, combined with high pressure CO2 12 MPa, 22ºC	Color did not show significant modifications. Thermally processed did affect L*, a*, b* parameters, decreasing their values.	Combined treatment induced a significant reduction of firmness about 92%, compared with fresh-cut carrot. Similar results than when thermally processed.	(Ferrentino, 2015b)
Cherries	33 kHz, 60 W, 10 / 20 / 30 / 40 / 60 min	TCD increased when > 30 min. 20 min treatment was the most effective to maintain color red brightness for 15 days.	Significant decrease in firmness after when samples treated for more than 20 min.	(Muzaffar, 2016)
Strawberries	33 kHz, 60 W, 10 / 20 / 30 / 40 / 60 min	Loss of brightness L* when exceeded 30 min of treatment.	Fruit firmness was better retained throughout all refrigerated storage if samples had been previously sonicated.	(Gani, 2016)
Apple slices	40 kHz, 1 / 2 min, combined with ascorbic acid, citric acid, NaCl or Ca-ascorbate	US alone did not help to prevent browning. When used with antibrowning solutions, especially with Ca-ascorbate, US enhanced this effect on some apple varieties.	N / A	(Putnik, 2017)

Fruit / vegetable	US conditions	Color	Texture	Source
Straw mushroom	40 kHz, 300 W, 3, 10, 30 min	No significant reduction of browning was observed when samples were treated by US for 3 or 30 min. 10-min US treatment significantly improved the storage life to 72 h keeping straw mushrooms with stable color without spoilage.	US retained the straw mushrooms firmness. 3-min US treatment at 95% RH led to the maximum firmness retention of 1.90 N.	(Li, 2017)
Romaine lettuce	25 kHz, 26 W, 1 / 2 / 3 min	Hue angle decreased in all samples, indicating that enzymatic browning was not affected by US.	Samples processed by US exhibited higher firmness (maximum force, N) than the control (water washed) did right after treatment and during storage.	(Yu, 2016)
Mirabelle plums	30 kHz, 100 W, 0 /15 / 30 / 45 / 60 min, pulsed / continuous	Highest changes in control. US preserved color better.	US helped maintaining firmness. Pulsed gives higher firmness than continuous.	(Hashemi, 2018a)
Cucumber	20 kHz, 200 W, 10 min	Combined with controlled atmosphere, US substantially improved the appearance of the cucumber samples up to 25 days and preserved the original green color.	Ultrasound treatment significantly retained the firmness. A decrease of 35.60% when applying US was observed compared with 56.78% of the control.	(Feng, 2018)
Melon	40 kHz, 500 W, 5 min	N /A	Firmness, adhesiveness, cohesiveness, guminess and chewiness increased after US processing.	(do Rosário, 2018)

TCD, total color difference (TCD value of 4 is considered a clearly distinguishable color difference to the average person); US, ultrasounds.

5. Conclusions

Ultrasound is a mild technology that has been studied with the aim to reduce microbial load of food, and its application in fresh and fresh-cut fruits and vegetables has potential interest for manufacturers, as it is versatile and reasonably easy to use. It has been reported to be relatively effective as an antimicrobial agent, and its effects can be improved if it is combined with other physical technologies, such as mild temperatures or supercritical CO_2, or with chemical agents, including organic acids and essential oils, ozone, or slightly acidic electrolyzed water. A part from being able to reduce pathogenic and alterative microbiota in F&V, US may have a consequence on other key features, such as color or texture, or components, namely phenols or vitamins. Overall, it seems that results of US processing on F&V do not follow general trend, as they depend on several parameters related with treatment conditions and matrix. Targeted microorganisms may not respond equally in the same conditions, and reductions may also vary depending on parameters stated before. Accounting on this review's information and knowing are capable to achieve the desired outcomes, each case should be studied and scaled-up individually in order to preserve safety, quality and nutrition values of fresh and fresh-cut F&V.

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Conflict of interests

The authors declare no conflict of interests.

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Chapter 5

Investigating the effects of sonication as a disinfection technology for strawberries and its impact in their overall quality

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The results of this chapter were part of a poster presented in **I Congreso de Jóvenes Investigadores en Ciencias Alimentarias** (Universidad de Almería, 2018)

Sonication is the technology that uses ultrasounds at a frequency beyond 18 kHz. Due to an effect called cavitation, in which microbubbles implode generating temperatures of 5000 °C, it has been reported to have antimicrobial effects. In this study, ultrasound (US) for 1 and 5 min was applied to strawberries, in order to reduce artificially inoculated *Listeria innocua* and native microbiota (including total aerobic mesophylls, yeasts and molds) without affecting their quality. A 200 ppm NaOCl washing treatment, alone (2 min) or in combination with US (1 and 5 min) was added as a positive control. A water treatment (2 min) was added as a negative control. Results showed that only treatments that used NaOCl (both alone and in combination with US) were able to not only significantly reduce *L. innocua* in strawberries but also in washing water. The other US treatment were not able to reduce pathogenic population in water, making it unsafe for its recirculation in the next washing processes in the fruit industry. The US treatments were also unable to reduce native microbiota in strawberries. Physicochemical (pH, total soluble solids, titratable acidity, color, firmness), and biochemical (antioxidant capacity, total phenolic content and total anthocyanin content) were not markedly affected by any of the treatments.

Sonication, quality, strawberries, non-thermal technologies, *Listeria innocua*

1 Introduction

Strawberries are one of the most common fruits in the Mediterranean diet, and one of the most investigated fruits because of their nutritional and nutraceutical properties, related with their contents in vitamin C and richness in a wide range of bioactive compounds such as polyphenols, flavonoids, anthocyanins and tannins (Giampieri, 2012). However, according to the European Food Safety Authority, the frozen strawberries may pose a risk for human health due to the presence of pathogenic agents *Salmonella* spp. and human norovirus. Since then, the efforts have focused on finding suitable sanitizers for these fruits.

Chlorine is typically used in fruit industry as a cheap and sufficiently effective disinfection agent. However, the associate problems (toxic vapors and toxic by-products such as trihalomethanes), make imperative to find alternatives to it, capable of achieving similar microbial reductions while maintaining the quality of the fruit. In this regard, and typically in the juice industry, sonication is being used for reducing microbial loads of the processed products. The ultrasound (US) includes those sound waves with a frequency higher than 20 kHz (Patist, 2008). US mechanism of action is based on the acoustic cavitation that make the created microbubbles to intensely collapse, generating a high temperature and pressures. This collapse affects the membrane of microorganisms, disruption of the cell components and DNA injuries (Birmpa, 2013).

Application of US has been successful in reducing microbial loads of the tested fruits (orange, guava, lime, katsuri, grapes, tomato, melon), vegetables and cereals, as reviewed in (Bhat, 2015). In fact, and as reviewed by (Lafarga, 2019), a number of works have proven US to effectively reduce microbial loads of naturally occurring microorganisms and decrease artificially inoculated pathogens in strawberries after sonication in times that ranged from 0 to 60 min. However, US still can affect the overall composition in a negative way; textural and sensorial properties may be compromised, especially in delicate products such as strawberries.

For this reason, further studies in the used of US applied to strawberries should be conducted, focusing not only on microbial reductions but also in quality maintenance after the treatments. In this work, the efficacy of US for 1 or 5 min against artificially inoculated *Listeria innocua* and against strawberry natural microbiota was studied, including the effect it had in decontaminating washing water for its further recirculation. Moreover, some quality and biochemical parameters of strawberries were determined in order to evaluated the main effects this technology has when applied to strawberries.

2 Materials and methods

2.1 Materials

Strawberries (*Fragaria* x *ananassa*) were purchased from local distributors. Calix and leaves were carefully removed before the treatment.

Triptone soy broth (TSB), triptone soy agar (TSA), Palcam base agar, yeast extract, plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), potassium bisulfate, sodium chloride and peptone were purchased from Biokar Diagnostics (Allonne, France).

Ascorbic acid, gallic acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol, acetone, chlorhidric acid (37%), sodium acetate, sodium hydroxide, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were purchased from Panreac (Llinars del Vallès, Spain).

2.2 Experimental design

Two types of experiments were carried out. On one hand, an experiment was conducted in artificially inoculated strawberries to determine *Listeria innocua* populations after the treatments. For this, *L. innocua* strain CECT-940 (*Colección Española de Cultivos Tipo*, Burjassot, Spain) was used in this study. It was cultured and inoculated in strawberries (to reach a theoretical initial concentration of 2×10^7 CFU / g) according to the methodology described in (Nicolau-Lapeña, 2019). On the other hand, the experiment in non-inoculated strawberries was replicated three times, two to ascertain the effect of washing treatments on epiphytic microbiota and one to perform the quality and nutritional determinations. The experiments were done two times, with 3 repetitions each.

The tested treatments for both experiments included the following: sonication 40 kHz, 120 W (Ultrasound HD, Selecta) for 1 min (US-1min) or 5 min US-5min and, in tap water or in a 200 ppm chlorine solution (adjusted to pH 6.5 with citric acid 1 M) (NaOCl+US-1min or NaOCl+US-5min). A chlorine control (NaOCl) was added to compare the effect of the tested treatments with this sanitizer alone. After the hypochlorite treatment, fruits were rinsed in 2 L of tap water. Moreover, a tap water (W) was added as a control in order to verify whether reductions could be due to the physical removal of microorganisms by water itself or if further reductions could be achieved by the use of a germicidal effect of PA. For washing treatments, 20 fruits were submerged for the necessary time, and were kept to dry at room temperature. Fruits. Free chlorine concentration was checked with an ion specific meter Hanna Instruments HI 95734-11 (Rhode Island, USA) and peracetic acid concentration was determined by titration.

Moreover, in the experiments with non-inoculated strawberries, microbiological and quality analysis were performed. For biochemical determinations, an aliquot of each replication was frozen with liquid nitrogen, milled using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain) and stored at -80 °C until analysis.

2.3 Microbial determinations

Microbial determinations were carried out both for strawberries and for washing water after the treatments, as described previously in similar works (Nicolau-Lapeña, 2019, 2020) (Chapter 1 and Chapter 2). Results were expressed as log CFU / g or log CFU / mL depending on the material analyzed (fruit or water, respectively). Detection limits were 1.3 log CFU / g and 1.7 log CFU / mL, respectively.

2.4 Physicochemical and biochemical characterization

Determination of physicochemical properties was carried out in non-inoculated strawberries. As aforementioned, two repetitions were carried out. However, only in one batch the non-treated strawberries were used for this purpose.

All the physicochemical and biochemical parameters were determined according to the described in (Nicolau-Lapeña, 2019, 2020) (Chapter 1 and Chapter 2). As quality parameters, pH, total soluble solids (TSS, ° Brix) and titratable acidity (TA, mg mallic acid / L juice) were determined. Moreover, CIE L*a*b* color coordinates, and firmness measured by compression (N) and punction (N) were determined. Biochemical characterization included the determination of the antioxidant activity by DPPH · (mg ascorbic acid equivalents (AAE) / 100 g fresh weight (FW)) and ferric reducing antioxidant power (FRAP, mg AAE / 100 g FW), total phenolic content (TPC, mg gallic acid equivalents (GAE) / 100 g FW) and total anthocyanin content (TAC, mg pelargonidin-3-glucosyde / 100 g FW).

2.5 Statistical analysis

Results are expressed by mean ± standard deviation (SD) of 6 repetitions. All data were checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analysis was carried out using JMP 13 (SAS Institute Inc., Cary, USA).

3 Results

3.1 Sonication effect on selected microorganisms

For all the treatments, the **pH** values were 6.62 ± 0.11 and 6.61 ± 0.06 for water and NaOCl solution respectively, and **oxido-reduction potential** values were 877.5 ± 13.4 and 882.3 ± 8.3 for water and NaOCl solution respectively.

The artificial inoculation of ***L. innocua*** on strawberries resulted on a population of 3.1×10^7 and 1.6×10^7 CFU / g strawberry, for replica 1 and 2 respectively. The effects of the selected treatments in *L. innocua* populations, both in strawberries and in washing water are shown in Figure 28.

The control treatment, that consisted only in washing the strawberries with water for 2 min, reduced the population by 2 logs. This could be attributed to a physical effect caused by the immersion of the product in agitated water, which leads to a dragging of the microorganisms from the surface. This is also proven by the presence of *L. innocua* in water, that was 4 log CFU / mL for both replicas. When NaOCl was used, the 2 min washing did not differ in the reductions of *L. innocua* in the strawberries when compared to control washing: 2-log reductions were achieved. The difference was found in the washing water, where *L. innocua* population was below the limit of detection for the two reps. However, the use of US in the disinfection of the strawberries did not exert any additional effect to the water washing or to the NaOCl disinfection: no differences were found between the treatments with US (for any of the times studied) and their non-US counterparts. Only US-1min and NaOCl+US-5 min for the second replica were significantly different. The effect in washing water was mainly attributed to the NaOCl sanitation effect rather than to the US application: only the treatments in which NaOCl was used proved to reduce *L. innocua* populations in washing water to below the detection limit. Other authors have reported similar reductions of artificially inoculated pathogen microorganisms on strawberry. For instance, Birmpa (2013) observed reductions of *Escherichia coli, Staphylococcus aureus*, and

Salmonella enteritidis of 3.0 ± 0.7, 2.1 ± 0.6 and 5.5 ± 0.1 log CFU / g respectively, and do Rosário (2017) showed reductions in *S.* Enterica populations by 1.0 ± 0.3 log CFU / g. However, as in this article, those reductions could be well attributed to the dragging effect caused by water. It is possible though, that this effect is enhanced by ultrasonic waves, as they can help in detaching the microorganisms from the fruit surface. In fact and as extensively reviewed (Nicolau-Lapeña, 2019; Piyasena, 2003), and despite the fame of US to possess antimicrobial effects, ultrasound alone is not feasible to achieve significant log reductions both in the product and in the washing water. To prevent cross-contamination caused by contaminated washing water for next fruit sanitation batches, other technologies or chemical products are suggested to be combined with the use of ultrasound.

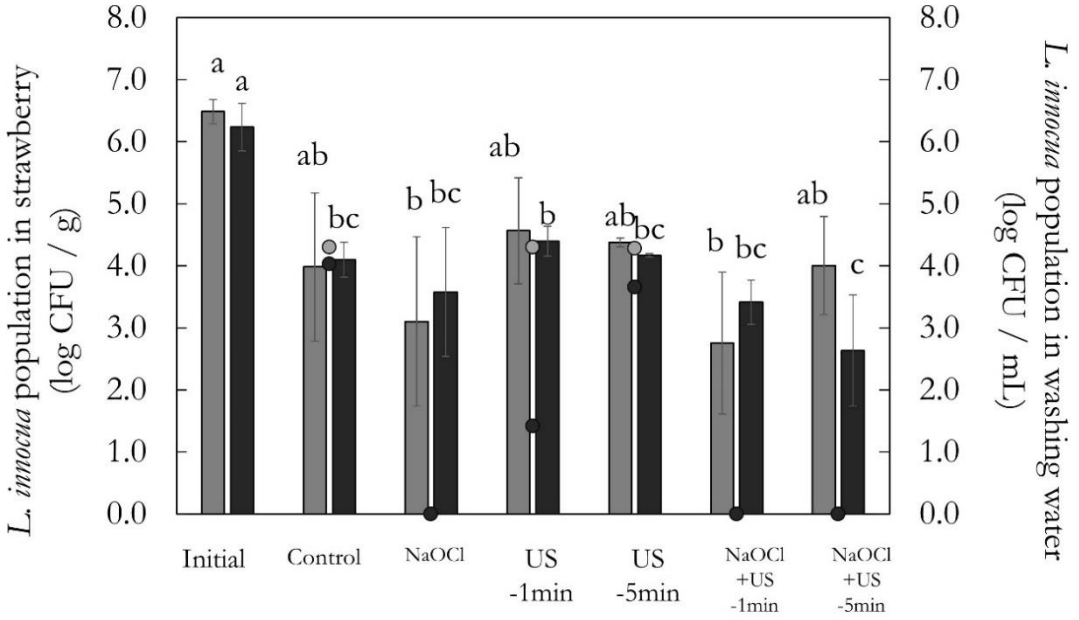

Figure 28. *L. innocua* populations in strawberry (bars, log CFU / g) and in washing water (circles, log CFU / g) before and after the treatments, for the replica 1 (■) and the replica 2 (■). Results are the mean values ± standard deviation (n = 3). Different letters indicate statistically significant differences ($p < 0.05$) between the treatments in the same replica, according to Tukey's test.

The effect of the selected treatments on the **native microbiota** of strawberries is also shown for the two different replications carried out in this study, as there can exist differences in the nature of the microbiota of different batches, cultivars and years (Baugher, 2016; Jensen, 2013), so sensitivity to the treatments could also be different. In this regard, the initial counts were only different for yeasts: 2.3 ± 1.1 and 3.4 ± 0.2 in the first and second replica. Fruits from the two replicates had similar initial counts of TAM (3.7 ± 0.2 and 4.1 ± 0.3 log CFU / g) and molds (3.3 ± 0.8 and 3.5 ± 0.1 log CFU / g) (**Figure 29**).

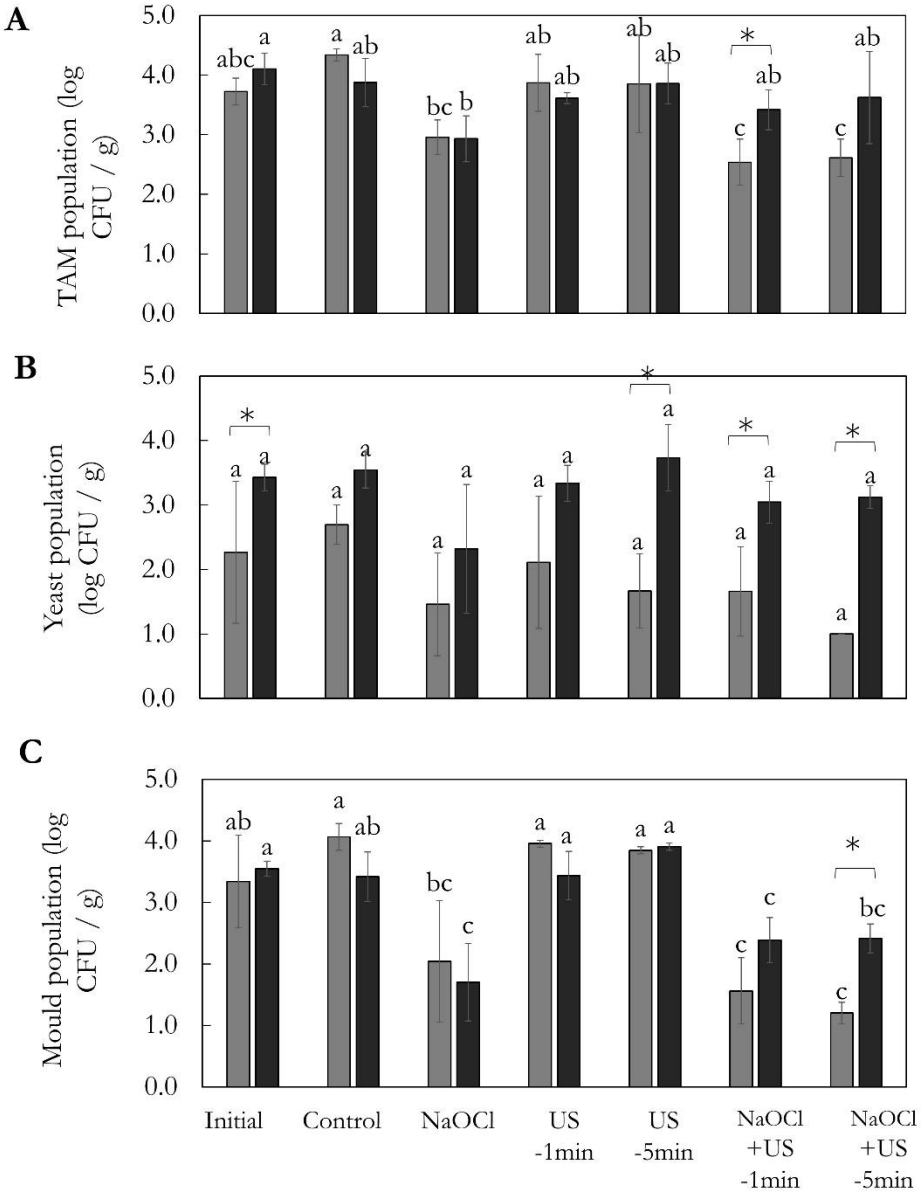

Figure 29. (**A**) Total aerobic mesophylls, (**B**) yeasts and (**C**) mold populations in strawberry (log CFU / g) before and after the treatments, for the replica 1 (■) and the replica 2 (■). Results are the mean values ± standard deviation (n = 3). Different letters indicate statistically significant differences ($p < 0.05$) between the treatments in the same replica, according to Tukey's test. Asterisk marks indicate statistically significant differences ($p < 0.05$) between the two replicas, according to T-student's test.

The effect of washing strawberries with water (control) was not effective in reducing the populations of TAM, yeasts nor molds. This was attributed to the higher attachment of the natural microbiota on the surface of strawberry, as it has grown and developed together with the strawberry, rather than being artificially inoculated the day before. In TAM counts, only NaOCl treatment for 2 min was able to significantly reduce the population by 1 log in the second replica. All the other treatments were unable to significantly reduce TAM populations. Regarding yeast counts, it seemed that reductions were higher in the first replica, achieving 1 log reductions in some of the cases (NaOCl and NaOCl+US-5min). However, and as the initial population had already revealed, the nature of the yeasts present in the strawberries was different. This is supported by the lack of reductions in the second rep. None of the

treatments, except for a non-significant reduction of 1 log when using NaOCl, was able to exert a remarkable reduction. Finally, mold population was highly influenced by NaOCl, which alone or in combination with US for 1 or 5 min caused a significant reduction of at least 1 log in the replica 2, and only when combined with US, also in replica 1. However, as it was reflexed in all of the counts of native microbiota, the ultrasound did not perform any outstanding effect. As reported in the literature, to achieve any reductions in native microbiota, longer times of 40 to 60 min are needed for this purpose. For instance, Gani (2016) reported a reduction of TAM and yeasts and molds of 3.6 ± 0.1 and 2.0 ± 0.1 log CFU / g, respectively, after sonication for 45 min. In other matrices, reductions of TAM in *calçots* (*Allium cepa* L.) after sonication for the same amount of time was 1.0 log CFU / g (Zudaire, 2018). In contrast, shorter treatment times were applied in our study. This could be the reason for the negligible impact of US in these populations. However, at industrial level, it is not feasible to perform such long (> 10 min) treatments, not only for the typical working timings, but also because the immersion in water for that amount of time could be detrimental for the product quality.

3.2 Physicochemical and biochemical characterization

Some features that characterize the fruit are **pH, total soluble solids (TSS) and titratable acidity (TA)**. Initial values for these parameters are 3.6 ± 0.1, 6.6 ± 0.1 °B and 4.8 ± 0.2 mg citric acid / L for replica 1, and 3.5 ± 0.1, 6.6 ± 0.1 °B and 7.4 ± 0.2 mg citric acid / L for replica 2. Although variations between batches can be observed, all the values are within the reported range for this parameters in strawberries (Ayala-Zavala, 2004). Changes in these parameters can be found in **Figure 30**.

Despite the statistical analysis revealed significant differences attributed to the application of some of the treatments, this did not affect the general quality, as all the values after the treatments were within the range of the acceptable for these products. In general, ultrasound has proven not to affect these parameters. In various studies, after 5 to 10 min sonication of red pepper, grapes or melon, no changes were observed (Alexandre, 2013; Bal, 2017; do Rosário, 2017). In strawberries, the application of US for 5 min combined with 0.075 mg / mL ozone resulted in no differences in pH immediately after the treatment, but reduced its decrease during storage period when compared to non-treated strawberries.

Color and texture are important feature for fruit, as they contribute to their appearance which is the main contributor to buying intention (Ragaert, 2004). In the case of this study, color parameters L*a*b* were not affected by any of the treatments (**Table 23**). Strawberries from the replica 1 had a more purplish color, as b* value was lower than in replica 2, whose strawberries were more orangish.

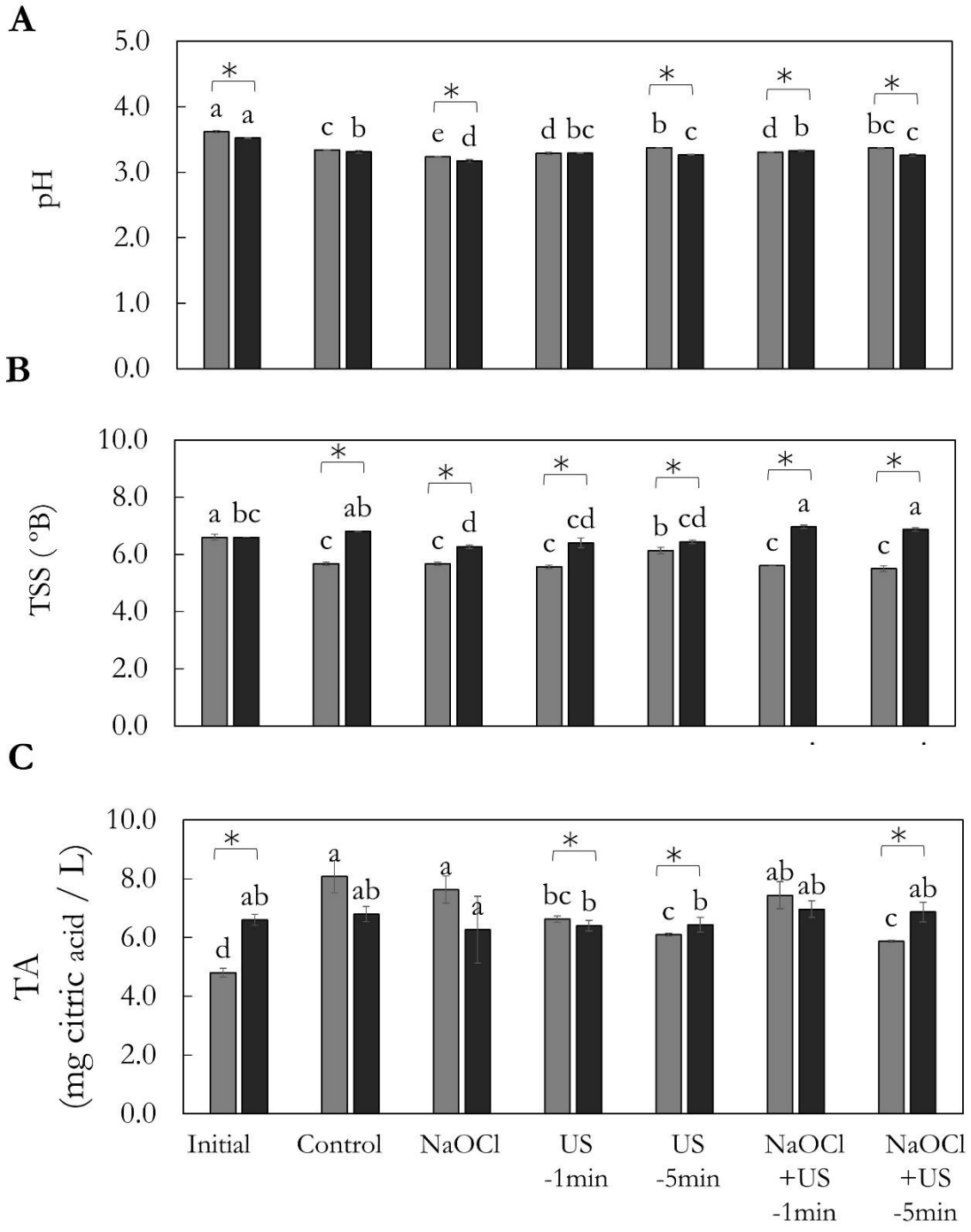

Figure 30. (**A**) pH, (**B**) total soluble solids (TSS, °B) and (**C**) titratable acidity (TA, mg citric acid / L) before and after the treatments, for the replica 1 (■) and the replica 2 (■). Results are the mean values ± standard deviation (n = 3). Different letters indicate statistically significant differences ($p < 0.05$) between the treatments in the same replica, according to Tukey's test. Asterisk marks indicate statistically significant differences ($p < 0.05$) between the two replicas, according to T-student's test.

Table 23. Color parameters, expressed as CIE L*a*b* coordinates, antioxidant activity by FRAP and DPPH· methods (mg AAE / 100 g FW), and total phenolic content (TPC, mg GAE / 100 g FW) of strawberries before and after the treatments, for replica 1 (R1) and replica 2 (R2). Different letters indicate statistically significant differences ($p < 0.05$) between the treatments in the same replica, according to Tukey's test. Asterisk marks indicate statistically significant differences ($p < 0.05$) between the two replicas, according to T-student's test.

	L*	
	R1	R2
Initial		47.2 ± 3.9
Control	39.8 ± 3.0	45.7 ± 3.7 *
NaOCl	39.8 ± 3.8	44.1 ± 3.1 *
US - 1 min	39.6 ± 3.7	47.0 ± 4.8 *
US - 5 min	42.3 ± 2.2	46.2 ± 3.3 *
NaOCl+US-1 min	40.8 ± 2.5	48.9 ± 4.5 *
NaOCl+US-5 min	40.0 ± 4.7	46.2 ± 4.3 *

	a*	
	R1	R2
Initial	-	31.4 ± 2.0
Control	32.9 ± 1.9	31.5 ± 1.6
NaOCl	32.9 ± 2.5	31.0 ± 2.4
US - 1 min	33.0 ± 2.5	31.2 ± 2.8 *
US - 5 min	34.4 ± 1.7	32.2 ± 1.9
NaOCl+US-1 min	34.1 ± 1.0	31.0 ± 3.0 *
NaOCl+US-5 min	32.6 ± 1.8	31.1 ± 2.6

	b*	
	R1	R2
Initial	-	33.9 ± 5.0
Control	22.6 ± 4.8	33.3 ± 5.8 *
NaOCl	23.1 ± 5.0	30.7 ± 3.5 *
US - 1 min	24.0 ± 6.1	33.9 ± 6.0 *
US - 5 min	26.5 ± 3.7	34.6 ± 4.6 *
NaOCl+US-1 min	25.0 ± 3.6	35.3 ± 5.1 *
NaOCl+US-5 min	22.8 ± 6.6	32.6 ± 5.5 *

	FRAP (mg AAE / 100 g FW)	
	R1	R2
Initial	-	140.2 ± 8.0
Control	144.1 ± 3.8	130.5 ± 2.4
NaOCl	180.2 ± 3.5	125.5 ± 8.6 *
US-1 min	183.9 ± 0.4	137.8 ± 1.4 *
US-5 min	152.8 ± 10.7	123.1 ± 21.0
NaOCl+US-1 min	170.1 ± 0.7	119.4 ± 3.6 *
NaOCl+US-5 min	174.2 ± 8.4	116.3 ± 2.9 *

	DPPH· (mg AAE / 100 g FW)	
	R1	R2
Initial	-	107.2 ± 1.5
Control	84.3 ± 21.7	102.2 ± 5.9 *
NaOCl	48.1 ± 1.4	102.3 ± 4.6
US - 1 min	78.6 ± 23.0	110.5 ± 4.6
US - 5 min	64.5 ± 15.7	99.7 ± 13.9
NaOCl+US-1 min	63.3 ± 32.4	94.4 ± 7.8
NaOCl+US-5 min	80.1 ± 0.1	95.5 ± 0.8 *

	TPC (mg GAE / 100 g FW)	
	R1	R2
Initial	-	75.5 ± 3.9
Control	126.0 ± 29.5	69.9 ± 1.0 *
NaOCl	129.4 ± 11.3	71.0 ± 3.4 *
US - 1 min	137.0 ± 9.0	74.3 ± 1.4 *
US - 5 min	112.4 ± 5.0	65.4 ± 7.7 *
NaOCl+US-1 min	110.8 ± 0.5	62.2 ± 5.1 *
NaOCl+US-5 min	110.6 ± 1.5	64.7 ± 5.8 *

Textural analysis by compression and punction also revealed no differences caused by any of the treatments, and such values were in the range of those reported in the literature (Duvetter, 2005) (**Figure 31**). Nevertheless, it was observed that strawberries from the first replica were significantly firmer than they were in the second replica. This could have been influenced by their maturity stage, as an advanced degree of maturity usually results in a loss of firmness due to the degradation of pectic walls and other processes (Toivonen, 2008).

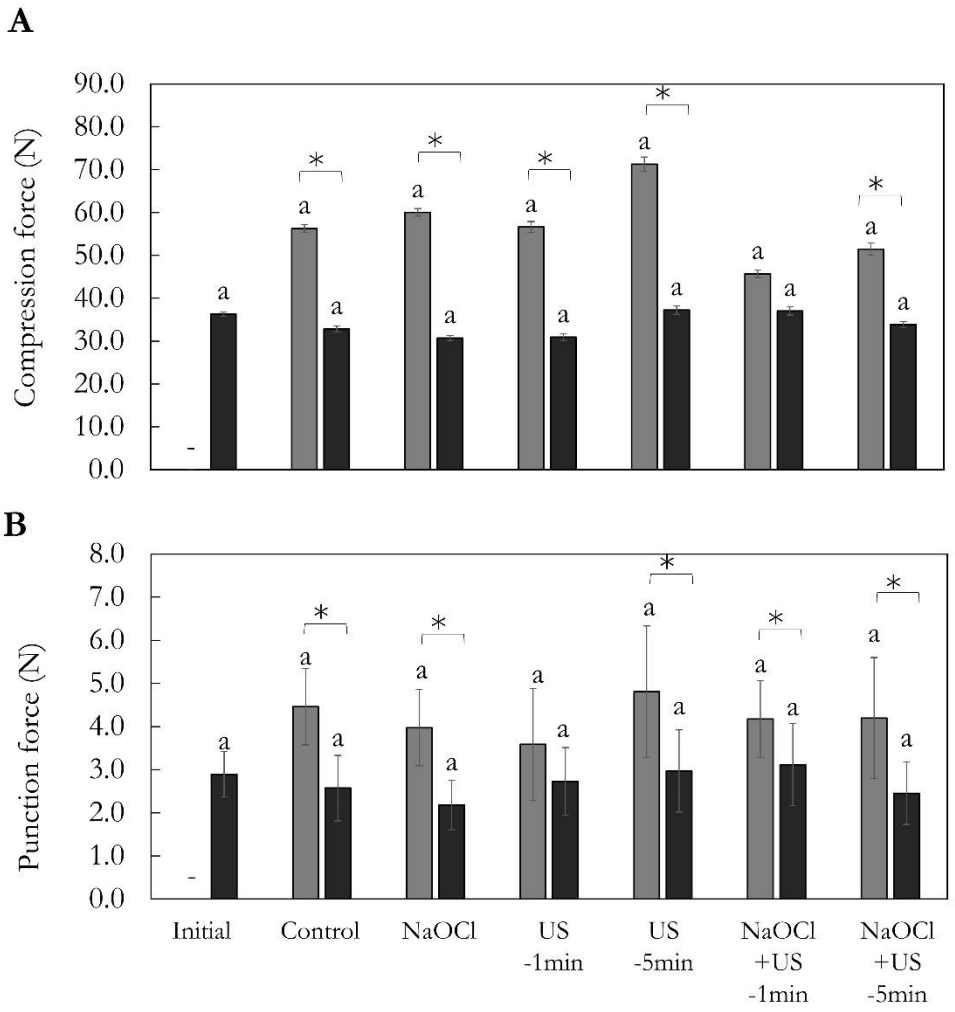

Figure 31. Firmness of strawberries evaluated by (**A**) compression and (**B**) punction before and after the treatments, for the replica 1 (■) and the replica 2 (■). Results are the mean values ± standard deviation (n = 3). Different letters indicate statistically significant differences ($p < 0.05$) between the treatments in the same replica, according to Tukey's test. Asterisk marks indicate statistically significant differences ($p < 0.05$) between the two replicas, according to T-student's test.

The **antioxidant activity** of the strawberries subjected to the different treatments was also evaluated by using DPPH· and FRAP methods. As it can be seen in **Table 23** no effect was observed for any of the tested treatments. The antioxidant values were similar after water, NaOCl, US or a combination of NaOCl for different times. DPPH· values were higher for the first replica than they were for the second replica, averaging 167.5 ± 15.8 and 127.6 ± 9.0 mg AAE / 100 g FW, respectively. Contrarily, FRAP values were lower in the first replica than they were in the second replica, averaging 69.8 ± 13.7 and 101.7 ± 5.8 mg AAE / 100 g FW, respectively. This indicates that each batch of strawberries contained different amounts or typologies of antioxidant molecules, each with a different mechanism. The direct relationship between the FRAP values with the **total phenolic content (TPC)** of the studied strawberries suggests that phenolic contents may be accountant for this kind of antioxidant activity. TPC values were also lower in replica 2 when compared to replica 1, averaging 69.0 ± 5.1 and 121.0 ± 11.3 mg GAE / 100 g FW, respectively. These values are close to those reported by our group in other studies (Chapter 1 and Chapter 2). However, in other works done with strawberries, authors reported that the treatment of the fruit with US for 10 to 60 min significantly increased both the antioxidant activities and the TPC values. It may be discussed, though, that the effect of US consisted in making

the antioxidants more available (by the rupture of the vacuole membranes in which they typically are stored) rather than an increase in their concentration (Ghafoor, 2009; Virot, 2010).

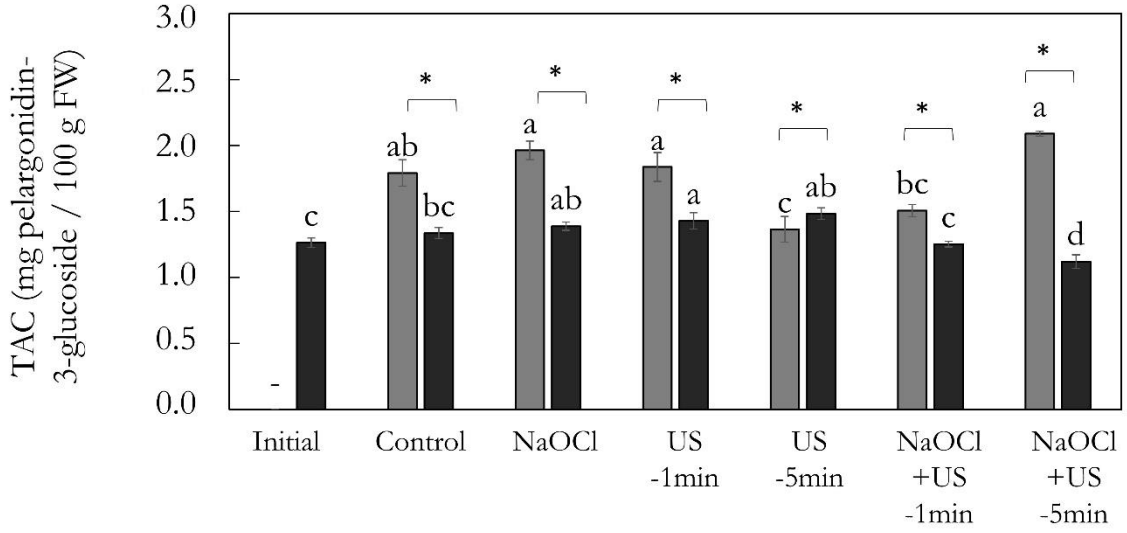

Figure 32. Total anthocyanin content (TAC, mg pelargonidin-3-glucoside / 100 g FW) before and after the treatments, for the replica 1 (■) and the replica 2 (■). Results are the mean values ± standard deviation (n = 3). Different letters indicate statistically significant differences ($p < 0.05$) between the treatments in the same replica, according to Tukey's test. Asterisk marks indicate statistically significant differences ($p < 0.05$) between the two replicas, according to T-student's test.

Anthocyanins were also evaluated, which are the components that mainly give the characteristic red color to strawberries. As it is shown in **Figure 32**, their concentration was maintained after most of the treatments, and ranged between 1.4 ± 0.1 and 2.1 ± 0.2 mg pelargonidin-3-glucosyde / 100 g FW, or 1.1 ± 0.5 and 1.5 ± 0.1 mg pelargonidin-3-glucosyde / 100 g FW in replica 1 or 2, respectively. Similar to what happened with TPC values, TAC values were also higher in replica 1 than they were in replica 2. However, the application of US did not exert a significant effect when compared to the same treatment without US. Alike in this study, Bal (2017) observed no significant effect of US treatment on anthocyanin content of grapes. However, TAC content of those grapes processed with US tended to increase during storage when compared to the non-sonicated ones. It is possible that higher sonication times are needed to achieve such effect.

To sum up, sonication (neither NaOCl which was the positive control in these trials) did not perform any relevant change (detrimental or improvement) for the physicochemical or biochemical quality.

4. Conclusions

All in all, the results obtained in this study reveal that sonication for 1 or 5 min was not effective in reducing native microbiota (including total aerobic mesophylls, yeasts and molds) or artificially inoculated *L. innocua* when compared to NaOCl, which was added in this study as a positive reference. Also, only NaOCl sanitation was able to reduce *L. innocua* counts in washing water below the detection limit. Moreover, any of the treatments showed a noteworthy effect in the quality parameters evaluated immediately after the immersion. The results obtained expose the lack of effectiveness of ultrasound as a sanitation technology when used alone. For this, the authors suggest its combination with other physical (e.g. high temperature, ultraviolet) or chemical (e.g. peroxide hydrogen, peracetic acid) to elucidate if it may, somehow, improve the efficacy of the suggested methods alone.

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Conflict of interests

The authors declare no conflict of interests.

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Chapter 6

Exploring thermosonication as non-chemical disinfection technology for strawberries

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The combination of ultrasound with mild temperatures, namely thermosonication, has been widely studied in the fruit and vegetable fruit juice industry with the objective to reduce pathogen populations. However, its application in fresh fruits has been less well-studied. The scope of this work was to study the efficacy of the combination of sonication at 35 or 130 kHz with three temperature treatments: 20, 50 and 55 ºC, on the population of artificially inoculated *Listeria innocua* in strawberries, and on their overall quality. Prior *in vitro* results showed that temperature was the main factor in decreasing *L. innocua* population: a maximum of 3.8-log reductions was obtained with sonication at 130 kHz and 55 ºC for 15 min. Treatment of artificially inoculated strawberries at 55 °C for 15 min resulted in 7.2 ± 0.1 and 6.0 ± 1.4 log reductions in non-sonicated and sonicated samples, respectively, and remaining population in washing water was below the limit of detection. Despite this, it was discarded for it led to a non-fresh like aspect of the strawberries. Treatments combining – or not – sonication at 130 kHz with mild temperatures (50 and 55 ºC) for 5 or 10 min were able to decrease about 3-log units of artificially inoculated *L. innocua* in strawberries and about 2-log units of total aerobic mesophilic and yeasts and molds populations naturally occurring in strawberries. Thermosonication treatments did not exert a detrimental impact on fruit quality, except for those at the higher temperatures and times, which caused a change in color to more purplish and a little softening of the strawberries, which were proposed to be assessed for further processing other than fresh commercialization. Overall, the impact of sonication in fresh strawberries needs to be further investigated to find the adequate conditions to enhance the effects of temperature itself.

Ultrasound, quality, *Listeria innocua,* antioxidant, fresh fruit, epiphytic microbiota

1 Introduction

Sonication consists on the use of ultrasonic waves at a frequency beyond 18 kHz with a specific intensity and amplitude (Bevilacqua, 2018). Microorganism lethality caused by sonication is attributed to transient cavitation, a phenomenon consisting of a large number of microscopic bubbles that originate from cycles of pressure (Pérez-Andrés, 2018). The collapse of generated bubbles causes spots with extremely high pressure and temperature, destroying the cellular envelopes and other components, thus reducing the viable microorganisms (Leong, 2017). Also, if fresh and fresh-cut fruits are treated with ultrasounds, chemical and structural changes affecting quality and nutritional values of the product may occur (Leong, 2017). However, as reviewed by Nicolau-Lapeña (2019), it is necessary to combine ultrasounds with other physical or chemical methods to achieve successful sanitation of fresh and fresh-cut fruits. In particular, thermosonication, or the combination of heat with ultrasounds, is one of the most effective approaches to inactivate microbes for industrial purposes (Chandrapala, 2012). When ultrasounds are combined with heat, the rate of sterilization of foods can be accelerated, thus lessening both the duration and intensity of thermal treatment and the resultant damage. The advantages of ultrasound over heat pasteurization include: the minimizing of flavor loss, especially in sweet juices; greater homogeneity; and significant energy and cost savings (Piyasena, 2003).

The need for sanitation procedures in fresh fruit industry becomes evident in the many foodborne illnesses and outbreaks occurring annually (Doyle, 2015). Additionally, several outbreaks caused by *Listeria monocytogenes* have been recently linked to fresh produce contamination around the world (Zhu, 2017). Listeriosis is causing from mild gastroenteritis to severe disease conditions (septicemia, encephalitis, meningitis, abortions and stillbirths) and resulting in a high fatality rate in immune-compromised populations (Swaminathan, 2007). Inactivation of *L. monocytogenes* has already been described in other studies, both *in vitro* (Franco-Vega, 2015) and *in vivo* in different products such as milk (Bermúdez-Aguirre, 2009) and fruit juices (Anaya-Esparza, 2017). Although strawberries are considered a low risk product, (lessa, 2005) demonstrated that L. monocytogenes was capable of surviving but not growth on the surface of fresh intact or cut strawberries throughout the expected shelf life. Growth of *L. monocytogenes* has been demonstrated in a number of vegetables under refrigerated and ambient and in non-acidic fruits (Penteado, 2004). Growth has also been demonstrated on the outer surface of acidic fruits such as tomatoes (Honjoh, 2016) and peeled oranges (Caggia, 2009) when stored at greater than 20 ºC.

In this paper, sonication and thermosonication effects on the survival of *L. innocua* (used as a *L. monocytogenes* surrogate) were investigated both *in vitro* and *in vivo* in strawberries, as well as their impact on quality parameters of the treated fruits, in order to produce safer products while maintaining their quality.

2 Materials and methods

2.1 Materials

Strawberries (*Fragaria* × *ananassa*) were purchased from local providers the same day of the experiment, and stored at 4 °C until it was carried out.

Triptone soy broth (TSB), triptone soy agar (TSA), Palcam base agar and Palcam selective supplement for *Listeria* spp., yeast extract, plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), sodium chloride and peptone were obtained from Biokar Diagnostics (Allonne, France). Dey-Engley broth was obtained from Honeywell Fluka (Madrid, Spain).

2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and sodium carbonate were acquired from Sigma-Aldrich (Steinheim, Germany). Methanol, chlorhidric acid (37 %), sodium acetate, sodium hydroxide, ferric chloride hexahydrate and Folin Ciocalteau's reagent were procured by Panreac (Llinars del Vallès, Spain).

2.2 Methods

2.2.1 *Listeria innocua* culture preparation

Listeria innocua strain CECT-910 (*Colección Española de Cultivos Tipo*, Burjassot, Spain) was used as a surrogate of *L. monocytogenes* in this study (Francis, 1997). Cultures were prepared as described in Nicolau-Lapeña (2019), and the concentrated suspension obtained contained 1.2×10^{10} CFU / mL, approximately.

2.2.2 Survival curves of *L. innocua* under different combinations of temperature and sonication

The inactivation of *L. innocua* when exposed to mild temperatures (50 and 55 ºC) combined or not with sonication (35 or 135 kHz) was investigated and compared to the behavior of *L. innocua* at 20 ºC, also combined or not with sonication. For this, 500 mL of sterile water were prepared in a glass beaker (3 repetitions) were immersed in a TRANSSONIC TI-H-20 bath (Elma Schmidbauer GmbH, Singen, Germany) set to the selected temperature. Once the water in the beaker had reached the studied temperature, an adequate volume of the prepared *L. innocua* suspension was added to reach a concentration of 10^7 CFU / mL. Nine different treatments were carried out twice (2 replicates), combining three temperatures (20, 50, or 55 ºC) with three sonication conditions (no sonication, sonication at 35 kHz or 130 kHz) during 15 min. For each sampling time (0, 1, 2, 5, and 15 min), 5 mL of each repetition were poured in a sterile tube, and 1 mL was added to 9 mL of Dey-Engley media to evaluate the presence or absence of *L. innocua*. Tubes were immediately cooled by immersion in water iced. An additional beaker with 500 mL of water was used to monitor the temperature using a DualTemp Pro Insertion-Infrared Thermometer (Dostmann, Reicholzheim, Germany).

2.2.3 Survival of *L. innocua* artificially inoculated in strawberries under different combinations of temperature and sonication

The effect of the combination of sonication at 130 kHz with mild temperature (50 and 55 ºC, thermosonication) was evaluated and compared to the effect of sonication at 130 kHz at 20 ºC. For this, strawberries were inoculated the day before the assay with a suspension containing 10^{10} CFU / mL of *L. innocua*, by pipetting 50 µL in small droplets on the surface. Once dried, strawberries were stored overnight at 4 ± 1 ºC.

Six treatments were performed, combining three temperatures (20, 50, and 55 ºC) and two sonication conditions (with and without sonication). For this, 500 mL of water were poured in each of the four

glass beakers, which were immersed in the ultrasonic bath (TRANSSONIC TI-H-20) set to the selected temperature. Once the water reached the studied temperature, 12 strawberries were simultaneously immersed on it. Each sampling time (1, 2, 5, or 10 min), 3 strawberries were collected from one beaker using a net and immersed in chilled water for 10 s, to lower the temperature and avoid further *L. innocua* inactivation. Strawberries were let to dry at room temperature on disinfected grids. Also, 5 mL of water were poured in a sterile tube to count *L. innocua* that could have been dragged to water, and 1 mL was added to 9 mL of Dey-Engley media to evaluate the presence or absence of *L. innocua* in wash water. To prevent further population changes, tubes were kept in ice until count procedure. This experiment was carried out twice (2 replicates, n=6).

2.2.4 Determination of *L. innocua* population

***L. innocua* population in the suspension or in water.** Concentration of *L. innocua* after the treatments was determined by serially dilution in saline peptone (SP, peptone 1 g / L and NaCl, 8.5 g / L) and plating in TSAYE (triptone soy agar supplemented with 6 g / L of yeast extract, 2.5 g / L glucose and 2.5 g / L K_2HPO_4) for concentrate suspension, or in selective Palcam agar for both, concentrate suspension and studied water. Plates were incubated at 37 ± 1 ºC for 22 ± 2 h for TSAYE and 48 ± 2 h for Palcam. Results were expressed as log CFU / mL. When counts were below the limit of detection (0.7 log units), and presence was confirmed by Dey-Engley color change after incubation at 37 ± 1 ºC for 24 or 48 h, an arbitrary value of half detection limit of detection was assigned.

***L. innocua* population in strawberries**. One strawberry per replicate was used for microbiological analysis (3 repetitions per treatment, 2 replications of the experiment, n=6). Populations were determined by plate count on selective Palcam medium in duplicate, as it has been previously described in (Nicolau-Lapeña, 2019). Results were expressed as log CFU / fruit, and detection limit was 1.3 log units.

2.2.5 Evaluation of the effect of selected combinations of temperature and sonication in strawberry quality parameters

Based on the results previously obtained, the following treatments were selected to study their effect on fruit quality: sonication and non-sonication at 50 ºC for 5 and 10 min, and sonication and non-sonication at 55 ºC for 5 min. Results were compared with non-sonicated treatments at 20 ºC for 5 and 10 min. Treatments were performed as described in section 2.2.3., using non-inoculated strawberries, and when the fruits were dry, the following quality determinations were performed: total aerobic mesophylls (TAM), yeasts and molds (Y&M), firmness and color. Also, an aliquot of the samples was frozen with liquid nitrogen, milled using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain) and stored at -80 °C for further biochemical analysis: antioxidant capacity, total phenolic content (TPC) and total anthocyanin content (TAC). The experiment was performed once, with 3 repetitions (n=3).

2.2.5.1 Epiphytic microbiota

For the determination of total aerobic mesophylls (TAM) and yeasts and molds (Y&M), 25 ± 1 g per repetition (n=3), taken from pieces of 2 strawberries, were diluted 1:10 in peptone buffered solution in a sterile filter bag (BagPage®,Interscience BagSystem, Saint Nom, France) and homogenized in a paddle blender for 90 s at 9 stroke/s. Aliquots were serially diluted in SP. For TAM, samples were plated in PCA and incubated at 30 ± 1 ºC for 3 days. For Y&M, samples were plated in DRBC and incubated at 25 ± 1 ºC for 5 days. Results were expressed as log CFU / g, and detection limit was 1.7 log CFU / g.

2.2.5.2 Firmness and color

Firmness changes were evaluated on 10 strawberries per repetition (n=3) using the TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England). Compression test consisted of compressing a strawberry half to 6.0 mm using 2 parallel plates, and recording the maximum force required. Penetration test was performed with a 4 mm cylindrical probe, measuring the maximum force encountered when the probe when entering 8.0 mm deep into the tissue. Both tests were run at 5 mm/s speed with a trigger force of 0.1 N.

Color of 10 strawberries per repetition (n=3) was measured on 3 points of each sample using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan) with a D65 illuminant and 10° observer angle. The instrument was calibrated using a standard white reflector plate. Color is expressed as CIE L* a* b* coordinates.

2.2.5.3 Biochemical analysis

Antioxidant activity of strawberries was assessed by ferric reducing antioxidant power (FRAP) and DPPH scavenging activity assays, as described in Nicolau-Lapeña (2019). Results were expressed as mg ascorbic acid equivalents (AAE) / 100 g FW of 3 repetitions (n=3).

The **total phenolic content** (TPC) was assessed by Folin Ciocalteau method on the same extract used for antioxidant activity determination, following the procedure described by Nicolau-Lapeña (2019). Results were expressed as mg gallic acid equivalents (GAE) / 100 g FW of 3 repetitions (n=3).

Anthocyanin extracts and quantification were carried out in triplicate (n=3) according to the method described by (Meyers, 2003). Anthocyanin content was expressed as mg of cianidine-3-glucosyde / 100 g FW.

2.3 Statistical analysis

All data were checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analyses were carried on using JMP 13 (SAS Institute Inc., Cary, USA).

3 Results

3.1 Survival curves of *L. innocua* under different combinations of temperature and sonication

Initial population of *L. innocua* in the water suspension was 7.2 ± 0.1 log CFU / mL (**Figure 33**). At 20 ºC (**Figure 33**A), no changes during sonication for 15 min were observed, either at 35 or at 130 kHz. Similarly, at 50 ºC (**Figure 33**B), population did not significantly change during the 15 min treatment, regardless of sonication conditions. The survival curves at 55 ºC (**Figure 33**C) were significantly different from those at 20 and 50 ºC from min 2, when population of *L. innocua* started to decrease. Inactivation was more acute after 15 min, reaching final population of 3.8 ± 0.9 log CFU / mL in non-sonicated and sonicated at 130 kHz treatments. When the two ultrasound conditions combined with 55 ºC were compared, 130 kHz showed higher efficacy than 35 kHz did (final populations of 3.2 ± 0.3 and 4.8 ± 0.1 log CFU / mL, respectively). However, the ANOVA test revealed that the significance of the factor 'Ultrasound' could be considered negligible when compared to that of 'Temperature' and the interaction of 'Temperature × Time'.

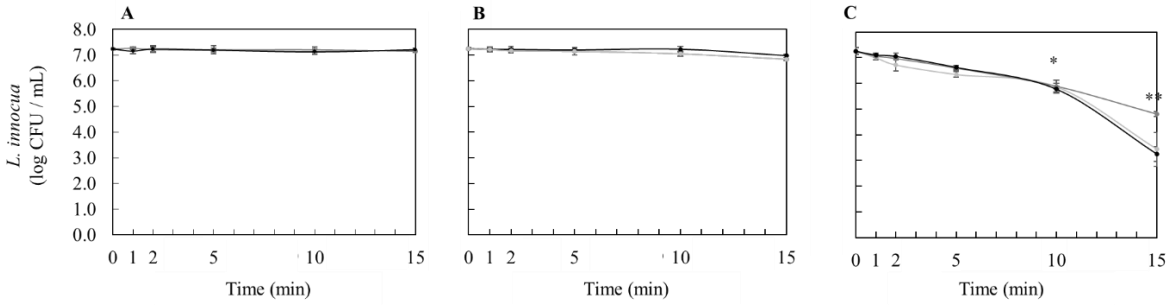

Figure 33. Survival curves of *Listeria innocua* in water at 20 °C (A), 50 °C (B), and 55 °C (C) without sonication (■), sonication at 35 kHz (■) or sonication at 130 kHz (■). Values are the mean ± standard deviation (n=3). Asterisk mark represents sampling times at which the population significantly decreased ($p < 0.05$) in comparison to the previous treatments according to Tukkey's HSD test.

3.2 Reductions of *L. innocua* artificially inoculated in strawberries under different combinations of temperature and sonication

Initial population of *L. innocua* in strawberries was 7.2 ± 0.4 log CFU / fruit after (data not shown). After two minutes, *L. innocua* population was significantly reduced at least 2.0 log units by all combinations of temperature and sonication conditions (**Figure 34**A). At 20 ºC, a significant difference attributed to sonication was revealed in *L. innocua* reductions after 15 min, which was 1.8 ± 0.8 and 3.0 ± 0.7 log CFU / strawberry in non-sonicated and sonicated samples, respectively. At 2 min of treatment, there were marked differences between sonicated and non-sonicated samples at 50 and 55 ºC. While non-sonicated samples had a decrease in population of 4.8 ± 1.5 log CFU / strawberry, the population decreased one more log when sonication was combined with the mild temperatures, achieving 6.0 ± 1.4 log CFU / strawberry reductions. However, the impact of ultrasound was concealed in the following times at the mild temperatures studied, factor that showed more significancy than ultrasound did.

Regarding population of *L. innocua* that was transferred to water (**Figure 34**B), no difference was found between sonication and non-sonication treatments, and the differences in viable populations could essentially be attributed to the temperature. At 20 ºC and after 2 min treatment *L. innocua* population

in the wash water was 4.3 ± 0.5 and 3.5 ± 1.0 log CFU / mL in non-sonicated and sonicated treatments, respectively. After 15 min, population decreased to 2.4 ± 0.8 and 1.7 ± 0.4 log CFU / mL. However, the final population in water was similar regardless the sonication treatment. Significantly lower *L. innocua* counts in washing water were found at 50 and 55 °C when compared to those at 20 °C, with most of counts below the detection limit. Contrarily, no differences in the population of *L. innocua* in the wash water were observed between 50 and 55 ºC, which ranged between 1.6 after 2 min to below the detection limit after 15 min, regardless the sonication treatment.

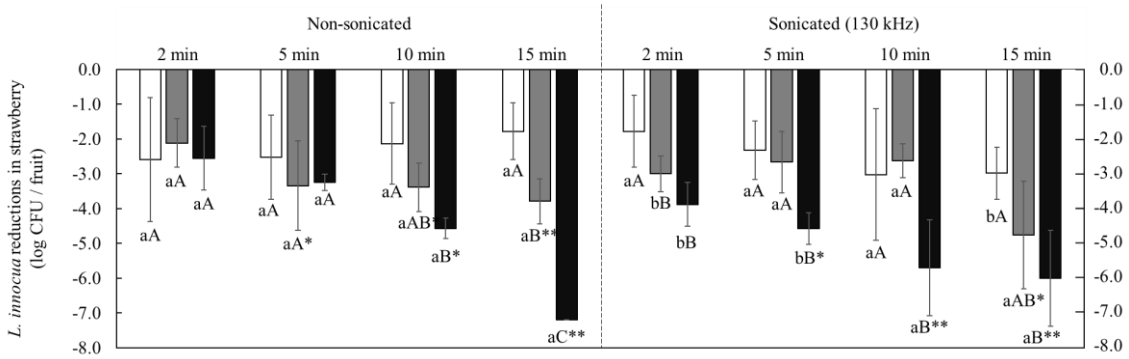

Figure 34. Reductions in artificially inoculated *Listeria innocua* in strawberry with or without sonication (130 kHz), at 20 °C (■), 50 °C (■), or 55 °C (■). Values are the mean ± standard deviation (n=3). Lowercase letters show statistical differences ($p < 0.05$) between sonication (non-sonicated or sonicated) within the same temperature and treatment time, and capital letters show statistical differences between temperatures (20, 50, or 55 °C) within the same sonication condition and treatment time. Asterisk mark represents sampling times at which the population significantly decreased ($p < 0.05$) in comparison to the previous times in the same combination sonication – temperature, according to Tukkey's HSD test.

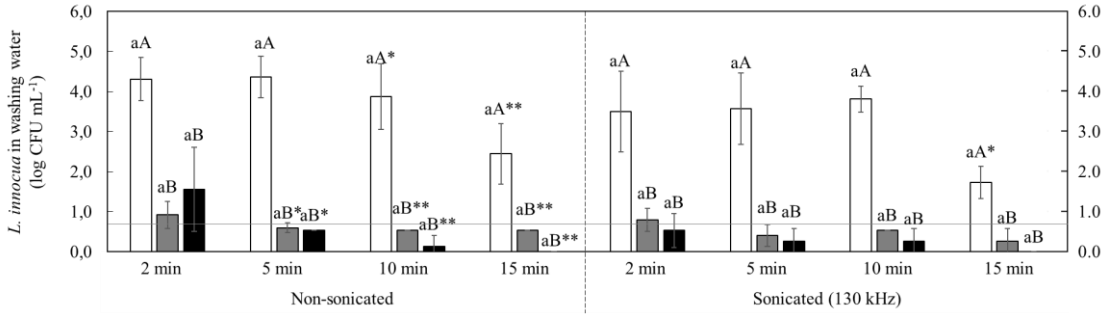

Figure 35. *Listeria innocua* populations remaining in water after the treatments with or without sonication (130 kHz), at 20 °C (■), 50 °C (■), or 55 °C (■). Values are the mean ± standard deviation (n=3). Lowercase letters show statistical differences ($p < 0.05$) between sonication (non-sonicated or sonicated) within the same temperature and treatment time, and capital letters show statistical differences between temperatures (20, 50, or 55 °C) within the same sonication condition and treatment time. Asterisk mark represents sampling times at which the population significantly decreased ($p < 0.05$) in comparison to the previous times in the same combination sonication – temperature, according to Tukkey's HSD test. Grey line represents the detection limit.

Based on the results of efficacy in reduction of *L. innocua* and the visual aspect of the samples after treatment, selected treatments were 50 °C for 5 and 10 min, and 55 °C for 5 min, sonicated at 130 kHz or not. A 20 °C non-sonicated treatment for 5 and 10 min was used as a control. The treatments at 55 ºC for 10 and 15 min and 50 ºC for 15 min were rejected due to the loss of quality, which was visually assessed and showed that strawberries acquired a cooked aspect, presented exudation and overcame a loss of firmness.

3.3 Effect of the selected combinations of temperature and sonication on strawberry quality parameters

3.3.1 Epiphytic microbiota

The initial microbial load of the strawberries was 4.6 ± 0.5 and 3.5 ± 0.1 log CFU / g for total aerobic mesophilic (TAM) and yeasts and molds (Y&M), respectively (**Table 24**). Washing of strawberries in water at 20 ºC did not cause a significant reduction in both populations, regardless the sonication or the treatment time. Besides, 5 min treatment at 50 or 55 ºC revealed no significant changes in TAM population of non-sonicated strawberries. The other treatments carried out at the mild temperatures (including non-sonicated for 10 min at 50 ºC, sonicated for 5 or 10 min at 50 ºC, and sonicated for 5 min at 55 ºC) resulted in the decrease in TAM populations, which achieved values averaging 2.6 ± 0.2 log CFU / g. Regarding Y&M, counts in strawberries immersed at 20 ºC for 5 or 10 min did not show significant differences compared to untreated samples. At 50 and 55 ºC, and regardless of the time or sonication, Y&M counts decreased significantly to 1.6 ± 0.2 log CFU / g.

Table 24. Total aerobic mesophilic (TAM) and yeasts and moulds (Y&M) in strawberries before and after the treatments. Values are the mean of 3 reps ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments (Tukkey's test).

			TAM (log CFU / g)	**Y&M** (log CFU / g)
Untreated			4.6 ± 0.5 ^{a}	3.5 ± 0.1 ^{a}
20 ºC	Non sonicated	5 min	4.6 ± 0.7 ^{a}	3.6 ± 0.2 ^{a}
		10 min	4.3 ± 0.5 ^{ab}	3.3 ± 0.1 ^{a}
50 ºC	Non sonicated	5 min	3.9 ± 0.6 ^{abcd}	1.8 ± 0.5 ^{b}
		10 min	2.5 ± 0.5 ^{de}	1.6 ± 0.3 ^{b}
	Sonicated	5 min	2.3 ± 0.6 ^{e}	1.7 ± 0.1 ^{b}
		10 min	2.8 ± 0.7 ^{bcde}	1.4 ± 0.1 ^{b}
55 ºC	Non sonicated	5 min	4.2 ± 0.1 ^{ab}	1.5 ± 0.2 ^{b}
	Sonicated	5 min	2.6 ± 0.7 ^{cde}	1.4 ± 0.1 ^{b}

3.3.2 Firmness and color

Strawberry firmness prior to any treatment was 41.0 ± 8.2 and 3.3 ± 0.6 N tested by compression and penetration tests, respectively (**Table 25**). Sonication was not a significant factor influencing texture. Although firmness of the fruits after treatments seemed to decrease, the statistical analysis revealed no significant differences between them when firmness was measured by compression test. With penetration test, only samples sonicated at 50 ºC for 5 min and non-sonicated at 55 ºC for 5 min showed significantly different values from the samples untreated, being 1.9 ± 0.1 and 2.0 ± 0.2 N, respectively.

Strawberry color before treatments expressed as CIE-Lab coordinates was 42.3 ± 2.7 L*, 33.4 ± 1.3 a*, and 30.4 ± 3.9 b* (**Table 25**). After treatments, luminosity expressed by the coordinate L*, did not change significantly. Also, sonication was not a significant factor influencing color. However, at higher temperatures and longer times, lower a* and b* values were observed, implying a change in red: from orangey to more purplish at the end. For instance, sonicated samples at 50 ºC for 10 min presented

30.2 ± 0.7 a* and 23.7 ± 1.3 b* values, and sonicated samples at 55 ºC for 10 min presented 31.6 ± 0.5 a* and 24.7 ± 0.4 b* values.

Table 25. Firmness assayed by compression and puncture tests and color expressed as CIE Lab coordinates of strawberries before and after the treatments. Values are the mean of 3 reps ± standard deviation. Different letters show statistically significant differences ($p < 0.05$) between treatments (Tukkey's test).

			Firmness		Color		
	Sonication	Time (min)	Compression (N)	Puncture (N)	L*	a*	b*
Untreated			41.0 ± 8.2 ^{a}	3.3 ± 0.6 ^{a}	42.3 ± 2.7 ^{a}	33.4 ± 1.3 ^{ab}	30.4 ± 3.9 ^{ab}
20 ºC	No	5	43.5 ± 1.1 ^{a}	2.9 ± 0.2 ^{ab}	41.1 ± 0.5 ^{a}	35.0 ± 0.3 ^{a}	31.4 ± 1.3 ^{a}
		10	34.0 ± 3.2 ^{a}	2.1 ± 0.1 ^{abc}	39.3 ± 0.8 ^{a}	34.7 ± 0.2 ^{a}	28.5 ± 0.7 ^{ab}
50 ºC	No	5	34.3 ± 6.0 ^{a}	2.5 ± 0.3 ^{abc}	41.5 ± 2.4 ^{a}	32.5 ± 0.5 ^{b}	27.9 ± 3.3 ^{ab}
		10	37.2 ± 7.2 ^{a}	2.2 ± 0.6 ^{abc}	40.4 ± 1.6 ^{a}	32.1 ± 0.5 ^{b}	26.6 ± 2.1 ^{ab}
	Sonicated	5	32.6 ± 6.5 ^{a}	1.9 ± 0.1 ^{c}	39.9 ± 2.0 ^{a}	32.0 ± 0.5 ^{b}	24.3 ± 1.1 ^{b}
		10	29.7 ± 4.3 ^{a}	2.2 ± 0.2 ^{abc}	41.0 ± 0.9 ^{a}	30.2 ± 0.7 ^{c}	23.7 ± 1.3 ^{b}
55 ºC	No	5	29.3 ± 4.7 ^{a}	2.0 ± 0.2 ^{bc}	37.9 ± 1.0 ^{a}	31.7 ± 0.7 ^{b}	24.1 ± 0.8 ^{b}
	Sonicated	5	32.6 ± 6.4 ^{a}	2.3 ± 0.4 ^{abc}	40.1 ± 0.4 ^{a}	31.6 ± 0.5 ^{bc}	24.7 ± 0.4 ^{b}

3.3.3 Biochemical analyses

The **TPC** and **TAC** values of untreated strawberries were 95.1 ± 13.1 mg GAE / 100 g FW and 2.4 ± 0.1mg cianidine-3-glucosyde / 100 g FW, respectively. Thermosonication was not detrimental for nutritional quality of the product. In this regard, TPC values of the treated samples (which ranged from 88.1 ± 2.3 mg GAE / 100 g FW in non-sonicated strawberries washed for 10 min at 20 ºC to 114.5 ± 1.3 mg GAE / 100 g FW in non-sonicated strawberries treated at 55 ºC for 5 min) were not statistically different from that of treatments. Similarly, TAC values after thermosonication were also not statistically different from the untreated and ranged from 1.7 ± 0.3 mg cianidine-3-glucosyde / 100 g FW in non-sonicated strawberries washed for 5 min to 2.9 ± 0.4 mg cianidine-3-glucosyde / 100 g FW in sonicated samples treated at 50 ºC for 5 min and in non-sonicated samples at 55 ºC for 5 min.

4 Discussion

The effects of combination of mild temperatures with sonication against *L. innocua* both *in vitro* and on fresh strawberries were investigated in this work, as several studies have indicated that sonication alone is not successful to inactivate *L. monocytogenes* sufficiently unless combined with other techniques, including mild temperatures (> 60 ºC) (Bahrami, 2020).

In the present study, mild temperatures of 50 and 55 ºC were selected in order to maintain quality parameters in a fruit that is sensible to heat due to the lack of peel, applied. At ***in vitro conditions***, only the treatment at 55 ºC combined with ultrasound at 35 or 130 kHz over 10 min was effective to significantly reduce *L. innocua* population. When ultrasound was combined with 50 ºC treatment for 15 min, only a 0.3 log reduction was observed. Possibly, the selected combination for ultrasound and temperature parameters was not enough to inactivate *L. innocua* populations, and a higher time would have been needed to see a significant effect. At 55 ºC, 130 kHz showed higher efficacy than 35 kHz did but no differences were observed when no sonication was applied. In contrast, Franco-Vega (2015)

found that the treatment time to achieve the same reduction in *L. monocytogenes* counts in tryptic soy broth pH 6 was halved with thermosonication at 55 to 65 °C when compared with thermal inactivation at the same temperatures alone. Similarly, Muñoz (2012) reported a significant decrease (1.1 log units) in *L. innocua* counts after sonicating at 20 kHz a buffered solution containing this microorganism for 2 min. Indeed, efficacy of ultrasound not only depends on the temperature to which it is combined, but on a number of factors, including wave frequency, power and treatment time (Lafarga, 2019). For this, and as the observed in vitro reductions of *L. innocua* at 55 °C were higher when 130 kHz were used (achieving 4.0-log reductions compared to the 2.4-log reductions observed at 35 kHz), this frequency was the one selected for further investigations in this study.

To apply this technology ***in vivo*,** i.e. to a fruit matrix for its processing, and according to Patras (2010), it is not possible to predict the effect of a thermal treatment on retention of bioactive compounds, so it becomes necessary to evaluate each case individually. Heat processing is usually used in the fruit processing industry for liquids and fluids, such as juices, nectars, purées and jams. Though, as Lafarga (2019) recently reviewed, mild heat treatments are more appropriate for fresh and minimally processed strawberries, due to the changes that high temperatures could cause to the fruit. For this, in the present study, sonication was suggested as a technology to be combined with mild temperatures for sanitization of strawberries, assuming there would be an additive effect that could help to decrease the temperatures and the time needed to achieve the same results that could be obtained with a more aggressive treatment. For this, a screening test using **the *L. innocua* counts remaining in strawberry and in wash water after treatments** as a criterion was employed to select the best combinations 'Ultrasound' × 'Temperature' × 'Time'. In this regard, when studying the effect of sonication at 20 ºC, it was revealed that sonication was able to significantly reduce *L. innocua* counts in strawberries more than in non-sonicated samples. This could be attributed to the detachment of the microorganisms from the surface of the strawberry to the water induced by cavitation processes, as bubbles generated collapse near the fruit surface and may separate the microorganism from it (Nicolau-Lapeña, 2019). Indeed, ultrasound capacity to remove bacterial cells from the surface has been recognized, as it influences the flagella and fimbriae of the bacteria, as well as their attachment ability (Tan, 2017). However, some studies failed in significantly decontaminate food products with only the application of sonication. For instance, 30 or 45 min sonication times were needed to significantly reduce *E. coli*, *Staphylococcus aureus* and *L. innocua* populations in strawberries and lettuce, respectively (Birmpa, 2013). Also, Anese (2015) reported that *L. monocytogenes* count in waste-water of fresh-cut lettuce wash was reduced by 5-log after continuous sonication for 5 min, but it should be noted that the temperature at the end of the study reached 60 ºC, so the effect was the result of the combination of mild temperature with ultrasound. Our study also demonstrated that higher temperatures and times (50 and 55 °C for more than 10 min) exert a significant effect in decreasing *L. innocua* populations both in strawberry and wash water (where a population lower than the detection limit was reached). However, one of the problems of washing strawberries is that remaining moisture in the fruit can make them more exposed to fungal growth (Baicu, 2018). For this and for the timing in fruit processing industries, these washing times are often excessive for fresh and fresh-cut fruits and vegetables. Furthermore, sometimes the additional time is not related with an additional and significant pathogenic reduction, as other authors have reported that ultrasound treatment for 10 minutes can achieve the maximal removal of attached bacteria from fruits and vegetable surfaces without significant damage to the fresh produce quality (Bilek, 2013). According to them, exposure to ultrasound treatment maximum 10 min is potentially adequate for decontamination of fresh produce, while minimizing the impact on the nutritional and organoleptic properties of fresh produce. Huang (2017) also reported that treatment times longer than 10 min did not significantly enhance the removal of *E. coli, L. innocua*, and *Pseudomonas fluorescens* from the inoculated lettuce leaf samples sonicated at 20 ºC with a frequency of 45 kHz. Reductions in such *L. innocua*

populations were 2.2 ± 0.1 log CFU / cm². In the present study, and at 20 ºC, more than 15 min were needed to reach levels of 2.1 ± 0.5 log CFU / mL in wash water, which makes evident the need to combine ultrasounds with mild temperatures. It must be noted that a higher efficacy against *L. innocua* was observed when applying sonication with 50 °C to strawberries rather than what happened *in vitro* at the same temperature. One possible hypothesis proposed for this behavior is that the higher efficacy of thermosonication treatment on *L. innocua* on the strawberry may be related to the time spent on its surface: the acidic conditions and the overnight period at 4 °C after the artificial inoculation may have increased its susceptibility. Despite this, careful use of mild temperatures is always advised, as the main concern of mild thermal processing it that it can sub-lethally injure the bacteria, which in turn, may allow them to recover and grow during storage afterwards.

Based on the effects that different combinations of temperature, sonication and time had on *L. innocua* populations in treated strawberries, a selection of 6 treatments was carried out for further experiments involving quality determination. At 50 ºC, sonication or no sonication treatments for 5 or 10 min were selected, whilst at 55 ºC, only 5 min treatment time was selected, with or without sonication (as longer treatments gave strawberries were deleterious for strawberry quality, as explained in section 3.2). Treatments at 20 ºC were carried out as a reference, to see the effect that sonication or immersion in water for a certain time had on the different parameters evaluated afterwards. Similar to what happened with *L. innocua* population, treatments performed at 20 ºC – regardless sonication– did not have an effect on TAM and Y&M counts. By this way, ultrasound at 20 kHz frequency and 30 – 90 W power for 5 or 10 min did not result in significant decreases in TAM and Y&M counts in strawberries when compared to non-treated control (Aday, 2013). The fact that the TAM and Y&M reductions did not depend on sonication could be explained by the stronger attachment to the strawberry surface these microorganisms have acquired compared with that of *L. innocua*; the latter was artificially inoculated the day before the experiment, whilst TAM and Y&M have accompanied the fruit for a longer time and may have become more attached to it, forming biofilms or internalized in stomata. In this case, temperature was the factor which had significant effect in reducing epiphytic populations in strawberry. Even this strategy has been studied as an alternative to prevent microbial spoilage in fresh and processed strawberry products, sonication alone does not exert a great impact in epiphytic microbiota of these fruits, as reviewed in Lafarga (2019). However, both in the present study and as reviewed in the literature, when combined with mild temperatures, a greater effect was detected regardless the sonication. For instance, and related to temperature, hot water treatment of strawberries (45 ºC for 5 min) prevented the incidence of decay during storage at 4 ºC for 9 days and then transferred to 16 ºC for 3 days (Vicente, 2002).

The decrease in **firmness** contributes to fruit susceptibility to decay. Therefore, any treatment able to delay softening is potentially helpful to extend postharvest life of strawberries (Vicente, 2002). Immediately after the treatments in which mild temperatures, combined or not with sonication, were applied, firmness evaluated by compression test was not significantly affected. Variations in this parameter, attributed to the different shapes and widths fruit pieces, could be a reason for the lack of significance. In contrast, when analyzing the results of penetration test, some samples showed differences from the controls. It is believed that the penetration approach is more suitable when different shapes are studied, as it evaluates the force made with the tip of the probe in a smaller area, while compression test uses a larger area in which the contact surface may vary between samples. There is no consensus about the effect of heat and sonication may have on strawberries. For instance, some authors reported that heat treated fruit remained firmer than control (Vicente, 2002), but it should be noted that they applied dry heat instead of immersing strawberries in hot water. Other studies performed with the same immersion method that in the present study, also reported contradictory

results: heat treatment in water at 45 ºC for 15 min enhanced firmness in 'Tudla' strawberry fruit (García, 1995), while the same treatment affected firmness of 'Pájaro' fruit negatively (Lara, 2006). The softening of strawberries immediately after some treatments in the present study should be carefully considered if they are for fresh market. However, the suitability of those fruits to be processed as frozen products or sold as an ingredient for other products should be assessed.

Color and appearance are quality attributes that mainly affect whether a fruit product is accepted or rejected; therefore, this is one of the most critical quality attributes (Barrett, 2010). In this study, the color parameters described for untreated strawberries, which are in accordance to that reported in the literature, represent a brilliant red color. However, strawberries acquired a more purplish color after treatments that included mild temperatures. Some authors reported that ultrasound application (20 or 33 kHz, at 60 W) improved color of treated strawberries (Gani, 2016), while others described the changes in color after heat treatment up to 90 ºC for 15 min as barely visible in strawberry purée (Marszałek, 2015). Özsen (2012) reported that a* and b* values decreased in strawberry pulp with higher temperatures: at 60 ºC, these parameters had decreased a 13.3 and 9.3 %, respectively. In the present study, the variations were 6.0 and 24.4 % for a* and b*, respectively. The changes in color of strawberries could be attributed to the variations in anthocyanin content, as these compounds are accountable for it (Alvarez-Suarez, 2014).

Values of **TPC and TAC** of strawberries used for the treatments are in accordance for those already reported in strawberries from the same origin (Nicolau-Lapeña, 2019; Nicolau-Lapeña, 2020). In the present study, TAC and TPC values did not overcome major changes after heat treatment with or without sonication. On the contrary, some authors have reported a decrease in anthocyanin content after exposing strawberries or strawberry juice to heat treatments from 60 to 90 ºC (Odriozola-Serrano, 2009; Özsen, 2012). Also, the TPC and the TAC values, together with tannins and vitamin C, are the ones which mainly give the antioxidant properties to strawberries (Fierascu, 2020). The absence of major changes in these compounds' contents after treatments could be the reason why antioxidant capacity expressed by DPPH· values was maintained for all the treatments and, excepting for one treatment, also by the FRAP values. Another possible explanation could be that, while some antioxidant molecules are lost with heat treatments, formation of new antioxidants could occur during this process (Alvarez-Suarez, 2014). In fact, sonication is likely to alter quality during time; cavitation can produce radicals that can initiate the degradation of the products as well as trigger chain reactions (Bahrami, 2020). Sonication could also lead to the breakup of some cell walls of the strawberry, releasing the molecules inside. Moreover, heat treatment could trigger or inactivate phenylalanine ammonia lyase (PAL), an enzyme involved in the biosynthesis of the polyphenol compounds such as flavonoids, phenylpropanoids, and lignin in plants (Kumar, 2001). For this, it should be advisable to perform more investigation including the evolution of quality parameters during time.

5 Conclusions

Overall, the present study suggests that sonication plays a minor role in reducing *L. innocua* counts, both *in vitro* (studied in a suspension) and *in vivo* (in artificially inoculated strawberries), at least at the conditions tested. The main effects that increased with treatment time could mainly be attributed to temperature.

Maximum reduction of *L. innocua* in strawberries was achieved by thermosonication at 130 kHz and 55 ºC for 10 min, accounting for 4.0-log unit reduction. However, this treatment caused a decrease in the freshness appearance of the strawberries. In this regard, fate of treated strawberries should be assessed for further commercialization, e.g. in frozen, for purées, or as an ingredient for other products. Fortunately, thermosonication at 50 °C for 5 or 10 min, or at 55 °C for 5 min did not cause major changes in strawberry quality and biochemical parameters analyzed, except for a slight darkening and softening observed after the strongest treatments.

This research extends our knowledge of the application of thermosonication for strawberry sanitation. This technique could be a good approach to produce strawberries that accomplish safety and quality standards, but more research is needed to reach its full potentiality.

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Conflict of interests

The authors declare no conflict of interests.

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Chapter 7

Combination of sonication with anti-browning treatments as a strategy to increase shelf-life of fresh-cut potato (cv. Monalisa)

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Two antioxidant solutions, a patented mixture based on vitamin C and other compounds (Natureseal ®, MV) and green tea extract (GT), were proposed to prevent browning in sliced potatoes. Combination with ultrasound (US) at two frequencies (35 and 130 kHz) was also evaluated, but no significant enhancement of the effects was detected with US. In MV-treated samples, respiration rate and dry matter were higher than they were in the control (CK) or GT-treated samples. Membrane integrity nor MDA content were significantly affected by the addition of MV or GT, but in MV-samples the antioxidant activity was increased 6-fold at the beginning of the storage, but decreased after 9 days. A yellowish coloration on the surface of GT samples was observed, attributed to the coloration of the tea concentrate. In the conditions studied, GT was not able to delay browning in potato slices. Contrarily, MV solution preserved the original colour of the sliced potatoes during the 9-day storage at 4 ºC

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Sliced potato, antioxidant, shelf-life, green tea, ultrasound

1 Introduction

Potatoes are the most important tuber crop in the world and they hold the third place in the rank of important human consumption crops (Torero, 2018). In addition to their low-fat content, potatoes also supply dietary fibres, carbohydrates and high-quality proteins and minerals (Seijo-Rodríguez, 2018). As an outcome of consumers' lifestyle changes, and in response to their demand for fresh, healthy and easy-to-prepare vegetables, a wide assortment of minimally processed fruits and vegetables have been developed, including sliced potatoes (Ramos, 2013).

The different culinary methods that exist for potatoes make these sliced potatoes a good product to be sold as ready-to-cook, but they have two main drawbacks: processing operations - peeling, cutting - may add susceptibility to quality deterioration and shelf-life is averagely limited to 5-7 days (Ierna, 2017). It is known that once cut, potatoes undergo colour changes, induced by the formation of intensely coloured products, as a result of enzymatic browning (Mareçek, 2013). To avoid this deterioration in colour, sulphites are usually added. However, this may lead to reactions in sensitive consumers, so certain products have been commercially developed in order to delay browning in fresh-cut potatoes. One example is Natureseal®, based on organic acids, vitamins and minerals, which claims to maintain the product colour during storage. Green tea extract has also been suggested for this purpose; due to its antioxidant capacity it has been reported to inhibit polyphenol oxidase (PPO) activity, which is the enzyme behind the browning reactions (Nirmal, 2011; Soysal, 2009).

On the other hand, ultrasound technology has been explored as a potential technology to induce an enhancement in the penetration of the antioxidant solutions in the vegetable tissue, and hence, increase their effect (Nicolau-Lapeña, 2019b). This effect is attributed to the cavitation bubbles created by the sonic waves. Their asymmetric implosions generate microjets in the direction of the solid surface, which can affect mass transfer, enhancing the penetration on the fruit surface (Carcel, 2012).

The aim of this study was to investigate a method consisting of a combination of antioxidant solutions and US technology to reduce the mechanisms that shorten sliced potato shelf-life. The paper focuses on this patented mixture of vitamins and minerals (primarily based on vitamin C) and green tea extract, which were proposed as antioxidant solutions. The effect of antioxidant treatments, combined or not with ultrasonication, was evaluated by physicochemical, microbiological, enzymatic and nutritional parameters during cold storage.

2 Materials and methods

2.1 Materials

Potatoes (cv. Monalisa) were purchased from a local supermarket. Natureseal® (mixture of vitamins and minerals, based on vitamin C and sulphite free) was kindly provided by Eurotech (Barcelona, Spain) and green tea was acquired from a local provider.

Peptone, plate count agar (PCA), and dichloran rose bengale chloramphenicol agar (DRBC), were obtained from Biokar Diagnostics (Allonne, France).

Ascorbic, and gallic acids, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), polyvinyl pirrolidone (PVPP), cistein, pyrocatechol, and guayacol were acquired from Sigma-Aldrich (Steinheim, Germany). Sodium hypochlorite, peroxide hydrogen, methanol, sodium chloride, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were procured from Panreac (Llinars del Vallès, Spain).

2.2 Methods

2.2.1 Experimental design

Potatoes were processed and sliced as described below, and two solutions were used in order to delay surface browning during storage and to increase shelf-life. Natureseal® 7.5 % (w:v) (MV) and green tea 5 % (w:v) (GT) were used as potential antioxidants and enzyme inhibitors (**Figure 36**), according to previous studies (Bobo, 2014). Tap water was used as a control (CK). Ultrasounds (US) at two frequencies, 35 or 130 kHz, were applied with the intention of increasing the penetration of the two solutions into the potato slice. A non-sonicated trial was carried out in order to ascertain whether ultrasound had an enhancing influence on the anti-browning effect of each solution (NS, GT or CK). In total, 9 treatments were carried out: CK with or without 35 or 130 kHz US (CK-0, CK-35, CK-130), and the same for MV (MV-0, MV-35, MV-130) and GT (GT-0, GT-35, GT-130).

Once processed, trays containing the sliced potatoes were stored at 4 ºC. Sampling was done on days 0, 2, 4, 7, and 9 (D0, D2, D4, D7, D9). Some determinations were made in the fresh product, including respiration rate, color, total aerobic mesophylls (TAM), total aerobic psychrophiles (TAP), yeasts and molds (Y&M), dry matter and membrane integrity. Aliquots of samples for each treatment were frozen with liquid nitrogen, milled using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain) and stored at -80 °C for further biochemical analysis, which included antioxidant activity, total phenolic compounds (TPC), malonildialdehyde (MDA) content and free sugar content.

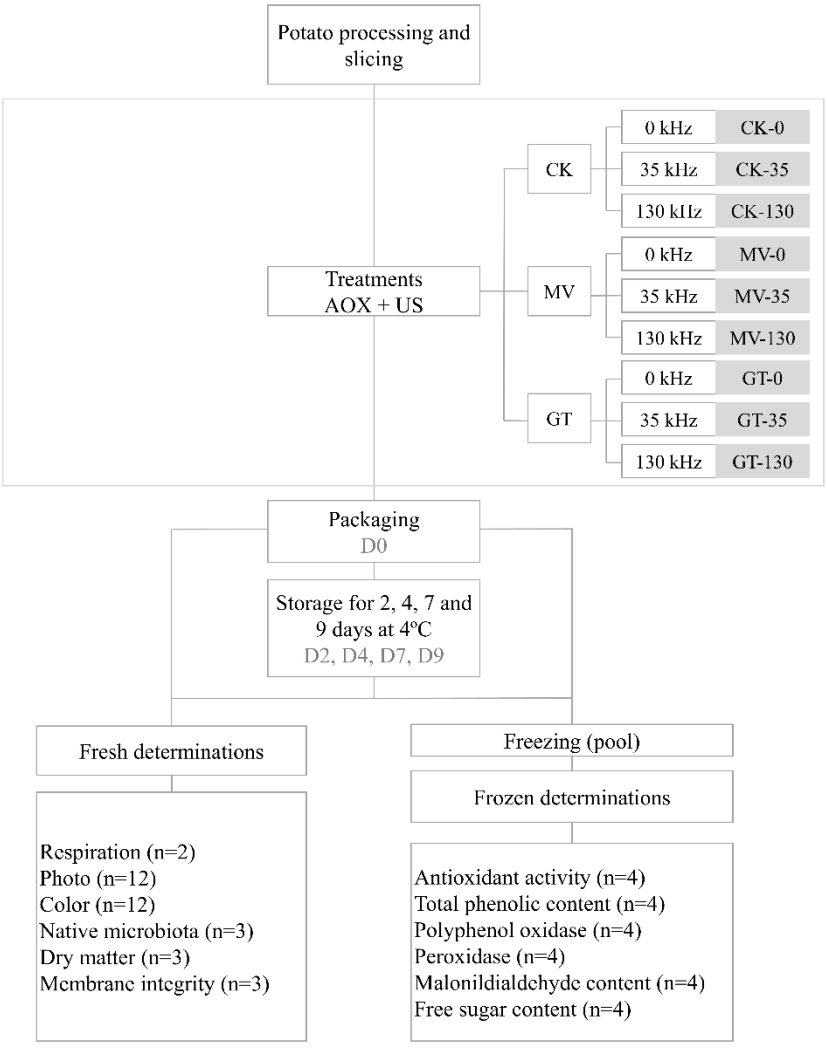

Figure 36. Experimental design

2.2.2 Preparation and characterisation of antioxidant solutions

MV solution was prepared to reach a final concentration of 7.5 % (w:v) diluting the commercial product in distilled water. GT solution was prepared from a green tea concentrate brewed in the ratio of 1:6 (GT: water; w:v), which was prepared with distilled water at 75 °C and sonicated for 10 min, 75 kHz, continuous, 20 % power, to enhance antioxidant extraction (Das & Eun, 2018). After filtration and centrifugation to eliminate suspended solids, the extract was diluted to a final concentration of 5 % as Bobo (2014) established as the optimal concentration to inhibit potato PPO. The solution was kept at 4 ºC until assay. To characterise the potential efficacy of both solutions, the antioxidant activity was determined by DPPH· assay, explained in section 2.2.10 and the ability to inhibit PPO activity was determined with a microplate colorimetric method, as described in section 2.2.12.

2.2.3 Potato processing

Potatoes were washed with tap water at 4 ± 1 ºC, disinfected in a 200 mg / L chlorine solution (pH 6.5) for 2 min in a ratio of 1:3 potato: solution (w:v), and rinsed with tap water for 2 min in the same proportion. After peeling using potato abrasive peeler PI-20 (Sammic, Spain) for 1.5 min, potatoes were cut into 5 mm width slices with automatic slicer Robot-Coupe CL-50 Ultra (Bourgogne, France). Excess of water was removed for 40 s using a centrifuge Marrodan PR47248 (Navarra, Spain). Then, slices were immersed in glass pots containing the treatment solutions – tap water (CK), 7.5 % Natureseal ® (MV) and 5 % green tea (GT) – in a ratio of 1:2 potato: solution (w:v) and agitated for 2 min. When required, continuous mode, 100 % power, 35 or 130 kHz US was applied simultaneously to the immersion. After treatment, the slices were centrifuged to drain the excess water for 40 s, and 100 ± 2 g of product were weighed in a 1,000 cm^3 polypropylene tray. Packages were sealed with an impermeable polypropylene film and a 100 μm hole was made manually and the packaged product was stored at 4 ºC.

2.2.4 Respiration rate and analysis of internal gas composition

Respiration rate (RR) of potato slices was determined immediately after the processing. For this, 100 ± 2 g of sliced potatoes were put inside a hermetic plastic pot and stored at 4 ºC. After 4 and 24 h, O_2 and CO_2 concentrations were measured using headspace gas analyser CheckMate 3 (Dansensor, Spain). Respiration rate was calculated following Equation 1.

$$\mathbf{RR\ (\mu mol\ /\ kg \cdot s)} = \frac{[CO2]f - [CO2]i \cdot (Vt - V0) \cdot 0.01}{W \cdot (tf - t0)} \qquad \text{Eq. 1}$$

where $[CO_2]_f - [CO_2]_i$ is the change in concentration between measurements (moles of gas using the equivalence at standard conditions of 1 mole equals 22.4 L), V_t is the total volume of the container (600 mL), V_0 is the volume of the potatoes (mL), $t_f - t_i$ is the time difference between measurements (s), and W is the weight of the potatoes in the container (kg).

Evolution of internal gas composition in sample trays was followed by measuring O_2 and CO_2 concentration on each storage day (D).

2.2.5 Color analysis

The surface color of 4 slices per tray and per treatment was measured (n=12) on three random places of the surface of each slice by using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Color was expressed as CIE L* a* b* coordinates, using a D65 illuminant and 10° observer angle.

2.2.6 Microbial quality

To study the evolution of alterative microbiota, 10 ± 1 g of sliced potatoes were placed in a sterile filter bag (400 mL BagPage®, Interscience BagSystem, Saint Nom, France) and diluted with buffered peptone water 1:10 (w:v). It was mashed in a paddle blender (MiniMix, Interscience, France) for 2 min at 9 strokes / s. Aliquots of the mixture were serially diluted on saline peptone and plated in duplicate on PCA for total aerobic mesophylls (TAM) and total aerobic psychrophiles (TAP) and on Dichloran-rose bengal chloramphenicol (DRBC) for yeasts and molds (Y&M). Plates were incubated at 30 ± 1 ºC for 3 days for TAM, at 4 ± 1 ºC for 10 days for TAP and at 25 ± 1 ºC for 3 to 5 days for

Y&M. Three repetitions were made for each treatment. Results were expressed as log CFU / g and the detection limit was 5 CFU / g.

2.2.7 Dry matter

Dry matter of samples was calculated using Equation 3 after drying dice of 5 mm^3 for 24 h at 105 ºC until a constant weight.

$$\textbf{\% dry matter} = (\text{dry weight} / \text{fresh weight}) \cdot 100 \qquad \text{Eq. 3}$$

2.2.8 Membrane permeability

Membrane permeability was expressed as electrical conductivity as previously reported by Liu (2019) with some modifications. Briefly, a 12 mm diameter circle per slice was cut and washed with distilled water three times. Two slices per tray and in triplicate (n=6) were determined per each treatment. The circles were dried using filter paper and immersed in 30 mL of boiling distilled water for 15 min. After removing slices, the water was cooled down and its electrical conductivity was measured using a conductimeter Testo 240 (Tarragona, Spain), and the results expressed as mS / m.

2.2.9 Malonildialdehyde (MDA) content

Determinations of MDA content in potato slices were carried out employing the supernatant obtained from a mixture of 1.0 ± 0.1 g with 8 mL 0.1 % TCA (w:v), followed by homogenisation for 10 min and a centrifugation at 20,000 × *g* for 10 min at 20 ºC. For the reaction, 0.5 mL of the extracts were transferred to 1.5 mL of 20 % TCA (w:v) and to 0.5 % TBA (w:v) in 20 % TCA. Tubes were incubated for 30 min in a thermal plate at 90 ºC, and the reaction was stopped by immersing the tubes in ice for 5 min. Absorbance at 532 and 600 nm were read using GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). Four replicates were determined for each treatment, only at D0 and D9, and MDA content, expressed as μmol kg^{-1} (in FW basis) was calculated following Equation 4.

$$\textbf{MDA content (μmol / kg)} = (2 \cdot (m + V) \cdot (\Delta Abs_{TBA} - \Delta Abs_{TCA}) / (m \cdot b \cdot E) \qquad \text{Eq. 4}$$

where m is the mass of the sample (kg), V is the extract volume (mL), ΔAbs_{TBA} is the difference between absorbance at 532 and 600 nm for samples that reacted with 0.5 % TBA in 20 % TCA. ΔAbs_{TCA} is for samples mixed with 20 % TCA, b is the optical distance and E is the MDA extinction coefficient (15.5 M^{-1} m^{-1}).

2.2.10 Reducing sugar content

Total reducing sugars, including sucrose, D-glucose and D-fructose, were determined on frozen slices (n=4). Reducing sugar content was determined spectrophotometrically with PowerWave HT (Biotek, Vermont, United States), following the instructions of the Kit 12819 from Biosystems (Barcelona, Spain) and expressed in g / kg FW.

2.2.11 Antioxidant activity (AC)

Antioxidant activity of the frozen samples was assessed using two methods: ferric reducing antioxidant power (FRAP) and DPPH· scavenging activity assays, as described previously in Nicolau-Lapeña (2019a). Standard curves were prepared with ascorbic acid (AA) for both methods, and

processed the same as with the samples. Results were expressed as AA equivalents (AAE) in mmol / kg (DW basis). Four repetitions were measured for each treatment (n=4).

2.2.12 Total phenolic content (TPC)

The TPC was determined by the Folin-Ciocalteau method, as described previously in Nicolau-Lapeña et al. (2019a). Standard curve with gallic acid was prepared, and results were expressed as gallic acid equivalents (GAE) in mmol / kg (DW basis). Four repetitions were measured for each treatment (n=4).

2.2.13 Polyphenol oxidase (PPO) and peroxidase (POD) enzymatic activity

The enzymatic extraction was carried out by mixing 5.0 ± 0.5 g of the frozen product with 0.5 g PVPP and 10 mL 0.1 M phosphate buffer solution pH 6 (PBS) with 0.05 mM cysteine in an ultraturrax Ultra-turrax ® Tube drive P control (IKA, Staufen, Germany) for 1.5 min at 5,000 rpm. After filtration and centrifugation at 20,000 × *g* for 10 min at 4 ºC, supernatant was maintained in ice. The supernatant was the crude extract.

The capacity of MV and GT solutions to inhibit potato PPO activity (%) was determined *in vitro* by microplate assay. For this, 65 µL of each antioxidant were poured into different wells, and 65 µL of potato crude extract were added to them. As a control (to calculate the 100 % enzymatic activity), 65 µL of distilled water were poured into different wells and mixed with 65 µL of potato crude extract. Then, 65 µL of 0.2 M pyrocathecol in PBS were added. After 10 min of incubation at 37 °C, absorbance was read at 400 nm. Inhibition was expressed as a percentage of potato PPO inhibition (Bobo, 2014; Masuda et al., 2005).

To evaluate the effect of the treatments and US in potato PPO y POD activity *in vivo* (in food matrix), the following analyses were also done:

PPO activity determination was carried out by adding 20 µL of the sample to 300 µL of 0.2 M pyrocathecol in PBS. Absorbance at 400 nm was read every 9 s for 3 min using a microplate spectrophotometer.

POD activity determination was carried out by adding 20 µL of the sample to 200 µL of 10 mM guayacol solution in PBS and 100 µL of 10 mM H_2O_2 solution in PBS. Absorbance at 470 nm was read every 9 s for 10 min using the same spectrophotometer.

Their enzymatic activities (PPO and POD) were calculated based on the linear portion of the plotted curve. Four replicates were done, and results were expressed as the increment in optical density (ΔOD 10^6) / kg · s (in protein basis).

3 Results and discussion

3.1 Antioxidant solution characterisation

Antioxidant activities of the proposed solutions MV and GT were determined by DPPH· inhibition assay, and MV solution antioxidant activity was found to be 3.8-fold higher than GT solution. Moreover, PPO inhibition was higher for MV solution than it was for GT solution, being 95.7 and 71.5 %, respectively. Despite the MV solution showing an inherent higher potential in *in vitro* studies, the two solutions were applied to the potato at the following concentrations: MV was prepared following the manufacturer's indications, and GT was prepared at 5 % according to Bobo (2014), as a higher concentration could have affected the sensorial properties of the samples negatively.

3.2 Respiration rate and O_2 and CO_2 concentrations during storage

Respiration rate (RR) of sliced potatoes averaged 1.8 ± 0.1, 2.8 ± 0.3, and 2.4 ± 0.1 μmol CO_2 / kg · s, for CK, MV, and GT treatments, respectively. These values are in agreement with the literature data for potatoes (Fennir et al., 2003).

US application involves cell wall disruption and it may imply certain stress. However, no significant differences were observed in RR between sliced potatoes regardless the sonication conditions. Regarding changes in the internal atmosphere (Data not shown), potato slices treated with MV showed significant changes in O_2 (12.7 ± 1.2, 6.0 ± 0.7, 2.1 ± 1.3, and 1.0 ± 0.9 % at D2, D4, D7, and D9, respectively) and CO_2 (5.7 ± 0.4, 12.2 ± 0.6, 17.1 ± 0.6, and 18.2 ± 1.0 % at D2, D4, D7, and D9, respectively) concentrations, respectively. Contrarily, in GT and CK samples O2 and CO2 exchange was slower. In CK samples, the internal O_2 (14.3 ± 0.4, 11.1 ± 0.5, 10.2 ± 0., and 9.7 ± 0.7 % at D2, D4, D7, and D9, respectively) and CO_2 (3.6 ± 0.2, 6.8 ± 0.3, 8.6 ± 0.2, and 10.1 ± 0.5 % at D2, D4, D7, and D9, respectively) in CK samples changed gradually and achieved balance at D9. Regarding GT potatoes, composition balance in trays was reached between D4 and D7, and composition in O_2 (13.6 ± 0.3, 10.8 ± 0.3, 6.8 ± 1.5, 6.9 ± 1.0 %, at D2, D4, D7, and D9, respectively) and CO_2 (3.9 ± 0.2, 7.1 ± 0.2, 9.8 ± 0.8, and 10.8 ± 0.7 %, at D2, D4, D7, and D9, respectively) reached a balance between D4 and D7. The stability values reached in gas composition inside the trays with CK and GT treated potatoes were close to the recommendations given by Farber et al. (2003), who stablished that percentages of 1-3 % O_2 and 6-9 % CO_2 were optimal for potato storage. This internal gas combination is reported to be the most suitable for products like tubercles, in order to maintain quality and delay the processes that typically degrade the product, such as colour alterations, changes in texture or microbial growth. Petri (2008) reported that immersion in ascorbic acid solutions (0.5 %) decreased the RR of fresh-cut potato by scavenging oxygen, which affected the enzymes of the oxidative phosphorylation pathway. The differences between samples form GT or CK treatments and MV behaviour could be explained in part by the different pH values of the solutions and their effect on the potato metabolism. It is suggested that ascorbic acid content in MV and the pH of the solution (3.2) could be related to the higher RR of these samples. The stress posed by immersion of MV samples in such acidic solution could have quickened their metabolic and respiration processes (Tudela, 2002).

3.3 Microbial quality evolution

The evaluation of epiphytic microbiota in potato slices revealed that populations of Y&M were below the detection limit for all the antioxidants and US frequencies used, both immediately after the treatments and during storage. Populations of TAM and TAP in the sliced product before the

treatments were 2.2 ± 0.1 and 1.8 ± 0.3 log CFU / g, respectively. After the treatments (**Figure 37**), populations were maintained in CK samples, whereas MV treatment showed a slight sanitizing effect. Conversely, after GT application, populations increased in the same numbers for both TAM and TAP. For all the treatments and days, a strong correlation was observed (0.7293 – 0.8879 R^2) between TAM and TAP counts – except for MV-35 and MV-130 treatments. A bacteriostatic effect of MV was patent mostly against TAP, maintaining such populations below 1.84 ± 0.6 and 2.4 ± 0.6 log CFU / g for MV-35 and MV-130, respectively. A possible explanation of this effect involves the low pH of the MV solution and the subsequent acidification of the surface of sliced potatoes. At such pH conditions, that are lower than the growth range of most TAM (between 4.2–7.5), most microorganisms common in food products are not able to grow (Ray, 2004). Few reports have been found regarding antimicrobial activity of MV, as it is described as an antioxidant product and it is therefore used for this purpose. *Salmonella enterica* was not found on fresh-cut cantaloupes washed with hot water (76 °C) for 3 min with MV 8.5 % (w/v) during the 21 days of study (Alicea, 2018). In CK-0, a growth was observed during storage, and counts at D9 were 4.5 ± 0.5 log CFU / g. No significant differences were observed when comparing US application, either at 35 or at 130 kHz. A similar growth trend was detected in potato slices treated with GT, achieving values averaging 4.0 ± 0.5 log CFU / g for both TAM and TAP. The antimicrobial characteristics of GT extract in food have been previously described (Perumalla, 2011). However, in this study and at concentrations used, GT did not exert a significant effect against epiphytic microbiota.

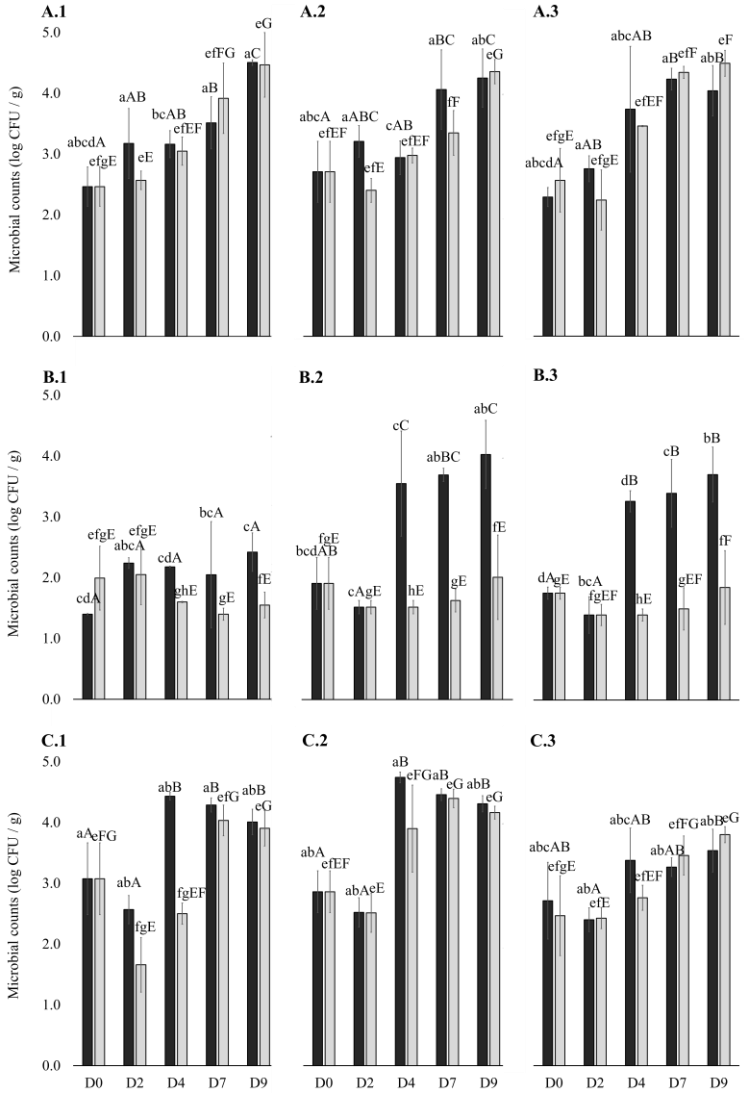

Figure 37. Microbial counts (log CFU / g) of total aerobic mesophylls (TAM, ■), total aerobic psychrophiles (TAP,□), of control (CK, **A**), mix of vitamins (MV, **B**), and green tea (GT, **C**) without ultrasound 0 kHz (**1**) or with ultrasound at 35 kHz (**2**) or 130 kHz (**3**). Values are the mean ± standard deviation (n=3). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.05$).

3.4 Dry matter and free sugar content

Dry matter and free sugar content are important parameters of potato quality, as they are used as reference values for further processing, especially frying (Wayumba et al., 2019). **Figure 38** presents the results obtained for these two parameters at D0 and D9. Sugar content was only evaluated at the beginning and the end of the storage, and dry matter values did not present practical differences between samples or days during storage. At the end of the storage, dry matter was maintained at 17.3 ± 1.1, 19.7 ± 1.2, and 16.8 ± 1.6 % for each treatment (CK, MV and GT, respectively), and differences between sonication conditions were not significant. Dry matter was slightly higher in samples in which MV was applied, possibly because of the higher concentration in soluble solids of the MV solution. Sugar content did not significantly differ between treatments, but it was affected by storage time. While reducing sugars at D0 were under 8.2 ± 0.5 g / kg (except for MV 130 in which values of 9.3 ± 0.1 g /

kg were determined), at D9 there was an increase ranging from 1.2- to 1.8-fold of their initial sugar content, which reached values between 10.6 ± 0.4 to 12.1 ± 0.3 g / kg. Storage at temperatures lower than 4 °C or higher than 8 °C is related to sugar accumulation or sweetening for sprout, respectively (Medeiros Vinci, 2012). As both parameters (sugar and dry matter) are directly correlated with the formation of acrylamide in fried potatoes, potatoes should be stored at 6 °C to keep their values low (Halford, 2012).

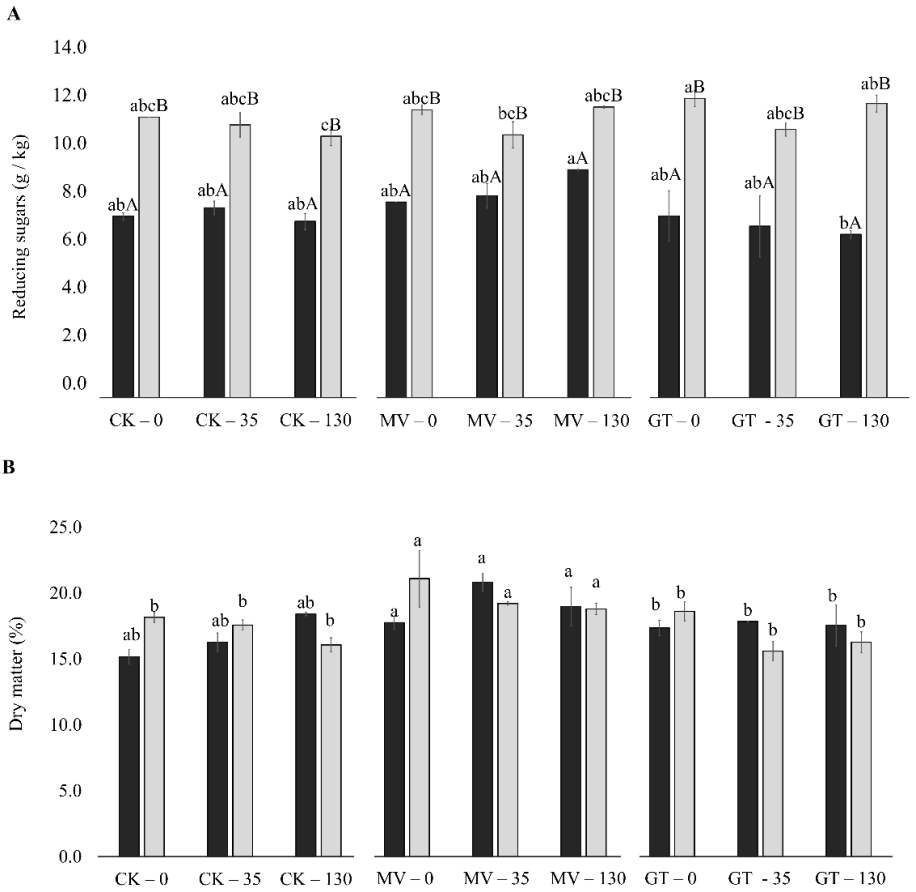

Figure 38. Reducing sugars (g / kg, in FW basis) (**A**) and dry matter (%) (**B**) at day 0 (■) and day 9 (■). Values are the mean ± standard deviation (n=3). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.05$).

3.5 Malonildialdehyde (MDA) content and membrane integrity

Slicing operations may affect membrane integrity of cell tissue, and subsequent oxidative damages may occur. MDA is an indicator of the degree of oxidative stress of the plant cell (Hodges, 2004), and electrical conductivity is usually correlated with the integrity of the cell membrane (Jiankang, 2007). In the present study, initial values of MDA content ranged from 14.7 ± 1.2 to 21.7 ± 0.6 μmol / kg (**Figure 39**). No pattern was observed involving antioxidant solutions and US frequencies that can explain variations in initial content. After 9-day storage, MV treated potatoes showed a higher increase in MDA values, when compared to CK and GT. Contrary to what Liu (2019) reported, there was a sharp rise in the conductivity of CK treated potatoes, even though conductivity immediately after

the processing was lower in these samples (**Figure 39**). The treatments MV-35, and GT-35 helped in maintaining membrane conductivity (below 100 mS / m), which can be related with a better maintenance of membrane integrity when using this frequency (35 kHz) in combination of both antioxidants.

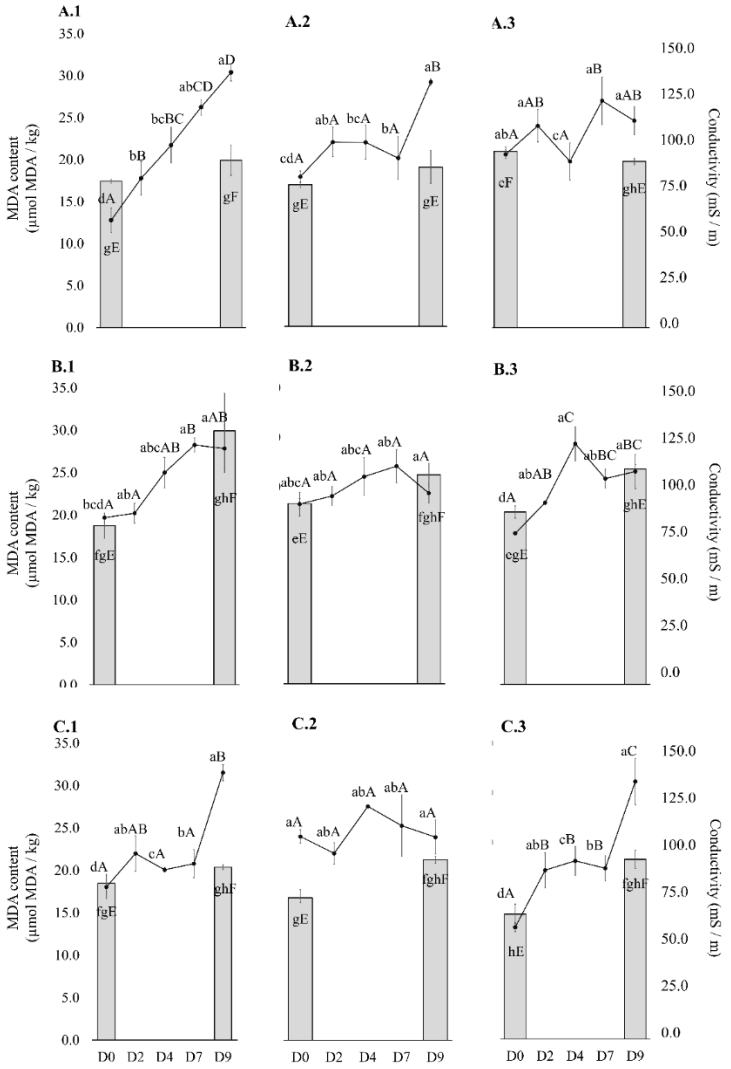

Figure 39. Malonildihaldeyide content (MDA, µmol / kg) (**bars**) and conductivity (mS / m) (**lines**) of control (CK, **A**), mix of vitamins (MV, **B**), and green tea (GT, **C**) without ultrasound 0 kHz (**1**) or with ultrasound at 35 kHz (**2**) or 130 kHz (**3**). Values are the mean ± standard deviation (n=4). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.05$).

3.6 Antioxidant capacity (AC) and total phenolic content (TPC)

AC was calculated by the methods of DPPH· and FRAP. As a strong correlation has been found between the two variables ($R^2 = 0.9622$), only DPPH· results are shown in **Figure 40**, which also presents TPC results. Initial AC and TPC values of CK potatoes averaged 5.5 ± 1.0 and 1.7 ± 0.1 mmol / kg, respectively. These values differ to what was observed by Serpen (2009) and Albishi (2013). Variations were attributed to differences in potato cultivars, the maturity of the tubers or previous storage conditions (Seijo-Rodríguez, 2018). Statistical differences were found between MV treated

potatoes and the other treatments (initial AC and TPC contents in such samples was significantly higher), but regarding US conditions, no differences were observed. Initial AC and TPC values in MV samples averaged 39.1 ± 1.1 and 3.8 ± 0.4 mmol / kg, respectively. GT treated potatoes did not reach such levels of AC, the values (averaging at the beginning of the storage 6.6 ± 0.8 mmol / kg) being 6 times lower than they were for MV. Differences in TPC figures were mainly attributed to an overestimation of TPC in MV samples rather than biological variances. In fact, MV is primarily composed of organic acids, including ascorbic acid, which is a reducing compound (non-phenolic antioxidant) that also reduces the Folin Ciocalteau reagent to form a blue colouring alkaline pH (Lester, 2012). A decrease in TPC values for MV treatments was observed for all US treatments, which could also be attributed to the reduction of ascorbic acid present in MV to dehydroascorbic acid during time, reducing, in turn, the interferences caused in Folin-Ciocalteau method. For CK and GT samples, AC and TPC values were well maintained during storage.

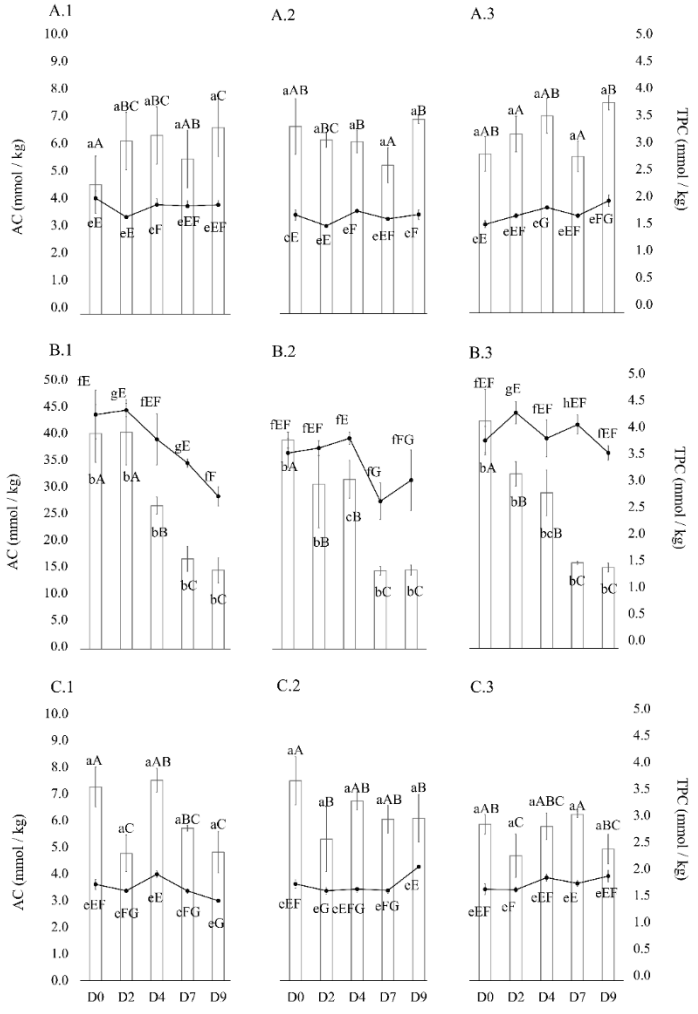

Figure 40. Antioxidant capacity (ascorbic acid equivalents, mmol / kg, in DW basis) (**bars**) and total phenolic content (gallic acid equivalents, mmol / kg, in DW basis) (**lines**) of control (CK, **A**), mix of vitamins (MV, **B**), and green tea (GT, **C**) without ultrasound 0 kHz (**1**) or with ultrasound at 35 kHz (**2**) or 130 kHz (**3**). Values are the mean ± standard deviation (n=4). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.05$).

3.7 Colour

Colour of potatoes was expressed by L* and a* coordinates (**Table 26**). MV treated potatoes were clearer and brighter to eyesight (**Figure 41**).

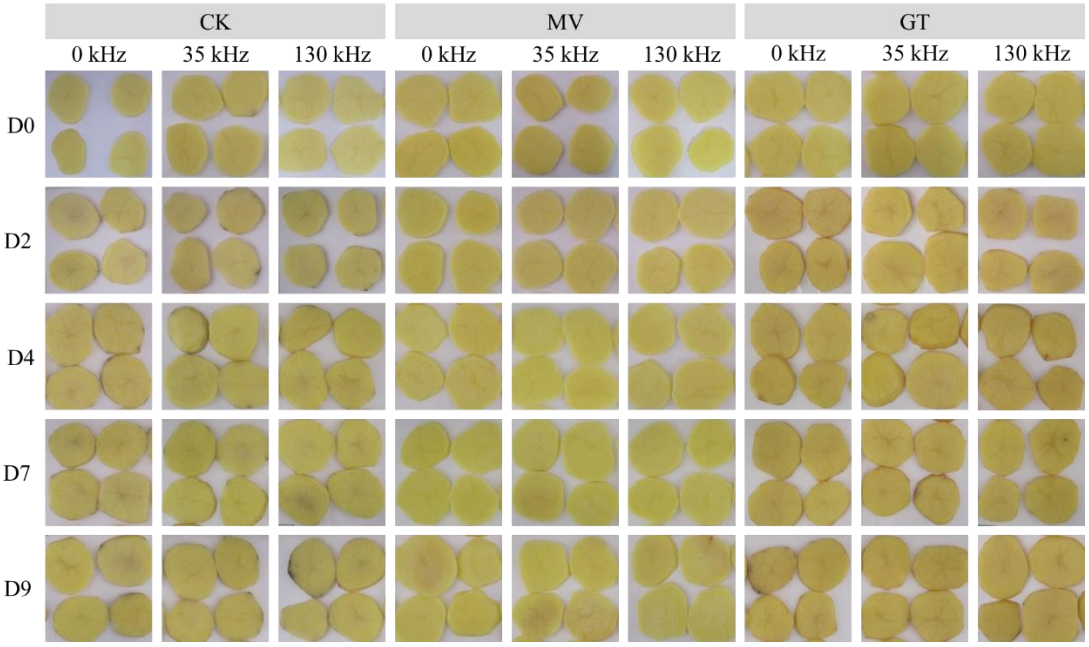

Figure 41. Images of the fresh-cut potatoes in control (CK), with mix of vitamins (MV) or green tea (GT) with or without ultrasound at 35 or 130 kHz, during storage at 4 ºC for 9 days (D0, D2, D4, D7, D9).

However, L* values that indicate luminosity did not show statistical differences between MV and CK samples, which averaged 69.5 ± 0.5 and did not present significant differences throughout storage. Only GT potatoes showed a lower luminosity when compared to the others, which, starting from D0 and continuing during the storage, averaged 68.3 ± 0.3. The a* parameter indicates the green (-) to red (+) dimension of the colour. At the beginning, a* values ranged from -6.3 ± 0.2 to -6.9 ± 0.1, there was homogeneity between all the treatments and no differences in a* values were found between US conditions. These values increased during storage, separating in two marked groups at D4. On one hand, CK and MV, in which the a* change was slight, averaged -4.5 ± 0.4. On the other, GT, in which a significant increase in a* occurred, reaching -3.2 ± 0.4, turned into a more brownish colour, rather than yellow-green. As can be seen in the photos (**Figure 41**) and also reflected in the lower luminosity and higher a* values of the samples, GT slices acquired a brownish colour. That was attributed to the natural pigmentation of the green tea solution that imbibed on the potato slice surface and remained slightly visible on it. As aforementioned, the composition of the patented antioxidant is based mainly on ascorbic acid, which has already been used successfully in potato slices to prevent browning by Abubakr (2016). Ierna (2017) also applied a mix of ascorbic acid and citric acid on fresh-cut potatoes, preventing the browning until 9-day of storage. The proposed mechanism involves the acids, that could have reverted the oxidized quinones and their derivatives into phenolic substances, preventing their subsequent polymerization to form brownish melanoid pigments (Li, 2017). Regarding green tea, it is rich in polyphenols (including catechins, teaflavins and tearubigines), substances that can possess strong antioxidant properties (Amarowicz, 2003) and have been reported to inhibit PPO activity, and further browning in fruits (e.g. in apple and apple juice up to 96% when using 3 g / L)(Klimczak, 2017; Soysal, 2009). Even so, in the present study, GT solution was not able to control browning in potato slices in the conditions established.

Table 26. Time evolution of L* and a* values of potatoes processed with antioxidants combined with US, expressed as the mean of 10 replicates ± standard deviation. Different lowercase letters mean significant differences (p-value < 0.05) between treatments in the same D, and different capital letters mean significant differences (p-value < 0.05) between days within the same treatment.

	L*				
Treatment	**D0**	**D2**	**D4**	**D7**	**D9**
CK-0	69.8 ± 1.3 ^{aA}	70.1 ± 0.6 ^{aA}	71.4 ± 0.6 ^{aA}	69.1 ± 0.8 ^{aA}	69.7 ± 2.1 ^{aA}
CK-35	70.4 ± 1.8 ^{aA}	71.2 ± 0.9 ^{aA}	71.1 ± 0.7 ^{aA}	70.0 ± 1.3 ^{aA}	69.4 ± 0.7 ^{aA}
CK-130	69.3 ± 0.2 ^{aA}	69.3 ± 0.5 ^{aA}	70.5 ± 0.9 ^{aA}	69.8 ± 1.2 ^{aA}	69.9 ± 1.9 ^{aA}
NS-0	69.6 ± 0.6 ^{aA}	67.8 ± 1.1 ^{bA}	70.8 ± 1.2 ^{aA}	68.4 ± 2.4 ^{aA}	70.9 ± 0.9 ^{aA}
NS-35	69.0 ± 0.3 ^{aA}	66.8 ± 0.8 ^{bB}	69.1 ± 0.9 ^{aA}	68.8 ± 1.2 ^{aAB}	70.2 ± 0.4 ^{aA}
NS-130	69.6 ± 0.7 ^{aA}	66.8 ± 1.2 ^{bB}	70.4 ± 1.2 ^{aA}	70.2 ± 0.8 ^{aA}	70.1 ± 0.5 ^{aA}
GT-0	68.3 ± 1.5 ^{bA}	67.8 ± 1.0 ^{bA}	68.5 ± 0.5 ^{bA}	67.5 ± 1.2 ^{aA}	67.6 ± 1.4 ^{bA}
GT-35	67.6 ± 1.3 ^{bA}	68.2 ± 1.2 ^{bA}	69.6 ± 0.4 ^{bA}	68.6 ± 1.1 ^{aA}	66.6 ± 2.4 ^{bA}
GT-130	68.2 ± 0.8 ^{bA}	68.0 ± 1.5 ^{bA}	67.6 ± 1.7 ^{bA}	67.6 ± 2.3 ^{aA}	68.2 ± 0.9 ^{bA}
	a*				
Treatment	**D0**	**D2**	**D4**	**D7**	**D9**
CK-0	-6.5 ± 0.2 ^{abcA}	-5.9 ± 0.1 ^{cdAB}	-5.4 ± 0.4 ^{bABC}	-5.1 ± 0.2 ^{bBC}	-4.5 ± 0.9 ^{bC}
CK-35	-6.9 ± 0.1 ^{bA}	-6.3 ± 0.3 ^{cdeB}	-5.2 ± 0.2 ^{bC}	-5.6 ± 0.2 ^{bC}	-4.2 ± 0.2 ^{bD}
CK-130	-6.5 ± 0.1 ^{abcA}	-5.7 ± 0.1 ^{cAB}	-5.1 ± 0.2 ^{bBC}	-5.3 ± 0.4 ^{bBC}	-4.7 ± 0.7 ^{bC}
NS-0	-6.9 ± 0.1 ^{bA}	-6.9 ± 0.1 ^{eA}	-6.8 ± 0.3 ^{cA}	-5.9 ± 0.6 ^{bB}	-4.0 +- 0.1 ^{bC}
NS-35	-6.8 ± 0.2 ^{bcA}	-6.5 ± 0.1 ^{eAB}	-6.4 ± 0.1 ^{cAB}	-5.9 ± 0.1 ^{bAB}	-4.9 ± 0.6 ^{bC}
NS-130	-6.7 ± 0.1 ^{abcA}	-6.6 ± 0.1 ^{deA}	-6.7 ± 0.3 ^{cA}	-5.9 ± 0.6 ^{bAB}	-5.0 ± 0.4 ^{bB}
GT-0	-6.4 ± 0.2 ^{abA}	-2.5 ± 0.4 ^{abC}	-3.4 ± 0.4 ^{aB}	-3.7 ± 0.4 ^{aB}	-3.7 ± 0.1 ^{aD}
GT-35	-6.3 ± 0.2 ^{aA}	-3.7 ± 0.3 ^{bBC}	-3.8 ± 0.3 ^{aB}	-3.6 ± 0.1 ^{aBC}	-2.9 ± 0.4 ^{aC}
GT-130	-6.5 ± 0.3 ^{abcA}	-3.9 ± 0.5 ^{bB}	-3.5 ± 0.4 ^{aB}	-3.6 ± 0.6 ^{aB}	-2.9 ± 0.4 ^{aB}

3.8 Polyphenol oxidase (PPO) and peroxidase (POD) activity

Initial values of PPO activity ranged between 461.9 ± 32.5 and 855.5 ± 56.4 ΔOD 10^6 / kg ·s (**Figure 42**). The higher values presented in PPO activity of GT samples were attributed to the increased content of phenolic compounds after the addition of GT, which act as a substrate for PPO (Tsouvaltzis, 2017). MV treated samples showed the lowest PPO activity, which started ranging between 461.9 ± 32.5 and 763.5 ± 45.5 ΔOD 10^6 kg · s at D0, and ended ranging from 236.2 ± 10.7 to 312.1 ± 29.4 at D9. This could be explained by the bonding of the organic acids in MV with the phenolic compounds or with PPO, thus creating inactive complexes. The acids that are present in MV could also contribute to a pH decrease, making PPO less effective in its suboptimal pH (Tsouvaltzis, 2017).

POD activity at D0 ranged between 5,696.1 ± 323.6 and 8,443.0 ± 821.6 ΔOD 10^6 / kg · s. A clear effect of antioxidants or US conditions could not be identified. Even though initial activities were significantly different between the samples, a general decrease of such values was observed during storage, making POD activities homogeneous in all samples at the end of the 9-day storage. The initial higher values of POD and PPO activity in GT-130, and also in PPO in MV-130, could be attributed to the sonication and the subsequent liberation of the enzymes (O'Donnell, 2010). The posterior

decrease could be explained by the effect of MV – by means of pH decrease - and GT – for its antioxidant compounds – as explained before.

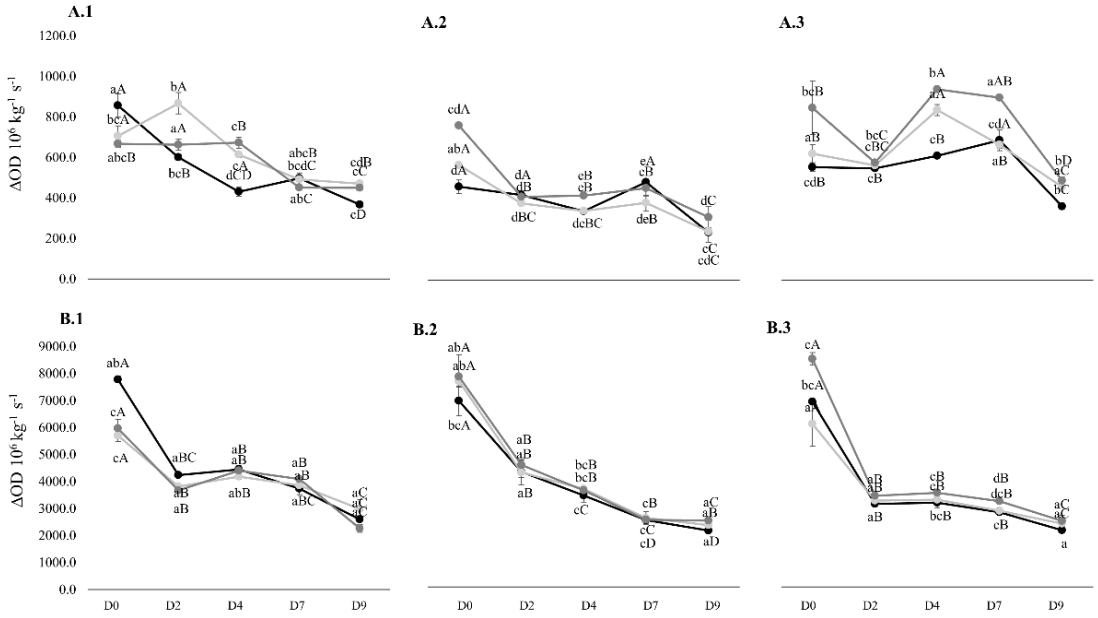

Figure 42. Polyphenol oxidase (PPO, **A**) and peroxidase (POD, **B**) activities in potato (ΔOD 10^6 / kg · s, in protein basis) of (CK, 1), mix of vitamins (MV, 2), and green tea (GT, 3) combined with US at 0 kHz (■), 35 kHz (■), 130 kHz (■). Values are the mean ± standard deviation (n=4). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.05$).

3.9 Overall view

A Principal Component Analysis (PCA) was performed in order to provide an overall view of the effects that the different treatments, CK, MV, and GT, and the different US conditions applied, 0, 35, or 130 kHz, relating all the studied variables. In both PCAs, D0 and D9, scores were distributed similarly. There was one separated group, formed by MV samples, and another two groups that overlap, CK and GT.

At D0 (**Figure 43**A), the sum of variability explained by principal component 1 (PC1) and principal component 2 (PC2) was 73.4 %. MV samples were located in one side of the PC1, and CK and GT samples were located in the other side of the PC1, in two groups that had an intersection, meaning that a similar trend was found in those samples. MV samples were characterized by a high dry matter content and high antioxidant activity expressed as DPPH· inhibition and FRAP. A direct connection between membrane conductivity and MDA content in PC1 was also observable, as the more stressed and disrupted the potato tissue was, the higher those values were. An indirect relationship was found between these parameters and TAP and TAM counts, which were higher for GT and CK samples. Variances found between MV and GT could be attributed to the differences of both antioxidants. MV solution is mainly composed of ascorbic acid, whose antimicrobial effects relay on the free radicals formation during autoxidation of the acid (Tajkarimi, 2011), and on the low pH, that makes it difficult for bacteria to survive as it alters some metabolic cycles (Angós, 2008). GT extract consists mainly of phenolic compounds, which basically interact with membrane-dependent processes (cell signaling

cycle, arachidonic acid metabolism, cell proliferation, apoptosis and mitochondrial functionality) (Taylor, 2005).

At D9 (**Figure 43**B), the sum of variability explained by PC1 and PC2 was 74.1 %. Groups were distributed similarly to D0. Gas concentration appeared to be a significant variable, and related with TAM and TAP counts. Indeed, a reduced O_2 pressure in the package acts as a reducer of metabolism rate of microorganisms (Zahra, 2016). As happened in D0, GT was positively related to a higher a* value, indicating a more brownish colour, given by the solution. Also, MV was characterized by high antioxidant values expressed by DPPH· inhibition, and higher content in dry matter, which was attributed to the organic acids present in the solution. High L* values also correlated with MV samples, which verified that MV could maintain potato slices brighter after the 9-day storage. As a general rule, the more O_2 concentration in contact with the surface of potatoes, the more polyphenols will be oxidized by PPO and converted into brownish molecules that will change the colour of the samples (Deng, 2018). However, some authors discussed the direct implication of the main components that are usually related to browning: PPO, POD, hydrogen peroxide, ascorbic acid content, and initial phenolics content as well as total and individual phenolics accumulation. According to Cantos (2002), none of these parameters per separate was able to fully explain the browning behavior of fresh-cut potatoes. Moreover, they suggested that to completely understand the possible limiting factors in the development of browning in fresh-cut potatoes, more studies involving other important aspects (lipid composition, calcium content, protease activity, agronomic practices, etc.) are required.

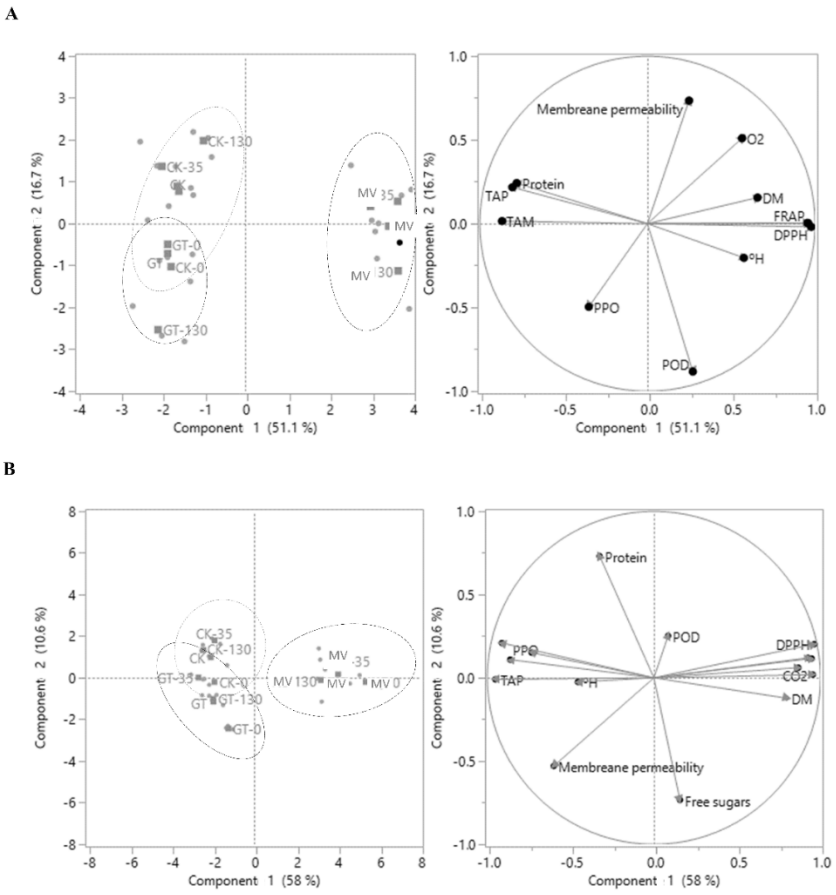

Figure 43. Principal component analysis of the relationship between variables studied, at day 0 (**A**) and day 9 (**B**).

4 Conclusions

In this study, two anti-browning solutions, a patented mix based on vitamin C (MV) and green tea (GT) extract, were evaluated to prevent browning and prolong the shelf-life of packaged fresh-cut potatoes stored at 4 ºC. Ultrasound was proposed as a technology to enhance the penetration of both solutions into the potato tissue. However, ultrasound did not exert a remarkable effect in any of the treatments in the conditions evaluated.

In *in vitro* assays, green tea presented potential to prevent enzymatic browning and oxidation. However, it was not that effective when used *in vivo* in potatoes immersed in a 5% GT solution under the conditions established. In fact, surface darkening occurred in the early stages of the storage because the solution had some colour and no significant improvement, such as microbial counts, antioxidant activity, or reducing sugars, was observed when compared to the control. Nevertheless, green tea extract has been reported to have a potential in increasing shelf-life of fresh-cut fruits and vegetables, so other conditions, such as different packaging techniques or the application of modified atmospheres could be studied.

Conversely, the visual quality of potatoes immersed in MV was acceptable after 9 days of storage, and no browning had developed. Antioxidant capacity and total phenolic content was higher in MV samples than it was in the control samples. Gas composition of samples immersed in MV solution should be further studied in order to ascertain the causes of the rapid gas exchange observed. Furthermore, this treatment was able to exert a higher control on the psychrophilic microorganisms, which otherwise, could grow at 4 ºC. Overall, NatureSeal® proved to be a good product to prevent sliced potato deterioration.

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Conflict of interests

The authors declare no conflict of interests.

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Antioxidant and antimicrobial activities of ginseng extract, ferulic acid and noni juice, in the evaluation of their potential to be incorporated in food.

Chapter 8

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Ginseng extract (GE), ferulic acid (FA), and a fermented noni juice powder (FNJP), were investigated for their antioxidant and antimicrobial activities *in vitro*. Half inhibitory concentration (IC_{50}) was 29.87, 0.45 and 3.82 mg / mL, for GE, FA, and FNJP, respectively. The capacity of the three extracts to inhibit polyphenol oxidase from three vegetable matrices, and to delay lipid peroxidation of three fats was also evaluated. The extracts' antimicrobial activity was studied on thirteen bacterial strains using the disk diffusion assay and the microdilution assay. Minimal inhibitory concentration (MIC) values determined with the latter included 5.5 mg / mL of GE for *Listeria monocytogenes*, 1.7 mg / mL of FA for *Staphylococcus aureus, L. monocytogenes* 1/2 and 4b, and 4.2 mg / mL of FNJP for *Bacillus cereus*. The changes in lag phase, growth rate and asymptotic value of the bacteria growing under different concentrations of the three compounds were described. The results obtained suggest the potential of GE, FA and FNJP for its further application in food industries

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Growth modelisation, microorganism, lipid peroxidation, natural source, bacteriostatic, bactericide

1 Introduction

Food quality and safety maintenance during shelf-life is a major concern for both producers and consumers. In order to extend shelf-life and facilitate access to low-cost and palatable foods, the food industry has relied on the use of fats, sugars, and chemical aids for decades. However, despite chemical additives being safe in commercial doses, consumers tend to demand more natural alternatives. Plants are excellent sources of active molecules, possessing antioxidant and/or antimicrobial properties (Negi, 2012). Plant-derived extracts, including essential oils or volatile compounds, are used in many areas of the food industry to prevent microbial growth and undesirable quality changes during storage (Nikmaram, 2018; Olatunde, 2018).

In a search for more sustainable and natural options, alternative plant-based extracts are being explored to answer consumer requests. There are still plenty of plant-derived or plant by-products that must be investigated in order to develop potential and functional ingredients, or additives for food products. High-value plants such as ginseng (*Panax ginseng* L.) and its derived compounds have been extensively described for their health-promoting properties (Kim, 2017). According to the European Food Safety Authority (EFSA), ginseng is allowed to be used as a herbal medicinal product, to combat fatigue and asthenia and/or "strengthen the human body, supply of lacking energy, and positive life force, antioxidant" (Comittee on Herbal Medicinal Products, 2013; EFSA, 2008). Bacteriostatic and bactericidal effects have also been reported by some authors (Kachur, 2016). The antimicrobial bioactivities were mainly attributed to ginsenosides, which are about thirty different saponin-type, triterpenoid glycosides (Santangelo, 2019). These compounds, which are part of the defence mechanism of the plant, also give the pharmacological properties attributed to ginseng: modulating blood pressure, metabolism, and inflammatory and immune functions (Leung, 2010).

Other promising plant-based organic compounds include ferulic acid ([E]-3-[4-hydroxy-3-methoxy-phenyl] prop-2-enoic acid; FA) as an ubiquitous phenolic acid present in plant tissues (Mattila, 2002). FA is approved as a food additive in Japan, where it can be used as an antioxidant, while natural extracts with high contents of FA are permitted in the US and most European countries to prevent lipid peroxidation of foods (Quitmann, 2014). Its antimicrobial properties have also been explored against the pathogenic bacteria *Escherichia coli* and *Salmonella* Typhimurium *in vitro* (Pacheco-Ordaz, 2017) and in some ready-to-eat food (Takahashi, 2013).

Finally, novel plants are under exploration as novel sources of bioactive compounds with potential food applications. Noni plant (*Morinda citrifolia* L.) has received the status of a novel food ingredient, which is defined as food that had not been consumed to a significant degree by humans in the EU before 15 May 1997. This is when the first regulation on novel food came into force, with the approval of the European Union Novel Foods Regulation (Regulation (EC) No 2015/2283). The use of noni plant purée and concentrates is allowed in a number of foods as an ingredient. Its functional action is attributed to flavonoids and polyphenols present in the fruit (Gironés-Vilaplana, 2014). Its antioxidant, antimicrobial, and immune-enhancing properties have been reviewed by several authors (Abou, 2017; Almeida, 2019). Alternative food applications, including the use of noni fruit extract as sanitizer in washing steps for romaine lettuce, spinach, and kale, have demonstrated reductions of *L. monocytogenes* between 1.47-3.38 log CFU / g (Kang, 2019).

Considering the potential of the three matrices described previously, this study aims to contribute to this growing area of research by exploring the antioxidant and antimicrobial activities of ginseng extract (GE), pure *trans*-ferulic acid (FA), and fermented juice extract powder (FNJP). This study makes an original contribution to the current knowledge, including the lipid peroxidation prevention of three

different fats, and the polyphenol oxidase inhibition efficacy of the three compounds, tested *in vitro*. Moreover, to deepen the understanding of its antimicrobial effects, the changes in the growth parameters of 13 bacterial strains were evaluated. A thorough understanding of the possibilities of these plant-derived compounds would be useful in their further application in food.

2 Experimental

2.1 Materials

Commercial ginseng extract containing 1 % was purchased from EPSA (Torrent, España) and trans-ferulic acid (≥ 99% purity) was obtained from Sigma-Aldrich (ref. W518301, Steinheim, Germany). Noni juice extract was kindly provided by the University of Nayarit, Mexico, and was prepared as described by Ulloa, González-Tapia, Rosas-Ulloa, Ramírez-Ramírez, & Ulloa-Rangel (2015).

Scopoletin, ursolic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, K_2HPO_4 and KH_2PO_4, 2-polyvinyl pirrolidone (PVPP), cistein, pyrocatechol, guayacol and streptomycin were acquired from Sigma-Aldrich (Steinheim, Germany). Peroxide hydrogen, methanol, were procured from Panreac (Llinars del Vallès, Spain). Triptone soy broth (TSB) and Müeller-Hinton broth (MHB) were purchased from Biokar Diagnostics (Allonne, France).

2.2 Methods

2.2.1 Determination of scopoletin, ursolic acid and rutin in fermented noni juice powder

Concentrations of scopoletin, ursolic acid and rutin in freeze-dried fermented noni juice FNJP were determined by UPLC-MS, using Acquity UPLC-Xevo TQS (Waters) by the Scientific and Technical Service of Chromatographic Techniques and Mass Spectrometry (TCEM) of the University of Lleida. Briefly, 50 mg of freeze-dried material was diluted and filtered with a PTFE hydrophylic 0.22μm filter. Addition standard method was used to identify and quantify the compounds. UPLC was performed using Acquity UPLC ® HSS T3 1.8 μm, 100 x 2.1 mm column, injecting 2.5 μL of sample at 10 ºC, in an isotherm column at 30 ºC, with two mobile phases: (A) water, methanol and formic acid (1.5:98:0.5 v:v:v), and (B) methanol and formic acid (99.5:0.5 v:v) at 0.3 mL / min. They were performed in a gradient as follows: from 0 to 0.51 min 95 % A and 5 % B, from 0.51 to 3.50, up to 100 % B, from 3.51 to 5.50, at 100 % B, and finally, back at initial conditions for 8.00 min. Mass spectrometry was done with an ESI with negative ion mode, 2 kV capillarity, source and desolvation temperatures of 120 and 450 ºC, respectively. Cone and desolvation gas flow were 150 and 1000 L/h, respectively, and collision gas flow was 0.15 mL / min. Results were obtained by comparing the peaks on the chromatogram with the peaks produced by the added standards.

2.2.2 Antioxidant properties

2.2.2.1 Half maximal inhibitory concentration (IC_{50})

The compounds were serially diluted in distilled water in concentrations ranging from a stock solution (which, according to preliminary studies, corresponded to their maximum solubility in water) of 33.0 mg / mL GE, 10.0 mg / mL FA, and 100.0 mg / mL FNJP, to 0 mg / mL. Then, 0.1 mL of the diluted samples were added to 1.4 mL of 0.1 mM DPPH· solution. Methanol was used as a blank. After incubation at room temperature for 60 min in the dark, absorbance at 515 nm was read using a GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). For IC_{50} calculation, the percentage of inhibition calculated using Eq. 1 was plotted against extract

concentration. IC_{50} corresponds to the necessary concentration of an extract to achieve 50 % of inhibition (Eq. 2) (Kumawat, 2012),

$$\% I = [(A_b\text{-}A_s) / A_b] \cdot 100 \qquad \text{Eq. 1}$$

$$\% I = m \cdot C + n;\ IC_{50} = (50\text{-}n)/m \qquad \text{Eq 2.}$$

where % I is the inhibition in percentage, A_b and A_s are the absorbance of the blank and the sample, respectively. m is the slope of the lineal adjustment when representing % I in front of C (mg / mL), concentration, and n is the intercept.

2.2.2.2. Polyphenol oxidase (PPO) activity inhibition

To evaluate GE, FA and FNJP as natural inhibitors of enzymatic browning, determination of the enzyme inhibition was evaluated on different model systems containing PPO from different matrices, on apple, potato, and mushroom, following the method reported by (Bobo, 2014, Masuda 2015) with some modifications. PPO extraction was carried out by mixing 5.0 ± 0.5 g of frozen apple, potato, or mushroom with 0.5 g PVPP and 10 mL 0.1 M phosphate buffer solution pH 6 (PBS) with 0.05 mM cysteine in an Ultra-turrax® Tube drive P control (IKA, Staufen, Germany) for 1.5 min at 5,000 rpm. After filtration using a sterile cloth and centrifugation at 20,000 × *g* for 10 min at 4 ºC, the supernatant was kept in ice.

PPO inhibitory activity was assessed spectrophotometrically. For each compound, three concentrations were tested: 33.0, 25.0, and 16.5 mg / mL GE; 7.5, 5.0, and 2.5 mg / mL FA; and 100.0, 75.0, and 50.0 mg / mL FNJP. Briefly, 65 µL of PPO extract were incubated for 10 min with or without 65 µL of the solutions to be analysed. Then, 65 µL of 0.2 M pyrocatechol in PBS were added. After 10 min of incubation at 37 °C, absorbance was read at 400 nm. Inhibition was expressed as % of the PPO activity using Equation 3.

$$\% \text{ Inhibition} = [(A_0 - A_E) / A_0] \cdot 100 \qquad \text{Eq. 3}$$

where A_0 is the absorbance at 400 nm after enzymatic reaction alone, and A_E is the absorbance at 400 nm after the enzymatic reaction in presence of the extract studied.

2.2.2.3 Prevention of lipid peroxidation

The effect of GE, FA and FNJP in the prevention of lipid peroxidation on olive oil, sunflower oil and butter was evaluated by the Oxidation stability of oils and fats –Rancimat method, following the recommendations of the manufacturer (Rancimat, Metrohm). Samples were prepared immediately before the test by homogenizing olive oil, sunflower oil, or butter with 10 mg / mL of each compound, using an Ultra-Turrax T-25 homogenizer (IKA Works GmbH & Co, Staufen, Germany) operating at 14,000 rpm. Samples were tested per quadruplicate (n=4) in a Rancimat 743 apparatus for oils and fats (Metrohm, Germany), at a temperature of 110 °C and using a gas flow of 10 L/h. Induction time was calculated using Rancimat 743 software 1.1.

2.2.3 Antimicrobial effects

2.2.3.1 Strain and inoculum preparation for antimicrobial effect assays

The antimicrobial effect of each compound was tested against 13 strains (**Table 27**). Strains were grown for 22 ± 2 h in 50 mL of triptone soy broth (TSB), which was supplemented with 6 g / L of yeast extract, 2.5 g / L of glucose, and 2.5 g / L of K_2HPO_4 (TSBYE) for *Listeria monocytogenes* – at 37 ± 1 °C in a rotatory shaker set at 150 rpm.

Table 27. Bacterial strains used for the antimicrobial analyses.

Specie		Collection number
Listeria monocytogenes	4b	CECT[1]-935
Listeria monocytogenes	1/2 a	Isoleated in Lab
Listeria monocytogenes	1/2	CECT-4031
Salmonella enterica subsp. Enterica	Typhimurium	CECT-4594
Salmonella enterica subsp. Enterica	Agona	ATCC[2] BAA-707
Salmonella entérica subsp. Enterica	Montevideo	ATCC BAA-710
Salmonella enterica subsp. Enterica	Gaminara	ATCC BAA-711
Escherichia. coli (virulent factor deleted)	O157:H7	NCTC[3]-12900
Escherichia. coli		CECT-516
Staphylococcus. aureus		CECT-435
Bacillus cereus		CECT-131
Enterococcus faecalis		CECT-795
Enterobacter aerogenes		CECT-684

[1] Colección Española de Cultivos Tipo
[2] American Type Culture Collection
[3] National Collection of Type Cultures

2.2.3.2 Antimicrobial activity: Disk diffusion test

The disk diffusion test to investigate the susceptibility of bacteria to selected antimicrobials was performed to test serial 2-fold dilutions of the compounds, starting with stock solutions of 33.0, 20.0, and 100.0 mg / mL of GE, FA, and FNJP, respectively. Plates with a thin layer of TSB or TSBYE (for *L. monocytogenes* strains) were prepared in advance. Then, 5 mL of semi-solid TSB or TSBYE agar were prepared in glass tubes, where 50 μL of the inoculum, prepared as described in section 2.2.3.1 were added. After homogenization, the semi-solid agar was poured onto the plates to spread the microorganism over the entire surface. When it was solid, nine paper disks (6 mm diameter) per plate were placed separately on the agar. Then, 5 μL of the concentrations to study were discharged on each disk. Negative and positive controls were distilled water (no extract present) and streptomycin 1 mg/mL respectively. When more than 9 solutions were needed to be tested, two plates were used. Each extract and concentration was tested in triplicate (three plates). After 1 h at room temperature, plates were incubated at 37 ºC for 22 ± 2 h. The antimicrobial effect was stated when inhibition halos or zones with no microbial growth were observed.

2.2.3.3 Effect of extracts on the kinetic parameters of studied strains and determination of the minimal inhibitory concentration (MIC)

The MIC of the different extracts for each strain was tested using the microdilution method (CLSI, 2012). The inoculum of the 13 tested microorganisms was prepared as described in section 2.2.3.1 and diluted to 7.5×10^5 CFU / mL in Mueller-Hinton Broth Cation Adjusted (MHB-CA) with 25 mg / L Ca^{2+} and 12.5 mg / L Mg^{2+}. A stock solution that contained 33.0, 10.0, and 100.0 mg / mL, of GIN, FA, and FNJP, respectively, was prepared in sterile water under sterile conditions. From this, 5 more concentrations were prepared by making 2-fold serial dilutions. Then, 100 μL of the inoculum was poured into each microplate well, containing 50 μL of each compound at the prepared concentrations. Negative and positive controls were distilled water (no extract present) and streptomycin 1000 ppm respectively. A blank for each concentration, consisting of MHB-CA without inoculum, but with the corresponding concentration of the compound, was set in order to correct the compounds' color basis. The plate was incubated for 48 h at 37 ºC in a PowerWave HT (Biotek, Vermont, United States). Absorbance at 620 nm was read every 30 min (Andrews, 2001).

For the bacterial growth experiment, primary models were fitted using the DMFit 3.5 Excel add-in provided by ComBase predictive modelling tool (https://www.combase.cc) and growth parameters

(lag time, growth rate, and maximum optical density) were determined using the re-parameterized Gompertz model described by Zwietering *et al.* (1990) based on Equation 4.

$$y = A \exp\left\{- exp\left(\frac{\mu \max e}{A}(\lambda - t) + 1\right)\right\} \quad \text{Eq. 4}$$

where y represents the absorbance at time t, μ_m is the maximum growth rate (OD × 10^3 / min), t is the incubation time (min), λ corresponds to lag time (min), and A is the asymptotic value (or maximum growth, OD units).

To determine the minimum inhibitory concentration (MIC) of the three compounds, the value recorded was the lowest concentration of the agent that completely inhibited the growth of each bacterial strain studied (EUCAST, 2003).

2.3 Statistical analysis

Results were expressed as mean ± standard deviation (SD). All data was checked for significant differences by applying the analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analysis was carried out using JMP 13 (SAS Institute Inc., Cary, USA).

3 Results and discussion

3.1 Antioxidant properties

3.1.1 Half maximal inhibitory concentration (IC_{50})

Ginseng extract ginsenosides of 1 % showed an IC_{50} value of 29.87 mg / mL. The results of the antioxidant capacity of GE, reported so far, are contradictory. Indeed, Kim (2002) observed that 2.0 mg / mL of a red ginseng extract completely inhibited DPPH· radical. Results were comparable to those reported by Kitts (2000), which showed a powerful antioxidant activity of a North American ginseng extract. In turn, Jung (2006) reported an IC_{50} of a wild ginseng extract in water or methanol of around 30 mg / mL, which is in line with our results. Ginseng extracts have been tested on some food products for their antioxidant properties. For instance, 2 % red ginseng extract was added to milk and yoghurt in order to increase their antioxidant capacities by a combined action of the ginsenosides and phenolic compounds that are present in the extract (Park, 2018). Moreover, Kim (2019) added 0.5 and 1.0 % of red ginseng extract to cheese. Although the authors reported color and texture changes, the antioxidant capacity of the cheese increased, and the acceptance of the product was not affected. The evidence of the studies on the effects of ginseng on the human body suggests that the ingestion of ginseng extracts with food, could not only be advantageous for the properties and shelf-life of the food itself, but also for the consumer's health. As an example, ginsenoside Rg3 acted as a mechanism for antiaging in human dermal fibroblasts (Lee, 2018), and ginsenoside Rg1 promoted the activity of antioxidant proteins by reducing reactive oxygen species (ROS) and delaying apoptosis (Gao, 2019).

FA showed an IC_{50} value of 0.45 mg / mL, which was the lowest of the three extracts studied. FA has repeatedly been reported as a powerful antioxidant. It is important to highlight that the FA studied in the current paper was chemically synthesized and its purity was of an analytical grade. Its action mode is primarily related to scavenging of free radicals by combining with reactive molecules. This makes the initiation of the complex cascade reaction that leads to the generation of further free radicals difficult (Rice-Evans, 1996). This compound may also act as a hydrogen donor, giving atoms directly to the radicals (Zduńska, 2018). Actually, that is the main reason why FA has been used for cholesterol control, prevention against thrombosis and atherosclerosis, anti-inflammatory effects, cancer, and as an anti-ageing agent (Kumar, 2014; Ou, 2004).

The characterisation of FNJP showed an IC_{50} value of 3.82 mg / mL. The antioxidant activity of this extract is mainly related to the active biomolecules of the fruit, such as phenolic acids, flavonoids, phytoestrogens, and vitamin C (Chang, 2018). Values of pH and total soluble solids of FNJP were 3.16 ± 0.05 and 5.5 g / L, respectively. In addition, concentrations of 333.5 μg / g scopoletin and 346.5 μg / g of rutin were also determined by UPLC-MS, which have been reported as the main bioactive compounds present in noni fruit (Almeida, 2019). Gironés-Vilaplana et al. (2014) reported higher IC_{50} values (25 mg / mL) than those obtained in the present study using DPPH· assay. This could be attributed to the differences in phenolic contents related to maturity or origin of the fruit. This not only affects the juice yield but also the total quantities of phenolic compounds, condensed tannins, flavonoids and scopoletin (Assanga, 2014), or the fermentation conditions of the juice: longer times lead to lower antioxidant content in the final product (Yang, 2007). As assayed *in vitro* in cells, noni juice has shown that its antioxidant capacity may prevent cancer incidence, as it decreases intracellular ROS generation and mitochondrial membrane potential in breast cancer cell lines (Sharma, 2015). The studies also report a decrease in the level of lipid peroxidation and an increase in catalase activity in cervical cancer cell lines (Gupta, 2013).

This section has reviewed the antioxidant capacities of the three studied compounds, showing their potential for being used as valuable natural additives to prevent oxidation and to increase the shelf-life of food.

3.1.1 Polyphenol oxidase (PPO) activity inhibition

PPO catalyzes two type of reactions, hydroxylation of monophenols to diphenols, which results in colorless products, and oxidation of diphenols to quinines, which gives colored products (Ioannou, 2013). Finding ways to inhibit such reactions is relevant to food processors, because this enzyme has been directly related to browning reactions in fruits and vegetables that decrease consumer acceptance (Sulaiman, 2015). The percentage of inhibition of the PPO activity in potato, apple, or mushroom is shown in **Table 28**. FA could only be studied at doses lower than 7.5 mg/mL, because it was not possible to read its absorbance at 400 nm at higher concentrations.

Table 28. Inhibition of potato-, apple- or mushroom-derived polyphenol oxidase (PPO) activity, caused by ginseng extract (GE), ferulic acid (FA), and lyophilized of a spontaneously fermented noni juice (FNJP). Different letters indicate significant differences ($p < 0.05$) among extract tested concentration according to a Tukkey's Honest Significant Difference test

Extract	Concentration (mg/mL)	Inhibition of potato PPO (%)	Inhibition of apple PPO (%)	Inhibition of mushroom PPO (%)
GE	33.0	No inhibition	33.9 ± 2.5 [a]	No inhibition
	25.0	No inhibition	24.2 ± 2.6 [b]	No inhibition
	16.5	No inhibition	16.3 ± 1.9 [c]	No inhibition
FA	7.5	37.8 ± 1.0 [a]	41.7 ± 1.4 [a]	73.6 ± 4.29 [a]
	5.0	35.8 ± 1.4 [a]	35.3 ± 9.9 [b]	42.9 ± 0.05 [b]
	2.5	36.3 ± 7.4 [a]	21.2 ± 1.9 [b]	39.4 ± 1.07 [b]
FNJP	100.0	86.3 ± 7.7 [a]	71.7 ± 6.3 [a]	89.5 ± 7.6 [a]
	75.0	78.5 ± 1.0 [a]	86.3 ± 5.8 [ab]	74.0 ± 0.4 [a]
	50.0	86.7 ± 5.9 [a]	95.1 ± 1.3 [b]	59.1 ± 2.4 [a]

There is a lack of information about the effect that ginseng extract or ginsenosides can exert on PPO. In this study, GE did not show inhibition of potato and mushroom derived PPO. An increase in GE concentration may conceivably trigger the inhibition effect on the PPO obtained from these matrices. For this reason, and to increase solubility of GE, other suitable solvents should be tried. Contrarily, when testing 33 mg / mL of GE with apple-PPO, a 33.9 ± 2.5 % inhibition of the enzymatic activity was observed. The mechanisms of this inhibitory effect have not yet been described. To have a better understanding, it would be advisable to assess other matrices and the kinetics of this interaction.

Inhibition of PPO attributed to FA was matrix-dependent, and it has been stated that it can also be variety-dependent (Liao, 2020). Higher inhibitory activities were observed for the enzymes extracted from mushroom, obtaining a decrease in PPO activity of 73.6 ± 4.29 % at a concentration of 7.5 mg / mL. For potato and apple derived PPO, the same concentration showed a 37.8 ± 1.0 and 41.7 ± 1.4, respectively. In the case of FA, a higher inhibition was observed at higher doses. (1967) suggested that FA acted as a competitive inhibitor of apple-derived PPO, preventing the substrate from binding to the enzyme by occupying its place in the active site. Nirmal (2009) added that hydroxyl group of FA also had a role in the decrease in the activity of PPO, by its electron donating to intermediate quinone. The results obtained show that FA has potential to be used in a food matrix to inhibit enzymatic browning. Nevertheless, interactions between FA, PPO, and other food components must be taken into account because they can determine the concentration and the effect of FA in food. As reported by Sukhonthara (2016), concentrations of 390 mg / L only reached a decrease of 15 % in PPO activities

when added to potato and apple purees, indicating that higher amounts of FA were needed to prevent enzymatic browning. FA was also used in Chinese water chest-nut to prevent yellowing, inhibiting not only PPO, but also preventing the increase of other compounds naturally present in chest-nut that contribute to its yellowing process, namely eriodyctiol and naringenin (Song, 2019). In addition, Liao (2020) showed that there was no direct relationship between the inhibition of browning in pear puree and the inhibition of PPO.

FNJP reduced the PPO activity from the three matrices (Table 2). Maximum inhibition was observed in PPO from mushroom, being 89.5 ± 7.6 % when FNJP was used at 100 mg/mL. Percentage of inhibition of potato and apple PPO was not concentration-dependent. A saturation of the inhibitory effect of FNJP on PPO could explain this independence. The mechanisms underlying the decrease in PPO activity exerted by NJE are not yet described in the literature. A likely explanation is that noni fruit possesses plenty of antioxidant compounds that may promote this effect (Almeida, 2019). Nevertheless, there is a need for a deeper understanding on the role played by its characteristic active compounds, such as scopoletin or rutin.

In this study, FA and FNJP are revealed to be effective in inhibiting a remarkable percentage of PPO activity. However, in vitro studies give an idea of the potential of the compounds to be used in food matrices, and browning reactions are not only PPO dependent. For this reason, for certain foods, specific experiments must be carried out prior to application.

3.1.2 Lipid peroxidation prevention

None of the extracts were able to delay the oxidation of sunflower oil or butter at the tested concentration (**Table 29**). When tested in olive oil, only FA increased the induction time to 37.57 ± 4.86 h, compared to the control (with no extract) which had an induction time of 15.13 ± 0.09 h. The effectiveness of FA might be explained by its chemical structure: its hydroxyl group can provide protons and inhibit the formation of free radicals, delaying the rate of oxidation. In fact, FA has been reported to have protective effects in linseed oil (Kyselka, 2017) and soybean oil (Luo, 2012). In this study, GE did not reveal any effect on the lipid peroxidation of the tested matrices. However, some authors reported a decrease in lipid peroxidation of up to 90 % when adding 0.5 to 2 % of red ginseng extract to milk (v:v) (Jung, 2020). Regarding FNJP, although no effect was observed in our study at the concentration used, its puree was used at concentrations ranging from 2 to 6 % in beef patties, which showed a delay in their lipid peroxidation in time, when compared to the control samples (Tapp, 2012).

Table 29. Effect of **10 mg/mL** of ginseng extract (GE), ferulic acid (FA) and fermented noni juice powder (FNJP) on the peroxidation of three different fats, expressed as induction time (h). Values are the mean ± standard deviation of 4 reps. Different letters indicate significant differences ($p < 0.05$) among extract tested according to a Tukkey's Honest Significant Difference test

Extract	Sunflower oil	Olive oil	Butter
-	2.42 ± 0.18 ^{a}	15.13 ± 0.09 ^{b}	1.46 ± 0.31 ^{ab}
GE	2.43 ± 0.08 ^{a}	14.76 ± 0.13 ^{b}	1.76 ± 0.10 ^{a}
FA	2.10 ± 0.91^{a}	37.57 ± 4.86 ^{a}	1.50 ± 0.31 ^{ab}
FNJP	1.81 ± 0.19 ^{a}	14.31 ± 0.18 ^{b}	1.11 ± 0.83 ^{b}

3.2 Antimicrobial effect of GE, AF and LFNJ

3.2.1 Disk diffusion test

None of the extracts cause inhibition halos in any of the tested strains (Data not shown). As will be described in section 3.2.2., the microdilution method showed growth inhibition, so the methodology used for the determination of antimicrobial activity is crucial. In other studies, the microdilution method was also more sensitive than the disk diffusion was (Scorzoni, 2007). Disk diffusion methods cannot be used to determine MICs, because the amount of the substance that is diffused in the agar is unknown, so it is not possible to relate the inhibition halo with a determined inhibitory concentration (Balouiri, 2016). These methods may serve as a screening method to ascertain whether the studied compounds have antimicrobial activity or not. The absence of an inhibitory effect in our study could be explained by the low diffusion of the diluted substances in the agar, or to the higher difficulty that the active compounds encounter in order to be in contact with the microorganism when compared to a broth dilution method, in which this contact is direct (Rios, 1988).

3.2.2 Effect of studied substances on the kinetic parameters of studied strains and determination of the minimal inhibitory concentration (MIC)

The growth curves of the microorganisms grown under the presence of different concentrations of the compounds were adjusted to the 3-parametric Gompertz equation, which has been proved to be a good mathematical model to describe biological parameters of microorganism growth (Pla, 2015). In the present study, the coefficient of determination (R^2) was always higher than 0.800 and averaged 0.988. Kinetic growth parameters of studied microorganisms in relation to the presence of GE in the growth medium are shown in **Table 30**. GE had the greatest impact on *L. monocytogenes* serovar 1/2a, in which lag phase (λ) was 3-fold longer at 2.7 mg / mL, and MIC value (**Table 33**) was 5.5 mg / mL GE, when its growth was completely inhibited. In contrast, *L. monocytogenes* serovars 1/2 and 4b lag phase was affected only at the highest GE concentrations. These also slightly decreased the maximum growth rate (μ) and asymptotic value (A). Norajit (2012) suggested that ginsenosides may induce the lysis of *L. monocytogenes*. The other Gram-positive bacteria studied, *S. aureus* or *E. faecalis,* were not significantly affected by GE. In most of the works published, GE was used as a compound that, when ingested or taken as a medical treatment, is able to diminish some of the virulent mode of action of the microorganisms. These include its ability to attach to the human gut cell, or its effect of inhibiting cytokines and chemoquines responsible for the inflammation when the body is infected by bacteria (Iqbal, 2020; Szczuka, 2019). In this work, the assessment of the compounds to inhibit the growth of microorganisms in a possible direct food application is taken into account. Even though none of the tested strains of *S. enterica* were affected by GE, growth parameters of *B. cereus* were modified when GE was used at a concentration of 11.0 mg / mL. *E. aerogenes* and toxigenic *E. coli* respective lag phases (λ) were also longer when applying GE at 1.4 mg/mL, when compared to the control. Pina-Pérez (2018) investigated the effect that different heat treatments applied to GE had on the viability of bacteria. They reported that microwaved GE had a greater effect on microorganism growth than non-heated GE when using concentrations ranging from 10 to 100 mg / mL, especially on *E. coli* O157:H7, followed by *Cronobacter sakazakii*. Other authors also reported that heating GE to 100 °C for 2 or 16 h was related to an increase of its antimicrobial activity against *S. aureus* and *B. cereus* (Na, 2017). To date, the applications of ginseng extract or its derivatives as antimicrobial agents for food preservation are scarce. Norajit (2012) developed an alginate coating with ginseng extract, which was tested against *Staphylococcus epidermidis, Bacillus subtilis* and *L. monocytogenes*. The results suggested that the incorporation of ginseng extracts into edible films could be used to control food pathogens and improve shelf life in food systems.

Table 30. Effecs of ginseng extract (GE) on lag time (λ, min), maximum growth rate (μ, ΔD· 10^3/s), and asymptotic value (A, optical density) of the modelized growth of foodborne bacterial strains. For each strain and kinetic parameter, different letters indicate significant differences ($p < 0.05$) among extract tested concentration according to a Tukkey's Honest Significant Difference test.

Microorganism	Strain	P	Control	0.7 mg/mL	1.4 mg/mL	2.7 mg/mL	5.5 mg/mL	11.0 mg/mL
***Listeria monocytogenes* 4b**	CECT -935	λ	283.6 ± 3.7 ^{a}	248.6 ± 12.3 ^{ab}	241.4 ± 8.9 ^{b}	253.0 ± 23.1 ^{b}	269.8 ± 3.7 ^{ab}	236.4 ± 6.2 ^{b}
		μ	0.66 ± 0.02 ^{a}	0.60 ± 0.05 ^{ab}	0.58 ± 0.03 ^{abc}	0.51 ± 0.04 ^{bc}	0.48 ± 0.02 ^{cd}	0.41 ± 0.01 ^{d}
		A	0.18 ± 0.00 ^{ab}	0.20 ± 0.00 ^{a}	0.19 ± 0.01 ^{a}	0.19 ± 0.02 ^{a}	0.15 ± 0.02 ^{bc}	0.14 ± 0.00 ^{c}
Listeria monocytogenes 1/2 a	Lab	λ	422.4 ± 33.8 ^{a}	530.7 ± 187.7 ^{a}	633.4 ± 167.5 ^{a}	1261.5 ± 510.5 ^{b}	c.i.	c.i.
		μ	0.46 ± 0.08 ^{a}	0.28 ± 0.14 ^{a}	0.28 ± 0.25 ^{a}	0.46 ± 0.01 ^{a}	c.i.	c.i.
		A	0.17 ± 0.02 ^{a}	0.16 ± 0.05 ^{a}	0.14 ± 0.01 ^{a}	0.08 ± 0.09 ^{a}	c.i.	c.i.
Listeria monocytogenes 1/2	CECT -4031	λ	337.1 ± 10.1 ^{ab}	336.3 ± 8.8 ^{ab}	315.0 ± 17.2 ^{abc}	291.1 ± 4.6 ^{bc}	285.9 ± 8.3 ^{c}	253.7 ± 25.2 ^{c}
		μ	0.99 ± 0.06 ^{a}	0.97 ± 0.15 ^{a}	0.83 ± 0.07 ^{ab}	0.75 ± 0.06 ^{ab}	0.65 ± 0.09 ^{b}	0.42 ± 0.12 ^{c}
		A	0.22 ± 0.02 ^{a}	0.25 ± 0.03 ^{a}	0.23 ± 0.03 ^{a}	0.22 ± 0.01 ^{a}	0.20 ± 0.02 ^{a}	0.20 ± 0.03 ^{a}
***Salmonella enterica* subsp. Enterica Typhimurium**	CECT -4594	λ	30.0 ± 0.1 ^{a}	29.7 ± 2.1 ^{a}	32.7 ± 8.0 ^{a}	37.2 ± 7.0	35.0 ± 2.1 ^{a}	34.7 ± 4.6 ^{a}
		μ	0.96 ± 0.05 ^{a}	0.76 ± 0.5 ^{a}	1.10 ± 0.7 ^{a}	1.37 ± 0.3 ^{a}	1.32 ± 0.05 ^{a}	1.37 ± 0.13 ^{a}
		A	0.29 ± 0.09 ^{a}	0.21 ± 0.02 ^{a}	0.25 ± 0.06 ^{a}	0.23 ± 0.04 ^{a}	0.20 ± 0.02 ^{a}	0.23 ± 0.01 ^{a}
***Salmonella enterica* subsp. Enterica Agona**	ATCC BAA-707	λ	171.2 ± 5.2 ^{a}	161.0 ± 4.3 ^{a}	159.6 ± 8.3 ^{a}	164.7 ± 2.9 ^{a}	164.6 ± 2.3 ^{a}	162.8 ± 3.13 ^{a}
		μ	1.13 ± 0.08 ^{ab}	1.28 ± 0.12 ^{a}	1.09 ± 0.12 ^{ab}	1.06 ± 0.08 ^{ab}	1.00 ± 0.03 ^{b}	1.01 ± 0.03 ^{b}
		A	0.19 ± 0.00 ^{a}	0.19 ± 0.01^{a}	0.19 ± 0.01 ^{a}	0.17 ± 0.01 ^{b}	0.17 ± 0.01 ^{b}	0.18 ± 0.01 ^{ab}
***Salmonella entérica* subsp. Enterica Montevideo**	ATCC BAA-710	λ	120.7 ± 17.8 ^{a}	137.2 ± 3.5 ^{a}	141.8 ± 6.4 ^{a}	130.3 ± 1.2 ^{a}	131.0 ± 23.5 ^{a}	148.3 ± 2.0 ^{a}
		μ	0.90 ± 0.03 ^{a}	0.94 ± 0.06 ^{a}	0.99 ± 0.05 ^{a}	0.94 ± 0.02 ^{a}	0.96 ± 0.04 ^{a}	0.96 ± 0.04 ^{a}
		A	0.22 ± 0.01 ^{a}	0.20 ± 0.01 ^{a}	0.19 ± 0.01 ^{a}	0.18 ± 0.01 ^{a}	0.19 ± 0.04 ^{a}	0.18 ± 0.01 ^{a}
***Salmonella enterica* subsp. Enterica Gaminara**	ATCC BAA-711	λ	169.0 ± 9.2 ^{a}	165.5 ± 2.6 ^{a}	162.5 ± 0.3 ^{a}	172.1 ± 10.8 ^{a}	177.5 ± 8.1 ^{a}	177.3 ± 7.4 ^{a}
		μ	1.75 ± 0.53 ^{a}	1.48 ± 0.38 ^{a}	1.32 ± 0.14 ^{a}	1.55 ± 0.15 ^{a}	1.68 ± 0.13 ^{a}	1.49 ± 0.15 ^{a}
		A	0.27 ± 0.08 ^{a}	0.26 ± 0.03 ^{a}	0.25 ± 0.02 ^{a}	0.27 ± 0.03 ^{a}	0.24 ± 0.01 ^{a}	0.24 ± 0.02 ^{a}
***Escherichia coli* (virulent factor deleted)**	NCTC -12900	λ	131.8 ± 7.2 ^{a}	130.2 ± 7.1 ^{a}	112.8 ± 1.9 ^{bc}	115.9 ± 6.1 ^{c}	122.1 ± 2.5 ^{ab}	130. ± 0.0 ^{ab}
		μ	2.46 ± 0.83 ^{a}	1.39 ± 0.05 ^{b}	1.41 ± 0.12 ^{b}	1.46 ± 0.14 ^{b}	1.36 ± 0.10 ^{b}	1.34 ± 0.02 ^{b}
		A	0.42 ± 0.02 ^{a}	0.49 ± 0.04 ^{a}	0.42 ± 0.03 ^{a}	0.42 ± 0.06 ^{a}	0.43 ± 0.04 ^{a}	0.31 ± 0.00 ^{a}
Escherichia coli	CECT -516	λ	61.1 ± 9.7 ^{a}	106.6 ± 4.5 ^{ab}	112.0 ± 5.9 ^{b}	110.5 ± 1.4 ^{b}	113.1 ± 1.3 ^{b}	111.9 ± 4.0 ^{b}
		μ	1.42 ± 0.14 ^{a}	0.71 ± 0.01 ^{d}	1.02 ± 0.02 ^{bc}	1.23 ± 0.12 ^{ab}	0.95 ± 0.10 ^{cd}	0.90 ± 0.06 ^{cd}
		A	0.31 ± 0.02 ^{a}	0.23 ± 0.01 ^{b}	0.25 ± 0.01 ^{b}	0.27 ± 0.04 ^{ab}	0.23 ± 0.03 ^{b}	0.20 ± 0.01 ^{b}
Staphylococcus aureus	CECT - 435	λ	413.9 ± 27.3 ^{a}	404.3 ± 7.0 ^{ab}	377.2 ± 3.0 ^{bc}	426.9 ± 3.0 ^{a}	423.1 ± 8.1 ^{a}	359.0 ± 3.5 ^{c}
		μ	0.55 ± 0.1 ^{a}	0.59 ± 0.03 ^{a}	0.53 ± 0.04 ^{a}	0.59 ± 0.07 a	0.55 ± 0.05 ^{a}	0.44 ± 0.01 ^{a}
		A	0.18 ± 0.02 ^{a}	0.17 ± 0.01 ^{ab}	0.15 ± 0.01 ^{ab}	0.15 ± 0.01 ^{ab}	0.14 ± 0.01 ^{b}	0.15 ± 0.01 ^{ab}
Bacillus cereus	CECT -131	λ	64.6 ± 1.8 ^{b}	59.5 ± 8.7 ^{b}	69.1 ± 5.6 ^{b}	80.1 ± 2.2 ^{b}	83.6 ± 15.5 ^{b}	155.0 ± 26.0 ^{a}
		μ	1.03 ± 0.30 ^{ab}	0.89 ± 0.24 ^{ab}	1.12 ± 0.04 ^{a}	1.14 ± 0.04 ^{a}	1.03 ± 0.12 ^{ab}	0.59 ± 0.10 ^{b}
		A	0.22 ± 0.01 ^{a}	0.23 ± 0.02 ^{a}	0.21 ± 0.01 ^{a}	0.21 ± 0.01 ^{a}	0.21 ± 0.01 ^{a}	0.15 ± 0.04 ^{b}
Enterococcus faecalis	CECT -795	λ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		μ	0.74 ± 0.06 ^{ab}	0.78 ± 0.09 ^{a}	0.80 ± 0.02 ^{a}	0.75 ± 0.01 ^{ab}	0.64 ± 0.01 ^{b}	0.64 ± 0.02 ^{b}
		A	0.22 ± 0.01 ^{a}	0.23 ± 0.01 ^{a}	0.22 ± 0.01 ^{a}	0.23 ± 0.01 ^{a}	0.23 ± 0.01 ^{a}	0.24 ± 0.03 ^{a}
Enterobacter aerogenes	CECT -684	λ	26.1 ± 15.4 ^{a}	25.0 ± 23.2 ^{a}	48.7 ± 1.5 ^{b}	49.0 ± 19.8 ^{b}	50.0 ± 16.4 ^{b}	60.0 ± 0.0 ^{b}
		μ	2.09 ± 0.23 ^{a}	2.11 ± 0.08 ^{a}	2.07 ± 0.08 ^{a}	2.05 ± 0.07 ^{a}	1.67 ± 0.09 ^{b}	1.40 ± 0.04 ^{b}
		A	0.97 ± 0.01 ^{a}	0.98 ± 0.06 ^{a}	1.02 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.88 ± 0.01 ^{b}	0.87 ± 0.03 ^{b}

c.i.: complete inhibition

Regarding FA (**Table 31**), *L. monocytogenes* 4b and 1/2 growth were significantly affected, as FA extended their lag time (λ), and decreased their maximum growth rate (μ) and asymptotic value (A) at concentrations of 0.8 mg / mL. These strains were completely inhibited with 1.7 mg / mL FA, (MIC

value, **Table 33**) and *L. monocytogenes* 1/2a MIC was 2.5 mg / mL FA. For other Gram-positive bacteria studied, *S. aureus* and *E. faecalis* at 1.7 mg / mL, or *B. cereus*, MIC value was higher, 3.3 mg / mL FA. FA also had an antimicrobial effect on Gram-negative bacteria, such as *E. aerogenes*, whose maximum growth rate (μ) and asymptotic value (A) were reduced significantly at concentrations ≥ 0.8 mg / mL FA. 3.3 mg / mL were needed to completely inhibit its growth (MIC value). Although FA had no effect on *S.* Typhiurium, MIC values for *S.* Montevideo and *S.* Gaminara were 2.5 mg / mL, and that of *S.* Agona was 3.3 mg / mL FA. Both strains of *E. coli* were also completely inhibited by 3.3 mg/mL FA. The MICs found in literature for *Salmonella* Typhi and *E. coli* were 20 mmol / L (3.9 mg / mL) (Pacheco-Ordaz, 2017). Pernin (2018) also tested the antimicrobial effect of FA against *L. monocytogenes* and reported that the MIC was 13.6 mmol / L (2.6 mg / mL). These values are similar to the MICs reported in the present study. Small differences could be explained by the existing differences linked to strain resistance to a certain compound, as has already been stated in the present study. When a number of phenolic compounds are studied, their mode of action consists of a combination of two mechanisms: the acidic dissociation and the intercalation in the phospholipid membrane of the bacteria. The effect of dissociation of the acid, causing the acidification of the cell cytoplasm, the efflux of K^+ ions and the eventual death of the microorganisms, is combined with the intercalation of the acid in the phospholipid layers of the membrane. This disturbs the Van der Waals interactions and inhibits the substrate transport of key enzymes (Pernin, 2019). Our study used a media, MHB-CA, whose pH is 7, but according to Miyague (2015), MIC values can decrease at lower pH values. For instance, they found that at pH 5, MIC was 2.5 mmol / L (0.5 mg / mL), while in contrast, it was 10 mmol / L (1.9 mg / mL) at pH 7. That could be explained by a combination of hurdle barriers against *L. monocytogenes* growth. The antimicrobial effect of FA has been studied in some food matrices by Takahashi (2013, 2015) who evaluated the effect of FA in smoked salmon, cheese and coleslaw at a concentration of 1.5 mg / mL in coleslaw and observed reductions ≥ 1.5 log CFU / g in the counts of *L. monocytogenes* after 5 days.

Table 31. Effecs of ferulic acid (FA) on lag time (λ, min), maximum growth rate (μ, $\Delta D \cdot 10^3$/s), and asymptotic value (A, optical density) of modelized growth of foodborne bacterial strains. For each strain and kinetic parameter, different letters indicate significant differences ($p < 0.05$) among extract tested concentration according to a Tukkey's Honest Significant Difference test

Microorganism	Strain	P	Control	0.2 mg/mL	0.4 mg/mL	0.8 mg/mL	1.7 mg/mL	2.5 mg/mL	3.3 mg/mL
***Listeria monocytogenes* 4b**	CECT - 935	λ	398.5 ± 2.8 ^{a}	335.9 ± 37.9 ^{a}	343.1 ± 37.8 ^{a}	473.9 ± 15.8 ^{b}	c.i.	c.i.	c.i.
		μ	1.03 ± 0.12 ^{a}	0.27 ± 0.06 ^{b}	0.20 ± 0.01 ^{bc}	0.08 ± 0.03 ^{c}	c.i.	c.i.	c.i.
		A	0.25 ± 0.01 ^{a}	0.13 ± 0.02 ^{b}	0.11 ± 0.01 ^{b}	0.06 ± 0.01 ^{c}	c.i.	c.i.	c.i.
Listeria monocytogenes 1/2 a	Lab	λ	356.4 ± 11.6 ^{a}	371.4 ± 48.5 ^{a}	372.1 ± 48.7 ^{a}	466.1 ± 87.6 ^{a}	492.1 ± 43.3 ^{a}	c.i.	c.i.
		μ	1.19 ± 0.19 ^{a}	0.53 ± 0.07 ^{b}	0.53 ± 0.07 ^{b}	0.61 ± 0.11 ^{b}	0.55 ± 0.20 ^{b}	c.i.	c.i.
		A	0.33 ± 0.02 ^{ab}	0.36 ± 0.04 ^{a}	0.36 ± 0.04 ^{a}	0.22 ± 0.08 ^{bc}	0.17 ± 0.01 ^{c}	c.i.	c.i.
Listeria monocytogenes 1/2	CECT-4031	λ	432.5 ± 4.3 ^{a}	446.6 ± 9.1 ^{a}	530.9 ± 11.5 ^{a}	1240.0 ± 143.0 ^{b}	c.i.	c.i.	c.i.
		μ	0.63 ± 0.06 ^{a}	0.30 ± 0.01 ^{b}	0.20 ± 0.01 ^{c}	0.03 ± 0.01 ^{d}	c.i.	c.i.	c.i.
		A	0.17 ± 0.01 ^{a}	0.11 ± 0.01 ^{b}	0.08 ± 0.01 ^{c}	0.03 ± 0.01 ^{d}	c.i.	c.i.	c.i.
***Salmonella enterica* subsp. Enterica Typhimurium**	CECT - 4594	λ	127.5 ± 8.3 ^{a}	113.4 ± 24.2 ^{a}	112.6 ± 29.1 ^{a}	127.9 ± 17.7 ^{a}	144.1 ± 3.9 ^{ab}	172.3 ± 29.8 ^{ab}	1932. ± 31.3 ^{b}
		μ	1.76 ± 0.50 ^{a}	1.32 ± 0.41 ^{a}	1.40 ± 0.90 ^{a}	1.46 ± 0.59 ^{a}	1.45 ± 0.62 ^{a}	1.17 ± 0.45 ^{a}	0.87 ± 0.23 ^{a}
		A	0.29 ± 0.05 ^{a}	0.29 ± 0.07 ^{a}	0.23 ± 0.07 ^{a}	0.21 ± 0.04 ^{a}	0.20 ± 0.04 ^{a}	0.17 ± 0.05 ^{a}	0.15 ± 0.05 ^{a}
***Salmonella enterica* subsp. Enterica Agona**	ATCC BAA-707	λ	181.8 ± 4.7 ^{a}	179.9 ± 5.6 ^{a}	155.2 ± 29.2 ^{a}	168.8 ± 5.5 ^{a}	197.3 ± 20.4 ^{a}	1129.88 ± 476.2 ^{b}	c.i.
		μ	1.16 ± 0.02 ^{a}	0.89 ± 0.19 ^{a}	0.84 ± 0.17 ^{ab}	0.69 ± 0.10 ^{ab}	0.57 ± 0.53 ^{ab}	0.20 ± 0.11 ^{b}	c.i.
		A	0.15 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}	0.11 ± 0.01 ^{a}	0.10 ± 0.01 ^{a}	0.10 ± 0.05 ^{a}	0.14 ± 0.04 ^{a}	c.i.

Microorganism	Strain	P	Control	0.2 mg/mL	0.4 mg/mL	0.8 mg/mL	1.7 mg/mL	2.5 mg/mL	3.3 mg/mL
Salmonella entérica **subsp. Enterica Montevideo**	ATCC BAA-710	λ	105.9 ± 5.1 ^{a}	141.8 ± 3.2 ^{a}	127.0 ± 6.7 ^{a}	149.9 ± 25.6 ^{a}	137.0 ± 40.3 ^{a}	c.i.	c.i.
		μ	0.80 ± 0.04 ^{a}	0.89 ± 0.03 ^{a}	0.76 ± 0.06 ^{a}	0.74 ± 0.03 ^{ab}	0.36 ± 0.03 ^{b}	c.i.	c.i.
		A	0.23 ± 0.01 ^{a}	0.14 ± 0.01 ^{b}	0.14 ± 0.01 ^{b}	0.13 ± 0.01 ^{bc}	0.12 ± 0.01 ^{c}	c.i.	c.i.
Salmonella enterica **subsp. Enterica Gaminara**	ATCC BAA-711	λ	165.5 ± 13.6 ^{a}	181.7 ± 6.3 ^{ab}	180.7 ± 4.1 ^{ab}	194.4 ± 1.6 ^{b}	182.5 ± 9.9 ^{ab}	c.i.	c.i.
		μ	1.38 ± 0.54 ^{a}	0.87 ± 0.10 ^{ab}	0.95 ± 0.04 ^{ab}	0.86 ± 0.24 ^{ab}	0.60 ± 0.02 ^{b}	c.i.	c.i.
		A	0.41 ± 0.13 ^{a}	0.20 ± 0.01 ^{b}	0.20 ± 0.01 ^{b}	0.17 ± 0.01 ^{b}	0.15 ± 0.01 ^{b}	c.i.	c.i.
Escherichia coli **(virulent factor deleted)**	NCTC - 12900	λ	141.5 ± 24.2 ^{a}	143.8 ± 0.8 ^{a}	131.7 ± 39.5 ^{a}	203.4 ± 3.0 ^{ab}	247.0 ± 21.1 ^{b}	254.3 ± 34.1 ^{b}	c.i.
		μ	2.19 ± 0.87 ^{a}	1.20 ± 0.02 ^{abc}	1.03 ± 0.29 ^{bc}	1.40 ± 0.05 ^{ab}	0.71 ± 0.07 ^{bc}	0.26 ± 0.04 ^{c}	c.i.
		A	0.43 ± 0.05 ^{b}	0.57 ± 0.05 ^{a}	0.34 ± 0.09 ^{bc}	0.39 ± 0.01 ^{b}	0.24 ± 0.03 ^{cd}	0.11 ± 0.03 ^{d}	c.i.
Escherichia coli	CECT - 516	λ	126.2 ± 0.3 ^{a}	112.3 ± 32.4 ^{a}	71.3 ± 13.7 ^{a}	95.5 ± 59.6 ^{a}	136.3 ± 10.0 ^{a}	143.4 ± 6.2 ^{a}	c.i.
		μ	2.26 ± 0.01 ^{a}	1.44 ± 0.14 ^{ab}	1.24 ± 0.11 ^{b}	1.60 ± 0.65 ^{ab}	1.97 ± 0.32 ^{ab}	1.44 ± 0.37 ^{ab}	c.i.
		A	0.32 ± 0.01 ^{a}	0.36 ± 0.01 ^{a}	0.34 ± 0.01 ^{a}	0.29 ± 0.04 ^{a}	0.21 ± 0.03 ^{a}	0.18 ± 0.04 ^{a}	c.i.
Staphylococcus aureus	CECT-435	λ	236.5 ± 2.2 ^{a}	236.6 ± 17.8 ^{a}	237.0 ± 18.2 ^{a}	328.9 ± 70.0 ^{b}	c.i.	c.i.	c.i.
		μ	1.50 ± 0.02 ^{a}	1.17 ± 0.15 ^{b}	0.91 ± 0.10 ^{b}	0.37 ± 0.01 ^{c}	c.i.	c.i.	c.i.
		A	0.43 ± 0.01 ^{a}	0.35 ± 0.01 ^{b}	0.29 ± 0.02 ^{c}	0.13 ± 0.05 ^{d}	c.i.	c.i.	c.i.
Bacillus cereus	CECT - 131	λ	263.8 ± 1.0 ^{a}	865.3 ± 100.6 ^{a}	1112.9 ± 183.8 ^{ab}	1178.8 ± 42.8 ^{ab}	1425.7 ± 289.9 ^{ab}	2183.8 ± 187.9 ^{b}	c.i.
		μ	0.48 ± 0.12 ^{a}	0.63 ± 0.35 ^{a}	0.77 ± 0.32 ^{a}	0.84 ± 0.39 ^{a}	0.45 ± 0.21 ^{a}	0.57 ± 0.13 ^{a}	c.i.
		A	0.74 ± 0.22 ^{a}	0.79 ± 0.20 ^{a}	0.81 ± 0.19 ^{a}	0.79 ± 0.27 ^{a}	0.63 ± 0.37 ^{a}	0.38 ± 0.13 ^{b}	c.i.
Enterococcus faecalis	CECT - 795	λ	761.4 ± 32.3 ^{a}	689.4 ± 102.7 ^{a}	502.3 ± 44.8 ^{a}	492.5 ± 177.2 ^{a}	c.i.	c.i.	c.i.
		μ	0.80 ± 0.10 ^{a}	0.43 ± 0.29 ^{a}	0.37 ± 0.10 ^{a}	0.44 ± 0.15 ^{a}	c.i.	c.i.	c.i.
		A	0.18 ± 0.03 ^{a}	0.19 ± 0.06 ^{a}	0.16 ± 0.01 ^{a}	0.12 ± 0.04 ^{a}	c.i.	c.i.	c.i.
Enterobacter aerogenes	CECT - 684	λ	185.0 ± 24.7 ^{a}	204.2 ± 19.1 ^{a}	182.9 ± 39.3 ^{a}	174.1 ± 14.8 ^{a}	154.3 ± 27.2 ^{a}	152.9 ± 46.5 ^{a}	c.i.
		μ	2.25 ± 0.59 ^{a}	2.08 ± 0.24 ^{ab}	1.66 ± 0.22 ^{ab}	1.37 ± 0.11 ^{bc}	0.69 ± 0.06 ^{c}	0.60 ± 0.13 ^{c}	c.i.
		A	0.64 ± 0.14 ^{a}	0.74 ± 0.07 ^{a}	0.65 ± 0.05 ^{a}	0.59 ± 0.06 ^{a}	0.18 ± 0.05 ^{b}	0.15 ± 0.05 ^{b}	c.i.

c.i.: complete inhibition

Finally, FNJP also showed antimicrobial activity against most of the pathogenic bacteria studied (**Table 32**). Concentrations of FNJP of 2.1 mg / mL led to a decrease in the maximum growth rate (μ) of *L. monocytogenes* 4b, 1/2a and 1/2, *S.* Typhimurium*, S.* Agona*,* and *B. cereus*. MIC value (**Table 33**) of all strains of *L. monocytogenes* and *S. Typhimurium* was 16.6 mg / mL FNJP. For the other serovars of *S. enterica*, Agona, Montevideo and Gaminara, 33.3 mg / mL were needed to completely inhibit their growth (MICs). The same concentration was the MIC found for *E. coli* CECT-516 and O157:H7, and *E. faecalis*. Lower concentrations of FNJP were needed to completely inhibit the growth of *B. cereus*, (4.1 mg / mL) and *S. aureus* (16.6 mg / mL). However, this compound was non-effective against *E. aerogenes* at the concentrations tested. Other authors have studied the effect of noni derivates on other species of *Staphylococcus*, but focusing only on the infective mechanisms of the bacteria, and not on their ability to grow (De La Cruz-Sánchez, 2019). In fact, noni is used in traditional medicine of some Asian countries for its antimicrobial properties, mostly attributed to its main coumarin, named scopoletin. As stated before, the FNJP used in this study had 333.5 μg / g scopoletin. It is suggested that this coumarin interacts with the membrane of microorganisms, destroying its integrity and increasing its permeability, leading to cell death (Yang, 2016). The antimicrobial effect could also be attributed to the low pH values of the extract, which are below the growth limits of most pathogens. Methanolic extracts of noni fruit have also shown *in vitro* antimicrobial activity against *Pseudomonas aeruginosa, Proteus morganii, S. aureus, B. subtilis, E. coli, Salmonella* spp.*,* and *Shigella* spp. (Rosyida, 2019). Noni extract has already been proposed for washing fresh-cut kale, lettuce, and spinach, with reductions of *L. monocytogenes* ATCC

19111 and 19115 ranging from 1.47 to 3.38 log CFU/g, depending on the roughness of the surface of the product (Kang, 2019).

Table 32. Effects of the fermented noni juice powder (FNJP) on lag time (λ, min). Maximum growth rate (μ, $\Delta D \cdot 10^3/s$). and asymptotic value (A, optical density) of the modelized growth of foodborne bacterial strains. For each strain and kinetic parameter, different letters indicate significant differences ($p < 0.05$) among extract tested concentration according to a Tukkey's Honest Significant Difference test.

Microorganism	Strain	P	Control	2.1 mg/mL	4.2mg/mL	8.3 mg/mL	16.7 mg/mL	33.3 mg/mL
***Listeria monocytogenes* 4b**	CECT -935	λ	283.6 ± 3.7 ^{b}	216.7 ± 8.0 ^{a}	213.4 ± 18.3 ^{a}	308.2 ± 23.6 ^{b}	c.i.	c.i.
		μ	0.66 ± 0.02 ^{a}	0.49 ± 0.06 ^{b}	0.46 ± 0.07 ^{b}	0.26 ± 0.04 ^{c}	c.i.	c.i.
		A	0.18 ± 0.00 ^{a}	0.17 ± 0.00 ^{a}	0.18 ± 0.02 ^{a}	0.09 ± 0.03 ^{b}	c.i.	c.i.
Listeria monocytogenes 1/2 a	Lab	λ	422.4 ± 33.8 ^{a}	487.0 ± 14.4 ^{a}	495.7 ± 15.2 ^{a}	737.4 ± 11.6 ^{b}	c.i.	c.i.
		μ	0.46 ± 0.08 ^{a}	0.29 ± 0.07 ^{b}	0.32 ± 0.03 ^{ab}	0.17 ± 0.01 ^{c}	c.i.	c.i.
		A	0.17 ± 0.02 ^{ab}	0.18 ± 0.03 ^{ab}	0.19 ± 0.01 ^{a}	0.13 ± 0.02 ^{b}	c.i.	c.i.
Listeria monocytogenes 1/2	CECT -4031	λ	337.1 ± 10.1 ^{a}	257.4 ± 25.4 ^{b}	281.9 ± 6.1 ^{b}	418.7 ± 14.8 ^{c}	c.i.	c.i.
		μ	0.99 ± 0.06 ^{a}	0.69 ± 0.14 ^{b}	0.61 ± 0.24 ^{b}	0.51 ± 0.13 ^{b}	c.i.	c.i.
		A	0.22 ± 0.02 ^{a}	0.27 ± 0.05 ^{b}	0.26 ± 0.05 ^{b}	0.18 ± 0.04 ^{b}	c.i.	c.i.
***Salmonella enterica* subsp. Enterica Typhimurium**	CECT -4594	λ	30.0 ± 0.1 ^{a}	30.0 ± 0.1 ^{a}	30.0 ± 0.1 ^{a}	38.5 ± 2.7 ^{b}	c.i.	c.i.
		μ	0.95 ± 0.05 ^{a}	0.6 ± 0.05 ^{b}	0.44 ± 0.01 ^{c}	0.20 ± 0.03 ^{d}	c.i.	c.i.
		A	0.29 ± 0.09 ^{a}	0.19 ± 0.09 ^{ab}	0.15 ± 0.01 ^{b}	0.09 ± 0.00 ^{b}	c.i.	c.i.
***Salmonella enterica* subsp. Enterica Agona**	ATCC BAA-707	λ	171.2 ± 5.2 ^{a}	156.2 ± 1.4 ^{a}	156.6 ± 4.1 ^{a}	233.4 ± 2.1 ^{a}	268.1 ± 14.4 ^{b}	c.i.
		μ	1.13 ± 0.08 ^{a}	0.88 ± 0.13 ^{b}	0.85 ± 0.01 ^{b}	0.69 ± 0.06 ^{b}	0.37 ± 0.03 ^{c}	c.i.
		A	0.19 ± 0.00 ^{a}	0.17 ± 0.01 ^{b}	0.15 ± 0.00 ^{c}	0.13 ± 0.00 ^{d}	0.06 ± 0.01 ^{e}	c.i.
***Salmonella entérica* subsp. Enterica Montevideo**	ATCC BAA-710	λ	120.7 ± 17.8 ^{a}	136.6 ± 15.0 ^{a}	125.6 ± 3.8 ^{a}	209.3 ± 5.5 ^{a}	243.2 ± 13.1 ^{b}	c.i.
		μ	0.90 ± 0.03 ^{a}	0.83 ± 0.06 ^{a}	0.77 ± 0.03 ^{a}	0.90 ± 0.05 ^{a}	0.50 ± 1.13 ^{b}	c.i.
		A	0.22 ± 0.01 ^{a}	0.16 ± 0.01 ^{b}	0.15 ± 0.01 ^{b}	0.15 ± 0.01 ^{b}	0.1 ± 0.02 ^{c}	c.i.
***Salmonella enterica* subsp. Enterica Gaminara**	ATCC BAA-711	λ	169.0 ± 9.2 ^{a}	149.9 ± 15.5 ^{a}	144.2 ± 14.6 ^{a}	259.1 ± 2.0 ^{a}	309.1 ± 14.8 ^{b}	c.i.
		μ	1.75 ± 0.53 ^{a}	1.19 ± 0.13 ^{ab}	0.93 ±± 0.10 ^{ab}	0.96 ± 0.12b ^{c}	0.41 ± 0.09 ^{c}	c.i.
		A	0.27 ± 0.08 ^{a}	0.24 ± 0.01 ^{a}	0.20 ± 0.02 ^{a}	0.23 ± 0.02 ^{a}	0.06 ± 0.01 ^{b}	c.i.
***Escherichia coli* (virulent factor deleted)**	NCTC -12900	λ	131.8 ± 7.2 ^{ab}	116.7 ± 26.4 ^{b}	157.8 ± 1.9 ^{a}	194.2 ± 7.3 ^{c}	c.i.	c.i.
		μ	2.46 ± 0.83 ^{a}	1.35 ± 0.09 ^{a}	1.68 ± 0.07 ^{a}	1.57 ± 0.04 ^{a}	c.i.	c.i.
		A	0.42 ± 0.02 ^{ab}	0.50 ± 0.02 ^{a}	0.47 ± 0.02 ^{bc}	0.38 ± 0.01 ^{c}	c.i.	c.i.
Escherichia coli	CECT -516	λ	61.1 ± 9.7 ^{a}	76.0 ± 32.7 ^{a}	142.0 ± 19.9 ^{b}	189.4 ± 6.8 ^{b}	205.2 ± 7.8 ^{c}	c.i.
		μ	1.42 ± 0.14 ^{abc}	1.07 ± 0.14 ^{bc}	2.02 ± 0.10 ^{ab}	2.07 ± 1.01 ^{b}	0.19 ± 0.02 ^{c}	c.i.
		A	0.31 ± 0.02 ^{a}	0.39 ± 0.03 ^{a}	0.44 ± 0.03 ^{a}	0.41 ± 0.17 ^{a}	0.04 ± 0.00 ^{b}	c.i.
Staphylococcus aureus	CECT - 435	λ	413.9 ± 27.3 ^{bc}	375.6 ± 4.1 ^{ab}	361.4 ± 20.2 ^{a}	463.2 ± 18.6 ^{c}	c.i.	c.i.
		μ	0.55 ± 0.11 ^{a}	0.46 ± 0.01 ^{ab}	0.42 ± 0.01 ^{ab}	0.28 ± 0.12b ^{c}	c.i.	c.i.
		A	0.18 ± 0.02 ^{a}	0.15 ± 0.01 ^{a}	0.13 ± 0.01 ^{a}	0.07 ± 0.03 ^{b}	c.i.	c.i.
Bacillus cereus	CECT -131	λ	64.4 ± 1.3 ^{a}	64.4 ± 1.3 ^{a}	c.i.	c.i.	c.i.	c.i.
		μ	1.03 ± 0.30 ^{a}	0.52 ± 0.30 ^{b}	c.i.	c.i.	c.i.	c.i.
		A	0.22 ± 0.01 ^{a}	0.15 ± 0.01 ^{b}	c.i.	c.i.	c.i.	c.i.
Enterococcus faecalis	CECT -795	λ	n.d.	n.d.	n.d.	n.d.	n.d.	c.i.
		μ	0.74 ± 0.06 ^{a}	0.63 ± 0.02 ^{ab}	0.47 ± 0.16 ^{ab}	0.39 ± 0.05 ^{b}	0.42 ± 0.01 ^{ab}	c.i.
		A	0.26 ± 0.01 ^{a}	0.26 ± 0.0 ^{a}	0.24 ± 0.01 ^{b}	0.24 ±. 0.01 ^{b}	0.24 ± 0.01 ^{b}	c.i.
Enterobacter aerogenes	CECT -684	λ	51.2 ± 20.1 ^{a}	61.2 ± 10.8 ^{ab}	74.8 ± 36.7 ^{ab}	119 ± 27.8 ^{ab}	153.7 ± 87.1 ^{ab}	772.7 ± 36.8 ^{c}
		μ	2.09 ± 0.23 ^{ab}	1.98 ± 0.28 ^{ab}	2.57 ± 0.15 ^{a}	2.21 ± 0.26 ^{ab}	1.66 ± 0.21 ^{b}	0.69 ± 0.45 ^{c}
		A	0.97 ± 0.01 ^{a}	0.97 ± 0.11 ^{a}	0.96 ± 0.01 ^{a}	0.97 ± 0.05 ^{a}	0.81 ± 0.15 ^{ab}	0.46 ± 0.28 ^{b}

c.i.: complete inhibition

It is important to note that studies *in vivo* should be carried out in order to test real conditions of these extracts, such as pH of the matrix, other nutrients or compounds that may interact with them, different surfaces, and water activities, amongst others. But as shown in this study, the three compounds

analyzed have antimicrobial activities that may be used for increasing the safety of food products. Antimicrobial data of these compounds may provide more information concerning different ways to combat the emergent resistance of bacteria, by using sources from a natural origin.

Table 33. MIC values (mg/mL) of ginseng extract (GE), ferulic acid (FA) and fermented noni juice powder (FNJP) for the bacterial strains studied.

Specie	Strain	GE	FA	FNJP
***Listeria monocytogenes* 4b**	CECT -935	> 11.0	1.7	16.7
L. monocytogenes 1/2 a	Lab	16.5	2.5	16.7
Listeria monocytogenes 1/2	CECT-4031	> 11.0	1.7	16.7
***Salmonella enterica* subsp. Enterica Typhimurium**	CECT -4594	> 11.0	> 3.3	16.7
***Salmonella enterica* subsp. Enterica Agona**	ATCC BAA-707	> 11.0	3.3	33.3
***Salmonella entérica* subsp. Enterica Montevideo**	ATCC BAA-710	> 11.0	2.5	33.3
***Salmonella enterica* subsp. Enterica Gaminara**	ATCC BAA-711	> 11.0	2.5	33.3
***Escherichia coli* (virulent factor deleted)**	NCTC -12900	> 11.0	3.3	16.7
Escherichia coli	CECT -516	> 11.0	3.3	33.3
Staphylococcus aureus	CECT- 435	> 11.0	1.7	16.7
Bacillus cereus	CECT -131	> 11.0	3.3	4.1
Enterococcus faecalis	CECT -795	> 11.0	3.3	33.3
Enterobacter aerogenes	CECT -684	> 11.0	3.3	> 33.3

4 Conclusions

In this article, the following *in vitro* properties of ginseng extract (GE), ferulic acid (FA), and a fermented noni juice powder (LFNJ) were studied: IC50, anti-lipid peroxidation, inhibition of PPO activity, and effect on lag time, maximum growth rate, and asymptotic value in the growth curves of 13 pathogenic strains.

GE decreased the activity of mushroom-derived PPO and caused the complete inhibition of *Listeria monocytogenes* 1/2a when used at 16.5 mg / mL. It also extended the lag phase of *E. aerogenes* and *E. coli* O157:H7 at 8.2 mg / mL. FA, in turn, showed potential to be used as an antioxidant, as its IC50 was 0.45 mg/mL and it showed a delay of lipid peroxidation in olive oil. FA also showed antimicrobial effects: the MIC value for *S. aureus,* and *L. monocytogenes* 4b and 1/2 was 5.0 mg / mL, and for *S.* Montevideo *and S*. Gaminara was 7.5 mg / mL. FNJP proved to be antioxidant and a natural inhibitor of PPO in apple, mushroom and potato. It also acted as an antimicrobial agent, including the complete inhibition of *B. cereus* at concentrations of 12.5 mg / mL.

As shown in this article, GE, FA and FNJP might constitute control strategies for food preservation. These products, which can be obtained from natural sources, may constitute an added value for the food products. However, data shown hereby has been obtained *in vitro*. As discussed above, further *in vivo* studies in food matrices should be carried out due to their complex composition and the interaction with the different intrinsic and extrinsic parameters, such as storage conditions, which may exert an effect on the activity of the compounds.

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Conflict of interests

The authors declare no conflict of interests.

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Chapter 9

Ferulic acid application to control growth of *Listeria monocytogenes* and *Salmonella enterica* on fresh-cut apples and melon, and its effect in quality parameters.

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The highest priority of fresh-cut processors is to ensure the safety of their products, as no further operations will reduce their pathogenic loads after sanitation / disinfection before they are eaten. *Listeria monocytogenes* is able to grow under fresh-cut fruit storage conditions, and *Salmonella* spp. has been associated to a number of outbreaks related with such products. There appears the need to find products capable to reduce microbial counts while maintaining quality of the product. In this regard, ferulic acid (FA), one of the most important phenolic acid in plants, has shown antimicrobial, antioxidant and many physiological functions in humans. This study aimed to test the efficacy of FA in fresh-cut apple and melon to prevent such pathogenic growth and determine the effect on fruit quality. For this purpose, three strains of *L. monocytogenes* and four strains of *S. enterica* were artificially inoculated in both fruits. Several concentrations of FA were tested and the results shown that they did not caused immediate decrease in such populations in fruit or in wash water. After 7 days of storage at 10 ºC, FA showed a higher effect against *L. monocytogenes* (averaging 4.2 ± 0.7 log CFU / g) than against *S. enterica* (averaging 1.9 ± 1.3 log CFU / g). The reductions were significantly different from the samples without FA, but not significant differences were found among the 3 tested concentrations. Comparison between immersion and spray applications of FA revealed that immersion was the best method. Although FA did not prevent the increase of browning index in apples, melon with FA did not show significant colour differences during storage at 4 ºC. FA did not inhibit the growth of total aerobic mesophylls and yeasts and moulds, but maintained overall quality of the fruits, including pH, total soluble solids and titratable acidity. Overall, FA could be used in fresh-cut apple and melon to prevent growth of *L. monocytogenes* without affecting physicochemical quality, delivering a product with increased antioxidant activity

Antimicrobial, antioxidant, shelf-life, pathogens, fruit, anti-browning

1 Introduction

Fresh-cut produce has been defined by the International Fresh-cut Produce Association as "any fruit, vegetable or their combination subjected to a physical alteration from its original form, remaining in a fresh state" (Grau-Rojas, 2010). The global demand for healthy, fresh, and sustainable products is leading to an expanding period in the fruit processing industry (Qadri, 2015). However, consumption of minimally processed fruits has been associated to concerns on their safety due to the emergence of several outbreaks of foodborne pathogens linked to their consumption (Pinela, 2015). Growth of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* has been previously demonstrated on fresh-cut apples (Abadias, 2011) and melon (Abadias, 2012). Moreover, *Salmonella* spp. and *L. monocytogenes* have been related to several outbreaks related to the consumption of apples and melons (CDCP, 2014; Callejón, 2015). Furthermore, shelf-life of fresh-cut fruits tends to be short, mainly due to browning caused by oxidation processes, loss of weight and loss of firmness (Wilson, 2019).

In food industry, the use of preservatives to inhibit microbial growth or to delay browning and ripening processes is critical to maintain consumer's safety and extend shelf-life of the product. Lately, with the emergence of bacteria resistance to chemical antibiotics, and the increasing mistrust of consumers towards chemical additives, there is a trend in the search for natural products with antioxidant and antimicrobial properties (Pernin, 2019).

Ferulic acid (FA, [E]-3-[4-hydroxy-3-methoxy-phenyl] prop-2-enoic acid) is an ubiquitous phytochemical phenolic acid, the most common of the cinnamic acid group (Mattila, 2002). FA is present in cell wall components as covalent side chains, conferring rigidity to vegetable cells (Kumar, 2014). It can be found in whole grains, spinach, parsley and cereal seeds, mainly wheat, oats, rye and barley (Zhao, 2008). The use of FA is approved as an antioxidant food additive in Japan, while natural extracts with high contents of FA are permitted in the US and most European countries to prevent lipid peroxidation of foods (Quitmann, 2014). Moreover, FA has been reported to have antimicrobial properties (Kumar, 2014). Its mode of action consists on leading irreversible changes in membrane properties, including charge, intra and extracellular permeability, and its physicochemical properties (Borges, 2013). Low minimum inhibitory concentrations against several pathogenic bacteria have been elucidated for this compound (Pacheco-Ordaz, 2017). Preliminary work has shown the minimum inhibitory concentration of this compound against several bacterial strains at *in vitro* conditions, which ranged from 3.3 to 1.7 mg FA / mL, and its antioxidant potential (IC_{50} 0.45 mg/mL) (Data not shown).

The objectives of this study were (i) to evaluate the effect of FA in controlling growth of *S. enterica* and *L. monocytogenes* on fresh-cut apple and melon, (ii) to determine the best application method (immersion or spray) and (iii) to study its action as an antioxidant agent was studied in fresh-cut fruits in order to delay browning or changes in colour, as well as its effect on other quality parameters, including pH, total phenolic content, firmness, and spoilage microbiota.

2 Materials and methods

2.1 Materials

Apple ('Golden Delicious') and melon ('Piel de sapo') fruits were obtained from local providers. *Trans*-Ferulic acid (trans-4-Hydroxy-3-methoxycinnamic acid, ≥ 99 %, W518301) was purchased to Sigma-Aldrich (Steinheim, Germany), and NatureSeal® was from Agricoat NatureSeal Ltd (Hungerford, United Kingdom).

Triptone soy broth (TSB), triptone soy agar (TSA), yeast extract, Palcam base agar and Palcam selective supplement for *Listeria*, xilose lysine deoxycholate agar (XLD), plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), potassium bisulfate, sodium chloride and peptone were purchased from Biokar Diagnostics (Allonne, France). Dey-Engley broth was obtained from Honeywell Fluka (Madrid, Spain).

Ascorbic acid, gallic acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol, ethanol, chlorhidric acid (37%), sodium acetate, sodium hydroxide, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were purchased from Panreac (Llinars del Valles, Spain).

2.2 Methods

2.2.1 Experimental design

In this study, four sets of experiments were performed (**Figure 44**). The first trial consisted on the screening of the *in vivo* antimicrobial activity of ferulic acid (FA) against three *Listeria monocytogenes* and four *Salmonella enterica* strains, that were individually artificially inoculated on apple and melon disks. In the second, the efficacy of FA two application methods (dipping or spraying) was compared as antimicrobial treatment against artificially inoculated *L. monocytogenes* or *S. enterica* strains on apple and melon disks. In the third experiment, the minimum FA concentration against artificially inoculated *L. monocytogenes* on apple and melon during storage was determined. All the trials with pathogens were done at 10 ºC for 7 days, in order to analyse the effect in a worse-case scenario of abusive storage temperature. Finally, the fourth set involved the evaluation of the quality parameters of uninoculated fresh-cut apple and melon treated with FA during storage at 4 ºC, mimicking the commercial conditions.

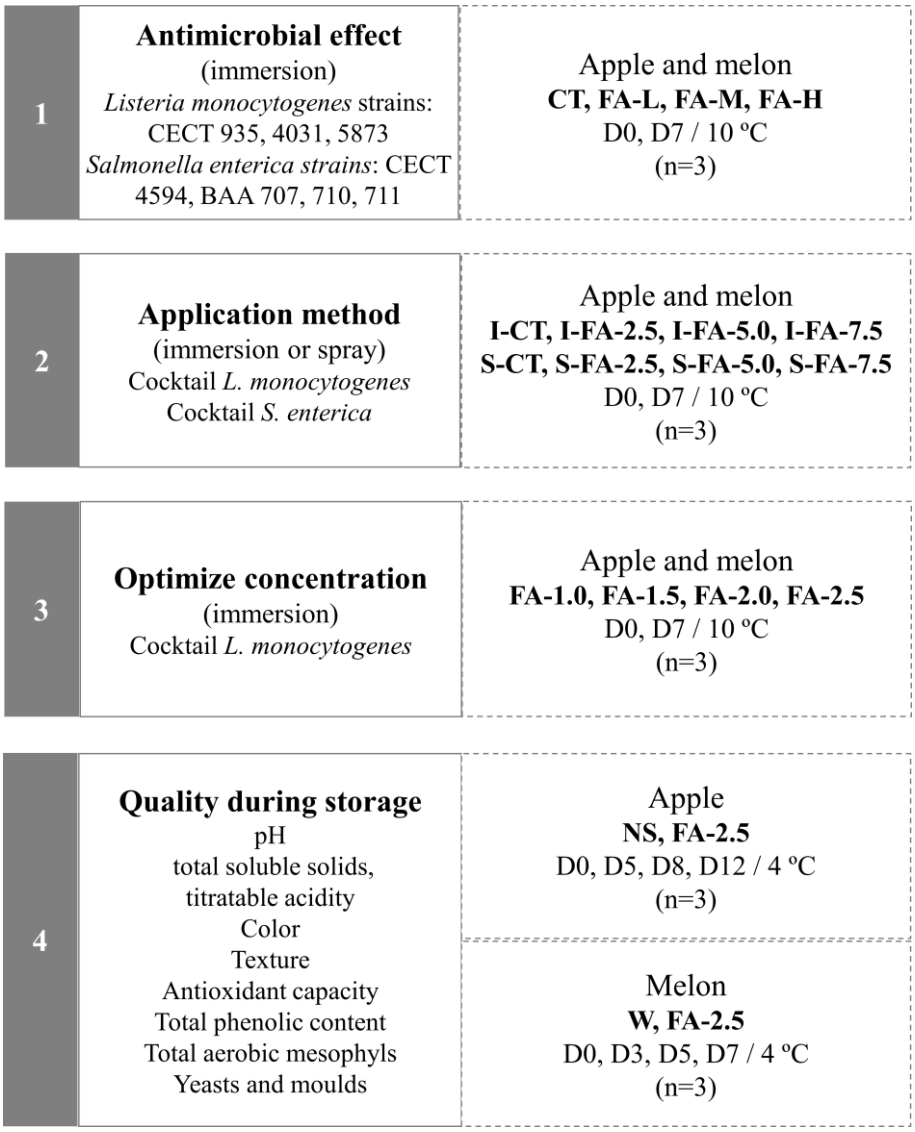

Figure 44. Experimental design

2.2.2 Effect of FA at different concentrations against pathogenic strains on fresh-cut apple and melon

Pathogenic bacteria strains. The bacterial strains used in this work included the serovars of *Salmonella enterica* subsp. *enterica*: Agona (ATCC BAA-707), Montevideo (ATCC BAA-710), Gaminara (ATCC BAA-711) and Typhimurium (CECT-4594) in addition to *L. monocytogenes* serovar 1/2 (CECT-4031), serovar 4b (CECT-935) and serovar 1/2a, which was previously isolated in our laboratory from a fresh-cut lettuce sample (Abadias, 2008).

Inoculum preparation. *S. enterica* strains were grown in 50 mL TSB, and *L. monocytogenes* strains in TSB supplemented with 6 g/L of yeast extract, 2.5 g / L glucose and 2.5 g / L K_2HPO_4 (TSBYE) for 24 h at 37 ºC, until stationary phase. The culture was centrifuged at 9800 × *g* for 10 min at 10 ºC. The pellet containing the bacteria was resuspended in 25 mL saline solution (NaCl, 8.5 g / L). The population of bacterial suspensions was determined by plating in TSA and XLD, or TSAYE and Palcam, respectively, and incubated for 24 h at 37 ºC.

Preparation of apple and melon. Apple and melon were rinsed with tap water and disinfected with ethanol 70 %. Fruits were peeled and cylinders of 1.2 Ø were taken off using a core borer and cut in 1.0 cm high with a sharp knife. Disks were obtained from different fruits to assure representativity of the samples.

Inoculation of fruit disks. Inoculation of the fruit disks was done by immersion in a previously prepared suspension containing about 1.5×10^7 CFU / mL of each strain. Concentration was checked by serial dilution in saline peptone (SP, peptone 1 g / L and NaCl, 8.5 g / L) and plating in TSA for *S. enterica* or TSAYE for *L. monocytogenes* and incubated 24 h at 37 ºC. A ratio 1:10 (fruit:inoculum volume) was used for the inoculation, to assure complete immersion of all pieces. After agitation at 150 rpm for 2 min, fruit pieces were dried over a lab rack in a biosafety level 2 laminar flow cabinet.

Treatments. For FA treatments, ten fruit disks were immersed in 500 mL of sterile distilled water containing FA in three different amounts. For each bacterial strain, three FA concentrations were selected, low, medium, and high (FA-L, FA-M, FA-H) (**Table 34**), according to the *in vitro* previously evaluated MIC values of FA against the strains studied in a preliminary study. Another treatment, consisting of distilled water without ferulic acid, was added as a treatment control for the treatments (CT). Six discs were also left untreated, for population reference. After treatment, fruit discs were left to dry in a biosafety level 2 cabinet during 1 h.

Table 34. Low, medium and high concentrations of FA used for each pathogenic strain.

Strain	FA treatment		
	FA-L (mg/mL)	**FA-M** (mg/mL)	**FA-H** (mg/mL)
S. Agona	10.0	12.5	15.0
S. Michigan	7.5	10.0	12.5
S. Montevideo	5.0	7.5	10.0
S. Typhimurium	7.5	10.0	12.5
L. monocytogenes 1/2	2.5	5.0	7.5
L. monocytogenes 4b	2.5	5.0	7.5
L. monocytogenes 1/2a	5.0	7.5	10.0

FA-L, lower concentration of ferulic acid; FA-M, medium concentration of ferulic acid; FA-H, higher concentration of ferulic acid tested.

Sampling dates. Two sampling dates were stablished. The first (D0), immediately after the treatments, to see if FA had bactericidal effect. The second, after 7 days (D7) at 10 ºC, being the disks stored in individual glass tubes, to evaluate the bacteriostatic effect of FA.

Microbiological assessment. For bacterial counts, 1 disk per repetition (n=3), weighing 1 g, approximately, was placed in an 80-mL sterile filter bag (BagPage®, Interscience BagSystem, Saint Nom, France) and mixed with 9 mL of buffered peptone water (BPW, Biokar Diagnostics). It was smashed in a paddle blender (Minimix® 100, Interscience, France) for 120 s at 6 strokes / s. Aliquots were serially diluted in SP and 20 µL were plated in duplicate plates of selective media. *L. monocytogenes* was plated in Palcam agar and incubated at 37 ± 1 ºC for 48 ± 2 h. *S. enterica* was plated in XLD agar and incubated at 37 ± 1 ºC for 24 ± 1 h. Detection limit was 250 CFU / g or disk. Results were transformed to log CFU / g or disk and expressed as reductions in population, calculated as described in (Equation 1),

$$\text{Microbial reduction (log CFU / g)}_t = (\text{Log } N_t / N_0) \qquad \text{Eq. 1}$$

where N_0 is the mean of the population of untreated discs (as a population reference), and N_d is the population of each treatment at sampling date (CFU / g).

2.2.3 Selection of application method: immersion or spray

Preparation of pathogenic strains and fruits. Preparation of apple and melon disks, and culture of pathogenic strains was done as described in section 2.2.2. For this trial, two bacterial cocktails were prepared, one of *S. enterica* strains and another one of *L. monocytogenes* strains, by mixing the 5 mL of the resuspended pellet of the cultured strains, respectively. Inoculation of apple and melon disks with the respective cocktails was performed as described in section 2.2.2.

Treatments. Two application methods were studied: immersion and spray. For spray treatments, disks were distributed in a lab rack, and sprayed with an airbrush model Hobby Air 707523 (Werther International, Reggio Emilia, Italy) over one surface for 2 s each. Afterwards, discs were turn out and were sprayed again. Three concentrations of FA were selected: 2.5, 5.0 and 7.0 mg / mL. Also, a water application with no FA was added as a treatment control (CT), resulting in 8 different treatments, as a combination of immersion (I) or spray (S) and each of the FA concentrations (CT, FA-2.5, FA-5.0, and FA-7.5). Also, inoculated and untreated batch of discs were included in the experiment and were the reference to compare reductions of population. Results were expressed as reduction of population when compared with the reference, as described in Equation 1. Sampling dates were stablished for D0 and D7. Populations were determined as explained in section 2.2.2.

Microbial count in wash water. Moreover, in the immersion treatment, a sample of water and FA wash water was analyzed after treatment for pathogenic bacterial count, in order to check any bactericidal effect on water. For this, 1 mL sample of water was mixed with 9 mL Dey-Engley neutralizing medium, and serial dilutions were plated and incubated in duplicate on XLD or Palcam, for 24 or 48 h, respectively, for *S. enterica* and *L. monocytogenes*. Results were expressed as log CFU / mL, and detection limit was 50 CFU / mL. Dey-Engley tubes were incubated at 37 ºC for 24 h to determine the absence / presence of both pathogens when population in wash-water was <50 CFU / mL. When presence was confirmed, a value corresponding to ½ detection limit was given.

2.2.4 Optimization of FA concentration against *L. monocytogenes*

This trial, the possibility to decrease FA concentration by maintaining the same antimicrobial efficacy was evaluated. For this, three concentrations were compared to FA-2.5: 1.0, 1.5, and 2.0 mg/mL FA (FA-1.0, FA-1.5, and FA-2.0) (n=3). Based on previous results, only *L. monocytogenes* cocktail on apple and melon was studied in this trial. Preparation of *L. monocytogenes* cocktail is described in section 2.2.3., and preparation of fruit disks, inoculation, and sampling times and procedure is described in section 2.2.2. FA was applied by immersion, due to results obtained in previous experiments.

2.2.5 Effect of FA application on the quality of fresh-cut apple and melon

Preparation of apple and melon pieces. Evaluation of commercial quality and shelf life of the fresh-cut apple and melon was performed in non-inoculated pieces. To prepare the fruit, the external surface of the fruits was disinfected in a 200-ppm chlorine solution (pH adjusted to 6.5 using citric acid 2 M) bath for 2 min, and rinsed with tap water for 2 min. Then, apples were peeled and 10 wedges of approximately 1 cm width were obtained with a 10-blaze apple slicer and corer. For melon, pieces of approximately 4 × 3 × 2 cm without peel were cut with a knife. Fruit pieces were immersed in treatment solutions immediately after cutting.

Treatments. FA treatments for apple and melon consisted on application of 2.5 mg/mL solution (FA-2.5) in a proportion 1:3 (fruit:solution) for 2 min. To study the antioxidant effect of FA, the commercial

antioxidant NatureSeal® was the control treatment for fresh-cut apple (NS). Apple slices were immersed in NatureSeal® at 4 % (w:v) for 2 min, following provider instructions. Tap water was the control treatment for fresh-cut melon (W), in which was immersed for 2 min. Fruit pieces were let dry over a filter paper at room temperature for 1 h until packaging.

Evaluation of FA in the samples. To determine the concentration of FA that remained in fruit disks after the immersion in the solutions, an extraction of the phenolic content was carried out by mixing 3.0 ± 0.1 g of the sample with 10 mL of a methanol solution 70 % (v:v). After agitation for 20 min, the mixture was centrifuged at 14,000 × g for 10 min, and the supernatant lyophilized. The supernatant was then resuspended in water, methanol and formic acid (1:98:1 v:v:v) and determined by UPLC-MS, using Acquity UPLC-Xevo TQS (Waters). UPLC was performed using Acquity UPLC ® HSS T3 1.8 μm, 150 x 2.1 mm column, injecting 5 μL of sample at 10 ºC, in a isotherm column at 40 ºC, with two mobile phases: (A) water, methanol and formic acid (1:98:1 v:v:v) and (B) methanol and formic acid (99:1.5 v:v) at 0.3 mL/min in a gradient as follows: from 0 to 0.51 min 80 % A and 20 % B, from 0.51 to 5.00 min, 20 % A and 80 % B, from 5.01 to 7.50, flow was increased to 0.4 mL min and mobile phases were 1 % A and 99 % B. Finally, back at initial conditions to 10.00 min. Mass spectrometry was done with a ESI with negative ion mode, 2 kV capillarity, source and desolvation temperatures, 120 and 450 ºC, respectively, desolvation gas flow was 1000 L / h, and collision gas flow was 0.15 mL / min. Multiple reaction monitoring of ferulic acid in channels 192.83 > 134.20 and 192.83 > 177.97, where collision energy was 15 eV and Cone was 30 V. Results were expressed as mg FA / g sample, and detection limit was 0.026 μg / g.

Storage conditions and sampling dates. Storage of fresh-cut apple and melon was done in 500 mL clamshell plastic containers. Approximately 130 g apple or 200 g melon were distributed in each container and kept at 4 ± 1 ºC until sampling date. The day of the treatment was the first sampling date (D0). Apple was analyzed at days 5, 8, and 12 after treatment (D5, D8, D12), and melon at days 3, 5, and 7 after treatment (D3, D5, D7).

Effect of treatments on fresh-cut fruit quality parameters. Each sampling date pH, titratable acidity, total soluble solids, color, firmness, total aerobic mesophilic microorganisms, and yeasts and molds were determined in triplicate samples (three clamshells). Also, an aliquot of the fruit pieces was frozen using liquid nitrogen, pulverized in a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain), and kept at – 80 ºC until further determinations of antioxidant capacity by FRAP and DPPH· methods, and total phenolic content.

For **pH, titratable acidity (TA)** and **total soluble solids (TSS)** determination, fruit pieces from 3 different containers (repetitions) were smashed in a blender to obtain their juice. For each replication, 25 mL of apple or melon juice were prepared, and determined twice. pH was determined using an electrode in a pH-meter model GLP22 (Crison Instruments SA, Barcelona, Spain). TA was measured by diluting 10 mL of juice with 10 mL of distilled water and titrated with 0.1M NaOH until pH 8.2 was reached. Results were expressed as mg malic acid / L for apple, or mg citric acid / L for melon. TSS was measured at 20 °C with a refractometer (Atago Co. Ltd., Tokyo, Japan), and the results expressed as °Brix.

To assess changes in **texture**, firmness was measured by the maximum penetration force using the TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England) on 10 samples per repetition (n=30). The firmness test was performed using a cylindrical probe (4 mm). Pre-test and test were both run at 5 mm / s speed and using a trigger force of 0.1 N, allowing the probe to enter 8.0 mm deep into the tissue, measuring the maximum force encountered.

Color of 10 fruit pieces per repetition was measured on 3 points of each sample (n=30) by using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Color was expressed as CIE L*, a*, and b* coordinates, using the D65 illuminant and a 10° observer angle. These values were used to calculate the browning index (BI) for fresh-cut apples, using the equation proposed by (Pathare, 2013) (Equation 2) and the total color difference (TCD) for fresh-cut melon (Equation 3):

$$BI = 100 \times \left(\frac{X-0.31}{0.17}\right) \text{ Eq. 2.1} \qquad \text{where} \quad X = \frac{(a* + 1.75\,L*)\times a*}{(5.645L* + a* - 3.012b*)} \text{ Eq. 2.2}$$

$$TCD = ((L^*_d - L^*_0)^2 + (a^*_d - a^*_0)^2 + (b^*_d - b^*_0)^2)^{0.5} \qquad \text{Eq. 3}$$

where d=value at sampling day and 0=initial value (value at D0).

To determine **total aerobic mesophilic microorganisms (TAM)** and **yeasts and moulds (Y&M)** counts, 10 ± 1 g of three different fruit pieces per repetition (n=3), to assure heterogeneity, were mixed with 90 mL PS in a sterile filter bag (BagPage®, Interscience BagSystem, Saint Nom, France) and homogenised using a paddle blender (Minimix®, Interscience, France) for 120 s at 12 strokes / s. Aliquots were serially diluted in SP and plated in duplicate plates. For TAM, samples were plated in PCA and incubated at 30 ± 1 ºC for 3 days. For Y&M, samples were plated in DRBC and incubated at 25 ± 1 ºC for 5 days. Detection limit was 50 CFU / g.

Antioxidant capacity was determined by the ferric reducing antioxidant power (FRAP) and DPPH· scavenging radical (n=3). **Total phenolic content** (TPC) was determined by Folin -Ciocalteau method (n=3). For the extraction, 3.0 ± 0.1 g were mixed with 10 mL of methanol 70 % (v/v) and homogenized in a vortex. Samples were immediately placed in a stirrer at 4 °C working at 195 rpm for 20 min and centrifuged using a Sigma-3-18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 13,500 × g for 20 min at 4 °C. Supernatant was then filtered and marked to 12.5 mL with methanol 70%. FRAP, DPPH· and TPC determinations were performed as described in Nicolau-Lapeña (2019). Results of antioxidant capacity by FRAP and DPPH· methods were expressed as mg of ascorbic acid equivalents / 100 g of fresh weight (mg AAE / 100 g FW). Results of TPC were expressed as mg of gallic acid equivalents / 100 g fresh weight (mg GAE / 100 g FW).

2.3 Statistical analysis

All data were checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analyses were carried on using JMP 13 (SAS Institute Inc., Cary, USA).

3 Results

3.1 Effect of FA at different concentrations against pathogenic strains on fresh-cut apple and melon

To study the effect of FA at three different concentrations against *S. enterica* and *L. monocytogenes*, 7 strains of these pathogens were artificially inoculated on apple and melon. The pH values of FA solutions did not differ significantly between concentrations and ranged from 3.9 ± 0.1 to 3.7 ± 0.1, from the less concentrated to the most concentrated (1.0 to 15.0 mg / mL). Quality main parameters (pH, TSS and TA) of the fruits used in these trials are shown in **Table 35**.

Table 35. Initial quality parameters of apple and melon used for the experiments: pH, total soluble solids (ºBrix), and titratable acidity (mg malic acid / 100 g FW for apple, or mg citric acid / 100 g FW for melon) (n=21).

Fruit	pH	Total soluble solids (º Brix)	Titratable acidity (mg malic acid / 100 g FW)
Apple	4.6 ± 0.3	14.6 ± 1.2	1.6 ± 0.2
Melon	5.7 ± 0.3	11.3 ± 0.8	1.3 ± 0.1

FW, fresh weight

Populations of untreated discs at D0 ranged from 5.5 ± 0.2 to 6.5 ± 0.1 in apple and averaged 6.3 ± 0.2 log CFU / g in melon (**Table 36**). In general, *L. monocytogenes* strains reached higher populations than *S. enterica* after 7 days of storage at 10 ºC. Only S. Agona decreased 1 log after 7 days of storage in apple, while it did not happen in melon, in which this strain increased 1.7 logs. In fact, differences in growth for each strain were observed between fruit matrices: higher growth was observed in melon disks when compared to apple disks.

The values of pathogen population at D0 and D7 in untreated samples (**Table 36**) were used as a population control to calculate the log reductions for each treatment shown in **Figure 45**. Immediately after the treatments, no significant reductions of *S. enterica* or *L. monocytogenes* were observed for any of the treatments at D0. Populations were reduced 0.4 ± 0.2 or 0.3 ± 0.1 log CFU/g in apple or melon disks, respectively, regardless the FA concentration (data not shown).

Table 36. Initial (D0) and final (D7, after 7 days of storage at 10 ºC) populations of pathogenic strains in untreated apple and melon (n=3).

Strain	Apple		Melon	
	Initial population (log CFU / g)	Final population (log CFU / g)	Initial population (log CFU / g)	Final population (log CFU / g)
S. Agona	6.2 ± 0.1	5.2 ± 0.9	6.2 ± 0.1	7.9 ± 0.1
S.. Michigan	5.5 ± 0.2	7.3 ± 0.1	6.4 ± 0.1	8.3 ± 0.1
S. Montevideo	6.3 ± 0.2	7.4 ± 0.2	6.3 ± 0.1	8.6 ± 0.1
S. Typhimurium	6.5 ± 0.1	7.2 ± 0.5	6.6 ± 0.1	8.6 ± 0.1
L. monocytogenes 1/2	5.6 ± 0.2	7.9 ± 0.5	6.4 ± 0.1	9.5 ± 0.1
L. monocytogenes 4b	5.8 ± 0.1	8.5 ± 0.1	6.3 ± 0.1	9.2 ± 0.1
L. monocytogenes 1/2a	5.8 ± 0.1	8.2 ± 0.1	6.2 ± 0.1	9.3 ± 0.1

After 7 days of storage (D7) (**Figure 45**) pathogen reductions in FA treated apple and melon were significantly higher than they were in fruit disks washed with water (control treatment, CT). This fact implied that, while pathogens in CT samples grew under storage conditions similarly to untreated sample, pathogens in FA samples did not grow that much, or even decreased when compared to D0.

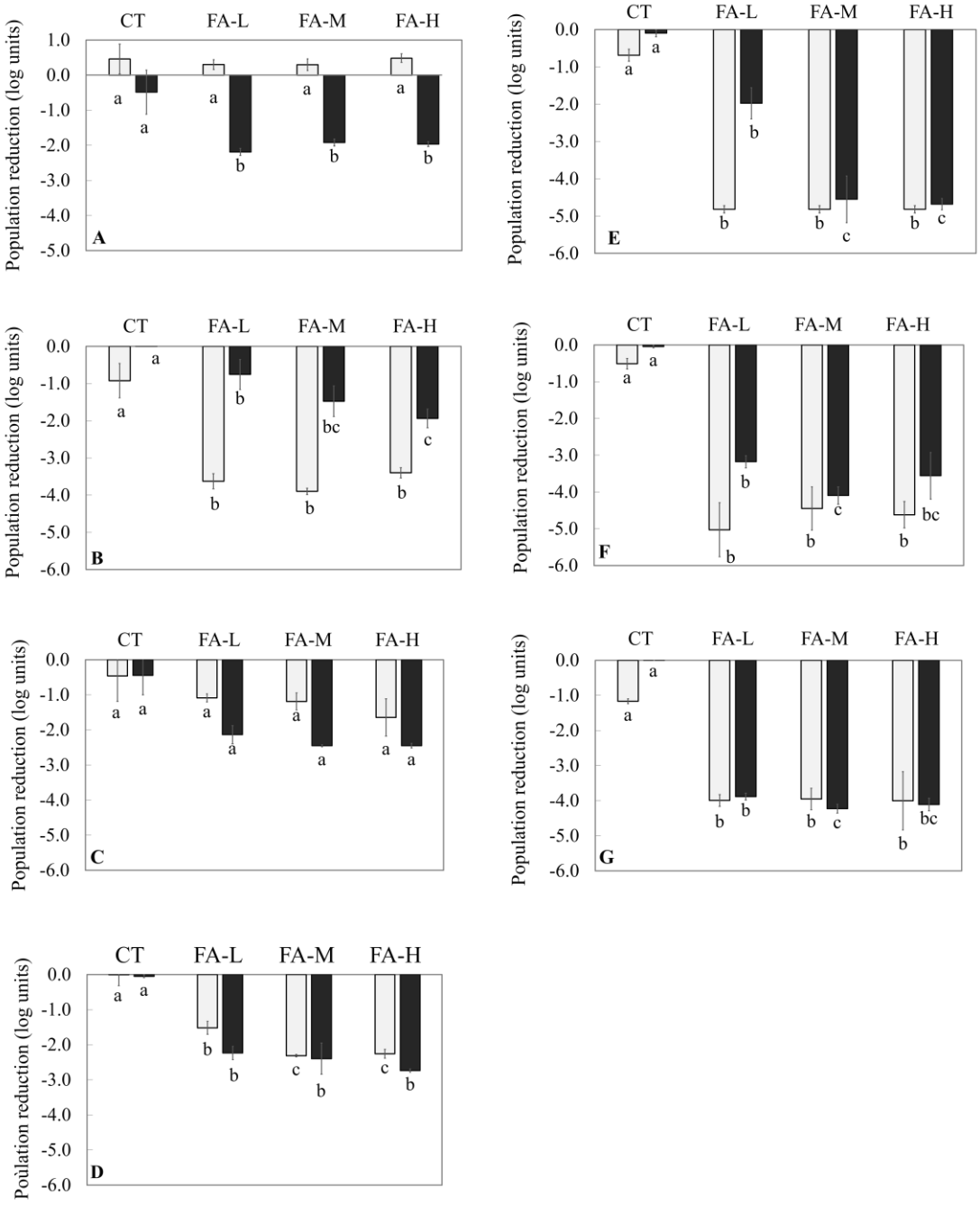

Figure 45. Reductions in counts of *S*. Agona (**A**), *S*. Montevideo (**B**), *S*. Gaminara (**C**), *S*. Typhimurium (**D**), *L. monocytogenes* serovar 1/2 (**E**), *L. monocytogenes* serovar 4b (**F**), and *L. monocytogenes* serovar 1/2a (**G**), in control treatment (CT), or in FA at low concentration (FA-L), at a medium concentration (FA-M) or at a high concentration (FA-H) compared to untreated samples, after 7 days of storage (D7) at 10 °C, in apple (□) and melon (■) plugs. Values are the mean ± standard deviation (n=3). Within the same fruit, different letters mean statistically significant differences between treatments ($p < 0.05$) according to Tukkey's HSD test.

Regarding *S. enterica* strains, *S.* Agona (**Figure 45**A) showed a different behaviour depending on the fruit matrix. As indicated above (Table 3), it decreased in untreated apple discs stored at 10 ºC, and the addition of FA did not enhance this decreasing effect. In melon, in contrast, this strain grew in the untreated sample, but decreased 2.0 ± 0.1 log in FA treated samples. *S.* Montevideo (**Figure 45**B) in apple was reduced by 3.6 ± 1.4 log by all FA treatments. In melon, a significant difference between FA-L and FA-H was observed, with reductions of 0.8 ± 0.4 and 1.9 ± 0.3 log, respectively. S. Gaminara (Figure 1C) strain was not affected by FA immersion. Differences between control treatment (CT) and FA treatments were not significant, in either apple or melon. *S.* Typhimurium (Figure 1D) population in samples treated with FA was reduced 1.5 ± 0.2 and 2.3 ± 0.1 log in apple, and between 2.2 ± 0.2 and 2.7 ± 0.1 log in melon, when compared to the untreated reference.

Concerning the effect of FA against *L. monocytogenes*, higher reductions than those obtained in *S. enterica* were observed, even higher concentrations were used for *S. enterica* (5.0 to 15.0 mg / mL FA) in comparison to those used for *L. monocytogenes* (2.5 to 10.0 mg/mL). Therefore, *L. monocytogenes* was more susceptible than *S. enterica* to FA. In comparison to the untreated reference, *L. monocytogenes* 1/2, 4b, and 1/2a growth after 7 days of storage at 10 ºC was reduced by 4.0 ± 0.2 log in apple, and ranged from 2.0 ± 0.4 to 4.7 ± 0.2, from 3.2 ± 0.2 to 3.6 ± 0.6, and from 3.9 ± 0.1 to 4.1 ± 0.2 log in melon, respectively.

3.2 Selection of the FA application method

The spray application was considered as an alternative FA application method on fruit surfaces, because of the advantages it would have in case of scaling up to industry. Three FA concentrations were selected for this experiment, based on the previous results. For *S. enterica*, the tested concentrations were decreased, because no difference was observed between FA-L and FA-H in section 3.1. So, in this case, a lower product concentration was studied, expecting the same effect. Moreover, concentrations > 7.5 mg / L clogged the nuzzle.

At D0, *S. enterica* and *L. monocytogenes* populations in the untreated apple and melon were 6.3 ± 0.1 and 6.1 ± 0.1 log, respectively. After treatment (D0), no remarkable differences were observed between application methods or FA concentrations, with reductions of each studied microorganism and fruit < 0.5 log units.

Regarding *S. enterica* cocktail (**Figure 46**A), the reduction of population in apple discs after 7 days of storage at 10 ºC was lower than 1 log unit regardless of the method of application and tested FA concentration. In melon, the application of FA by immersion (I-FA) caused a slightly higher reduction than application by spray (S-FA).

In contrast, reductions after 7 days of storage at 10 ºC caused by immersion application method against *L. monocytogenes* cocktail were significantly higher than those caused by spray application for each studied fruit (Figure 46B). Reductions were 2.1- and 2.8-fold higher in I-FA compared to S-FA, in apple and in melon, respectively. No differences were observed between FA-2.5, FA-5.0, or FA-7.5, in any of the cases.

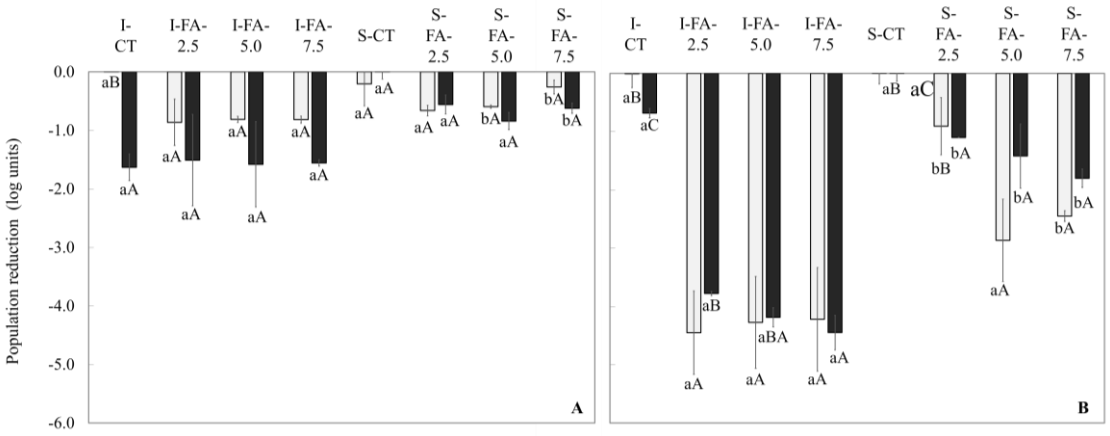

Figure 46. Reductions in counts of (**A**) *S. enterica*, and (**B**) *L. monocytogenes*, in comparison to untreated sample in apple (□) and melon (■) plugs, treated with different FA concentrations (2.5, 5.0, and 7.5 mg / mL) by immersion (I) or by spray (S) after 7 days of storage (D7) at 10 °C. Values are the mean ± standard deviation (n=3). Within the same fruit, different letters mean statistically significant differences between treatments ($p < 0.05$), according to Tukkey's HSD test.

The counts in the wash water after the treatment revealed the presence of 4.9 ± 0.1 log CFU / mL of *S. enterica* or *L. monocytogenes*. I-FA-2.5, I-FA-5.0 and I-FA-7.5 solutions contained the same concentration of microorganisms after treatments, confirming no bactericidal effect caused by FA at these concentrations (Data not shown). Due to its higher efficacy against *L. monocytogenes*, application of FA by immersion was selected for further experiments.

3.3 Optimization of the effective FA concentration against *L. monocytogenes*

As it has been described in sections 3.1. and 3.2, *L. monocytogenes* was effectively controlled by concentrations of FA above 2.5 mg/mL, while higher concentrations of FA were needed to reduce *S. enterica*. In this trial, the use of lower FA concentrations against *L. monocytogenes* was tested. Initial population of *L. monocytogenes* cocktail on apple and melon disks was 5.9 ± 0.3 and 6.2 ± 0.1, respectively (**Table 37**). Immediately after treatments, reduction of *L. monocytogenes* was 0.4 ± 0.1 log for both apple and melon. After 7 days of storage (D7) at 10 ºC, *L. monocytogenes* reductions in apple disks ranged from 3.7 ± .1 to 4.1 ± 0.1 log units, compared to the untreated reference. In contrast, in melon disks, *L. monocytogenes* reduction values were proportional to the FA concentration applied, and significantly affected its efficacy, with values ranging between 1.0 ± 0.1 and 3.9 ± 0.1 log.

Table 37. Population of *L. monocytogenes* cocktail in untreated apple and melon at initial (D0) and final (D7, after 7 days of storage at 10 ºC). Reductions of *L. monocytogenes* populations after FA treatments at different concentrations, in apple and melon (n=3).

		Apple		Melon	
		D0	**D7**	**D0**	**D7**
Population (log CFU / g)	**Untreated**	5.9 ± 0.3	7.9 ± 0.5	6.2 ± 0.1	9.3 ± 0.1
Reduction (log)	**FA-1.0**	0.4 ± 0.1 ^{a}	3.7 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	1.0 ± 0.1 ^{a}
	FA-1.5	0.4 ± 0.1 ^{a}	4.1 ± 0.1 ^{b}	0.5 ± 0.2 ^{a}	2.0 ± 0.2 ^{b}
	FA-2.0	0.5 ± 0.1 ^{a}	3.9 ± 0.1 ^{ab}	0.4 ± 0.1 ^{a}	3.3 ± 0.1 ^{c}
	FA-2.5	0.4 ± 0.1 ^{a}	3.7 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	3.9 ± 0.1 ^{d}

In the wash water, no bactericidal effect was observed. After the immersion of the fruit pieces, the remaining population in water averaged 4.7 ± 0.3 log CFU / mL (data not shown), and no significant differences were found between FA concentrations.

3.4 Impact of FA in the quality of fresh-cut apple and melon

The effect of FA selected dose (2.5 mg / L) in the quality of fresh-cut apple and melon was determined in non-inoculated samples, and fruit pieces were stored at 4 ºC to mimic commercial conditions, during 12 or 7 days, for apple or melon, respectively. The determination of the FA in the fresh-cut samples revealed that apple pieces contained 0.25 ± 0.04 mg FA / g FW, and melon pieces contained 1.22 ± 0.07 mg FA / g FW.

No significant differences were observed in **quality parameters** (pH, TSS and TA) of apple or melon during storage, regardless the treatment. In apple, values for these parameters were 4.3 ± 0.1, 11.6 ± 0.6 ºBrix, and 3.1 ± 0.3 g malic acid / L juice, respectively. In melon, these values were 5.9 ± 0.1, 11.4 ± 0.2 ºBrix, and 1.6 ± 0.1 g citric acid / L juice, respectively.

Firmness of fresh-cut apple after the treatments was 13.84 ± 0.24 N, and 10.09 ± 0.75 N, for Natureseal® treatment (NS) and 2.5 mg / mL FA (FA-2.5), respectively (**Table 38**). During storage, firmness of NS samples increased and firmness of FA-2.5 significantly decreased, achieving values up to 18.14 ± 1.36 and 6.85 ± 0.17 N, respectively at day 12 of storage. In fresh-cut melon, both water control (W) and 2.5 mg / mL FA (FA-2.5) samples had the same firmness values immediately after the immersion in the treatment solutions, which averaged 5.76 ± 0.09 N. However, firmness values significantly decreased up to 4.88 ± 0.02 N after 7 days of storage.

Table 38. Firmness values (N) of apple (NS or FA-2.5) and melon (W or FA-2.5) at different storage days (n=30). Values are the mean and the bars represent the standard deviation. Different lowercase letters indicate statistically significant differences between treatments in the same storage day and fruit ($p < 0.05$). Different capital letters indicate statistically significant differences between days within the same treatment ($p < 0.05$) according to Tukkey's HSD test.

Fruit	Day	**Firmness** (N)	
		Control*	**FA-2.5**
Apple	D0	13.84 ± 0.25 ^{aA}	10.09 ± 0.75 ^{bA}
	D5	15.49 ± 0.71 ^{aAB}	8.65 ± 0.58 ^{bAB}
	D8	19.56 ± 0.22 ^{aC}	8.07 ± 0.75 ^{bBC}
	D12	18.14 ± 1.36 ^{aBC}	6.85 ± 0.17 ^{bC}
Melon	D0	5.82 ± 0.35 ^{aA}	5.69 ± 0.17 ^{aA}
	D3	5.56 ± 0.06 ^{aA}	5.79 ± 0.20 ^{aA}
	D5	5.88 ± 0.31 ^{aA}	5.82 ± 0.35 ^{aA}
	D7	4.86 ± 0.17 ^{aB}	4.89 ± 0.15 ^{aB}

NS, Naturseal ® treatment; FA-2.5, ferulic acid at 2.5 mg / mL; W, water.

*Controls: NS for apple, W for melon.

Regarding color, the initial L*, a*, and b* values of apple plugs were 79.8 ± 0.5, 1.5 ± 0.3, and 19.4 ± 0.7, respectively (**Figure 48**). The changes observed led in a reduction in luminosity and an increase in reddish color, which can be expressed by browning index (BI) (**Figure 47**A). In NS samples, BI value was maintained during storage, but BI in FA-2.5 treated fresh-cut apples increased from 4.0 ± 0.3 to 10.7 ± 1.0. In melon pieces, initial L*, a*, and b* values were 69.8 ± 0.3, 1.1 ± 0.1, and 6.7 ± 0.1, respectively (**Figure 48**). Overall, although there were some variations during storage, no significant differences were observed in these values between W control and FA-2.5 in melon. At D7, TCD of samples averaged 1.4 ± 0.2 for both treatments (**Figure 47**B).

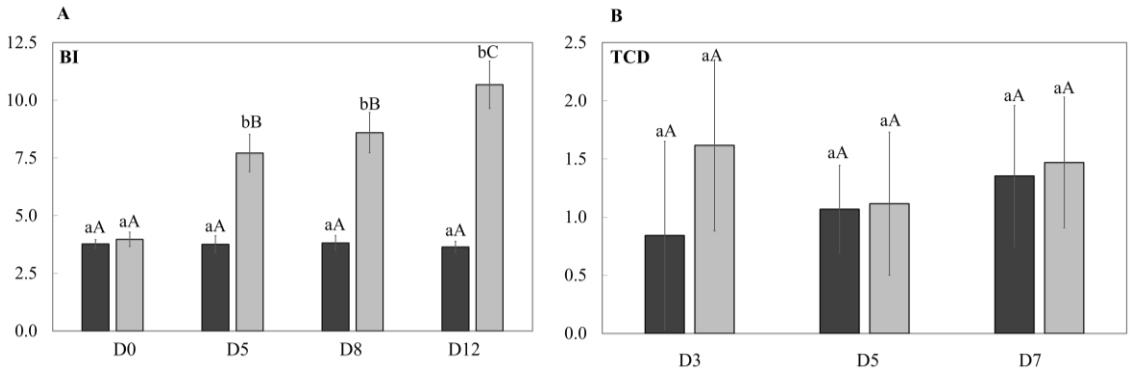

Figure 47. Browning index (BI) in Naturseal ® (NS, ▬) and 2.5 mg / mL FA (FA-2.5, ▬) treated fresh-cut apple (**A**), and total color difference (TCD) in water control (W, ▬) and 2.5 mg / mL FA (FA-2.5, ▬) treated fresh-cut melon (**B**) in trial 4 during storage at 4 ºC. Values are the mean ± standard deviation (n=3). Different lowercase letters mean statistically significant differences between treatments for the same day ($p < 0.05$). Different capital letters mean statistically significant differences between days within the same treatment ($p < 0.05$) according to Tukkey's HSD test.

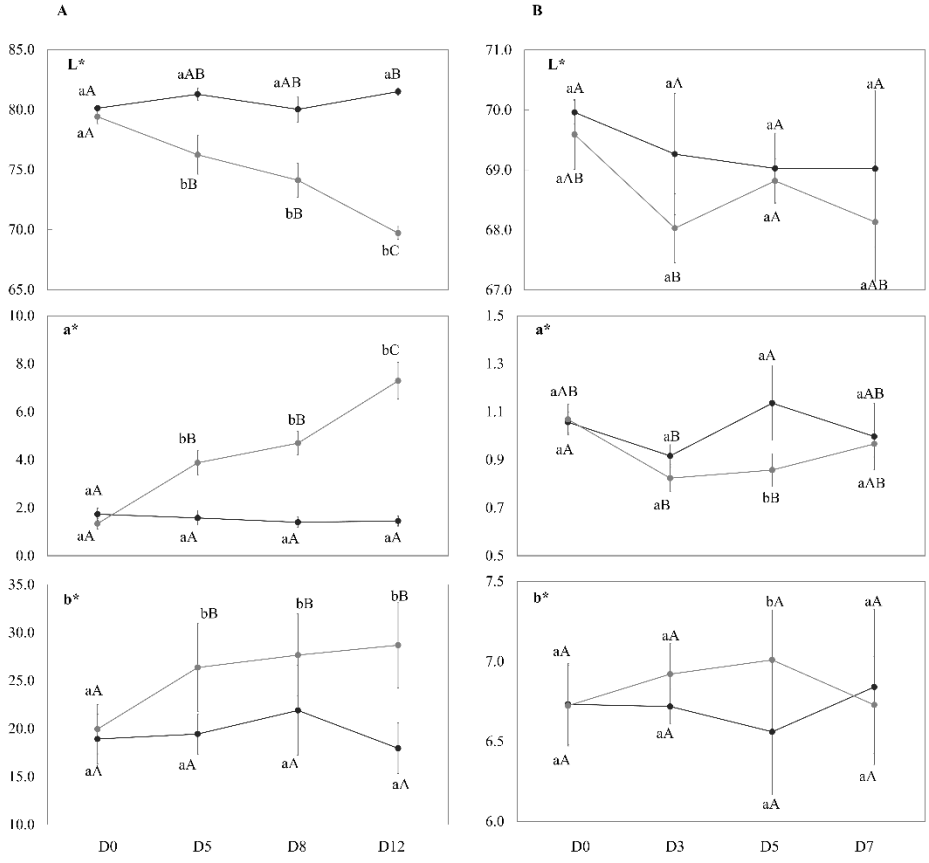

Figure 48. Changes in color values L*, a*, b* in NS (▬) and FA-2.5 (▬) fresh-cut apple (**A**), and L*, a*, b* in W (▬) and FA-2.5 (▬) fresh-cut melon (**B**) in trial 4 during storage at 4 ºC. Values are the mean ± standard deviation (n=3). Different lowercase letters mean statistically significant differences between treatments for the same day ($p < 0.05$). Different capital letters mean statistically significant differences between days within the same treatment ($p < 0.05$).

Control apple plugs treated with NS treatment showed significantly higher antioxidant values, both in DPPH· and in FRAP methods, than samples treated with FA-2.5 did (**Table 39**). During storage, DPPH· and FRAP significantly decreased, although in FA-2.5 such decrease was delayed. At the end of storage, antioxidant values of NS and FA-2.5 samples decreased by 37.3 and 25.7 %, respectively.

In melon, the addition of FA-2.5 to samples increased by 1.6-, 5.7-, and 3.2-fold their FRAP, DPPH· antioxidant capacities, and TPC values were also higher when compared to W samples (water control). There was a decrease in DPPH· and TPC values of fresh-cut melon during storage, achieving final values of 12.48 ± 0.42 mg AAE / 100 g FW and 6.61 ± 2.08 mg GAE / 100 g FW, respectively. Those values were also higher than those of the control samples, even at the end of the storage.

Table 39. Antioxidant capacity values by FRAP and DPPH· methods (mg AAE / 100 g FW) and total phenolic content (TPC) (mg GAE / 100 g FW) of apple (NS or FA-2.5) and melon (W or FA-2.5) at different storage days (n=3). Values are the mean and the bars represent the standard deviation. Different lowercase letters indicate statistically significant differences between treatments in the same storage day and fruit ($p < 0.05$). Different capital letters indicate statistically significant differences between days within the same treatment ($p < 0.05$) according to Tukkey's HSD test.

Fruit	Day	**FRAP** (mg AAE / 100 g FW)		**DPPH·** (mg AAE / 100 g FW)		**TPC** (mg GAE / 100 g FW)	
		Control*	**FA-2.5**	**Control***	**FA-2.5**	**Control***	**FA-2.5**
Apple	D0	169.06 ± 36.08 ^{aA}	51.06 ± 4.05 ^{bA}	151.90 ± 16.11 ^{aA}	51.47 ± 8.47 ^{bA}	186.11 ± 15.32 ^{aA}	67.54 ± 16.11 ^{bA}
	D5	142.51 ± 10.95 ^{aAB}	45.19 ± 3.14 ^{bA}	135.78 ± 8.07 ^{aAB}	48.15 ± 4.03 ^{bAB}	159.50 ± 16.11 ^{aAB}	60.72 ± 8.07 ^{bA}
	D8	134.97 ± 4.69 ^{aAB}	44.01 ± 2.10 ^{bAB}	116.82 ± 3.29 ^{aB}	45.65 ± 2.14 ^{bAB}	147.76 ± 28.80 ^{aAB}	60.57 ± 3.29 ^{bA}
	D12	110.75 ± 6.46 ^{aB}	36.41 ± 2.53 ^{bB}	88.65 ± 5.26 ^{aC}	36.50 ± 1.25 ^{bB}	119.80 ± 8.11 ^{aB}	55.73 ± 5.26 ^{bA}
Melon	D0	8.64 ± 0.24 ^{bAB}	14.24 ± 0.70 ^{aAB}	2.63 ±0.60 ^{bA}	14.99 ± 0.22 ^{aA}	14.03 ± 3.25 ^{bA}	44.61 ± 8.29 ^{aA}
	D3	8.81 ± 0.04 ^{bA}	13.46 ± 0.28 ^{aB}	2.38 ± 0.15 ^{bA}	15.30 ± 0.55 ^{aA}	10.27 ± 1.09 ^{bAB}	39.91 ± 2.29 ^{aA}
	D5	8.43 ± 0.09 ^{bBC}	13.58 ± 0.12 ^{aB}	3.20 ± 0.24 ^{bA}	15.45 ± 0.59 ^{aA}	9.64 ± 1.91 ^{bAB}	39.81 ± 1.82 ^{aA}
	D7	8.16 ± 0.06 ^{bC}	14.72 ± 0.05 ^{aA}	4.16 ± 0.14 ^{bB}	12.48 ± 0.42 ^{aB}	7.79 ± 0.54 ^{bB}	36.61 ± 2.08 ^{aA}

NS, Naturseal® treatment; FA-2.5, ferulic acid at 2.5 mg/mL; W, water; TPC, total phenolic content; AAE, ascorbic acid equivalents; GAE, gallic acid equivalents; FW, fresh weight.

*Controls: NS for apple, W for melon.

Initial **population of TAM** in fresh-cut apple was 2.4 ± 0.4 log CFU / g (**Figure 49**A). Immediately after NS and FA-2.5 treatments, TAM decreased to 2.1 ± 0.1 and 1.8 ± 0.5 log, respectively. During the 8 days of storage, counts increased similarly in both samples. In the case of fresh-cut apple, the treatment with FA showed a bacteriostatic effect, as populations did not significantly increase during this time. It was not until D12 of storage that, while TAM in FA-2.5 samples were maintained at 3.1 ± 0.1 log CFU / g, counts in NS samples achieved 4.5 ± 0.3 log CFU / g, respectively. In melon (**Figure 49**B), contrarily, although the initial TAM counts were 2.6 ± 0.1 log CFU / g, and after immersion of wedges in water control (W) or FA-2.5 solutions, counts decreased to 1.5 ± 0.2 and 1.4 ± 0.1 log, respectively, growth was not controlled by any of the treatments during storage. At D7, TAM counts in fresh-cut melon were 5.5 ± 0.3 log CFU / g, for both W or FA-2.5 treatments.

Initial **Y&M population** in apple wedges was 1.6±0.4 log CFU/g, and it did not significantly decrease after immersion in NS or FA-2.5 solutions (**Figure 49**C). Y&M counts increased during storage similarly for both treatments, and after 12 days at 4 ºC, it was 3.3 ± 0.5 and 3.0 ± 0.2 log, for NS and FA-2.5, respectively. Contrarily, Y&M populations in fresh-cut melon remained stable for the first 5 days of storage at 1.4 ± 0.1 (Figure 49D). After 7 days of storage, population in water control (W) samples grew up to 2.4 ± 0.1 log CFU / g, while it remained in FA-2.5 samples.

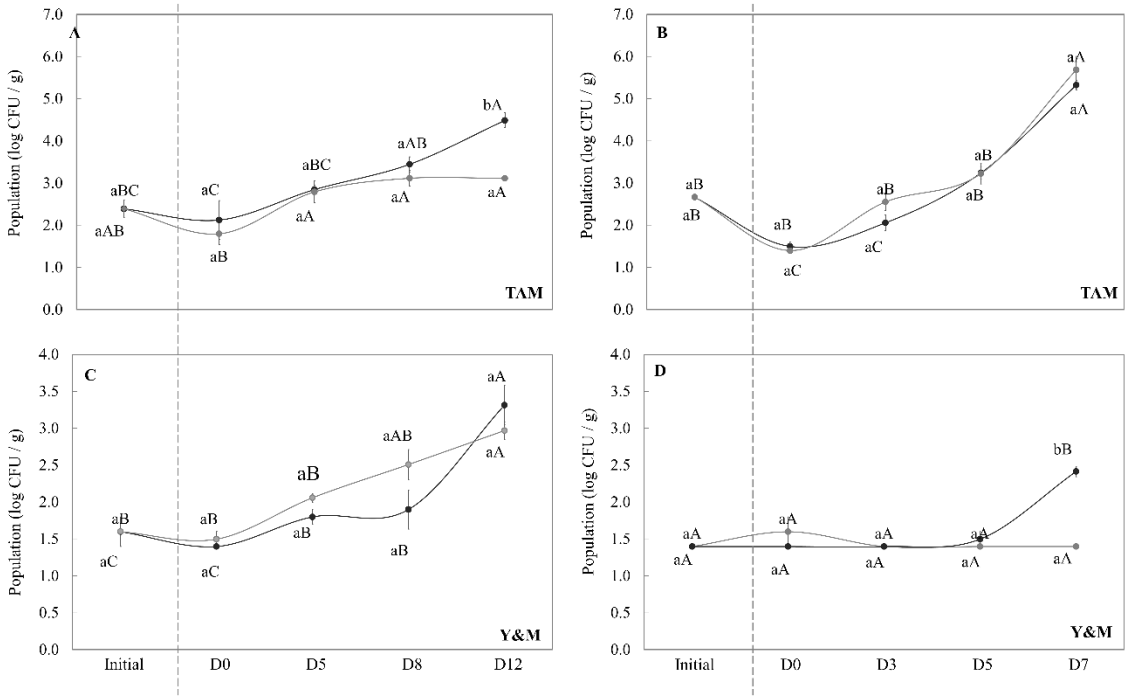

Figure 49. Counts of total aerobic mesophylls (TAM, A and B) and yeasts and moulds (Y&M, **C and D**) populations of Naturseal ® (NS, ▬) and 2.5 mg / mL FA (FA-2.5, ▬) treated fresh-cut apple (**A and C**) and of water control (W, ▬) and 2.5 mg / mL FA (FA-2.5, ▬) treated fresh-cut melon (**B and D**) in trial 4 during storage at 4 ºC. Values are the mean ± standard deviation (n=3). Different lowercase letters mean statistically significant differences between treatments for the same day ($p < 0.05$). Different capital letters mean statistically significant differences between days within the same treatment ($p < 0.05$) according to Tukkey's HSD test.

4 Discussion

In this paper, the antimicrobial effect of ferulic acid was studied against *L. monocytogenes* and *S. enterica* artificially inoculated in fresh-cut apple and melon. Results revealed no bactericide but bacteriostatic effect against the 7 strains used, after 7 days of storage at 10 ºC. For this, FA could be suggested as a solution to prevent pathogenic bacterial growth in fresh-cut products.

In general, all strains of each microorganism were affected in the same way by showing reductions around 2 to 4 log units. FA has been reported to have antimicrobial effect against *L. monocytogenes* (Borges, 2013; Pernin, 2019). In fact, in previous studies of the group, it was found that *L. monocytogenes* was more affected by FA than other tested strains such as *Bacillus cereus*, *Escherichia coli* or *Salmonella enterica* when tested *in vitro*. In this paper, the concentrations selected for the first trial were, at least 2 times higher than the MIC found in our previous studies (ranging from 1.7 to 3.3 mg / mL), because it has been observed that the concentration remaining in fruit tends to be lower than the concentration at which it is immersed. In fact, when fresh-cut apple and melon were immersed in a solution containing 2.5 mg FA / mL, the remaining content was 0.25 ± 0.04 and 1.22 ± 0.07 mg FA / g FW, respectively. In line with the results obtained in this paper, Takahashi (2013) reported no remarkable effect of FA on Gram negative bacteria, including *Salmonella* spp. The mode of action of the FA combines two mechanisms; the acidic and the lipophilic. The dissociation of the acid causes the acidification of the cell cytoplasm, the efflux of K^+ ions leading to the eventual death of the microorganisms. Also, the intercalation of the acid in the phospholipid layers of the membrane of the microorganism, causing a disturbance in the Van der Waals interactions and inhibiting the transport of the substrates for the key enzymes (Pernin, 2019).

In this study and based on previous results of the research group, three different concentrations of FA were tested for each strain. Except for and *S.* Typhimurium in fresh-cut apple, and *S.* Montevideo and *L. monocytogenes* 1/2a in fresh-cut melon, the antimicrobial effect observed was not concentration-dependent at the tested doses. Even though in the previous *in vitro* studies carried out in our lab indicated that the concentration chosen for FA-M treatment was the MIC for each strain, *in vivo* trials must consider more and interrelated conditions and variables, including the food characteristics, namely pH, natural antimicrobials, roughness of surface and adhesion capability of the cells to it, and extrinsic factors such as storage temperature. In the present study, we observed that FA had bacteriostatic effect, not bactericidal.

The decrease in *S. enterica* or *L. monocytogenes* after 7 days of storage at 10 ºC differed depending on the fruit studied. The difference in the behavior of the same concentrations in apple or melon could be related to the intrinsic properties of the sample, such as pH, acidity or the type of the characteristic acids. Apple and melon pH values were 4.6 ± 0.3 and 5.7 ± 0.3 (Table 2) and main acids are malic and citric, respectively. The higher pH and lower acidity of the melon may facilitate the growth of the microorganisms when compared to those values in apple. Therefore, pH is acting as a hurdle preventing growth of *L. monocytogenes* by itself in those samples, which made lower reduction values.

The concentrations of FA used against *L. monocytogenes* were reduced from 2.5 to 1.0 mg / mL as it was observed that concentrations of 2.5 were more effective against *L. monocytogenes* than they were against *S. enterica*. That reduction in FA concentration was accompanied with a reduction in its efficacy in apple, but not in melon. When FA was applied at concentrations higher than 2.5 mg / mL, the antibacterial effect was similar for all of them, independently of the concentration tested. One possible explanation is that the FA that remained on the surface of the apple, independently to the concentration in the washing solution, was the same, because there could be a maximum surface / FA attachment ratio that

was already reached at 2.5 mg / mL. This attachment ratio could depend on the porosity of the matrix. In fact, the difference in applying the same concentration of FA to different fruit matrices (apple and melon) was patent when determining the remaining FA in their surfaces: it was 6 times higher in melon than it was in apple. FA has also been tested for *L. monocytogenes* growth inhibition in food matrices other than fruit: Takahashi (2013) added 2 or 4 mg FA / g of cheese or salmon, respectively, and observed that artificially inoculated *L. monocytogenes* in those food did not grow as much as the non-FA control did (2 or 3 log units in FA-treated cheese or salmon, compared to 5 logs in non-treated samples, after the end of the storage). This represents the need to evaluate the effect against pathogens both *in vitro* and *in vivo*, as the target matrix characteristics may interfere or interact with the antimicrobial agent or the pathogen in several ways. In fact, Belgacem (2020) also found differences between matrices (apple, melon and pear) when investigating the effect of a pomegranate peel extract (PGE) on the growth of *L. monocytogenes*. At a tested concentration of 12 g PGE / L, *L. monocytogenes* was reduced by 1.24, 1.89, and 0.91 log units soon after treatment and by 3.81, 1.53, and 2.99 log units, after 7 days of storage on apple, pear and melon, respectively.

Also, two different application methods, immersion and spray, were evaluated. If spray and immersion had a comparable effect, less FA solution would be needed for practical application, which would constitute a reduction in the FA amount, lower treatment time and also there would be no need for a draining step. Other studies did not show differences in the effect of antimicrobial essential oils on lettuce between these two application methods in mesophilic, psychrotrophic, and coliform bacteria (Ponce, 2011). In the present study, however, the immersion application method was selected over the spraying, because it was more effective in inhibiting growth of pathogens, probably because of a greater impregnation of the product. Also, 2.5 mg / mL of FA proved to be effective against *L. monocytogenes* but also in *S. enterica*, so to assure the efficacy in both species, this concentration was selected to continue with the following experiments.

Finally, FA preserved the quality of fresh-cut products. Parameters like pH, TSS or TA did not vary, and were in accordance to those found in the literature (Iglesias, 2006; Kolayli, 2010). Regarding textural quality, the application of NS in apple resulted in an increase in firmness as shown in previous works (Giacalone, 2013). As also observed by Rössle (2009), Natureseal ® reduced firmness loss in consequence of crosslinking of both cell wall and middle-lamella pectin by calcium ions. Firmness of apple wedges decreased along storage in FA-treated samples. On the contrary, the firmness of samples in the control treatment (NS) was maintained or even increased and was significantly higher than FA treated fresh-cut apple. The composition of this product, which includes calcium salts, should reduce firmness loss in consequence of crosslinking of both cell wall and middle-lamella pectin by the calcium ions (Rössle, 2009). In melon, the treatment FA-2.5 did not maintain firmness, which decreased with time comparably to the W control. The main causes of texture loss were probably attributed to enzyme activities, such as galactosidase, endo- polygalacturonase, and / or exo-polygalacturonase, which solubilize pectin in cell walls of melon pieces (Aguayo, 2004).

Color is an important sensory attribute that provides an indication of freshness and flavor quality to the consumer (Barrett, 2010). Browning is a product alteration easily detected by consumers leading to the rejection of the product (Jaeger, 2018). BI was used as an indicator of color quality in fresh-cut apples, which are highly affected by these reactions (Lunadei, 2010). NS was selected as a commercial antioxidant treatment to use in the fresh-cut apple processing industry. FA is considered to be an antioxidant and it has been reported to inhibit the apple polyphenol oxidase activity (PPO), by acting as a competitive inhibitor of apple PPO, preventing the binding between substrate and enzyme by occupying the latter's active place (Shannon, 1967). In this study, FA did not show to act as an anti-browning agent as NS treatment did. Previous work reported that 2.5 mg / mL inhibited 21.2 ± 1.9 %

the apple PPO activity (Data not shown). Maybe, regardless its reported PPO inhibitory activity at *in vitro* conditions, more concentration is needed to increase its visible anti-browning effect in apple. We have to take into account that fruit was stored under air conditions (not in modified atmosphere) and oxygen could facilitate browning. For this, further investigations would be needed, including the use of modified atmospheres or the combination with FA for pathogenic control and NS for color preservation. In melon, the TCD values averaging 1.4 indicate that color was well maintained during storage, as a TCD higher than 3.5 would mean that a clear difference in color is noticed by the inexperienced viewer (Mokrzycki, 2011).

Epidemiological studies suggest that a high intake of food rich in antioxidants increases the antioxidant capacity of the plasma and reduces the risk of some diseases. Also, the antioxidant capacity of a fruit can increase its stability during storage and prevent detrimental changes, including variations in color (Hassimotto, 2005). Apples treated with NS had higher antioxidant capacity than they had with FA-2.5. TPC values were also significantly higher in NS samples than they were in FA-2.5 samples. As NS does not contain phenolic compounds, the higher TPC values could be attributed to an overestimation of TPC by interference caused by ascorbate, which is included in the composition of Natureseal®. Ascorbic acid is a reducing compound (non-phenolic antioxidant), which also reduces the Folin-Ciocalteau reagent to form a blue color in alkaline pH (Lester, 2012). On the other hand, in melon, FA-2.5 samples showed higher antioxidant values when compared to W control. In fact, FA has already been reported to be a powerful antioxidant (Zduńska, 2018). Moreover, FA helped to maintain the antioxidant capacity of melon during storage and also the TPC content remained constant.

The effect of FA on native microbiota of apple and melon was also studied. Immediately after treatments, populations of TAM slightly decreased, possibly because of the soaking in agitated water. However, only FA-2.5 treatment in apple was able to control TAM populations after 12 days of storage at 4 ºC. Regarding Y&M, FA was not effective in decreasing or controlling populations in apple or melon. Moreover, the reductions in natural microbiota were lower than they were in the artificially inoculated pathogens. Even some authors have reported that FA at concentrations higher than 250 ppm would have antimicrobial effect against *Saccharomyces cerevisiae* (Baranowski, 1980), there is not much literature on how FA may affect growth of yeasts and molds. Thus, more studies on effective concentrations and action modes should be carried out in the future. Although there is not a legislation determining the non-pathogenic native microbiota in fresh-cut products, the final concentrations of TAM reached 5 log units per gram, from which, 2 to 3 log units were Y&M. The high levels of microorganisms could alter the food's appearance, odor, texture, or taste, because of their biochemical activity as they grow in the food, that can include carbohydrate degradation into simpler sugars, organic acid oxidation or sugar fermentation (Sperber, 2009).

5 Conclusions

In this paper, the application of ferulic acid (FA) in fresh-cut apple and melon was evaluated. Immersion method was selected over spray application for FA, as it proved to have higher efficacy. Although no bactericidal effect after washing was found against the studied pathogenic microorganisms, *L. monocytogenes* and *S. enterica*, FA at 2.5 mg / L highly prevented growth of *L. monocytogenes* on fresh-cut apple and melon during storage at 10 ºC for 7 days, without affecting the quality evaluated in fresh-cut apple and melon stored at 4 ºC for 12 and 7 days, respectively. Some effect was found against *S. enterica*, but populations in fresh-cut fruit remained relatively high after storage at 10 ºC for 7 days. Moreover, the reported health impact that FA may exert, including anti-inflammatory, anti-thrombosis and anti-cancer activities, could contribute to enhance nutritional and functional properties of fresh-cut fruit, adding value to these products for the consumers' benefit.

Overall, FA effect in delaying the growth of pathogenic microorganisms, *L. monocytogenes* and *S. enterica*, would present this substance as a potential ingredient or additive to be used in fresh-cut products, in order to offer consumers safe and quality products. In a possible real application, the use of FA should be accompanied by the disinfection step, as no disinfection effect has been demonstrated in the studied conditions. However, legislation, scale up, and other pathogenic strains or fruit matrices should be also evaluated when developing commercial products using this compound.

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Conflict of interests

The authors declare no conflict of interests.

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Combination of ferulic acid with *Aloe vera* gel or alginate-based coatings for shelf-life prolongation of fresh-cut apples

Chapter 10

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Weight loss, microbial spoilage and enzymatic browning are the main quality-determining processes which limit the shelf-life of fresh-cut apples. In this study, two edible coatings based on Aloe vera gel (AV) and sodium alginate cross-linked with calcium lactate (AC), with the addition of 10 mg/mL ferulic acid (FA) as a functional ingredient, were developed in order to prolong the quality and safety of fresh-cut apple discs. Texture parameters, pH and Brix values and water activity did not undergo relevant changes related to the treatments. Except for weight loss, which was significantly lower for the coated samples, the addition of FA was found to be the most relevant factor for the other measured parameters, including the total phenolic content and the antioxidant activity measured by ferric reducing antioxidant power (FRAP). Browning was delayed by the addition of FA and also by the AV coating, while non-coated and alginate coated samples showed the highest values in early stages. Although no effect on *Saccharomyces cerevisiae* was observed, FA treatments and alginate were effective in reducing *Listeria monocytogenes* populations by 2.3 ± 0.4 log CFU/apple disc, which contributes to greater product safety.

Edible coatings, *Listeria monocytogenes*, browning, weight loss, preservation, fresh-cut fruit

1 Introduction

Fresh-cut apples are defined as pieces of apple that have been subjected to a physical alteration from their original form but remain in a fresh state. They are prepared for immediate consumption, packaged and should be stored under refrigerated conditions, thus providing convenience to consumers (Rojas-Graü, 2010). The fresh-cut industry is a growing food sector as the global demand for healthy, fresh and sustainable products increases (Qadri, 2015). However, health concerns have been associated with minimally processed fresh-cut produce because such produce does not undergo any treatment that inactivates all pathogens prior to consumption. Hence pathogens introduced at any point in the production chain may be present when the produce is consumed. Fresh produce was reported to be one of the main vehicles of food-borne outbreaks in Europe, accounting for 8 % of all food-borne outbreaks in 2011. The most common causative agents were *Salmonella* spp., norovirus and pathogenic *Escherichia coli* (Ölmez, 2016). Moreover, growth of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* has previously been demonstrated on fresh-cut apples (Abadias, 2011). Because of their fresh nature and increased surface as a consequence of cutting processes, these products are very sensitive to food quality change. Beside microbiological spoilage, deterioration can also occur by chemical and physical processes such as water loss, enzymatic changes (e.g. browning), oxidation and loss of cellular integrity (softening). Demands for quality retention for the benefit of longer and more global distribution chains as well as rising consumer expectations for high and lasting quality have to be addressed (Wilson, 2019).

The application of edible coatings is an approach to retard quality change during storage. These coatings act as a semipermeable barriers to O_2, CO_2 and water vapour, thus preventing amongst other things water loss, changes in firmness and oxidation (Raghav, 2016). Furthermore, they can act as a carrier for functional ingredients. For instance, ferulic acid is an organic compound that can be derived from sustainable sources such as cereal by-products (Dapčević-Hadnadev, 2018), has antimicrobial properties and is reported to be a powerful antioxidant (Ou, 2004). The combination of edible coatings and ferulic acid could help to overcome the major drawbacks of fresh-cut apples, thus increasing their shelf-life and consumer safety.

Research and development efforts are leading to an improvement of the functional characteristics of the coatings, which depends on the properties of the fruit to be preserved or enhanced (Dhall, 2013). Moreover, the food industry shows an increased interest in product innovation which brings specific health benefit and the additives used in the food industry should be as natural as possible. However, the application of FA in food is still being investigated, and its incorporation in edible coating has only been studied by Alves, Gonçalves & Rocha (2017) in a soy-protein isolate-based edible coating. In this study, two edible coatings were applied to fresh-cut apple discs. Firstly, *Aloe vera* gel which is a promising alternative to synthetic preservatives and has been found to have inherent antioxidant and antimicrobial properties (Asamenew, 2011; Estepa, 2004; Ray, 2013). Secondly, a recently improved sodium alginate coating which involves dipping the fruit in calcium lactate before dipping in alginate and then again in calcium lactate, so creating a more uniform coating on the fruit surface having specific barrier properties (Parreidt, 2019). Ferulic acid was separately tested as well as in combination with the edible coatings for its antioxidative effect.

2 Materials and methods

2.1 Materials

For the preparation of the edible coatings, the following materials were used: sodium alginate (Halal, Manugel GHB, FMC Biopolymer Co., Philadelphia, Pa, USA); glycerol (> 99 %, FC), calcium L-lactate hydrate (> 98 %), Tween 80 (polyoxyethylensorbitan monooleate), Span 80 (sorbitan monooleate) and ferulic acid (99 %) (all supplied by Sigma-Aldrich Chemie GmbH, Steinheim, Germany); sunflower oil and Aloe vera used for the coating formulations were obtained from the local market. Apples (Jonagold) were purchased in a local supermarket.

For the microbial assays, Ringer solution (Sigma-Aldrich), triptone soy agar (TSA), plate count agar (PCA) yeast and mould universal media (YMA) and Oxoid Chromogenic *Listeria* Agar (OCLA) (Merck KGaA EMD Millipore, Darmstad, Germany) with chromogenic supplement (Oxoid, Thermofisher Scientific, Waltham, Massachussetts) were used.

2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, polyvinyl pirrolidone (PVPP), cistein, pyrocatechol and guayacol were acquired from Sigma-Aldrich (Steinheim, Germany). Sodium hypochlorite, peroxide hydrogen, methanol, sodium chloride, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were procured from Panreac (Llinars del Vallès, Spain).

2.2 Methods

2.2.1 Experimental design

Preparation of food samples: Apples of the variety Jonagold were purchased from a local supermarket and were stored at 5 °C until being used. Before the experiments, they were washed, disinfected with a commercial solution consisting of ethanol 23 % and propan-1-ol 35 % (w/w) and rinsed with sterile water to remove excess of the solution. Apples were cut into discs having a diameter of 2 cm and a height of 1 cm. The average weight of each apple disc (3.0 ± 0.1 g) was determined by weighing 90 discs using a Sartorius ED-153-N balance (Sartorius AG, Göttingen, Germany). Samples were prepared immediately before coating in order to prevent browning.

Coating preparation and application: In this study two edible coatings were investigated - Aloe vera gel and sodium alginate coating. A non-coated control was prepared using distilled water. Ferulic acid was used as a plant-derived antioxidant, mainly to prevent browning.

Aloe vera gel (AV) was prepared immediately after harvesting *Aloe vera* L. leaves. They were washed and disinfected with a commercial solution consisting on ethanol 23 % and propan-1-ol 35 % (w/w) and rinsed with sterile water. The outer rind was separated from the parenchyma and the mucilaginous gel was filtered to remove the fibers (Khaliq, Ramzan, & Baloch, 2019). The resultant was mixed at speed 6 with a Thermomix TM5 (Wuppertal, Germany), pasteurised at 80 °C for 5 minutes at speed 2 and then immediately cooled. Glycerol 1 % (w/v, final solution basis) was added to a solution of 40 % AV gel prepared using sterile water.

The sodium alginate coating (AC) was prepared according to Parreidt (2019). It consisted of a solution of sodium alginate in sterile distilled water (1.25 %) (w/v, final solution basis), stirred at 70 °C until complete dissolution. The flexibility of the coating was increased by using 2 % (w/v) glycerol as a plasticizer. In addition, 1 % (w/v) of Span 80 and 0.2 % (w/v) of Tween 80 were used as surfactants and 0.2 % (w/v) sunflower oil was used to increase the water barrier properties. The solution was

homogenised with an Ultra-turrax ® (Miccra D-8, ART model Labortechnik GmbH&co, KG, Staufen, Germany) at 10,500 min^{-1} for 5 min, and degassed in an Bandelin Sonorex RK 225 H ultrasonic bath (Bandelin, Berlin, Germany) at a frequency of 35 kHz for 5 min. A calcium lactate solution 2 % (w/v, final solution basis) was prepared to induce the gelling and cross-linking reaction.

Ferulic acid (FA) was added once the coatings were prepared to give a concentration of 10 mg / mL.

In total, six different variations were studied: non-coated (NC-X), non-coated with ferulic acid (NC-FA), Aloe vera gel (AV-X), Aloe vera gel with ferulic acid (AV-FA), alginate coating (AC-X) and sodium alginate coating with ferulic acid (AC-FA).

The coatings were applied as follows: For NC and AV, apple discs were dipped into the respective solutions for 10 s and drained for 90 s. For AC, the procedure described by Parreidt et al. (2019) was used. This involved a 10 s dipping in calcium lactate, sodium alginate and calcium lactate (in this sequence) and subsequent draining for 90 s after each dipping. After the dipping and draining cycles all the samples were dried for 35 min in a laminar flow cabin.

Storage conditions: The coated apple discs were kept in sterile petri dishes, with cams in order to prevent a gas tight packaging. The samples were stored in a cool chamber at 5 ± 0.5 °C and 50 ± 5 % relative humidity for up to 7 days. For destructive tests, a number of sample sets equal to the number of sampling days were prepared. For non-destructive analyses, tests were carried out on the same sampling sets.

2.2.2 Physical characterisation

Coating thickness: The coating thickness was determined using a stereo microscope (Leica MZ16, Leica Mikrosysteme Vertrieb GmbH, Benheim, Germany) by taking five measurements at different points along the cross-section of five samples (n=5).

Weight loss: For weight loss measurement, samples were stored individually in sterile petri dishes. Each sampling day, five samples were weighed and the measurements were made in triplicate (n=15) using a laboratory balance (Sartorius Lab Instruments, GmbH & Co. KG, Goettingen, Germany). An ionizing blower (18V, AC, 2W, Sartorius Lab Instruments, GmbH & Co. KG, Goettingen, Germany) was used to increase the accuracy of the results and to avoid the static charge of the packaging material. The weight loss was calculated as the percentage weight loss on each sampling day (D_n) relative to the initial weight (D_0) (see Equation 1).

$$\text{Weight loss (\%)} = \frac{\text{Weight } (D0) - \text{weight } (Dn)}{\text{Weight } (D0)} \times 100 \quad \text{Eq. 1}$$

Water activity (a_w): The water activity was determined using a dew point water activity meter model Aqualab 4TE (Metergroup, Spain). Prior to the measurement (n=4), the meter was calibrated using suitable standards at room temperature.

Water vapour resistance (WVR): The water vapour resistance (WVR, s / cm) was calculated gravimetrically as described by Poverenov (2014) with some modifications from the reference method in order to imitate storage conditions (temperature, relative humidity) as given by Equation 2:

$$\text{WVR} = \left[\frac{\left(a_w - \frac{\%\,RH}{100}\right) \times p_{wv}}{R \times (275.15 + T)}\right] \times \left(\frac{A}{J}\right) \qquad \text{Eq. 2}$$

where, a_w is the water activity of the apple discs, % RH is the relative humidity of the climatic chamber (50%), p_{WV} is the saturated water vapour pressure at 5 ºC (8.58 mbar), R is the specific gas constant for water vapour (461.5 J /kg· K = 4.61 bar cm^3 /g K), T is the temperature of the climatic chamber (5

°C), A is the surface area of the food products (12.56 cm², considered as cylinders) and J is the gradient of the weight loss of the food product versus storage time (g / s). J and a_w were measured using devices that were previously described in this section.

Oxygen consumption: The oxygen consumption of the apple discs was measured in a specific oxygen measuring cell using three coated apple discs per repetition (2 replicates, 4 repetitions) (n=8). For this purpose, the samples were weighed and stored in the closed cylindrical cell which had a volume of approximately 133 mL (measured in a specific set-up procedure by determining the pressure difference between the cell and a reference vessel and final calculation via the gas equation). The oxygen concentration inside was measured with an Oxi 340i meter (WTW, Weilheim in Oberbayern, Germany) attached to a Clark Electrode. The oxygen consumption was calculated using Equation 3 and expressed as mg O_2 / 100 g fresh weight.

Consumed oxygen (mg O_2 / 100 g of fresh FW) =

$$\frac{0.0021 \times P \times [O2\ air - O2\ cell] \times V \times 32}{83.12 \times [273.15+T]} \times \frac{100}{W} \quad \text{Eq. 3}$$

where P is the atmospheric pressure when measured (mbar), O_2 is the oxygen concentration in the air or inside the hermetic cell (% saturation), V is the headspace volume (mL), T is the temperature when measured (ºC) and W is the weight of the three apple discs inside each cell (g).

2.2.3 Physicochemical properties

pH and Brix values: The pH and Brix values of the apple discs were measured at 20°C. For each parameter and sampling day, five replicate samples were analysed three times (n=15). The pH value was measured with a puncture probe for solid foods using an EC-30 pH meter (Phoenix Instruments, Garbsen, Germany). The Brix value was determined using a near infrared food scanner (SCiO, Consumer's Physics, Herzliya, Israel).

Measurement of colour changes: Colour changes were determined using a DigiEye imaging system (Luo, 2001) with an illumination cabinet and a diffuse D65 illuminator designed by VeriVide Ltd.. Images of apple discs were taken of five replicate samples (three repetitions, n=15) on each sampling day. The colour was measured with DigiPix software which was calibrated using a table of standard colours given by the supplier. A standard circle of 1.7 cm Ø was used to determine where the colour had to be measured. The software analysed the CIE L* a* b* coordinates, chroma and Hue angle of each measurement. The browning index (BI) was calculated using equations 4.1 and 4.2, according to Pathare, Opara, & Al-Said (2013).

$$BI = 100 \times \left(\frac{X-0.31}{0.17}\right) \quad \text{Eq. 4.1}$$

where

$$X = \frac{(a* + 1.75\,L*) \times a*}{(5.645L* + a* - 3.012b*)} \quad \text{Eq. 4.2}$$

Texture profile analysis (TPA): Texture profile analysis was performed using a TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England) and a compression platen probe P/75. The pre-test, test and post-test speeds were 0.5, 0.5 and 1.0 mm / s, the deformation was 20 % and the trigger force was 0.05 N. For each sampling day, five replicate samples were measured (three repetitions, n=15). The hardness, springiness, cohesiveness and chewiness were calculated according to Guiné (2011).

Antioxidant activity (AOX): The antioxidant activity of the coated samples was assessed by ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazy (DPPH) scavenging activity assays, as described by Nicolau-Lapeña (2019). A calibration curve was made using FA as a standard. The results are expressed as μg ferulic acid equivalents (FAE) / g FW as determined on four replicate samples with three repetitions (n=12).

Total phenolic content (TPC): The total phenolic content of the coated apples was assessed by the Folin Ciocalteau method on the same extract used for the antioxidant activity determination. The procedure used was as described by Nicolau-Lapeña (2019). A calibration curve was made using FA as a standard. The results were expressed as μg ferulic acid equivalents (FAE) / g FW as determined on four replicate samples with three repetitions (n=12).

Polyphenol oxidase (PPO) activity: The enzymatic activity was determined by *a spectrophotometric method*. The enzymatic extraction was carried out by mixing 5 ± 0.5 g of the frozen product with 0.5 g PVPP and 10 mL 0.1 M phosphate buffer solution at pH 6 (PBS) with 0.05 mM cysteine in an Ultra-turrax ® Tube drive P control (IKA, Staufen, Germany) for 1.5 min at 5,000 rpm. After filtration and centrifugation at 14000 rpm for 10 min at 4 ºC, the supernatant was stored in ice.

The PPO activity determination was carried out by adding 20 μL of the sample to 280 μL of 0.2 M pyrocathecol in PBS. The absorbance at 400 nm was read every 9 s for 3 min using a PowerWave HT microplate spectrophotometer (Biotek, Vermont, United States). To determine the enzymatic activity, the absorbance was plotted as a function of time and the slope of the linear regression was used to calculate the increment of optical density over time. Four replicate samples with three repetitions (n=12) were measured and the results were expressed as Δoptical density (ΔOD) / g apple · s.

2.2.4 Microbiological investigations

Total aerobic mesophylls count: TAM population counts were carried out after 1, 4 and 7 days after coating the apple discs. For this, 1 disc per repetition of three replicate samples (two repetitions, n=6) was homogenized with 10 mL of Ringer solution with 1 % Tween 80 in a Smasher AES Chemunex (Laboratorie Biomeriéux, Quebec, Canada) for 120 s at fast speed (620 strokes / min). Then, serial decimal dilutions were prepared. Plating was made in duplicate for each replication by inclusion pouring 1 mL into a Petri dish and adding 15 mL PCA. The plates were incubated for three days at 30ºC. The results are expressed as log CFU / g.

***S. cerevisiae* and *L. monocytogenes* culture preparation:** Strains for microbiological assays (*Listeria monocytogenes* DSMZ 15675 and *Saccharomyces cerevisiae* DSMZ 70449) were purchased from German Collection of Microorganisms and Cell Cultures GmbH. They were cultured overnight in TSB or YMB, at 37 °C and 25 °C respectively. **Artificial inoculation of apples with *S. cerevisiae* and *L. monocytogenes*:** In order to inoculate apple discs, a suspension having a concentration of 1×10^6 CFU / mL was prepared. For this, 20 mL of the overnight culture were centrifuged at 10 °C at 9,000 rpm for 10 min. The supernatant was discarded. Then 20 mL sterile distilled water were added and the pellet was resuspended and centrifuged again under the same conditions. After resuspension in 20 mL of sterile distilled water, an appropriate volume was poured into 1 L of sterile distilled water to achieve the final desired concentration for the bath. The apple discs were immersed in the microbial suspension (ratio 1:5), agitated for 2 min and then drained until a 5 % increase in weight was achieved. Finally, the samples were dried in a laminar flow cabinet at room temperature for 30 min.

Effect of the coatings on the microbial quality: The various coatings were applied as described in section 2.2.1. on previously inoculated (with *S. cerevisiae* or *L. monocytogenes*) apple discs. Then they were stored in groups of three replicate samples for further investigation. Three samples were used for

determining the initial microorganism concentration just after coating. Microbial sampling was performed twice with three replicates on each sampling day (n=6). For this purpose, one apple disc was homogenized with 47 mL of sterile Ringer solution with 1 % Tween 80 according to the procedure previously described for TAM. Plating was done with the corresponding media (OCLA supplemented with chromogenic *Listeria* selective supplement for *L. monocytogenes* and YMA for *S. cerevisiae*). Plates were incubated for 48 h at 37 °C for *L. monocytogenes* and for 72 h at 25 °C for *S. cerevisiae*. The results were expressed as CFU / g.

2.3 Statistical analysis

The experiments followed a complete factorial design, with two factors: coating (NC, AV, AC) and antioxidant (X, FA). All data were checked for significance of the factors by applying analysis of variance (ANOVA), where interactions were also studied. The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analyses were carried using JMP 13 software (SAS Institute Inc., Cary, USA).

3 Results

3.1 Physical characterisation of the coated samples

The pH of the coating formulations was just before their application on apple discs: AV formulations (AV-X, AV-FA) had similar pH values, averaging of 3.64 ± 0.70, while the coating with alginate (AC-X, AC-FA) resulted in higher pH values of 5.77 ± 0.02 and 4.68 ± 0.06 respectively. The coating thickness was only measured for the AC samples, as the application of AV gel with or without FA, and also the aqueous solution of FA alone, did not result in a visually observable film around the apple discs, but remained embedded in the surface. The thickness of the AC film was approx. 228.2 ± 15.5 μm, and no significant differences were observed in thickness values for AC-X and AC-FA.

Immediately after the treatments, the apple discs increased in weight due to the immersion in the solutions. After immersion in NC and AV, the apple discs gained 4.9 ± 0.6 % of their initial weight, while the AC-X and AC-FA coatings increased the weight by 16.2 ± 1.4 % and 15.3 ± 2.5 % respectively. After the coating application, the weight variation during storage of the apple discs (expressed as weight loss (%)) is shown in **Figure 50**A. The time-dependent course of the weight loss of the apple discs was similar for all the treatments. The presence of FA was not a significant factor for weight loss in samples coated with the different formulations. Non-coated samples showed the highest water loss after 7 days, being 11.3 ± 0.4 % and 10.2 ± 0.6 % for X and FA respectively. Samples coated with Aloe vera without ferulic acid (AV-X) did not show statistically significant differences compared to NC treatments. The samples with AV-FA, AC-X and AC-FA coatings showed significantly lower weight loss than NC-X, being 8.8 ± 1.3 %, 8.8 ± 1.2 % and 8.2 ± 0.3 % at the end of the storage period respectively.

The time-dependent change in the water activity of the samples is shown in **Table 40**. Most of the coated sample discs maintained their a_w values from the beginning, except for the discs with AV coatings which started at lower a_w values of 0.987 ± 0.002 and ended at 0.992 ± 0.001. The a_w values of the other samples ranged from 0.989 ± 0.001 to 0.994 ± 0.004, with no remarkable effect of the coating, FA addition or storage day.

For calculation of the water vapour resistance values, the gradient of the weight variation over the seven days of storage and the mean of the a_w values of each treatment were used. Apart for the AV-coated samples, no differences were observed during the storage period. Figure 50B shows the higher WVR

of all coated samples with or without ferulic acid, compared to the non-coated (NC-X) samples. The NC samples had the lowest resistance to water vapour transmission. The increase in WVR averaged 12.0 ± 0.5 % for AV-X, AC-X and AC-FA and 22.8 ± 0.6 % for AV-FA. These values were significantly higher than those of non-coated samples. The implied reason for this is the coating solution. In the case of non-coated samples and the Aloe vera coated samples, the presence of ferulic acid significantly contributed to the increase in WVR.

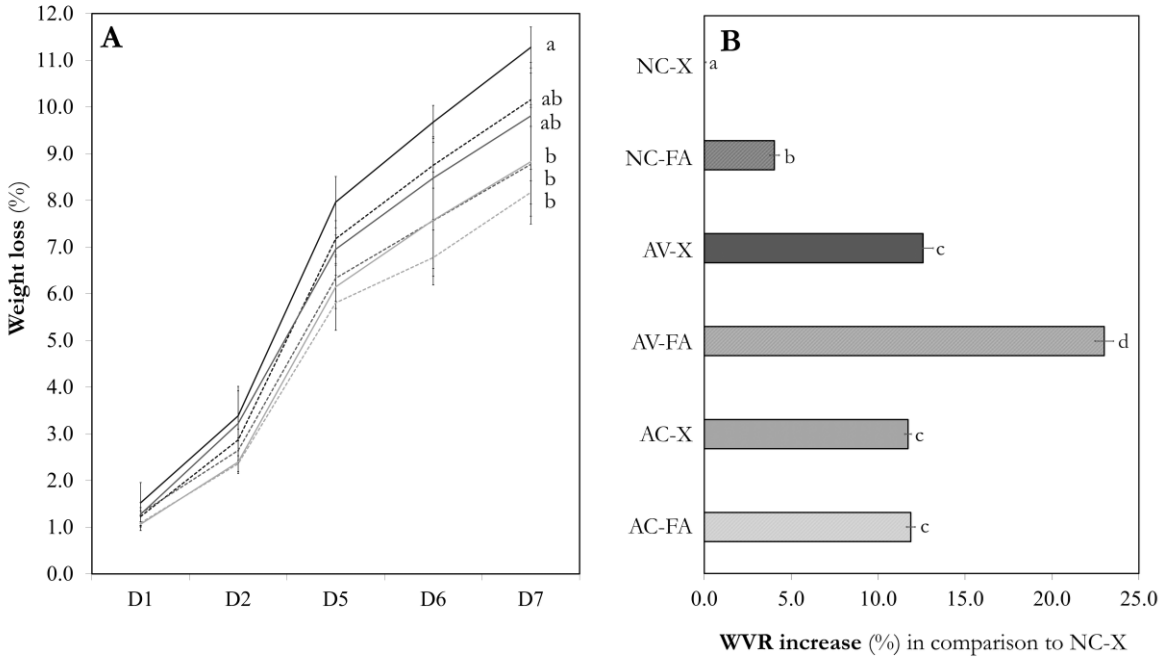

Figure 50. (**A**) Weight loss (%) of treated apple discs during 7 days of storage at 5 ± 0.5 ºC for non-coated samples (NC, ■), samples coated with Aloe vera gel (AV, ■) and samples coated with alginate and calcium lactate (AC, ■), both without ferulic acid (continuous line) and with ferulic acid (discontinuous line) (n=15). Different letters indicate statistically significant differences between treatments at the end of the storage period ($p < 0.05$). (**B**) Water vapour resistance of treated apple discs for non-coated samples (NC, ■), samples coated with Aloe vera gel (AV, ■) and samples coated with alginate and calcium lactate (AC, ■), both without ferulic acid (solid coloring) and with ferulic acid (discontinuous coloring) (n=4). Values are mean values and bars represent the standard deviation. Different letters indicate statistically significant differences between treatments ($p < 0.05$).

The apple samples' oxygen consumption (mg O_2 / 100 g FW) was measured for all coating variations (**Table 40**). According to the statistical analysis, FA was a significant factor affecting respiration. All samples containing FA consumed five times less oxygen, and the oxygen concentration in the measuring cell after the storage period was very similar to that at the beginning. In contrast, the oxygen concentration in the measuring cells containing apple discs coated with NC-X, AV-X and AC-X had respectively decreased by 83.0 ± 1.8, 105.1 ± 17.9 and 84.1 ± 19.3 mg O_2 / 100 g FW by the end of storage period.

Table 40. Values of water activity (a_w), at days 1, 4 and 7 (D1, D4, and D7), and oxygen consumption expressed as mg O_2 / 100 g fresh weight (FW), at days 1, 2, 5 and 7 (D1, D2, D5, and D7). Results are expressed as the mean values ± standard deviation, where a_w (n=4), and O_2 consumption (n=8). Different lowercase letters mean statistically significant differences between treatments within the same day, and different capital letters mean statistically significant differences between days for one treatment ($p < 0.05$)

	a_w			**Oxygen consumption** (mg O_2/ 100 g FW)			
	D1	**D4**	**D7**	**D1**	**D2**	**D5**	**D7**
NC-X	0.991 ± 0.002 ^{abA}	0.993 ± 0.003 ^{abA}	0.992 ± 0.002 ^{aA}	20.9 ± 0.9 ^{a}	31.6 ± 2.2 ^{b}	59.4 ± 5.7 ^{b}	83.0 ± 1.8 ^{b}
NC-FA	0.991 ± 0.005 ^{abA}	0.989 ± 0.001 ^{bA}	0.992 ± 0.001 ^{aA}	10.9 ± 2.0 ^{b}	13.3 ± 0.4 ^{d}	17.7 ± 0.8 ^{c}	20.2 ± 0.8 ^{c}
AV-X	0.986 ± 0.002 ^{bA}	0.989 ± 0.001 ^{bB}	0.992 ±0.001 ^{aC}	22.7 ± 2.4 ^{a}	56.1 ± 18.2 ^{a}	79.4 ± 8.3 ^{a}	105.1 ± 17.9 ^{a}
AV-FA	0.987 ± 0.002 ^{bA}	0.991 ± 0.002 ^{abB}	0.992 ± 0.001 ^{aB}	13.4 ± 0.1 ^{b}	15.4 ± 2.1 ^{cd}	21.1 ± 2.1 ^{c}	28.2 ± 7.5 ^{c}
AC-X	0.991 ± 0.001 ^{abA}	0.993 ± 0.001 ^{aA}	0.993 ± 0.002 ^{aA}	20.3 ± 1.1 ^{a}	29.9 ± 3.0 ^{bc}	62.5 ± 9.5 ^{b}	84.1 ± 19.3 ^{b}
AC-FA	0.994 ± 0.004 ^{aA}	0.993 ± 0.002 ^{aA}	0.992 ± 0.001 ^{aA}	12.6 ± 1.2 ^{b}	14.2 ± 0.2 ^{cd}	16.3 ± 2.5 ^{c}	17.3 ± 2.9 ^{c}

3.2 Physicochemical properties

The **pH and Brix** values of the coated apple samples were stable during storage. The average pH and Brix values of the apple discs (coated and uncoated) at the beginning of the storage period were 3.6 ± 0.2 and 11.4 ± 0.5 ° B respectively. The relevant values were 3.7 ± 0.2 and 10.8 ± 0.4 ° B after seven days of storage (data not shown).

Table 41. Values of pH, °Brix and texture profile analysis (TPA) of the apple discs. Results are presented by the mean values ± standard deviation (n=15).

	pH		**° Brix**		**Peak force** (N)	
	D0	**D7**	**D0**	**D7**	**D0**	**D7**
NC-X	3.9 ± 0.5 ^{a}	3.8 ± 0.4 ^{a}	11.3 ± 0.1 ^{a}	10.2 ± 1.5 ^{a}	167.9 ± 16.1 ^{a}	129.8 ± 12.4 ^{a}
NC-FA	3.5 ± 0.2 ^{a}	3.5 ± 0.1 ^{a}	10.8 ± 0.6 ^{a}	10.4 ± 0.6 ^{a}	164.9 ± 20.9 ^{a}	161.0 ± 13.0 ^{a}
AV-X	3.4 ± 0.1 ^{a}	3.6 ± 0.2 ^{a}	11.4 ± 0.1 ^{a}	10.9 ± 1.0 ^{a}	148.9 ± 12.1 ^{a}	158.6 ± 7.6 ^{a}
AV-FA	3.5 ± 0.2 ^{a}	3.5 ± 0.1 ^{a}	12.1 ± 0.7 ^{a}	11.1 ± 1.1 ^{a}	155.1 ± 3.9 ^{a}	147.1 ± 2.7 ^{a}
AC-X	3.7 ± 0.6 ^{a}	3.9 ± 0.3 ^{a}	11.7 ± 0.2 ^{a}	11.2 ± 1.6 ^{a}	157.1 ± 5.6 ^{a}	145.8 ± 3.8 ^{a}
AC-FA	3.7 ± 0.1 ^{a}	3.7 ± 0.1 ^{a}	11.0 ± 0.6 ^{a}	10.7 ± 1.1 ^{a}	177.8 ± 15.3 ^{a}	117.1 ± 13.1 ^{a}
	Cohesiveness ([ ])		**Resilience** ([ ])		**Chewiness** (N)	
	D0	**D7**	**D0**	**D7**	**D0**	**D7**
NC-X	0.6 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	65.3 ± 0.6 ^{a}	58.7 ± 10.9 ^{a}
NC-FA	0.5 ± 0.1 ^{a}	0.4 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	49.1 ± 0.1 ^{a}	54.7 ± 22.0 ^{a}
AV-X	0.6 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	0.4 ± 0.1 ^{a}	0.2 ± 0.1 ^{a}	49.1 ± 3.4 ^{a}	53.4 ± 18.4 ^{a}
AV-FA	0.6 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	0.4 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	40.8 ± 9.9 ^{a}	60.5 ± 13.3 ^{a}
AC-X	0.6 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	60.5 ± 4.7 ^{a}	54.1 ± 8.7 ^{a}
AC-FA	0.5 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	0.4 ± 0.1 ^{a}	38.1 ± 2.3 ^{a}	47.5 ± 6.7 ^{a}

The **colour** of the samples was evaluated using the browning index (BI). It was noticeable after the coating and drying process that the BI of the uncoated samples (NC-X) was 10.8 ± 1.1 (**Figure 51**). That value was maintained during the storage period. The initial BI values were 5.4 ± 0.8, 4.0 ± 2.7, 4.0 ± 1.5, 5.6 ± 2.1 and 3.7 ± 1.2 for the NC-FA, AV-X, AV-FA, AC-X and AC-FA samples respectively. These values were significantly lower than they were for NC-X samples. AC-FA samples reached a BI value of 10 at day 5 and the BI continued to increase after that. After six days of storage, all the other samples coated with FA and also AV-X showed significantly increased BI values, but none of them achieved values of 10. At the end of the storage period, the samples with the slowest browning reactions were found to be NC-FA, AV-X and AV-FA, with final BI values of 5.2 ± 2.3, 6.5 ± 2.7 and 6.6 ± 2.9 respectively.

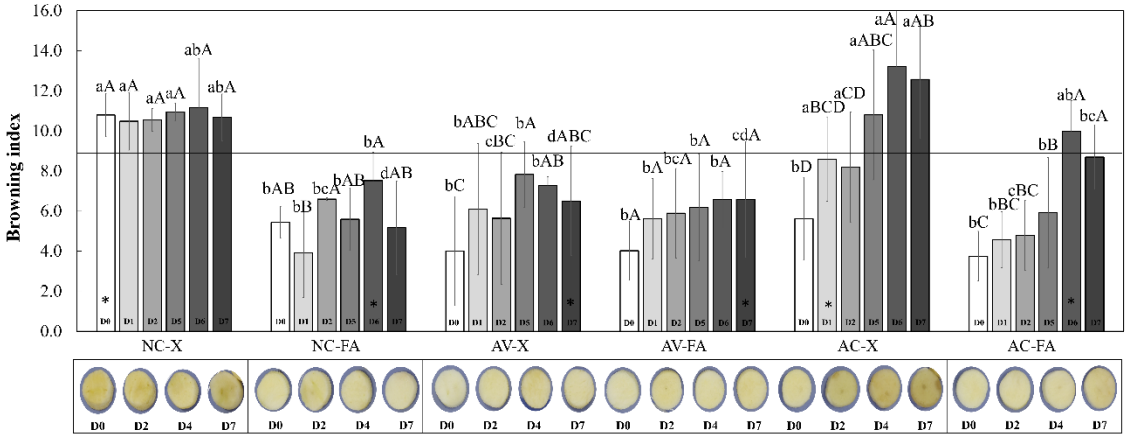

Figure 51. Browning index of treated apple discs for non-coated (NC) samples, samples coated with Aloe vera gel (AV) and samples coated with alginate and calcium lactate (AC), both without ferulic acid (X) and with ferulic acid (FA), after different days of storage at 5 ± 0.5 ºC (n=15). The images show the evolution of the apple surface browning. Values are mean values and the bars represent the standard deviation. Asterisks denote the day when significant browning was noticeable. Different lowercase letters indicate statistically significant differences between treatments for the same storage day ($p < 0.05$). Different capital letters indicate statistically significant differences between days within for same treatment ($p < 0.05$).

Texture profile analyses (TPA) did not reveal an effect on the apple discs that could be attributed to the coating or to the addition of FA. On average, the initial firmness, cohesiveness, resilience and chewiness values were 161.4 ± 21.7 N, 0.6 ± 0.1, 0.4 ± 0.1, 65.3 ± 18.3 % and 51.6 ± 12.7 N respectively (see Table 41 for detailed data). No relevant differences were observed between the treatments, and after 7 days the firmness, cohesiveness and resilience values had decreased by 11.3, 15.3 and 20.7 % respectively and the chewiness had increased by 6.2 %.

The impact of the AV and AC coatings on the **antioxidant properties** of the samples, with and without addition of FA, is shown in **Table 42**. AOX studied by DPPH· assay indicates that at the beginning of the study the AOX values of samples with different treatments were not statistically different. The exception was the AC-FA coated apple discs for which the AOX values were significantly higher, reaching 1751.9 ± 134.4 μg FAE / g FW. Although some deviations were found during storage (at D4 AOX values decreased for NC-X, NC-FA and AV-X coated samples), after seven days at 5 ºC the final DPPH· values were not significantly different to the initial values. Regarding the AOX capacity studied by FRAP assay, treatments with FA showed significantly higher AOX values than their counterparts without FA. Apart for a decrease in FRAP values for AC-X samples, all the other FRAP values were maintained during the storage period.

The total phenolic content (TPC) values are shown in **Table 42**. The highest values were observed for the NC-FA and AC-FA samples, being 23.7 ± 4.7 and 28.4 ± 0.8 μg FAE / g FW respectively, followed by 15.2 ± 2.2 and 17.9 ± 2.9 μg FAE / g FW for AV-X and AV-FA samples respectively. At the end of storage period, the differences in values between treatments were similar to those found at the beginning.

The **polyphenol oxidase activity** of the apple discs was determined after different days of storage (**Table 42**). The statistical analysis revealed that FA was a significant factor affecting the PPO activity on the first day of storage, with all samples with FA showing lower PPO activity. Even so, there was an evolution in PPO performance over time. At the end of the storage period, two different groups could be distinguished: those with lower activity, namely NC-X, and AV-X, averaging 458.0 ± 26.0 ΔOD / kg·s, and the others with higher activity of 631.6 ± 40.9 ΔOD / kg·s.

Table 42. DPPH·, FRAP and total phenolic content (TPC) values, expressed as µg ferulic acid equivalents (FAE) /g fresh weight (FW), at days 1, 4 and 7 (D1, D4, and D7). Results are expressed as the mean ± standard deviation (n=12). Polyphenol oxidase (PPO) activity values, expressed increase of optical density / kg · s (ΔOD / kg · s) at days 1, 4 and 7 (D1, D4, and D7). Results are expressed as the mean values ± standard deviation (n=4). Different lowercase letters mean statistically significant differences between treatments within the same day, and different capital letters mean statistically significant differences between days for one treatment ($p < 0.05$).

	DPPH· (µg FAE / g FW)			**FRAP** (µg FAE / g FW)		
	D1	**D4**	**D7**	**D1**	**D4**	**D7**
NC-X	559.0 ± 84.5 ^{aAB}	544.7 ± 46.2 ^{abB}	754.4 ± 49.1 ^{bA}	53.6 ± 7.6 ^{abA}	56.6 ± 11.7 ^{aA}	56.6 ± 6.4 ^{bA}
NC-FA	1359.8 ± 169.9 ^{aA}	861.0 ± 167.8 ^{abB}	1210.6 ± 201.6 ^{dA}	110.2 ± 15.8 ^{dA}	82.0 ± 8.9 ^{bB}	116.6 ± 10.5 ^{eA}
AV-X	929.7 ± 204.7 ^{aA}	733.4 ± 593 ^{abB}	933.6 ± 117.7 ^{cA}	65.0 ± 10.6 ^{bA}	53.5 ± 1.2 ^{aB}	66.7 ± 3.1 ^{cA}
AV-FA	980.9 ± 192.5 ^{aA}	1078.9 ± 274.9 ^{bA}	798.0 ± 80.1 ^{bcA}	86.8 ± 16.1 ^{cA}	91.7 ± 12.9 ^{bA}	91.0 ± 3.0 ^{dA}
AC-X	677.3 ± 147.3 ^{aA}	642.9 ± 168.8 ^{aA}	423.9 ± 79.3 ^{aA}	49.1 ± 8.1 ^{aA}	46.3 ± 7.9 ^{aAB}	40.2 ± 3.1 ^{aB}
AC-FA	1751.9 ± 134.4 ^{bA}	1209.4 ± 313.1 ^{cA}	1273.8 ± 144.1 ^{dA}	135.2 ± 22.1 ^{eA}	122.6 ± 23.9 ^{cA}	112.9 ± 16.7 ^{eA}
	TPC (µg FAE / g FW)			**PPO** (ΔOD / kg·s)		
	D1	**D4**	**D7**	**D1**	**D4**	**D7**
NC-X	9.8 ± 0.7 ^{bA}	10.9 ± 2.4 ^{aAB}	11.3 ± 1.4 ^{bB}	444.5 ± 62.0 ^{abAB}	381.6 ± 120.2 ^{cA}	476.4 ± 44.9 ^{bB}
NC-FA	23.7 ± 4.7 ^{dA}	15.5 ± 2.1 ^{bB}	24.6 ± 2.0 ^{eA}	289.8 ± 45.9 ^{bA}	551.2 ± 45.9 ^{abB}	592.3 ± 5.8 ^{abB}
AV-X	15.2 ± 2.2 ^{cA}	12.0 ± 1.2 ^{aB}	13.0 ± 0.3 ^{cAB}	367.6 ± 13.6 ^{bA}	535.9 ± 18.4 ^{abcB}	439.6 ± 64.9 ^{cB}
AV-FA	17.9 ± 2.9 ^{cA}	15.8 ± 2.9 ^{bA}	17.8 ± 1.2 ^{dA}	405.1 ± 89.5 ^{bA}	448.2 ± 109.3 ^{bcA}	687.8 ± 109.3 ^{aB}
AC-X	6.4 ± 0.9 ^{aA}	10.2 ± 0.2 ^{aC}	7.7 ± 0.2 ^{aB}	597.2 ± 22.3 ^{aA}	617.7 ± 22.3 ^{aA}	632.2 ± 95.9 ^{aA}
AC-FA	28.4 ± 0.8 ^{eA}	27.6 ± 3.0 ^{cA}	24.0 ± 1.0 ^{eB}	447.1 ± 67.8 ^{abA}	441.9 ± 72.5 ^{bcA}	614.2 ± 23.8 ^{aB}

The polyphenol oxidase activity of the apple discs was determined after different days of storage (**Table 42**). The statistical analysis revealed that FA was a significant factor affecting the PPO activity on the first day of storage, with all samples with FA showing lower PPO activity. Even so, there was an evolution in PPO performance over time. At the end of the storage period, two different groups could be distinguished: those with lower activity, namely NC-X, and AV-X, averaging 458.0 ± 26.0 ΔOD / kg·s, and the others with higher activity of 631.6 ± 40.9 ΔOD / kg·s.

3.3 Microbiological investigations

The **total aerobic mesophylls** count in the apple discs was measured at days 1, 4 and 7. After 1 day of storage, TAM populations ranged between 1.3 ± 0.7 and 3.4 ± 0.2 log CFU / g (Figure 52A). The presence of FA caused a decrease in TAM populations, and at the end of the storage period all FA samples had a two-fold lower count than X samples had.

The **initial *S. cerevisiae*** population in the apple discs after inoculation was 5.0×10^4 CFU / g. Immediately after the coating procedure, no changes in the population were found. Figure 52B shows, for each treatment, the variation in the *S. cerevisiae* population from day 0 to days 4 and 7 respectively. After 4 and 7 days, no significant growth or reduction in *S. cerevisiae* count was observed. Statistical analysis revealed no significant effect of the different coating variations on the population of *S. cerevisiae* in the apple discs. *S. cerevisiae* could only grow in apple discs coated with AC-X, reaching populations of 0.9 ± 0.4 log CFU / g.

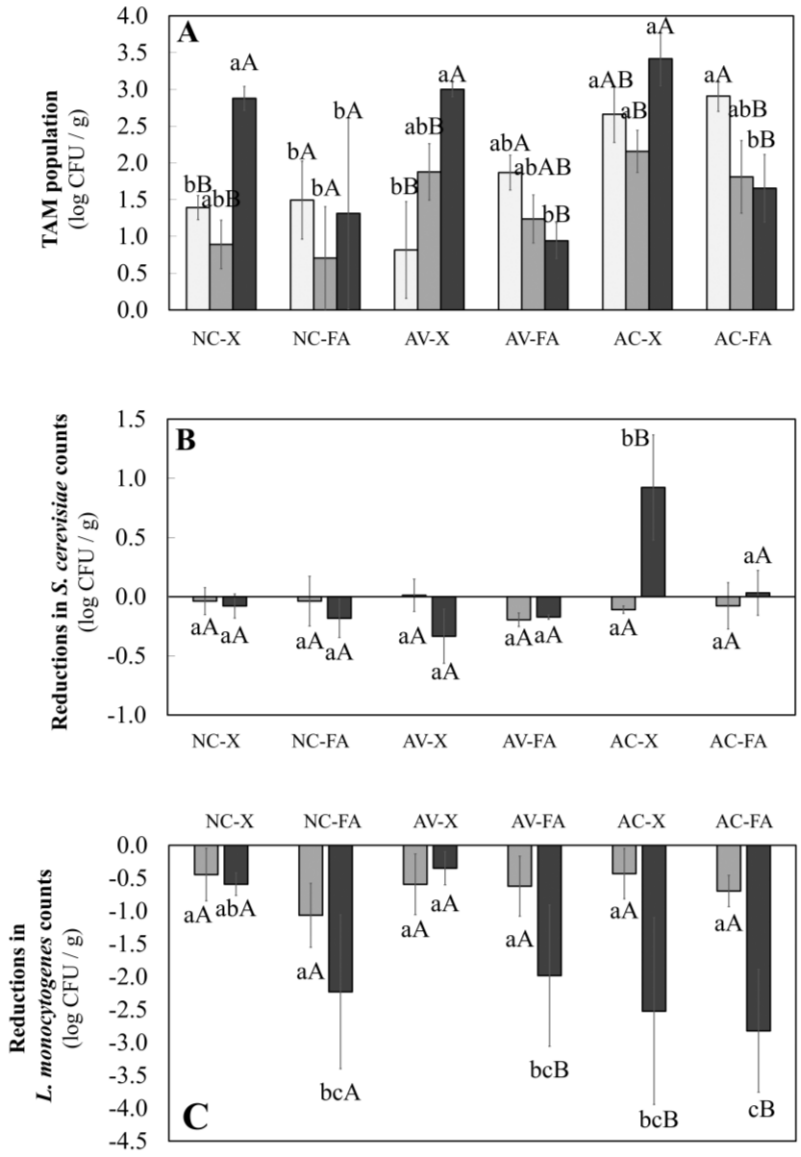

Figure 52. (A) Total aerobic mesophyll count for non-coated (NC) samples, samples coated with Aloe vera gel (AV) and samples coated with alginate and calcium lactate (AC), both without ferulic acid (X) and with ferulic acid (FA), after storage at 5 ± 0.5 ºC on day 1 (■), day 4 (■) and day 7 (■) (n=3). (B) *Saccharomyces cerevisiae* and (C) *Listeria monocytogenes* reduction, compared to the respective initial populations immediately after application of the coatings, after storage at 5 ºC on day 4 (■) and day 7 (■) (n=6). Values are mean values (log CFU / g) and bars represent the standard deviation. Different lowercase letters indicate statistically significant differences between treatments for the same storage day ($p < 0.05$). Different capital letters indicate statistically significant differences between days for the same treatment ($p < 0.05$).

Artificial inoculation of apple discs with cells of ***L. monocytogenes*** resulted in a population of 7×10^4 CFU / g. Immediately after the coating procedure, no changes to the population were found (Figure 52C). After a storage period of four days, the microbial count of *L. monocytogenes* decreased by 0.6 ± 0.2 log CFU / g for all treatments, including those without a coating or FA. After seven days of storage, no further inhibition was observed in the case of NC-X. The AV coating did not contribute to a decrease in the pathogen load, although some antimicrobial effect was expected for this treatment. Surprisingly, *L. monocytogenes* in AC-X samples showed a decrease comparable to the samples with FA. The samples coated with FA showed a microbial load reduction of 2.3 ± 0.4 log CFU / g compared to the first day populations.

4 Discussion

In this study, two edible coatings based on sodium alginate and Aloe vera, with and without the addition of ferulic acid, were investigated. The coating **thickness** in our study was higher than that reported by Rojas-Graü et al. (2007) and was 132.5 ± 20.5 μm for alginate coated fresh-cut apples. This may well be attributed to the double dipping in calcium lactate which implies two cross-linking steps. This could impact sensory aspects, for example the mouth-feel. However, the thickness of the coating is within the range published in the literature for edible coatings, namely < 300 μm (Pavlath, 2009).

The weight loss, colour change and microbial growth were the three main quality parameters chosen for investigation in this work. Weight loss is expected to occur during the shelf-life of fresh-cut fruit. The main cause is water loss, caused by the migration of water from the fruit to the ambient air (Soliva-Fortuny, 2003). In the case of the uncoated samples (NC-X) which served as a control, the weight loss was 11.3 ± 0.4 % after seven days of storage. Edible coatings can retard this process, by creating a semi-permeable barrier to water vapour. In this study, the treatments that offered the best solution for reducing the weight loss of apple discs (which at the end of storage averaged 8.6 ± 0.4 %) were AV-FA, AC-X and AC-FA. Aloe vera gel alone has also demonstrated its ability to reduce weight loss in white button mushrooms coated with 50 % AV gel (Mirshekari, 2019) and peaches coated with 20 % AV gel (Hazrati, 2017). AC has also been reported to decrease weight loss in fresh-cut apples (Olivas, 2007) and in other fruits such as plums (Valero, 2013). The measured a_w values agree with those reported in the literature (Schmidt, 2008). However, the fact that the a_w did not practically change while some of the samples (such as NC coated ones) had lost about 11 % of weight could be explained by the fact that a_w value represents the free, non-bound water of the product. It is possible that, even though a product losses a percentage of its weight in the form of water, the water activity is still not affected because more drying is necessary for this. For instance, the study carried out by Chambi (2016) shows that the gradients of moisture and aw of melon do not have the same slope, and that different moisture contents in the sample could show similar water activities. In the case of the present study, coatings were applied to the samples: AV gel presented a water activity of 0.9957 ± 0.02 and presented 1.36 ± 0.08 % dry matter. Alginate coating also presents a high content of unbound water, as it contains 2.25 % of soluble solids. In this regard, Parreidt (2019) indicated that coating strawberries with alginate coating increased the available, unbound water amount, observing non-dependent moisture contents and water activities in the samples, which could have had happened in our study. Polysaccharides are reported to have lower water vapour barrier properties than other materials used for edible coatings such as lipids and some proteins (Vargas, 2008). In this study, we observed significant differences in the water vapour resistance between the coated and uncoated samples. The coatings AV-X, AC-X and AC-VA increased the WVR by approximately 12.1 ± 0.5 % and the value for AV-FA was 22.8 ± 0.6 % higher than it was for NC-X samples. This may explain the reduction in weight loss of coated samples in this study. Although the presence of FA decreased the weight loss, albeit not significantly, of the samples compared to their non-FA equivalents, the presence of FA in the coatings increased the WVR of NC and AV coated samples compared to their counterparts without FA. This could be explained due to the fact that the WVR calculation takes into account not only the weight loss gradient (which was already different but not significantly) but also the a_w value. Meanwhile, in the case of CA, WVR was not altered by the presence of FA. The effect of FA in this case was negligible for both the weight loss and the water activity, not having an impact on this parameter. This effect of FA has not been described in the literature until now as FA has been reported to not easily disperse along the surface of fruit and it is recommended to be incorporated within an edible matrix (Alves, 2017). When FA is

added to a polymer coating, WVR values can increase because it facilitates the cross-linking of the polysaccharides (Cao, 2007).

Regarding **quality parameters**, the pH values of the apples used in this study agreed with those in the literature for this variety (3.2 - 3.9) but the Brix values were lower than the reported values (12 - 18 ºB) (Lammertyn, 1998). This could be related to the maturity of the fruit which affects the soluble solids content and other quality parameters such as the titratable acidity (Herregods, 1993). The study of the **texture** using TPA analysis was carried out because the parameters measured with this method have shown good correlation with sensory evaluations (Li, 2017). The observed variations in the investigated parameters are attributed to the action of pectic enzymes. After cutting operations, the mixing of substrates and enzymes (which normally are separated) initiates reactions which would normally not occur at such high rates (Toivonen, 2008). Even though edible coatings could help in preserving the turgor pressure of the vegetable tissue, a texture enhancer is often added to the coating. The most common enhancers are calcium salts. Calcium ions interact with pectic polymers to form a cross-linked network that increases the mechanical strength (Rojas-Graü, 2009). In fact, FA has been reported to maintain the firmness of blueberries which were dipped in an aqueous solution of 0.5 g FA / L and stored for 15 days. However, the underlying mechanism was not described (Xu, 2017). In another study, dipping apple slices into a formulation of ferulic acid at 4 g / L was found to be the most effective way to maintain their texture (Alves, 2017).

Colour maintenance was considered one of the main focuses of this study as it can affect consumers' buying intention, as it is an indicator of freshness and flavour quality (Barrett, 2010). The browning index (BI) is used as an indicator of colour changes in fresh-cut apples (Lunadei, 2010). In the case of the uncoated samples, the BI increased during the first 35 min and then remained constant throughout the storage period. As no coating and no antioxidant were applied, we considered the mean value as the standard browning that can occur in this variety of apples. The application of the AC-X coating was not able to delay browning for more than four days. Bertrand (2015) showed that the effect of an alginate coating 2 % (w:v) was negligible when compared with that of anti-browning agents incorporated into the coating on fresh-cut apple slices. The AV coating led to retarded browning and apple samples did not reach the browning levels of the control even after the 7th day of storage. Indeed, AV gel is reported to contain a number of antioxidant compounds, including aloe-emodin, anthraquinones and acemannan, which could contribute to colour preservation. Other reports have already described this effect on fresh and fresh-cut fruit and vegetables, including guava, plums and mango (Nasution, 2015; Pérez, 2016; Martínez-Romero, 2017). The samples to which FA was added showed good retention of colour, especially NC-FA and AV-FA, which retained their initial BI values for the 7 days. Ferulic acid has been reported to be a powerful antioxidant (Ggaf, 1992; Kumar, 2014) and has been used in edible coatings for that purpose (Fabra, 2011; Baraiya, 2016). Regarding the **antioxidant activity** of the coated samples, FA was the main factor that contributed to the increase in the AOX by DPPH· and FRAP values. The AV gel also caused an increase in the AOX values of coated apple discs, due to the high content of antioxidants. However, it must be noted here that, at D4 of storage period, AOX values by DPPH· and FRAP had decreased for NC-X, NC-FA and AV-X samples. The reason behind still needs to be clarified, as although there is a clear correlation between AOX and TPC in this paper, their variations cannot be explained by PPO activity. In fact, other authors have reported a decrease in antioxidant activities followed by an increase, depending on the matrix (Kevers, 2007) or the coating treatments (Oms-Oliu, 2008). In this regard, the phenolic and other antioxidant molecules such as organic acids have intricate pathways with a lot of enzymatic reactions involved. For this, variations in the enzymes (e.g. lipoxygenase, catalase, peroxidase) should be further assessed in order to identify their interactions with the different coatings and with the FA studied in

the present paper. The highest total phenolic contents were found in NC-FA and AC-FA samples, followed by AV-X and AV-FA samples. This is explained by the presence of FA, which is a phenolic acid, and the phenolic compounds in AV gel. Coatings such as alginate have no remarkable effect on the total phenolic compounds in treated apples (Cofelice, 2019). The observed increase in phenolic compounds in most of the coating variations could be attributed to activation of phenylalanine ammonia lyase after cutting the apples, with consequent formation of phenols as a defence mechanism against pathogens or water loss (Reyes, Villarreal, 2007). After cutting the plant tissue, enzymatic browning by polyphenol oxidase (PPO) is triggered by the decompartmentation of cells, which brings the enzyme and the substrates into contact. PPO has been directly related to the browning of fresh-cut apples but is dependent on the amount and type of polyphenols (Ferreira-Holderbaum, 2010). Shannon & Pratt (1967) proposed that FA may act as a competitive inhibitor of apple PPO, preventing the binding between substrate and enzyme by occupying the latter's active sites. Contrary to what was expected, the increase in PPO activity after seven days of storage for samples containing FA was 104.4, 69.8, and 37.4 % for NC-FA, AV-FA and CA-FA samples respectively, while the corresponding non-FA counterparts showed increases in PPO activity of 7.2, 19.8 and 5.9 %. This increase in PPO activity is not reflected in the BI of the samples, so we hypothesise that, despite the higher PPO activity, the browning could have been reduced by donation of an electron to the intermediate quinone of other compounds present in the matrix and in the coating (Nirmal & Benjakul, 2009).The oxygen consumption of a product can be determined by periodic measurement of the oxygen concentration within a hermetic cell (Saltveit, 2000). Edible coatings are reported to act as semi-permeable barriers for gases, decreasing the amount of O_2 and CO_2 that is exchanged between the product and the ambient environment, creating a modified atmosphere within the coating (Dhall, 2013). However, in this study we did not observe a reduction in the **oxygen concentration** due to the coating, but rather to the presence of FA: samples with FA did not consume as much O_2 as samples without FA. One hypothesis to explain this behaviour is that FA inhibits the PPO activity of the samples, and this enzyme does not use the available O_2. However, PPO activity increased more in FA samples than it did in samples without FA, so it was not inhibited by FA. To try to respond this decrease in oxygen consumption, we investigated the correlation between the oxygen consumption and the concentration of total aerobic bacteria in the fruit. Although AC samples showed the higher TAM at the beginning of the storage period, FA decreased their growth during the 7 days of storage. Samples without FA had significantly higher microbial populations needing oxygen for their metabolism. This led to the assumption that the microbial load mainly contributed to the observed oxygen consumption.

S. cerevisiae was studied because it is a food-borne yeast typically found in apple juice, arising from the processing of the fruit (Guerrero-Beltrán, 2005). Therefore, it was used as an example of an alternative microorganism that can occur in fresh-cut apples. After dipping in the coating solutions, the populations were maintained, indicating no microbiocidal effect of the coatings. In this study, the growth of *S. cerevisiae* was only observed in one set of samples (AC-X), which was attributed to the higher pH value of the coating (5.7) and the absence of FA, enabling suitable conditions for propagation of *S. cerevisiae*. In contrast, some authors have reported an inhibitory effect of FA against *S. cerevisiae* at doses above 250 ppm (Baranowski, 1980).

L. monocytogenes is a species of food-borne pathogenic bacteria that can even grow at low temperatures, and represents a problem for produce stored under refrigeration (4ºC) (Qadri, 2015). Even though there have been no outbreaks associated with *L. monocytogenes* and whole apples (Harris, 2003), the potential growth of food-borne pathogens is greater on fresh-cut produce than on produce with an intact peel because these products are minimally processed and there are more nutrients available on the cut surface (Conway, 2000). In this study, a decrease in the population of *L. monocytogenes*

was found in all the samples. We attributed this to the pH value of the apples which was lower than 3.9, the value reported as the limit for its growth (European Union Reference Laboratory for *Listeria monocytogenes, 2014)*. The Aloe vera coating was investigated in this study because of its reported antimicrobial activity, especially against epiphytic microbiota (Zapata, 2011). Under the experimental conditions used here, AV alone did not cause any decrease in the *L. monocytogenes* population. Various reasons could explain this, such as a low concentration of antimicrobial compounds (namely anthraquinones and aloe-emodin) in this batch of AV gel compared to those reported in literature (Pellizoni, 2016) or the resistance of the *L. monocytogenes* strain used in this study to those compounds. Unlike the Aloe vera coated samples, the alginate coating seemed to have an inhibiting effect *per se*, even though alginate has not been reported to have any significant bacteriostatic effect. The addition of FA, as expected, promoted a decrease in the population of *L. monocytogenes* on apple discs after seven days. FA has previously been reported to have antimicrobial effects (Pernin, 2019) against *L. monocytogenes*. The mode of action involves two mechanisms. Firstly, the intercellular dissociation of the acid causes acidification of the cell cytoplasm and the efflux of K^+ ions leading to the eventual death of the microorganisms. Secondly, the intercalation of the acid in the phospholipid layers of the membrane of the microorganism could also cause a disturbance of the Van der Waals interactions, inhibiting the transport of substrates used by the key enzymes of the microorganism (Pernin, 2019).

5 Conclusions

Browning and weight loss are two processes that contribute to quality changes in fresh-cut produce. They are also considered as freshness indicators by consumers. In this study, the application of Aloe vera or alginate coatings with the addition of ferulic acid were evaluated as ways to delay quality changes and ensure the safety of fresh-cut apples.

The application of Aloe vera gel and alginate-based coatings led to a decrease in weight loss of the samples. However, overall, the factor that had the greater impact on increasing the shelf-life of fresh-cut apples was the incorporation of ferulic acid. This compound, that can be derived from sustainable natural sources, Delayed the browning in the samples for 7 days (effect that was also achieved by Aloe vera gel alone) and controlled the growth of alterative microbiota and *Listeria monocytogenes* (effect that was also achieved by alginate coat alone). However, the relationship between some of the studied parameters, including the coatings with the respiration, PPO enzymatic activity or antioxidant evolution of the samples still needs to be deeper investigated.

Nevertheless, the addition of this compound to the edible-coatings has proven to be a promising approach for enhancing the quality and safety of fresh-cut produce.

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Conflict of interests

The authors declare no conflict of interests.

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Aloe vera gel: an update on its use as a functional edible coating to preserve fruits and vegetables

Chapter 11

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Aloe vera L. is a common succulent plant that has been used for centuries regarding their healing properties and health benefits. Nowadays, scientific investigations on its gel have gained more attention because of its interesting antioxidant and antimicrobial properties. Also, the food industry encounters the need to preserve safety and quality of fresh produce; fruits and vegetables are in high demand due to their reported health benefits, and fresh-cut products are a new trend that meets the restless needs of the society. Edible coatings are an effective way to maintain freshness of these products, extend their shelf life, and even act as an alternative to modified atmosphere packaging to be used in conventional packaging. *Aloe vera* gel is a natural hydrocolloid, composed mainly by polysaccharides, that has been applied in the last years on fruits and vegetables. It can act as a semipermeable barrier for gases and water vapor, decreasing the respiration and ripening processes of the fruit, thus maintaining weight, firmness and valuable compounds. Its antioxidant and antimicrobial properties make it also an interesting material for increasing the shelf-life of fruits and vegetables. This review aims to describe the preparation and preservation of *Aloe vera* gel as well as the properties and compounds that are effective against oxidation and microbial growth. Moreover, the recent findings of its use – with or without additives – as an edible coating on fruits and vegetables have been widely detailed, showing that *Aloe vera* gel is a promising preservative method in this industry.

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Antioxidant, antimicrobial, shelf-life, firmness, color, respiration, fresh-cut

1 Introduction: *Aloe vera*

Aloe vera L. is a succulent plant that belongs to the *Asphodelaceae*, a family of the genus Aloe, and has been used for *centuries* as a healing plant (Chase, 2016). It is a shrubby, perennial, green plant. Its leaves, arranged in a rosette pattern at the stem, have a high capacity to retain water, enabling the plant to survive harsh conditions (Iwu, 2014). It grows mainly in the dry regions of Africa, Asia, Europe and America (Amar, 2008). Among various species of Aloe, *Aloe vera* L. (AV) is considered to be the most popular, commercially important and the most potent one in the research field (Maan, 2018).

AV leaves have broadly three parts; (i) the rind, (ii) the yellow sap (*Aloe* latex), containing mainly anthraquinones, which are bitter and laxative, and (iii) the internal transparent mucilaginous jelly, called *Aloe vera* gel (AVG) (Eshun, 2004). AVG contains a large amount of bioactive compounds, including flavonoids, terpenoids, lectins, fatty acids, anthraquinones, mono- and polysaccharides, tannins, sterols, enzymes, salicylic acid, minerals (calcium, chromium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium and zinc) and vitamins (A, C, E, β-carotene, B1, B2, B3, B6, choline, B12, folic acid) (Hęś, 2019).

AVG is considered safe, but a few remarks regarding the complications must be done. When ingested in high doses, it can cause abdominal cramps or flatulence (Mulay, 2013), and it can also cause allergic responses by its application on skin of susceptible people (Surjushe, 2008). Despite this, the composition of AVG makes it valuable in different areas, outstanding pharmacy, skincare, and food preservation. There are a number of scientific papers reviewing the process to obtain AVG from the aloe plant (Eshun, 2004) and the uses of AVG in medical applications, including antiviral and antitumor activity, promotion of the immune system, wound healing, hepatoprotective and antidiabetic effects, laxative properties (Maan, 2018; Radha, 2015; Sahu, 2013), skincare (Maenthaisong, 2007; Richardson, 2005), dentistry (Wynn, 2005), and also of the biological effect of its individual components (Choi, 2003; Hęś, 2019). Furthermore, and as reviewed in (Ahlawat, 2011), the AVG or juice have many possible applications in the food industry as a functional ingredient or preservative: they have been added to dairy and baked products, fruits and vegetables juices or purées and, in general, to foods that have an adequate consistency, such as jams or jellies. When used as a preservative, the quality of the products was maintained during storage and microbial counts remained under control. For instance, AVG 20 % was added to mango nectar, and total bacterial counts decreased from 3.9 to 2.1 log CFU / mL, and maintained good quality attributes during 6 months of storage (Elbandy, 2014). Similarly in another study, aloe vera yoghurt was made with lactic acid bacteria and compared it with yoghurt prepared using dried skim milk and it was found that quality retention of aloe vera yoghurt at 5 °C for 15 days was better than the milk yoghurt (Lee, 1997).

As stated before, the aloe industry is flourishing worldwide. AVG has been recently proposed as an edible coating for whole or fresh-cut fruits and vegetables. Edible coatings are thin layers composed mainly by polysaccharides, proteins, or lipids that are formed directly on the fruit surface and act as a protective layer against chemical, physical or microbiological changes. In many cases, the selected raw materials have additional functional properties (e.g. antimicrobial, antioxidant) or are enriched with additives, which can contribute to enhance the product shelf life and safety. In addition to commercial purposes, the ingestion of the AVG as an ingredient for coated products should pose beneficial effects for human health besides those already reviewed for the fruits and vegetables themselves (e.g. cancer risk diminution, coronary diseases and cognitive decline prevention) (Blekkenhorst, 2018; Loef, 2012; Ruxton, 2006). AVG consumption has been related with a number of valuable effects: digestive diseases protection, antidiabetic effect, cardioprotective effect, antiinflammation, antimicrobial and prebiotic activity, cancer reduction, as well as bone protection (Foster, 2010; Sánchez, 2020).

Nowadays, fruits and vegetables are in high demand because of the healthy and nutritional values, but they have a limited shelf-life due to their perishable nature (Nath, 2007). Edible coatings provide a promising approach to prevent deterioration during storage, as they can act as a semipermeable barrier to O_2, CO_2 and water-vapor, thus preventing water loss, changes in firmness or oxidation, amongst others (Raghav, 2016). Misir (2014) highlighted the attractiveness and potential of the use of AVG as an edible coating to preserve fruits by minimizing the rate of respiration and maintaining quality attributes (color, flavor etc.). Moreover, AVG has antifungal and antibacterial properties, which provide a defensive barrier against microbial contamination of fruits and vegetables. Alternatives to synthetic preservatives for fruits and vegetables are needed to satisfy consumers' needs, and AVG seems to be a capable choice for that, as it is edible, invisible, odorless and does not affect the taste of products on which it is applied.

This review aims to provide a general view on the usage of AVG in food applications, specifically as an edible coating for prolonging the shelf-life of fruits and vegetables, combined or not with other functional additives in order to increase its efficacy. A general overview of the AVG composition and preparation for food coatings is done, and the recent studies of their impact on the physicochemical and nutritional quality as well as microbiological safety of fruits and vegetables are described.

2 Aloe vera gel: composition, preparation and preservation

According to Zhang (2018) AVG average composition consists of water (96 %) and dry matter (4 %), which contains organic acids (22.8 %), dietary fiber (18.8 %), polysaccharides (8.8 %), protein (4.7 %), lipids (2.7 %), and ashes (16.0 %) (w/w). The main compounds of each class can be found in **Table 43** in more detail, and were reviewed extensively by (Ahlawat, 2011; Gupta, 2017; Hęś, 2019).

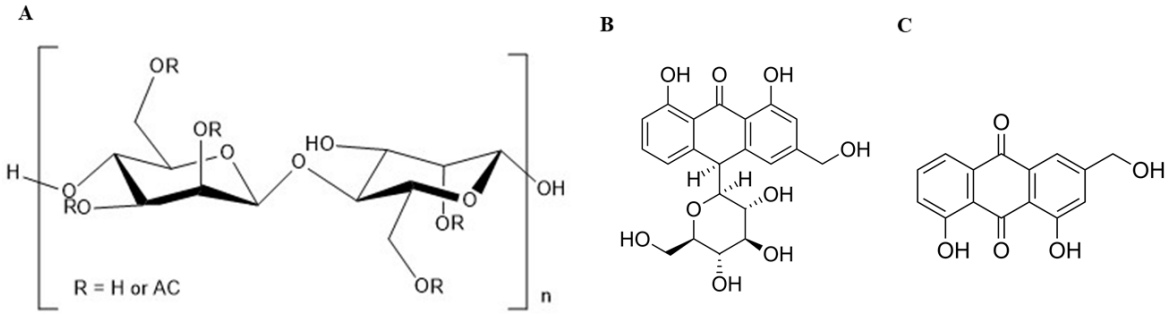

Figure 53. Active compounds characteristic of *Aloe vera* gel: (**A**) acemannan, (**B**) aloin, and (**C**) aloe emodin.

Polysaccharides contribute to bulk, body, viscosity, stability of emulsions and foams, water-holding capacity, and freeze-thaw stability (Ahmed, 2013). Amongst them, the mucopolysaccharide acemannan (**Figure 53**A), which is an acetylated mannose-rich polymer that functions as storage polysaccharide, is considered one of the most bioactive compounds of AV, thus having a lot of applications in the medicine field (Liu, 2019; Rodrigues, 2019). Lectins (aloctin A and B) are a group of glycoproteins that are characteristic for AV. They have shown antitumor effects in mice, by agglutinating carcinogenic cells (Akev, 2007). Aloe emodin and aloin (**Figure 53**B, **Figure 53**C) are the main anthraquinones in AVG, which mainly exhibit laxative properties (Salehi, 2018) and aloin has also shown antiviral activity against influenza (Huang, 2019). Even so, the beneficial properties of AVG are more likely to be due a synergistic action of the compounds – polysaccharides, glycoproteins, anthraquinones, polyphenols - rather than a single one (Hamman, 2008).

Table 43. Composition of *Aloe vera* gel (AVG) (Eshun, 2004; Ahlawat, 2011; Gupta, 2017; Hęś, 2019)

Class	Compounds
Polysaccharides	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectic substance, xylan, cellulose, hyaluronic acid
Saccharides	Mannose, glucose, L-rhamnose, aldopentose
Lipids	Arachidonic acid, γ-linolenic acid, steroids (campesterol, cholesterol, β-sitosterol), triglicerides, triterpenoid
Amino acids	Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, valine
Proteins	Lectins, lectin-like substance
Enzymes	Alkaline phosphatase, amylase, carboxypeptidase, catalase, cyclooxidase, cyclooxygenase, lipase, oxidase, phosphoenol, pyruvate carboxylase, superoxide dismutase
Minerals	Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, zinc
Vitamins	A, B1, B2, B6, C, β-carotene, choline, folic acid, α-tocopherol
Anthraquinones/anthrones	Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid
Chromones	8-C-glucosyl-(2'-O-cinnamoyl)-7-O-methylaloediol A, 8-C-glucosyl-(S)-aloesol, 8-C-glucosyl-7-O-methyl-(S)-aloesol, 8-C-glucosyl-7-Omethylaloediol, 8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, neoaloesin A
Hormones	Auxins, gibberellins
Other organic compounds	Lignins, potassium sorbate, salicylic acid, uric acid

The composition of AVG will depend on the cultivar, the season and also the method in which the gel is processed (Ni, 2004). For instance, acemannan can be affected by the pasteurization temperature and time conditions due to a decrease in its swelling properties – from 305 ± 3.7 mL / g at 65 ºC for 15 min, to 245 ± 2.9 mL / g at 85 ºC for 25 min –, water retention behavior – from 23.0 ± 0.2 to 15.9 ± 0.1 g H_2O/g at 65 ºC for 15 min or 85 ºC for 25 min, respectively – and fat adsorption capacity– which went from 33.20 ± 0.5 to 26.50 ± 0.4 when pasteurization conditions were 65 ºC, 15 min, or 85 ºC, 25 min, respectively –(Minjares-Fuentes, 2018). On the contrary, UV-C irradiation (24.2 mJ / cm^2 pH 3.5) could be an alternative to thermal treatments which has been suggested for efficiently preserving the characteristics of acemannan, as it reduces its degradation up to 27 % (Rodríguez-Rodríguez, 2019).

To prepare AVG for the edible coatings, most of the authors follow the same process with slight variations in the conditions and some steps (**Table 44**). First, a washing step is done to remove the external dirt of the leaves and chlorine to disinfect them. It is advisable that the processing of the leaves is completed within the first 36 h after the harvest, in order to avoid the decomposition of the biological activity (Ramachandra, 2008). Then, the leaf will be separated from the parenchyma to obtain the gel, which is then normally homogenized by blending with a shredder and filtered with a cloth to remove the fibers. On occasion, it can be filtered with active carbon to remove the anthraquinones, which

could have a laxative effect in dependency of the dose (Nasution, 2015). Sometimes, the pH is adjusted between 3 and 4, to stabilize and avoid enzymatic browning during storage. Then, the gel is pasteurized at temperatures ranging from 65 to 85 ºC for 10 s up to 30 min, with longer times at low temperatures. However, it is suggested that the best method for a gentle pasteurization is a high temperature for a short time (HTST), followed by flash cooling to 5 ºC (Eshun, 2004).

Table 44. Preparation of *Aloe vera* gel (AVG)

Cleaning	Filtration	Blending	pH adjustment	Pasteurization	Source
Water + blanching 100 ºC, 4 min	Activated carbon	n.d.	3.0, citric acid	85 ºC, 1 min + cool down 5 ºC	(Nasution, 2015)
n.d.	n.d.	n.d.	4.0, citric and ascorbic acid	70 ºC	(Ali, 2016)
Water + blanching 100 ºC, 4 min	Yes	Yes	3.0, citric acid	85 ºC, 1 min + cool down 5 ºC	(Chrysargyris, 2016)
Chlorine 0.03%	Yes	Yes	No	No	(Sogvar, 2016)
n.d.	Yes	Yes	No	No	(Martinez-Romero, 2018)
Chlorine 0.1 %, 3 min	Sterile muslim cloth	Yes	3.75, citric acid	65 ºC, 30 min	(Ali, 2019)
Yes	Yes	Yes	3.75, phosphoric acid	80 ºC, 10 s + cooling down 5 ºC	(Khaliq, 2019)
Chlorine 0.03 %	Yes	No	No	No	(Mirshekari, 2019)
Chlorine 2 %	Yes	Yes	No	No	(Rasouli, 2019)

According to some authors, processing may cause changes in the structure of the components of AVG, mainly attributed to the high temperatures. The rate in which acemannan undergoes a loss of mannosyl residues increases with advanced temperatures higher than 60 ºC and times longer than 1.6 h Femenia (2003). High temperatures over 70 ºC, can lead to a decrease in barbaloin and other polysaccharides from AVG (Sánchez-Machado, 2017), losses that would be detrimental to AVG bioactivities and functionality. Consistency and viscosity of the gel are also affected by high temperatures, as acetylated glucomannans are responsible for the plasticity of the gel (Hamman, 2008; Huang, 2019)A good approach to maintain the composition and the properties of AVG is the use of a non-thermal pasteurization, such as high hydrostatic pressure (HHP). Pressures of 300 to 500 MPa were applied for 1 and 3 min at 20 ºC on AVG, and although viable spore formers were detected by enrichment, the HHP treatment completely suppressed microbial growth during at least 90 days at 4 ºC (Reyes, 2012). Such treatments exert no major effect on gel properties or rheological activities (Opazo-Navarrete, 2012), and pressures below 500 MPa can increase antioxidant activity and aloin content of AVG (Vega-Gálvez, 2011).

Cool temperatures are advisable for AVG storage, for a better preservation of the color, vitamin C content, antioxidant potential and acemannan structure, amongst others (Saberian, 2013). Stored at room temperature, these parameters, pH, and viscosity are maintained for 2 days, while at refrigerating temperatures, AVG can last up to 5 days without any significant changes (Suriati, 2018).

As aforementioned, conditions in which extraction, preparation and storage of the AVG are carried out affect its composition and physical properties. To be used as an edible coating, it is important that AVG has the adequate plasticity and consistency, and is rich in the characteristic compounds giving functionality to prevent fruit and vegetable deterioration. For this, when processing *Aloe vera* leaves, it will be imperative to optimize and control the parameters that could affect somehow the quality and functionality of the final product.

3 *In vitro* properties with potential for food applications

3.1 Antioxidant activity

Most plant extracts possess antioxidant activity, acting as free radical scavengers or breaking chain oxygen reactions, due to their phenolic compounds and other bioactive molecules with this effect (Aqil, 2006; Wong, 2006). Antioxidant activity of AVG will not only play a role in reducing oxidative stress of the vegetable tissue on which it is applied as an edible coating, delaying the alteration of metabolic processes (i.e. ethylene production, relative electrical conductivity, transpiration, senescence and nutritional composition) (Hussein, 2019). When consumed, it will also pose a benefit to human health, as antioxidant activity of foods has been related with the prevention of some non-concomitant diseases such as coronary problems, obesity or Parkinson (Shahidi, 2015).

The IC_{50} – half maximal inhibitory concentration, which corresponds to the concentration of an inhibitor where the response is reduced by 50 %, and it is an indicator of the effectiveness of a substance in inhibiting a specific biochemical compound – of a methanolic extract of AVG was 572.14 μg / mL against DPPH·, 195.26 μg / mL against ABTS and 46.3 μg / mL inhibiting NO generation (Ray, 2013). Some of the bioactive compounds of AVG are aloe-emodin, which has been proven to inhibit oxidation of linolenic acid by 78 % (Abdul Qadir, 2017), or anthraquinones, that exert a protection against lipid peroxidation (Sánchez-Machado, 2017). Acemannan, specifically its acetyl and hydroxyl groups, has also been proven to have scavenging effects on free radicals, as well as chelating activity and reducing ability against iron ions (Liu, 2019). The age of the plant can also influence its antioxidant properties. For instance, AVG from 3-year-old plants had higher polyphenol content and antioxidant activity than that from 2-year-old AV, which was correlated with the DPPH· inhibition (Hu, 2003). Furthermore, the antioxidant capacity also depends on the different monosaccharides available in the plant. Kang (2014)reported that rhamnose and arabinose are the sugars with antioxidant activity within the polysaccharide structure.

In fact, the antioxidant properties of the aloe vera gel may vary amongst extracts or preparation methods, and the quantity and quality of the antioxidant content of the plant depend on the age of the plant, climate, or position of leaves on the stem, amongst others (Hęś, 2019). It is therefore advisable that processors of AVG consider all the factors in order to obtain with a high and consistent quality. For this, a further approach in the scientific field could include the prediction of the composition and physical properties of the AVG resulting from a certain prime matter and the adequate processing conditions.

3.2 Antimicrobial properties

Even though the antimicrobial activity is not the main reason why AVG is used, it has shown to be effective in inhibiting the growth of some microorganisms. The understanding of AVG's antimicrobial compounds and their action modes, as well as the target microorganisms and the effective concentrations, will be essential to guide the application of AVG to certain products with specific microorganism control requirements.

AVG has already shown some fungal control. For instance, Das (2011) isolated a protein from AVG with antifungal properties. Its structure is homologous to other antifungal proteins from plants, and it has proven to inhibit the growth of some *Candida* species, including *Candida krusei*, which can be found in food products. The concentration of this protein needed to reduce the growth halo to 50 % was 50.41 μM. Although AVG was not effective in inhibiting the growth of *Aspergillus carbonarius*, it was able to reduce ochratoxin production up to 75.69 %, depending on the concentration (Dammak, 2018), somehow affecting the mold's biosynthetic pathway for this toxin. The inhibition of the mycelium growth of *Botrytis cinerea, Penicillium digitatum, P. expansum* and *P. italicum* was well correlated with the concentration of AVG, and the gel also decreased the percentage of infected aloe leaves, that were artificially inoculated with these molds (Zapata, 2013). As an example, AVG 50 % showed a decrease of 11.3 % the mycelial growth ratio of the such molds. The authors found a correlation between mold inhibition and aloin content of the gels, and suggested this compound could be responsible for the antifungal activity, although its action mechanism has not yet been fully understood. It has been proposed that aloin and barbaloin affect the phospholipid membrane, leading to significant changes in the membrane physical properties, which affect the lipid/water interface in negatively charged phospholipids, with disruptions of the core of the bilayer (Estepa, 2004). Other authors have also reported antimicrobial activity of aloin against some foodborne pathogens, including, *Escherichia coli, Salmonella typhi* and *S. typhimurium, Staphylococcus aureus,* and *Vibrio chloreae*. In that study, minimum inhibitory concentrations of aloin ranged from 0.06 to 0.96 mM (Asamenew, 2011).

According to Hęś (2019), acemannan has also bactericidal, virucidal and fungicidal properties. For instance, it can decrease the adhesive properties of multiresistant strains of *Helicobacter pylori*, thus reducing its pathogenicity (Cellini, 2014). Acemannan has shown an inhibitory effect against both planktonic and sessile cells of foodborne pathogens *S. aureus* and *Pseudomonas aeruginosa*, which could contribute to the inhibition of biofilm formation (Cataldi, 2015). Salah (2017)reported that the efficacy of acemannan depends on the deacetylation degree, with a higher antimicrobial effect at lower deacetylation degree. In that study, it was also observed that Gram-negative bacteria (*E. coli, P. aeruginosa*) were more sensitive than Gram-positive bacteria (*S. aureus, E. faecalis*) to acemannan's biofilm inhibition activity.

Fewer are the reports on AVG used against viruses. Aloin, aloe emodin and other anthraquinone derivatives, such as rysophanol, aloetic acid, anthranol, anthracine, anthranon, barbaloin, ethereal oil, cinnemonic acid, isobarbaloin, and resistannol have been reported to have antiviral effects against influenza virus (Borges-Argáez, 2019) or hepatitis A virus, by inhibiting their polymerase activity (Parvez, 2019). However, as norovirus is the most prevalent virus in fruits and vegetables, especially in berries (EFSA, 2014), more information about the activity of AVG against it would be useful.

4 Application of AVG as edible coating for fruits and vegetables

Typically, AVG has been applied to whole or fresh-cut fruits and vegetables as edible coating at a postharvest level and prior to commercialization step. However, it should be noted promising results were also observed by coating fresh produce. For instance, Castillo (2012) incorporated a coating of AVG (66 %) by applying a fine mist until run off so that the entire leaf and fruit surfaces were wetted, one or one and seven days before harvest. After harvest and during storage, quality attributes of the grapes were maintained and rotten berries per cluster decreased from 13 % to 3 % in treated products. Similarly, the coating was applied to strawberries at preharvest, and the authors observed that aloe sprays reduced fruit weight loss, the incidence of decay, and fruit and calyx defects, while exhibited no effect on respiration and on certain quality attributes like antioxidant activity or color (Kafkaletou, 2018). At a postharvest level, immersion in the AVG preparation, with or without other ingredients or preservatives, is the preferred technique to create an edible coating for fruits and vegetables. However, AVG films formed by spraying have also been characterized, and were optimized to AVG 10 % for rambutan fruits for decreasing their respiration rate 15.08 mL O_2/kg · h to 5.77 mL O_2/ kg · h at 10 °C storage temperature (Darmawati, 2019).

4.1 Prevention of weight loss and firmness

Weight loss in fruits and vegetables mainly occurs due to water loss by transpiration and respiration, and it is more marked on fresh-cut fruits. As the water loss rate depends on the gradient between fruit tissue and the surroundings, AVG is able to reduce water loss by making a physical barrier around the fruit (Misir, 2014). Polysaccharides are suggested to be good non-fatty coatings for preventing water loss, and AVG has high contents of polysaccharides, which ordinarily provide homogenous edible coatings that are colorless, have an oily-free appearance and a minor caloric content (Hassan, 2018).

The effectiveness of AVG in reducing weight loss has been reported by a number of studies (**Table 45**). AVG at a concentration of 50% significantly reduced the weight loss, when compared to the non-coated samples, in rambutan fruit (Yingsanga, 2018), litchi (Ali, 2019), or white button mushroom (Mirshekari, 2019). But even lower concentrations were effective for this purpose: AVG 20% in grapes (Ali, 2016), in peach (Hazrati, 2017), or 4% to 6% AVG in bell pepper (Ullah, 2017) proved to be a good barrier for water loss.

This effect can be improved by adding other components to the AVG coating. For instance, carnauba wax was used at 0.1% to increase the water barrier properties of the film, as the hydrophobic characteristics of this lipidic component act along with the polysaccharides of the AVG (Pérez, 2016). Nasution (2015) used $CaCl_2$ 2% in fresh-cut guava, because the presence of calcium ions forms a complex (chelate) with the cell wall and middle lamella pectin, reinforcing the barrier properties. It also contributed on the maintenance of the texture by protecting the membrane integrity, leading to increased turgor cell pressure.

Not only with additives texture can be maintained. AVG, which is able to preserve moisture in the product, also contributes to delay the loss of firmness. The intracellular water in fruits and vegetables is maintained, keeping their natural turgor pressure. Retarding of decay and fruit softness has been described in all the works that studied firmness in fruits coated with AVG alone (**Table 45**). Firmness preservation can be explained by, on one hand, the barrier properties against O_2, which reduces the respiration rate of the product, thereby slowing the metabolic activity and ripening process (Asamenew, 2011). On the other hand, the reduction of the activity of the main cell wall degrading enzymes:

α-galactosidase, polygalacturonase, and pectinmethyl-esterase, attributed to a slow-down on the ripening processes and a decreased stress of the plant (Martínez-Rubio, 2015).

4.2 Prevention of color changes

Color and appearance affect consumers' buying or eating opinion. These attributes are the first getting consumer attention, and they often will contribute in the decision whether the product is accepted or rejected (Barrett, 2010). The colors are derived from natural pigments, namely chlorophylls (green), carotenoids (yellow to red), anthocyanins (blue-red), betalaines (red) or flavonoids (yellow). Colors that are not appropriate, such as dull-like or browning, may suggest a loss of freshness or lack of ripeness (Shewfelt, 1993).

To delay color changes in fruits and vegetables, AVG has been applied to whole and fresh-cut pieces (**Table 45**). In some cases, when used alone at different concentrations, e.g. 50 % or 100 % AVG on rambutan fruit (Yingsanga, 2018) or 5 %, 10 %, 15 % or 20 % AVG on tomatoes (Chrysargyris, 2016), it did not have a significant effect on color retention. It is possible that for certain fruits and vegetables, the only use of AVG is not enough to prevent the color change, so the addition of other active compounds to the coating may prove to be a good option. Exploration of new antioxidant extracts to be incorporated in AVG, especially those of natural origin or coming from food by-products, could be a benefit for AVG-coated products. Nowadays, AVG has already been used as a carrier for anti-browning agents, such as 4-hexylresorcinol 0.01 %, ascorbic acid 0.5 %, $CaCl_2$ 0.2 %, or 0.1 % cysteine in AVG 15 % in fresh-cut apple (Kumar, 2018), having synergistic effect, compared with the separate use of AVG or the additives. Other authors have also reported an improvement when adding some substances to AVG. For instance, Sogvar (2016) used AVG 50 %, carnauba wax 0.1 %, polisorbate 0.1 % and glycerol 0.1 % on fresh-cut mango, and found that change of color of AVG coated fruits was half of the change of control fruits, or Nasution (2015) that applied AVG 100 % with ascorbic acid 1.5 %, $CaCl_2$ 2 % or potassium sorbate 0.2 %, as well as their combinations on fresh-cut guava, in which the lower lightness and higher redness and yellowness - indicating higher browning – was observed in control fruits.

Nevertheless, AVG has also been used alone with positive results. Ali (2016) described good gloss and best visual effects when using 20 % AVG on grapes, which was attributed to a better preservation of anthocyanins. In green or red-yellow fruits, other pigments such as chlorophylls can be maintained, or carotenoid synthesis promoted, by the creation of a modified atmosphere with AVG (Nasution, 2015). The degradation of such pigments has been related with the oxidative stress, which occurs due to the presence of high amounts of reactive oxygen species. When creating a barrier between the fruit and the environment, AVG reduces the amount of oxygen available for degradation reactions (Yamauchi, 2014). Mirshekari (2019) also stated a browning delay when applying AVG 50 or 75 % on white button mushrooms, and Ali, (2019) reported a decrease in browning after storage of litchi fruit coated with AVG 50 % when compared to the control. Browning of fruits is attributed to the activity of polyphenol oxidase (PPO), that is enhanced when the fruit is cut, as the vegetal cell walls are disrupted and the enzyme can get easily in contact with oxygen and substrates (Kasim, 2015). An inhibition of PPO activity was observed for AVG-coated white mushrooms, in which PPO activities were 2.25-fold lower than in non-coated samples (Mirshekari, 2019), and for wax-apple coated with 75 % AVG, treatment that controlled the PPO activity after 6 days of storage when compared with non-treated samples (Supapvanich, 2016). Both of the studies mention the relationship between and the decrease in PPO activity, but the exact mechanism for which this happens needs further investigation. It has been theorized that one option for that is that AVG may act as a physical protection to the fruit tissue: as it has been reported by Ali (2019). In fact, in another study, AVG maintained significantly higher

membrane integrity of the guava, and PPO and peroxidase activities were 1.36-fold lower in comparison to the uncoated control (Hazrati, 2017). However, there is no information on how the characteristic components of AVG interact with the browning enzymes, and neither about the role of the phenolic compounds present in AVG, that may act as substrates or antioxidants for PPO (Fukumoto, 2000).

4.3 Impact of AVG coatings on respiration and ripening processes

Respiration, ripening and senescence are expected processes in fruits and vegetables. The rate at which they occur depends on many parameters, such as harvesting time, storage conditions (temperature, gas atmosphere) or mechanical damages e.g. by industrial cutting operations. Despite being inherent in the fruit, these processes lead to a progressive loss of quality (Irtiza, 2019). For instance, respiration and ripening lead to an increase in total soluble solids (TSS) and a decrease in acidity provided by organic acids, which will affect the sensory properties of the fruit (Thompson, 2010). Included in the reported advantages of edible coatings are their gas barrier properties. As suggested by Misir (2014), AVG acts as a semipermeable coating, by which the gas exchange between the fruit and the environment decreases, and so the respiration rate does.

Recent studies show the effect of AVG on respiration and ripening of fruits and vegetables. For instance, Mendy (2019) used AVG at concentrations ranging from 5 to 50 % on fresh-cut papaya, and observed that coated fruits had lower content of (TSS), 8.29 % in comparison to the non-coated control, 10.50 %. Furthermore, they reported that AVG 25 % was the optimal concentration for keeping titratable acidity (TA) higher than it was in control fruits. This was attributed to the lower metabolism of the fruit, and reduced use of the organic acids, thus maintaining TA values. Sogvar (2016) also observed this effect on strawberries when using AVG 33 % combined with 1 to 5 % ascorbic acid. Until day 12 of storage, the fruits kept lower TSS, from 7.5 to 9 ºBrix, compared to 10 ºBrix of the control, and pH was maintained. It was suggested that the higher maturation of the non-coated fruit had led to a solubilization of cell wall polyuronides and hemicellulose, thus increasing its TSS values when compared to coated fruits.

Moreover, membrane leakage can happen while fruit senescence, and incremented with tissue cutting or damage. In this regard, Ullah (2017)stated that AVG between 4 to 6 %, enriched with cinnamon oil, helped in reducing 10 to 20 % the membrane leakage of red bell pepper. Moreover, Ali (2019) showed that protection of membrane integrity was improved in litchi fruit covered with AVG 50 % itself. Even though, there are some contradictory studies where an increase of TA and TSS was observed in strawberries and tomatoes, respectively (Falah, 2018; Khatri, 2020). Due to the different characteristics of the matrices, the climacteric vs. non-climacteric nature, or the different formats in which they are presented (whole or fresh-cut), it is of high importance to evaluate the effect of each AVG formulation on the targeted matrix.

The majority of the studies seem to confirm that these effects on selected produce quality parameters are a consequence of the formed AVG layer, which decreases the gaseous exchange, and therefore, the ripening processes. The lower gas exchange induces lower O_2 and higher CO_2 concentrations in the internal atmosphere of the fruit, creating a modified atmosphere (Dhall, 2013). Pérez (2016) reported that fresh-cut mango coated with AVG 50 % had low rates of O_2 consumption and controlled CO_2 production, being 4.5 or 4.3 mL O_2 or CO_2 / kg · h compared to untreated samples, whose values were 7.0 or 6.3 mL gas / kg · h, for O_2 or CO_2, respectively. Contrarily, Chrysargyris (2016) showed that respiration rate of AVG 5 to 20 % coated tomatoes was not significantly different than it as for non-coated tomatoes. However, they also reported a decrease in ethylene production, which was 4.5 and 2.5 μL ethylene / kg · h, for non-coated and AVG 10 % coted tomatoes, which was related to the

slowdown of the ripening process. Martínez-Rubio (2015)observed similar results when using an AVG coat with 0.5 % citric acid and 0.5 % ascorbic acid on pomegranate arils. As control arils were also immersed in citric and ascorbic acid, this effect was attributed mainly to AVG. Also, essential oils have been added to AVG to enhance its barrier properties because of their lipophilic nature. It seems that the incorporation of essential oils increases the water vapor retention and the gas barrier by adding hydrophobicity to the coating and, but this effect depends on the coating composition, as the interactions between the components of AVG and the essential oil tend to be complex (Sánchez-González, 2011). E.g., Martínez-Romero (2018) found that diminution of respiration rates could be enhanced by adding rosehip oil to AVG coating for plums and prunes.

AVG coating has proved to be a good way to decrease the respiration rate of fruits, by creating a semipermeable layer that minimizes gas exchange, thus retarding maturation and keeping the quality parameters of the products. Future investigations should be focused on the optimization of the gas barrier properties without a major impact in the mouthfeel of the edible coating. Moreover, the addition of substances such as essential oils could be explored to improve gas and water vapor barriers, and subsequently further decrease the metabolic rate of the fruits and vegetables. In fact, essential oils have been a trend in the last decades not only for their abovementioned functionality in edible coatings but also for the antioxidant and antimicrobial activities they exert, showing potential to be more incorporated in AVG coatings completing a multipurpose.

4.4 Preservation of bioactive compounds and antioxidant activity

Fruits and vegetables are rich in bioactive compounds, such as polyphenols, including anthocyanins, carotenoids, vitamins, phytoestrogens, and glucosinolates. These compounds not only receive increased attention due to their potential health benefits, but also to the impact that they have on the color of the fruits and vegetables and their antioxidant activity, contributing to the plant defense mechanisms (Hazrati, 2017).

As a coating for fruits and vegetables, AVG has been reported to have an impact on the retention of these compounds (Table 4). In most of the cases, AVG has proved to be effective in maintaining TPC or FC values, or at least delaying their loss during storage, from AVG 20 % in tomatoes (Chrysargyris, 2016) to AVG 100 % in sweet cherries (Serrano, 2017). On the contrary, non-coated fruits show a decrease in the levels of total phenolic content (TPC) and flavonoid content (FC) during storage, and accordingly, their antioxidant activity. Sometimes, AVG-coating is not enough to maintain these values, and it is used as a carrier for other antioxidants. For instance, ascorbic acid in combination with AVG 33 % in strawberries resulted in higher levels of TPC and FC, which could not be maintained with AVG alone (Sogvar, 2016). Also, the addition of *Fragaria cretica* extract 1 % to AVG 50 % was more efficient in preserving these compounds than AVG alone.

Preservation of TPC and FC due to AVG can be explained by taking into account a sum of factors. First, the decrease in respiration rate and delayed senescence, which, on the one hand, increases the resistance of tissues against decay and thus enhances their antioxidant system, and, on the other hand, conserves the compartmentation of plant cells, leading to less oxidant-enzyme activities (Ali, 2019; Hassanpour, 2015). Resistance of tissues may also prevent the degradation of anthocyanins, which occurs due to breakdown of the vacuoles, making them more accessible to degradation enzymes (Jiang, 2018). Second, the gas barrier formed by AVG reduces the amount of available oxygen, thus avoiding the oxidation of phenols and flavonoids. The antioxidant activity is also carried out by aloe emodin, which also prevents the degradation of these compounds (Khaliq, 2019). And third, AVG has been reported to enhance phenylalanine ammonia lyase (PAL) activity - an enzyme involved in the biosynthesis of polyphenol compounds and flavonoids in plants -, although the mechanisms for which

this enzyme increases its activity are not fully understood (Hassanpour, 2015; Khaliq, 2019; Mirshekari, 2019).

Other bioactive compounds, especially carotenoids including lycopene or vitamins such as ascorbic acid, are also better preserved with AVG coatings (Table 4). As an example, litchi coated with AVG 50 % showed 1.46-fold higher ascorbic acid content than the non-coated control Ali (2019). Oxidative degradation is the main cause of their loss, and AVG provides a barrier against oxygen and thus controlling the auto-oxidation or enzymatic oxidation of such compounds (Leong, 2012).

4.5 Control of microbial spoilage

One of the issues that concerns fresh-cut fruits and vegetables is the microbial quality and safety. These products have a natural microbial load, and may have been contaminated by means of several ways, including harvesting practices, cross-contamination in the industry or handling (Kalia, 2006). Their processing does not include any pasteurizing step, indeed, the cutting and washing operations itself may increase the risk of microbial contamination and growth, due to the availability of nutrients from inside the vegetable cell (Bhagwat, 2006).

As it has been described in section 3.2, AVG has shown antimicrobial activities against bacteria, yeasts, and molds. However, in the past few years, its antimicrobial effect when applied as an edible coating on fruits and vegetables has not been widely studied. Hassanpour (2015) studied the effect of AVG 25 %, 50 % and 75 % on raspberries, and observed a reduction in the incidence of fungal decay on treated fruits, from 22.5 to 10.5 % of affected fruits, with no difference between the investigated concentrations. Also, (Ullah, 2017) coated bell pepper with 4 to 6 % of AVG and also reported a reduction in decay incidence and symptoms of fungal decay. Nasution (2015) used AVG 100 % on fresh-cut guava, and observed a delay of two days in the growth of total aerobic mesophylls, and also, the aerobic populations of coated guava were 2.5 log lower than in the control at the end of 14-day storage. The authors attributed this effect to the content on pyrocatechol, cinnamic acid and p-coumaric acid in AVG and to the enhanced tissue resistance against bacterial attack given by AVG, which strengthened the cell walls of the fruit. Additives, such as ascorbic acid 1.5 %, calcium chloride 2 % and potassium sorbate 0.2 % supported this effect, as the decrease in the pH creates an unfriendly environment for microorganisms, and the undissociated form of sorbic acid inhibited the growth of yeasts and molds. Other authors have also used citric and ascorbic acid in combination with AVG, in pomegranate arils and in strawberry fruit, respectively (Martínez-Rubio, 2015; Sogvar, 2016). In this case, Sogvar (2016) reported that AVG significantly reduced epyphitic microbiota counts, due to the saponins, anthraquinones and acemannan. After 18-day storage, total mesophyllic population decreased with the addition of AVG, from 3.63 to 3.31 log CFU / g and yeast and mold counts from 4.28 to 3.74 log CFU / g. When 5 % of ascorbic acid was added to the coating, aerobic mesophylls and yeasts and molds – 3.13 and 3.47 log CFU / g, respectively – were significantly lower than without this compound. Only one study, carried out by Pérez (2016), did not report any significant reduction of total aerobic mesophylls, yeasts or molds, when using a coating consisting on AVG 50%, carnauba wax 0.1 %, polysorbate 0.01 % and glycerol 1 % on mango.

The studies concerning the antimicrobial effect of AVG coatings on fruits and vegetables have focused on the epiphytic microbiota of the products. More research should be carried out to enlarge the knowledge about the effect that AVG coating could have on pathogenic microorganisms. In this regard, focusing on *Listeria monocytogenes* would be interesting, as these psychotropic bacteria may grow at lower temperatures that usually are recommended for this product's storage.

4.6 Other parameters

The effect of AVG on other quality parameters of fruits and vegetables, such as enzymatic activity, chilling injury or sensory attributes, have been little studied in the past few years.

The impact of AVG on the enzymatic activity of raspberries was studied by Hassanpour (2015). Berries were coated with three different percentages of AVG (25, 50 or 75 %), and superoxide dismutase was higher in all of them than it was in the non-coated control. Berries coated with AVG 50 % showed the highest activity of glutathione reductase and POD, and the highest content in reduced glutathione, which is one of the most abundant reducing thiols in the majority of cells, playing a role in the control of reactive oxygen species (Couto, 2016). Also, the ascorbate peroxidase enzyme was triggered in AVG 50 %, which is another of the major H_2O_2 scavenging enzymes in plant cells. Supporting this result, Ali (2016)also reported a lower content in H_2O_2 in litchi fruit coated with AVG 50% compared to the control, being 48 and 28 μmol / kg, respectively. Also, Mirshekari (2019) found similar results for white bottom mushrooms coated with AVG 50 %, that when compared to the control, H_2O_2 was 48 and 20 μmol / kg fresh weight, respectively, preventing then the peroxidation of the membranes.

Chilling injury was studied in oranges coated with AVG 30 % with glycerol 1 % by Rasouli (2019), but no difference was detected between them and the control samples in this regard. Contrarily, Ullah (2017) found less symptoms of chilling injury in bell pepper coated with AVG 4, 5, or 6 %, effect that was enhanced by arabic gum and cinnamon oil addition.

Regarding flavor, it is of high importance to carefully get rid of the latex in *Aloe vera* leaves, as its composition gives a bitter taste (Ramachandra, 2008). But if correctly prepared, and as it is exemplified by Martínez-Romero (2018), AVG coatings did not impart any undesirable flavor to the plums studied. Hazrati (2017) also reported that AVG coated peaches were more favorable in terms of color appearance, taste, chewing ability and the overall quality for the consumers when compared to non-coated ones. Nasution (2015) indicated that additives such as 1.5 % ascorbic acid, 2 % calcium chloride, and 0.2 % potassium sorbate enhanced the sensory evaluation of the fresh-cut guava coated with AVG.

From the studies aforesaid, it is patent that AVG used as an edible coating has many advantages, that should be deeper investigated. More studies focused on the effect of AVG on parameters mentioned in this section should be carried out in order to gain a better understanding of the effect that AVG can have on the shelf-life of fresh and fresh-cut produce.

Table 45. Effect of AVG on weight loss, texture and color of fruits and vegetables during storage.

AV coat	Fruit (T °C)	Weight loss	Texture	Color	Source
AVG 10%, 20%, or 30% (Control: water)	Grapes (0ºC)	Coated fruits significantly lost less weight than control fruits. Non-coated: 9% weight loss, AVG 10, 20, and 30%, 5, 3, and 4.5% weight loss, respectively.	AVG significantly reduced firmness losses, whereas losses >50% were detected in control grapes after 21 days of storage.	AVG provided good gloss. AVG 20% gave the best visual results.	(Ali, 2016)
AVG 5%, 10%, 15%, or 20% (Control: water)	Tomatoes (11ºC)	No significant effect.	At the end of the 14-day storage, firmness of control and AVG 15% coated tomatoes was 15.4 and 18.9 N, respectively.	No significant effect.	(Chrysargyris, 2016)
AVG 30% (Control: water)	Peach (1ºC)	AVG had a significant effect in reducing the weight loss. Control and AVG coated lost 5.5 and 4.0% of weight, respectively.	AVG coating resulted in less fimness loss. At the end of storage, control firmness was 4 kg·N/cm whereas AVG coated firmness was 5 kg·N/cm.	During all storage, AVG coated fruits had higher Hue angle value in comparison with control fruits, which was 0.8 and 0.5, respectively. Chroma values were significantly different, being 42 and 60 for control and AVG coated fruit, respectively.	(Hazrati, 2017)
AVG 100% (Control: water)	Sweet cherry (2ºC)	n.d.	Firmness loss was significantly delayed in AVG coated fruits. At the first storage day, firmness was 5.3 ± 0.1 N. After 14 days, firmness was 4.2 ± 0.1 and 4.8 ± 0.1 N for control and AVG coated fruits, respectively.	Color evolution, measured in decrease of Chroma index, was significantly delayed in AVG coated fruits. Initial chroma value was 3.76 ± 0.7. Final Chroma values were significantly different, 25.2 ± 1.7	(Serrano, 2017)

AV coat	Fruit (T °C)	Weight loss	Texture	Color	Source
				and 28.9 ± 0.7 for control and AVG coated fruits, respectively.	
AVG 50% (Control: water)	Litchi fruit (20°C)	After 8 days, weight loss was 2.56-fold less than control was.	n.d.	AVG coated fruits showed significantly reduced browning index (BI) during storage. Browning was delayed for two days compared with the control. At day 4, BI had increased 2.5 and 0.2 in non-coated and coated samples, respectively.	(Ali, 2019)
AVG 15%, AVG 25%, or AVG 50% (Control: water)	Fresh-cut papaya (28°C)	Weight was better maintained by coated fruits. Control fruits lost 6.5% weight.	AVG coated fruits maintained some degree of firmness up to last day of storage, especially AVG 50%. Control fruits showed the highest softening. At the end of storage, firmness was 12.65 and 52.29 N, for non-coated and AVG 50%, respectively.	n.d.	(Mendy, 2019)
AVG 25%, AVG 50%, or AVG 75% (Control: water)	White button mushroom (4°C)	AVG 50% and AVG 70% resulted in the lowest weight loss (3.5%) compared to the control (6.0%).	n.d.	All AVG delayed the development of postharvest browning. Browning was 20-25% lower in AVG 50 and AVG 75% than it was in the control.	(Mirshekari, 2019)

AV coat	Fruit (T °C)	Weight loss	Texture	Color	Source
AVG 100% + [ascorbic acid 1.5%, $CaCl_2$ 2%, potassium sorbate 0.2%, or a combination] (Control: non-coated)	Fresh-cut guava (5ºC)	Weight loss was lower in AVG coated fruit (9.9%) than it was in control fruit (15.1%). Addition of $CaCl_2$ 2% decreased the weight loss to 3.6%.	Hardness of all samples decreased, but it was more pronounced in control fruit than coated fruit. Addition of $CaCl_2$ in combination or not with ascorbic acid helped to retain hardness.	At the end of storage period (12 days), control samples had lower lightness and higher redness and yellowness, indicating higher browning. Amongst coated fruits, AVG 100% without additives had highest browning.	(Nasution, 2015)
AVG 50% + citric acid 0.5% + ascorbic acid 0.5% (Control: citric acid 0.5% + ascorbic acid 0.5%)	Pomegranate arils (3ºC)	n.d.	AVG coating significantly reduced firmness losses, whereas losses >20% were detected in control arils.	n.d.	(Martínez-Rubio, 2015)
AVG 50% + carnauba wax 0.1% + polisorbate 0.1% + glycerol 1% (Control: water)	Fresh-cut mango (4ºC)	AVG coating contributed to the significant reduction of the weight loss (1 %) when compared to control(2 %).	Firmness of AVG coated fruits was stabilized, and firmness values were significantly higher (28 N) than those of the control fruit (20 N).	Change of color of AVG coated fruits was half of the change of control fruits.	(Pérez, 2016)
AVG 33% + [ascorbic acid 0,	Strawberries (1ºC)	By the end of storage, weight loss of control, AVG and AVG + ascorbic acid	AVG and AVG + ascorbic acid coated fruits softened more slowly than control	n.d.	(Sogvar, 2016)

AV coat	Fruit (T °C)	Weight loss	Texture	Color	Source
1, 3 or 5%] (Control: water)		of strawberries was 21.3, 18.1 and 12.6%, respectively.	(1N at the end of storage). AVG + ascorbic acid retained texture more than AVG alone (up to 3.5 N compared to 2 N).		
AVG 100%, AVG 100% + rosehip oil 2% (Control: water)	Plums (2/20ºC)	AVG 100% + rosehip oil 2% had the lowest weight loss, being 13.08 ± 0.21 %, compared to non-coated or AVG 100%, which were 15.30 ± 0.37 and 15.32 ± 0.35, respectively.	AVG 100% coating retarded softening. At the end of 28-day storage, firmness was 8 and 12 N, for non-coated and AVG 100%, respectively	Hue values remained close to those obtained at the harvest, especially AVG 100% + rosehip oil 2%.	(Martínez-Romero, 2017)
AVG 25%, AVG 32.5%, or AVG 40% + CMC 0.5% + corn syrup 2.5% + ethanol (Control: non-coated)	Strawberry (4ºC/10ºC)	At 4 ºC, weight loss was higher in fruits coated with AVG 40% than it was in AVG 25%. At tropical conditions, all AVG reduced weight loss when compared to the control, extending shelf-life.	AVG 40% showed the most stable texture among treatments, extending the shelf-life up to 14 days.	n.d.	(Falah, 2018)

n.d. not determined

Table 46. Changes in total phenolic content (TPC), flavonoid content (FC), antioxidant capacity (AC) and other important bioactive compounds of fresh fruit and vegetables, when coated with AVG with or without additives

AV coat	Fruit (T °C)	Total phenolic content, flavonoid content and antioxidant capacity	Bioactive compounds[3]	Source
AVG 5%, 10%, 15%, or 20% (Control: water)	Tomatoes (11ºC)	**TPC**: It remained stable for all treatments except for AVG 20%, in which TPC increased 2-fold compared with others.	**Carotenoids**: Lycopene and β-carotene contents increased 20% more compared to the control fruits, at the end of 14-day storage. **AA**: Coating significantly delayed the decline of AA content in coated samples in comparison to control samples. At day 14, AA content was 0.06 and 0.08 mg / g FW for control and AVG 10%, respectively.	(Chrysargyris, 2016)
AVG 100% (Control: water)	Sweet cherry (2ºC)	**TPC**: It increased during storage, but it was delayed by the application of AVG 100%. At day 0, TPC was 130.24 ± 5.56 mg / 100 g FW, and 170.48 ± 7.25 mg / 100g FW after 28 days. **FC**: Increase in anthocyanin content was delayed by coating with AVG 100%. At day 0, TPC was 110 mg / 100 g FW, and 120 mg / 100g FW after 28 days.		(Serrano, 2017)
AVG 50% (Control: water)	Litchi fruit (20ºC)	**TPC**: On day 8 storage, TPC of AVG coated fruit was 1.66-fold higher than control.	**AA**: At the end of the storage, coated fruit showed 1.46-fold higher AA content than the control.	(Ali, 2019)

AV coat	Fruit (T °C)	Total phenolic content, flavonoid content and antioxidant capacity	Bioactive compounds[3]	Source
		FC: AVG coated fruit had significant higher content of anthocyanins throughout storage. On day 8, it was 2-folf higher in AVG fruit.		
AVG 15%, AVG 25%, or AVG 50% (Control: water)	Fresh-cut papaya (28ºC)	**TPC**: Coated fruits were able to maintain better the TPC than control fruits did. **AC**: Highest antioxidant activity as DPPH· scavenger was observed in AVG 50% after 9 days of storage. **FC**: Highest flavonoid content was observed in AVG 50% (12.9 mg/100 g) after 15-day storage.	**AA**: Highest content of AA was recorded on AVG 50% coated fruit, whereas the lowest content was found in control fruits. **Carotenoids**: At the end of storage, coated fruits were able to maintain higher carotenoid content than uncoated.	(Mendy, 2019)
AVG 25%, AVG 50%, or AVG 75% (Control: water)	White button mushroom (4ºC)	**TPC**: AVG resulted in higher levels of TPC than in controls during all storage assessments. TPC was 34.16 and 41.75 mg / 100 g FW for control and AVG 50 %, respectively. **AC**: AVG resulted in higher antioxidant activity measured as DPPH· scavenging than in controls during all storage assessments. DPPH· inhibition for control and AVG 50% was 38.96 and 48.73 % respectively		(Mirshekari, 2019)
AVG 30% + glycerol 1% (Control: non-coated)	Orange (4ºC)	**TPC**: No significant differences were observed between coated and uncoated fruits. Average values were 180.0 ± 9.21 mg gallic acid / 100 g FW for both treatments.	**AA**: The rate of decrease in vitamin C was higher in the control than in the coated samples. At the end of 80-day storage, vitamin C was 33 and 37 mg AA / 100 g FW, for control and coated samples, respectively.	(Rasouli, 2019)

AV coat	Fruit (T °C)	Total phenolic content, flavonoid content and antioxidant capacity	Bioactive compounds[3]	Source
AVG 33% + [ascorbic acid 0, 1, 3 or 5%] (Control: water)	Strawberries (1°C)	**TPC**: Highest TPC content was found in AVG 33% + ascorbic acid 5%. At the end of 18-day storage, TPC was 14, 27, and 35 mg gallic acid / kg FW, for control, AVG, and AVG + 5 % AA, respectively. **AC**: Antioxidant activity decreased in all the samples. AVG 33% alone could not maintain antioxidant activity, while the addition of ascorbic acid delayed its decrease. At the end of 18-day storage, AC was 63, 68, and 80 % for control, AVG, and AVG + 5 % AA, respectively. **FC**: Increase in anthocyanin content was greater in coated fruit than in control. AVG 33% + 3 or 5% ascorbic acid coated fruits had the highest anthocyanin content. At the end of 18-day storage, anthocyanin content was 120, 145 and 165 mg pelargonidin glucoside / 100 g FW for control, AVG, and AVG + 5 % AA, respectively.	**AA**: Vitamin C content decreased from 69.7 mg/kg to 26.9, 38.5, 40.8 50.0 and 55.4 mg/kg fresh weight during storage, for control, AVG 33% + 1, 3, or 5% AA, respectively.	(Sogvar, 2016)
AVG 1%, or AVG 1% + chitosan 1% (Control: water)	Tomatoes (4°C)	**TPC**: In AVG 1% + chitosan 1%, TPC was slowly induced during storage period of 42 days. **AC**: AVG 1% + chitosan 1% had significantly induced the DPPH· radical scavenging activity, as control fruits did not have at the end of storage.	**AA**: Lowest level of AA content was observed in control fruits (0.59 mg/g), whereas all coated samples had higher contents. **Lycopene**: The highest level of lycopene was achieved at 35-day of storage (66.4 nmol/g fresh weight), and AVG+ chitosan was the most effective coat to maintain it.	(Khatri, 2020)

n.d. not determined

5 Conclusions

Aloe vera L. has gotten the attention of many industrial branches, such as pharmacy, cosmetics and food. As reviewed herein, its gel has shown promising antioxidant activities and antimicrobial effects, attributed to its characteristic active components, which include aloin, aloe emodin and other anthraquinone as well as the mucopolysaccharide acemannan.

In the last years, *Aloe vera* gel has become subject of current research regarding its applicability as an edible coating for fresh and fresh-cut produce. The present study highlights the recent scientific observations published in this regard, remarking the effects aloe vera gel had in fruit and vegetable quality retention: maintenance of color, firmness, antioxidant values and vitamins, as well as ripening delay has been reported by many studies.

Overall, AVG seems to be a promising alternative for preserving fresh produce, used alone or together with other preservatives. Its use offers the consumers a natural and sustainable edible coating that can contribute to shelf-life prolongation or enhanced food safety, while promoting some health benefits on them.

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Conflict of interests

The authors declare no conflict of interests.

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Discussion

The need for alternative treatments to chemical or synthesis agents to ensure safety and maintain quality of F&V is more and more evident. Traditionally, chlorine has been the main selection for most of the disinfection processes. However, its risks (toxic vapors) and drawbacks (pH dependence, interaction with organic matter), have pushed industries and scientists to look for safer and effective substitutes. The search for *natural* or *eco-friendly* substances, as well as for effective sanitation technologies, is well braced by consumers and innovative industries. For this, **finding ways to achieve safety and quality standards for fruits and vegetables has been the main objective of the present thesis**. The suggested solutions have been evaluated from a scientific point of view and at lab-scale. This attention on the F&V specific problematic, and the way each treatment may solve it, is necessary for an initial screening and an evaluation of the overall suitability of each proposed solution. This is the context in which this thesis is suggested to be read. Despite the fact that in the discussion section of every chapter, as well as in the general discussion, we have pointed out the possible causes, mechanisms or pathways in which each change could be based, the elucidation of the mechanisms underlying the effects of each treatment was not the objective of the thesis. Moreover, there is still further work to do, to scale-up the selected substances or technologies to an industrial level, as well as estimating their cost-benefit ratio, the feasibility of their implantation in the processing, or their environmental impact.

The results of the present thesis have been divided in eleven chapters, which stand individually and can be read separately. However, their interrelationship is visible, and they have been done within the framework of two financed **projects**: (i) FRESAFE, which aimed to find alternatives to chlorine sanitation to reduce pathogenic load while maintaining quality of strawberries, and (ii) AGRIMAX, which aimed to develop the processes to treat and purify by-products of F&V industries, and apply them as functional ingredients in other foodstuffs.

To start with, Chapters 1 and 2 have allowed to select an effective sanitation method for strawberries, consisting on a combination of peracetic acid with ultraviolet light irradiation, which is not detrimental for the quality of the product. Then, Chapter 3 shows the impact that this treatment has on fresh, fresh-cut and frozen strawberries for their commercialization and shelf-life prolongation. Chapter 5 and 6 examine other technologies for strawberry disinfection, namely sonication and thermosonication, suggested after a thorough review that is presented in Chapter 4. This technology has also been proposed to enhance the antioxidant penetration of two antioxidants in sliced-potato in Chapter 7, in order to increase the product's shelf life. In line with the functional compounds' topic, three substances were evaluated in Chapter 8 for their antimicrobial and antioxidant properties, highlighting their suitability to be incorporated in F&V or other foodstuffs with improving purposes. From those substances, ferulic acid outstand and was selected to be applied in two fresh-cut matrices (apple and melon) in Chapter 9. Its incorporation within an edible coating on apple was evaluated in Chapter 10, and one of the coatings (*Aloe vera* gel) was selected after a literature review that is presented in Chapter 11.

Overall, the analysis of all the results has been grouped in **four thematic sections**: (1) strawberry sanitization, (2) sonication for fresh and fresh-cut F&V, (3) on the use of plant extracts, and (4) edible coatings as systems for shelf-life enhancement, that are discussed hereafter.

1 A promising alternative for strawberry sanitization

In many of the chapters of this thesis there is a reference to the EFSA's Scientific Opinion on the risk posed by *Salmonella* spp. and norovirus on frozen berries including strawberries (EFSA, 2014). And this is because it is a safety issue that must be addressed to prevent related cases and outbreaks. Collection of appropriate data and subsequent risk-based development of microbiological criteria to support improved control of such pathogens in frozen strawberries is of high importance. However, the reader will be aware that, in the thesis, of the five chapters that refer to strawberries, only one includes the control of *Salmonella enterica* (Chapter 2). The reason for this is that this work has been conducted in parallel to the development of another thesis, by Ortiz-Solà (2020a) to which the readers are referred for further information on *Salmonella* spp. and Norovirus on strawberries. In Ortiz-Solà (2020a), the reader can find information on the effect that different chemical agents, ozonation, and the sanitation effect that has been selected in this thesis have on *L. monocytogenes, S. enterica* and Norovirus populations immediately after the treatments. Moreover, a study on the fate of such pathogens during the evolution of fresh, fresh-cut and frozen strawberries after the selected sanitation treatment can also be found.Both works are complementary, as in the present thesis, the impact in *Listeria* population, natural microbiota and overall quality of strawberries are assessed.

When a sanitation or disinfection method works (reducing significantly populations of pathogenic and spoilage microbiota), **is this enough evidence to suggest it as a good approach for introducing it** in the production chain of the product? The importance to go a step further and investigate the effect the sanitation approach may have on other features of the product becomes evident. For instance, the overall quality, including color and firmness, is a parameter strictly correlated with consumers' acceptance (Ragaert, 2004). Moreover, antioxidant capacity will be related with the ability of the fruit to combat oxidative stress, and thus increase its shelf-life (Hodges, 2004), and phytochemicals' maintenance, including phenolics or vitamin C, is key to preserve the nutritive value to which F&V are generally related. In these chapters, epiphytic microbiota has also been object of study after the sanitation treatments, in order to see the effect in these spoilage microorganisms whose growth could be detrimental for the fruit (Brecht, 2004). Additionally, we have included *L. innocua* as a good surrogate of *L. monocytogenes*. *Listeria* spp. was taken as an example of a Gram-positive bacteria to study disinfection processes. Although *Listeria* spp. does not have the ability to grow on strawberry surface (Flessa, 2005), and for so, strawberries are considered to be low-risk for this pathogen, strawberries have been implicated in reports of foodborne listeriosis due to cross-contamination (Schlech, 1996). It is therefore that a maximum limit of *Listeria* spp. of 100 CFU / g in frozen fruits is stablished (Reg. (EC) 2073/2005).

1.1 Peracetic acid, ultraviolet light, and their combination: chemical and technological solutions for strawberry disinfection

The problems on the use of chlorine have been already described in many points in this document: pH dependance, organic matter interactions, and mostly, the emitted toxic by-products. Trends are going towards looking for alternatives to it, and in the Introduction section, a general overview of the main options that are being considered for this purpose has been conducted. On a screening of different chemical sanitizers to reduce *L. monocytogenes* and *S. enterica* on strawberries (**Table 47**)(Ortiz-Solà, 2020), peracetic acid (PA) at 40, 80 or 120 ppm, and hydrogen peroxide (H_2O_2) or lactic acid at 2.5 and 5 % caused similar or greater reductions in both pathogenic populations when compared to chlorine. However, and despite the high efficacy of 5 % H_2O_2 (that caused a reduction 5.4 ± 0.8 and 4.9 ± 0.0 log CFU / g reductions on *L. monocytogenes* and *S. enterica*, respectively), when used at such concentrations, it resulted in significant changes in key attributes (e.g. color and total anthocyanins

content) (Alexandre, 2013). The higher reductions observed by PA disinfection over lactic acid reductions, together with the reported advantages of its broad antimicrobial spectrum (bactericidal, virucidal, fungicidal, and sporicidal effectiveness as demonstrated in various industries) and its decomposition in non-toxic by products, led to further experiments with this disinfectant. A possible drawback could be its high cost, which is partly due to limited production capacity worldwide. But if the demand for PA increases, especially from the wastewater industry, the future mass production capacity might also be increased, thus lowering the cost (Kitis, 2004).

The first question that had to be answered was: **is it possible to decrease the treatment time from 2 to 1 min while maintaining its efficacy?** In Chapter 1, the effect of PA disinfection on *L. innocua* population was evaluated for 1 and 2 min, having chlorine as a sanitation control. The concentration of 120 ppm was not used in this study because in the regions that the use of PA is already regulated (USA), the concentration is limited to 80 ppm (FDA - CFR 173.315).

Table 47. Average reductions (log CFU / g) of *L. monocytogenes* and *S. enterica* after 2 min on strawberries. Means ± standard deviation followed by the same small letter indicate no significant differences among the different concentration tested for each treatment (P≤0.05; n=6). (Adapted from (Ortiz-Solà, 2020b).

Treatment	Concentration	Reduction (log CFU / g)			
		L. monocytogenes		*S. enterica*	
		Strawberry	Water	Strawberry	Water
PA	40 ppm	3.8 ± 0.0 ^{a}	≤ 1.7	4.1 ± 0.0 ^{a}	≤ 1.7
	80 ppm	3.8 ± 0.0 ^{a}	0.0	4.1 ± 0.0 ^{a}	0.0
	120 ppm	3.8 ± 0.0 ^{a}	0.0	4.1 ± 0.0 ^{a}	0.0
NaClO	100 ppm	2.4 ± 0.1 ^{b}	0.0	2.3 ± 0.1 ^{b}	0.0
H_2O_2	1 %	2.4 ± 0.9 ^{c}	2.7	2.3 ± 0.4 ^{c}	3.1
	2.5 %	3.8 ± 0.7 ^{b}	0.0	3.8 ± 0.9 ^{b}	0.0
	5 %	5.4 ± 0.8 ^{a}	0.0	4.9 ± 0.0 ^{a}	0.0
NaClO	100 ppm	2.9 ± 0.4 ^{bc}	0.0	2.7 ± 0.4 ^{bc}	0.0
Lactic acid	1 %	2.7 ± 0.6 ^{a}	≤ 1.7	2.7 ± 0.6 ^{a}	2.0
	2.5 %	3.0 ± 0.0 ^{a}	0.0	2.9 ± 0.6 ^{a}	0.0
	5 %	2.7 ± 0.9 ^{a}	0.0	2.8 ± 0.8 ^{a}	0.0
NaClO	100 ppm	1.3 ± 0.4 ^{b}	0.0	1.2 ± 0.4 ^{b}	0.0
Acetic acid	1 %	2.4 ± 0.6 ^{b}	0.0	3.1 ± 0.6 ^{b}	≤ 1.7
	2.5 %	2.5 ± 0.6 ^{a}	0.0	3.2 ± 0.0 ^{ab}	0.0
	5 %	2.8 ± 0.6 ^{a}	0.0	3.9 ± 0.6 ^{a}	0.0
NaClO	100 ppm	3.2 ± 0.3 ^{b}	0.0	2.9 ± 0.3 ^{b}	0.0
Citric acid	1 %	3.2 ± 0.2 ^{a}	≤ 1.7	3.3 ± 0.4 ^{ab}	2.0
	2.5 %	4.0 ± 0.6 ^{a}	2.5	3.8 ± 0.6 ^{a}	2.8
	5 %	4.2 ± 0.6 ^{a}	≤ 1.7	3.8 ± 0.0 ^{a}	≤ 1.7
NaClO	100 ppm	2.7 ± 0.4 ^{a}	0.0	2.5 ± 0.4 ^{b}	0.0

In grey, the treatments that showed significantly higher performance than chlorine (NaClO) did.

Prior to starting the experiments, there was the dilemma on **which was the best initial load** of *L. innocua* for artificial inoculation on strawberries. Contamination level of food has demonstrated to influence the effectiveness of a wide range of emerging technologies, including pulsed electric fields, ozone, or hydrostatic pressures and on a variety of microorganisms (Ferrario, 2018; Molinari, 2004; Yao, 2014). Molinari (2004) pointed out that it is the growth phase of the microorganisms, more than

its initial size, what mainly affects their susceptibility. In general, during the growth and cells division phase (exponential phase), the microorganisms are more sensitive to stress than in the lag or stationary phase. The choice of working with microorganisms on stationary phase in this thesis was both practical and logical. On one side, the culture could be left under incubation in a nutritive media and at optimum temperature overnight, and the day of the experiment (after 22 ± 1 h of inoculation) the microorganism would have reached the stationary phase. On the other side, the higher resistance of cells in their stationary phase represents a worst-case scenario for screening sanitizer efficacies. We decided that, although in most cases the contamination of fruits is not likely to reach levels of 10^6 - 10^7 CFU / g, this was the best way to detect differences between treatments. Otherwise, reductions of 2 or 3 logs would mean that the counts had reach the limit of detection, making it impossible to compare efficacies of the treatments.

Focusing on the investigation of PA as a sanitizer: its effect on *L. innocua* was marked when used at 40 for 2 min and 80 ppm for 1 or 2 min. In those treatments, reductions in strawberries were similar to those of chlorine (~ 5 log units). These were already promising results, but **for us, the reduction of microbial load in washing water was also a determining factor for two reasons**. First, the importance in the reduction of water consumption. Among the different industries, the food industry ranks third in wastewater discharge rates (after the chemical and the refinery industry) (Casani, 2005). By recycling and reusing waste water, discharge of water could be reduced from 20 to 50 % depending on the sector (Ölmez, 2009). But this is not possible if the recirculated washing water is spoiled with microorganisms coming from previously washed F&Vs. It is acknowledged that the wash water may serve as a contamination source and has great potential to result in cross-contamination on the products washed, so disinfectants are used mainly to maintain the water quality during the processing (Tomás-Callejas, 2012). According to Banach (2015), peracetic acid was selected one of the suitable disinfectants for washing water. In our study, despite not achieving the efficacy of chlorine, PA significantly reduced pathogens from wash water, and when used at 80 ppm, kept levels lower than 0.5 log CFU / mL. Other studies indicated that PA was less effective than chlorine in reducing the pathogens in tap water, but similar when reducing pathogenic load in process water (Ölmez, 2009). They noted that the efficacy of chlorine is affected by the organic compounds, whereas not that of the PA. For this second reason, **we believe that PA is a useful chemical sanitizer that can be implemented in processes that use washing water recirculation**.

On the other hand, we had at our disposition a water-assisted ultraviolet C (UV-C) light prototype. The UV-C light, as detailed in the Introduction section, is reported to be germicidal, and for this reason, it was suggested for further experiments in this thesis. The mentioned equipment was employed in all the washing treatments in Chapter 2 and 3. The first step in testing the efficacy of the system was conducted by testing different lamp configurations for different treatment times. The obtained results are presented in Chapter 2, in which is patent that the best lamp configuration was observed when 4 lamps were on. In that case, reductions of *L. innocua* (~5 log reductions) were over 2-log higher from those obtained in the water control, which were 2.4 ± 0.9 log CFU / fruit. It has been already highlighted in the related chapters the mutual benefits of combining UV-C light with the immersion of the product in water (agitation, microbial detachment from surface, avoidance of shadowing effect). The highest reduction in washing water, though, was observed only after 5 min treatment, in which *Salmonella* Typhimurium population was not detectable. **Although these results are promising, 5 min treatment could be excessive for strawberries**, as these fruits are extremely sensitive, and both excessive agitation and contact with water could led to deterioration, loss of firmness and increase of decay incidence. Typically, strawberries that are going to be commercialized fresh are not washed, and are directly packed in boxes in the field, to reduce handling damages. The general recommendation for

fresh and whole strawberries when marketed is for consumers' to thoroughly washing the fruit reduce microbial loads in the final step of the chain. It is then riskier when products are sold as 'ready-to-eat' (fresh-cut or frozen). In this case, it is the food industry the responsible to offer safe fruit, and thus, the importance to find effective sanitation methods.

The combination of both PA and WUV-C was the next logical step. In fact, a literature survey reveals that this combination is starting to prove its potential: in the last 4 years, at least 28 papers have been published on the efficacy of the combination (e.g. Ao (2020), Collazo (2019), Hassaballah (2019) and Lippman (2020)). In Chapter 2, the relatively novel approach of using UV-C light in a water media (with the exception of waste water), in which a chemical substance as PA can be incorporated, was investigated. As anticipated, the combination proved to be more effective than each treatment per separate: both in strawberries (between 5 and 7 log CFU / g reduction) and in washing water (resulting in the absence of *L. innocua*, and population below the quantification limit of *S.* typhimurium). These results were observed when using 40 or 80 ppm PA with 4 UV-C lamps for 2 min (allowing the decrease of treatment time of 5 min that was needed when using UV-C alone). **The presented results are encouraging, as the treatment of strawberries with this method allows washing water recirculation and results in safer strawberries** (considering that initial population of *L. monocytogenes* or *Salmonella* spp. would not be as high as 10^5 CFU / g, a 5-log reduction would result in the accomplishment on the that requires the absence of both pathogens / 25 g ready-to-eat product (Reg. (EC) 2073/2005). Moreover, studies carried out by Ortiz-Solà (2020a) that the same combination allows a reduction of murine norovirus (a surrogate of human norovirus) of 2 log TCID50 / mL, increasing safety on this strawberry problem as well.

Fewer is to be discussed about the disinfection effect on natural microbiota (TAM, and Y&M). Even if there is no regulation on the natural contamination in strawberries, neither fresh, fresh-cut nor frozen, the deterioration processes that such microbial can cause have already been described in the Introduction section. Those include texture softening, off-flavors, off-odors, fermentation, and visual decay. Despite the hopeful results observed in the efficacy of PA (Chapter 1), WUV-C, or their combination (Chapter 2) against *L. innocua* and *S. enterica*, the reductions observed against TAM and Y&M were, in most cases, similar to those achieved only with water (about 1 to 2 log reductions) (Chapters 1 and 2). The higher attachment of epiphytic microbiota to the fruit surface, together with the heterogeneity in the resistance against the selected treatments, are proposed as the main reasons for the lower reductions observed after the treatments. However, **one of our main concerns in this regard was whether their numbers would increase during cold storage Figure 54**. Three commercialization formats: (A) fresh, (B) fresh-cut, and (C) frozen strawberries in the study of their shel-life after WUV+PA treatment (Chapter 3).

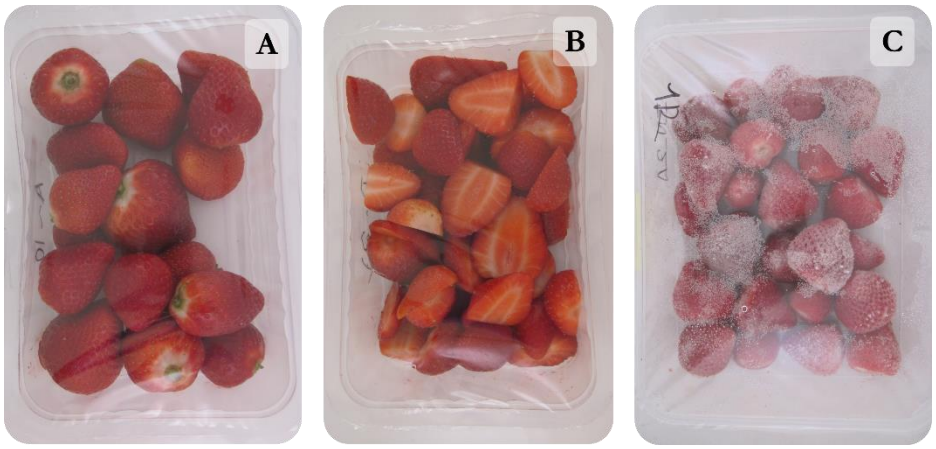


Figure 54. Three commercialization formats: (A) fresh, (B) fresh-cut, and (C) frozen strawberries in the study of their shel-life after WUV+PA treatment (Chapter 3)

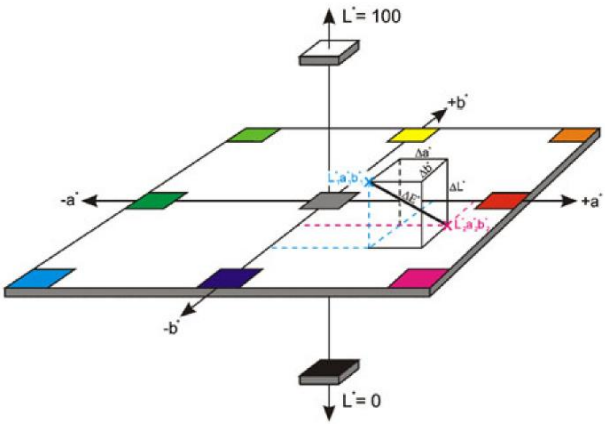
In Chapter 3, strawberries washed with WUV+PA treatment (the combination of UV-C light and PA 40 ppm for 2 min), TAM were reduced 0.5 log units, and Y&M were reduced between 0.5 and 0.8 log units, leaving populations of c.a. 1.4 and 1.3 log CFU / g, for TAM and Y&M, respectively. As expected, during frozen storage period (12 months, - 20 °C), counts were maintained. Generally, microorganisms such as bacteria would not experience sufficiently rapid cooling rates to induce cold shock. This means that the simple freezing of microorganisms in food is unlikely to be immediately injurious. However, they can progressively lose viability if their growth is prevented. Although it may occur, it may be of little consequence at usual frozen storage temperatures (Gill, 2014). What drew our attention was that, whereas TAM and Y&M counts increased in the control strawberries during the 11-day storage period at 4 ° C in dark and light cycles (mimicking storage conditions), this did not happen to WUV+PA and NaOCl treatments (in which populations even decrease). This fact is a positive outcome on the use of sanitizers in such fresh and fresh-cut strawberries. A possible explanation for these results may be the sub lethally injuries caused by the disinfectants, from which the microorganisms were not capable to recover at such conditions (Wu, 2008). There are, however, other possible explanations that relate the responses of the strawberry tissue to low doses of UV-C light. In this regard, UV has been shown to elicit a range of chemical responses in fresh produce ranging from antifungal enzymes to phytoalexins (which are antimicrobial and often antioxidative substances synthesized *de novo* by plants that accumulate rapidly at areas of pathogen infection). As our studies were not focused on finding the cause underlying this, such considerations could be the basis of new projects on the way of increasing safety of strawberries during storage.

1.2 The findings on the impact of peracetic and ultraviolet light on quality of strawberries

It is evident from the Introduction section that efforts must be done in increasing the shelf-life of the products in order to reduce food waste. Especially strawberries are high perishable products, as these fruits are soft and sensitive to a number of factors (Haffner, 2002). **One of the main causes of strawberry losses** are related with mold visible growth on their surface (especially *Botritys cinerea*– grey mold - and *Rhizopus* spp. and *Mucor* spp. – soft mold (Feliziani, 2016)). It has already been highlighted in the previous Section (1.1) that the proposed treatments delayed the growth of spoilage microbiota in strawberries, including molds, contributing to an increase in the maintenance of the quality of these fruits (Ragaert, 2007). Besides, consumers needs and expectations play an important

role on the attributes that make strawberries (and the other F&Vs) to remain in the market and that are considered adequate to consume. Consumers' demand for healthy, nutritive, and affordable products, and with marked organoleptic preferences. But despite these general trends, consumers preferences are not homogeneous. So, **what do consumers really ask for strawberries?** In a survey conducted by Wang (2017) to more than 1,000 participants, they found that there are mainly three kinds of strawberry consumers: (1) the balanced kind, for whom the strawberry traits have the same importance but are influenced by their cost (2) and the experience attribute sensitive consumers, who most value the internal characteristics of the strawberries they purchase regardless the cost (and the repetition of the purchase will be strongly affected by flavor and internal aspect of strawberries) and (3) the search attribute sensitive consumers, that are mainly influenced by the external aspect of the berries. Regardless the relative importance each kind of consumer gives to every attribute, what it is valued by all the three groups is: **an ideal external and internal intense red color, intense strawberry flavor, firm texture, and longer shelf-life**.

In this sense, the color of strawberries in Chapters 1, 2, 3, 5, and 6 was the intense red characteristic of mature fruits, as indicated by L* and a* coordinates (which refer to the luminosity and the redness of the color in the CIELab spectrum). The values for the coordinates averaged 40.0 ± 3.6 and 32.7 ± 0.2, respectively, which corresponds to the typical red color of strawberries (Kelly, 2019). Color was slightly affected by PA or WUV treatments, with the exception of PA 80 ppm, which affected in an apparent manner, but negatively, the color of strawberries. One analytical way to determine whether a color change is visible for the non-experienced eye is by calculating the total color difference (TCD) as explained in Chapter 1. Considering that the most precise measurements of color are performed in perceptually uniform color spaces as is CIELab, the calculation on the TCD can give us an idea of the noticeability of the change by the viewer (Mokrzycki, 2011) (**Figure 55**). Color of strawberries decreased in luminosity and a* values during storage, in the same manner for all the treatments, thus indicating that, despite WUV+PA does not improve the color of strawberries, at least it keeps the evolution similar to that of the control.



A standard observer sees the difference in color as follows:

TCD value	Interpretation
0 - 1	Observer does not notice the difference
1 – 2	Only experienced observer can notice the difference
2 – 3.5	Unexperienced observer may notice the difference
3.5 – 5	Clear difference in color is noticed
> 5	Observer notices two diferent colors

Figure 55. Graphical interpretation of the total color difference (TCD or ΔE) in the L*a*b* color space (From Mokrzycki (2011)).

In fact, **fresh-cut products tend to be more sensitive to the changes, as they have been submitted to stressing operations**: cutting and other minimal operations such as peeling. For this, it is surprising, but yet encouraging, that the fresh-cut strawberries even maintain higher L* and a* values than their whole counterparts. Another promising result involves firmness maintenance. This

parameter is reported to be a good indicator and predictor of strawberry quality and shelf life throughout the chain. Moreover, it has been found that the lower the strawberry firmness, the higher the fruit decay scores (Wageningen University and Research, 2018). Typically, the methods used to assess firmness in strawberry measure the force needed to penetrate, puncture or deform it. We used a texture analyzer (TA.XT), which is an advanced instrument that, although being expensive and time-consuming, gives reliable results (Doving, 2010). It has been stated in Chapters 1 and 2 that the firmness of strawberries immediately after the treatments did not decrease. However, it is **important to evaluate the progression of firmness during storage, as immersion in water and agitation can lead to increase in humidity and less of integrity of vegetable cells, accelerating the loss of firmness**. Surprisingly, firmness was maintained for 11 days at 4.5 ° C for both whole and fresh-cut strawberries. Regarding the frozen product, the benefits of cryo-freezing cell integrity have been explained in the Introduction section. In Chapter 3, cryo-freezing was considered as the freezing process, being the object of the study to evaluate the quality of the product during storage. After thawing the frozen strawberries, we observed a decrease in firmness. The lack of a slow-freezing process control prevents to ascertain wether this decrease could be more pronounced if cryo-freezing was not done. It is accepted, however, cryo-freezing decreases the size of the ice crystals, that otherwise would have been bigger, decreasing in turn the cell-wall damages and the loss of integrity and turgor of the fruit (Agoulon, 2012).

Moving to the impact strawberry consumption poses to human health, there is no need to say that as well as being a good source of vitamin C, dietary fiber, and minerals, berries contain high levels of natural polyphenol components that act as potent antioxidants (Giampieri, 2012). According to what was reviewed by Battino (2009), such bioactive compounds from strawberries have shown relevant outcomes in cardiovascular health improvement, control of cancer growth or control of blood glucose levels. **When implementing new sanitation treatments, it is essential to verify that they do not affect the main compounds present in the product.** For this, the immediate effect of PA and WUV were evaluated on several nutritional parameters, including antioxidant capacity (AC), total phenolic content (TPC), total anthocyanin content (TAC), total ascorbic acid (TAA) in Chapter 1 and Chapter 2. The effect that the combination of the treatments (WUV+PA) had on such parameters during shelf-life was studied in Chapter 3.

Two different methods were employed to estimate the AC of the samples after the treatments and during storage. The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) inhibition and the ferric reduction antioxidant power (FRAP). The first reaction is based in quenching free radicals by hydrogen donation and by electron transfer, whereas the second reaction is mainly based in electron transfer processes (Prior, 2005). Typically, studies on food employ two methods to obtain complementary information on their AC. The reasons behind the choice of DPPH· and FRAP as the employed methods were that they are both colorimetric, simple and do not require especial instrumentation. The AC of the strawberries used in the studies of the present thesis have shown DPPH· values between 105 to 608 mg AAE / g, and FRAP values ranging between 59 to 797 mg AAE / g. Such disparities in AC may be explained by the differences in varieties, cultivar or season (Alvarez-Suarez, 2014), but it is important to note that **none of the treatments** (NaOCl, PA, WUV, WUV+PA) neither the storage during 11 days at 4 °C **caused an important variation in the initial AC of each strawberry batch**, which in fact is quite encouraging. The exception was the slight decrease in DPPH· values in fresh-cut samples, which was attributed to the higher stress at which the fruits were submitted. A question on this topic asks whether DPPH· and FRAP values are related. We hypothesized that if the substances accountable for the AC in strawberries were present in similar proportions in all the batches, values obtained with both colorimetric methods would have a direct correlation. However, the lack of relationship found

between both parameters suggests that specific composition of strawberries affects their AC. In fact, it has been reported that the contribution of vitamin C to antioxidant capacity (AC) rounds 30 – 35 %, and other specific compounds like anthocyanins (namely pelargonidin-3-glucoside) may also account for up to 25 % of AC (Tulipani, 2008).

If we focus on vitamin C, strawberries are considered, amongst all the fruits, as one of the richest in vitamin C (Fierascu, 2020). Because L-ascorbic acid (AA) is easily converted (by oxidation) to L-dehydroascorbic acid (DHAA), both need to be determined in fruit to investigate the amount of vitamin C (Gil, 2006). This oxidation is reversible, and the problem will reside in the degradation of DHAA by oxidation or hydrolysis which, depending on the reactive oxygen species status, will result in different compounds (**Figure 56**) with no vitamin C activity (Parsons, 2011).

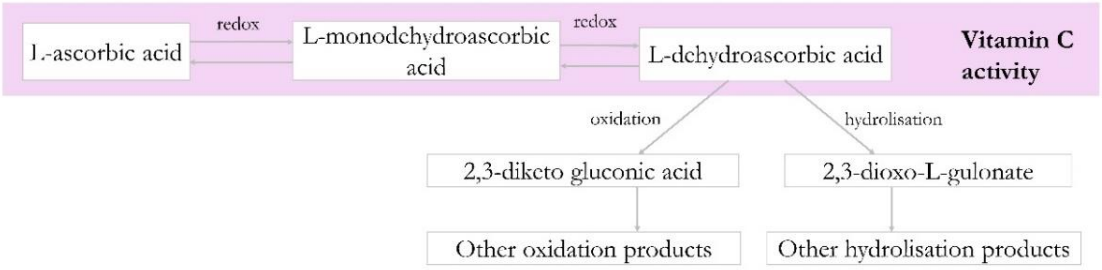


Figure 56. Reactions involving L-ascorbic acid and the different compounds with vitamin C activity in F&V.

The total ascorbic acid (including L-ascorbic and L-dehydroascorbic acid) content was determined by HPLC in strawberries before and after the NaOCl treatment, the WUV treatments for various times, and during storage after the treatment WUV+PA. Results on this part showed that vitamin C was retained after washing strawberries with all the proposed treatments, and that it is well maintained during fresh (11 days, 4 °C) or frozen (12 months, - 20 °C) storage periods. As has been commented, minimizing detrimental changes in the nutritional value is key in evaluating a new treatment method. The maintenance of vitamin C is considered, then, a valuable outcome on the use of WUV and PA in strawberry sanitation.

If we move to anthocyanin content, these are a kind of flavonoid, and are one the main constituents in the four classes of phenolic compounds we can find in strawberries. Pelargonidin-3-glucosyde is the main anthocyanin in strawberries, followed by cyanidin-3-glucoside or cyanidin-3-rutinoside (Fierascu, 2020). These compounds, accountable for the red color of strawberries, can be in different proportions, as well as the other phenolics. Although colorimetric methods used in the thesis give a general idea on the anthocyanin (Meyers, 2003) and phenolic content (Singleton, 1999), o**ne limitation of these spectrophotometric methods** is that they are more unreliable than HPLC methods: reactions can be different for the individual compounds within a family and they do not contribute equally to the sum of the colorimetric result (Tabart, 2010). Moreover, interferences with other substances such as ascorbic acid in the Folin-Ciocalteu (FC) method (as we have observed in Chapter 7) can occur (Lester, 2012). Such inconveniences lead to a possible under- or over-estimation of the results. In Chapter 2, a deeper investigation of the phenolic profile in strawberry by HPLC-DAD method was attempted, and 12 main compounds were identified, including the aforementioned anthocyanins. However, despite having this information, we could not find a general trend relating the AC with the specific composition of strawberries after the WUV treatments, neither with the changes occurring in the next 24 h. The key problem in this situation was the absence of the whole picture. Due to time and budget limitations, enzymes that have already been explained in the Introduction section and are involved in the phenolic and anthocyanin pathway, such as phenylalanine lyase, or other coenzymes were not evaluated.

Moreover, to better understand the impact of the WUV treatment on such compounds, a study at molecular level cold be conducted, in order to determine the reasons in the metabolism of strawberry underlying the changes.

However, and with the information at our disposal, it is worth noting that the total amount of anthocyanins and phenols (TAC and TPC) did not show significant changes after the WUV+PA treatment and neither during storage. The tentative values acquired, together with the slight differences observed in the phenolic profile, suggest that the combination of PA at 40 ppm in a tank irradiated with UV-C light does not cause any significant nutritional loss in strawberries. This, together with the **relatively high maintenance of the other evaluated parameters** (color, firmness, vitamin C and AC) after the treatment and during storage of whole, fresh-cut and frozen strawberries makes the proposed treatment worth studying. The sanitizing effect against *L. monocytogenes, S. enterica* and norovirus described in Section 1.1, and the confirmation that overall quality is not detrimentally affected, makes WUV+PA 40 a potential method for strawberry disinfection.

Nevertheless, **further research would be helpful:** scaling up the process, in order to increase the impact level of the innovation and to implement it in F&V industries to increase their safety for a healthier world. For this, the device should be adapted for the higher product load and possibly for a continuous flow, and studies on the decrease of effectivity of UV irradiation when organic matter in water increases (blocking the transmission of the light) are suggested as next investigations. Besides, it was not our objective to perform a cost-benefit balance on this system but to prove its effectiveness, so feasibility of its implementation is another must in this scaling up process.

2 Is sonication a solution?

The research on the ultrasound (US) technology applied with antimicrobial purposes on fruits and vegetables has increased in the few years. A quick bibliometric analysis in the database Scopus, using the key words: *sonication OR ultrasound, antimicrobial OR microorganisms, fruit* OR vegetable**, has revealed a sharp increase of publications on this topic since 2004 (**Figure 57**).

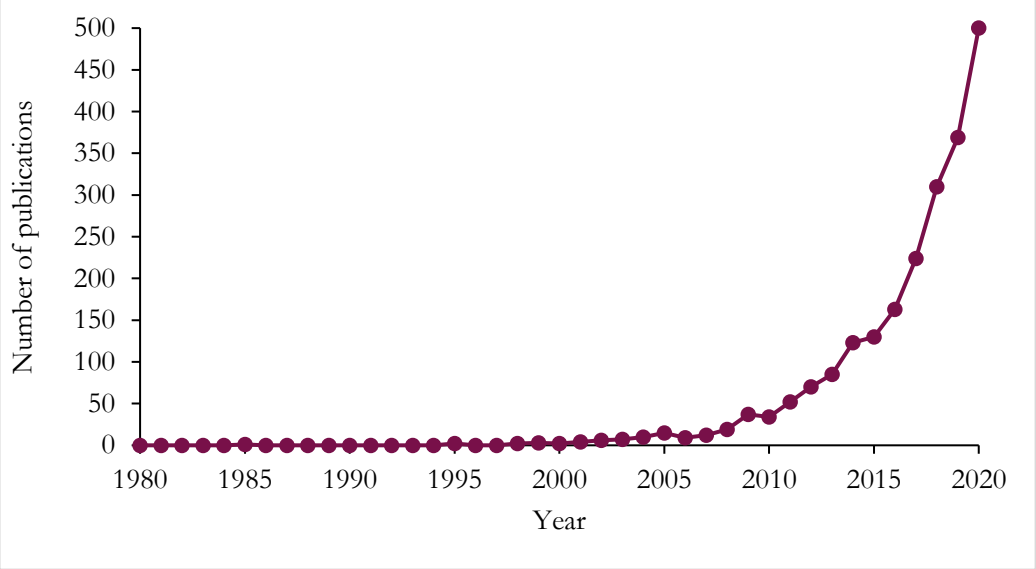


Figure 57. Time evolution on the number of publications per year in Scopus (bibliometric analysis using *sonication OR ultrasound, antimicrobial OR microorganisms, fruit* OR vegetable** as keywords).

As mentioned in the Introduction, US have been investigated for sanitation of food products, as the cavitation effect that occurs in the generated microbubbles is expected to make them collapse and

generate great temperatures and pressures in those spots, affecting microbial viability. However, when searching for practical examples and applications, we realized that US were mostly applied to liquid food matrices: milk and derivatives, and F&V juices (Dolas, 2019; Firouz, 2019; Salehi, 2020). This outcome was logical, as US waves can be vehiculated through liquid media. However, the literature on the application of US on the fresh and fresh-cut F&V field, although relatively high (71 out of the 502 papers published in 2020 contained *fresh* or *fresh-cut* as keywords), was not reviewed yet. One of the questions we wanted to answer with the search was **if US could be effective as a sanitizing or as a quality improving treatment for fresh and fresh-cut F&Vs**, question that was the focus of our work. With the review presented in Chapter 4, we tried to answer that question, taking an overall view of the latest publications on the topic. The review highlighted the main outcomes on the microbial effects (natural occurring microbiota and artificially inoculated pathogens), as well as on the quality parameters (color, firmness) and biochemical composition and antioxidant capacities of the treated F&Vs. At the light of the results, we considered adequate to incorporate into the review the combination of US with other chemical and physical techniques, as *per se*, US did not prove to be sufficiently effective.

Most of the studies reported that, when combining US with other techniques, a higher effect (additive or even synergic) was observed. The better results were reported for both, microbiological and quality aspects. Some further considerations, though, needed to be done. First, as similarly to what happened in Chapters 1 and 2 on the strawberry disinfection, **artificially inoculated microbiota was better reduced** than natural occurring microorganisms were. In a same direction that other authors have hypothesized, we believe that this could be attributed to the lower attachment inoculated cells have on the F&V surface (**Figure 58**). In this sense, cavitation occurring near the surface may exert an impact in this detachment, and real decreases in population would depend on the time contamination has occurred or and the interaction between microorganism – F&V surface (Takeuchi, 2000).

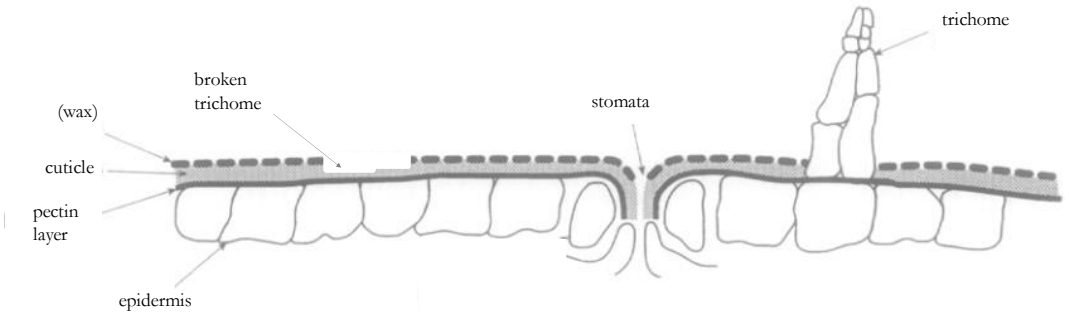


Figure 58. Diagram illustrating the surface layers of a plant (Adapted from Fahn (1990)). The inability of sanitizer treatments to inactivate cells on organic surfaces is readily explained by the protection cells receive by being entrapped within the surface structure (often stomata, broken trichomes, or cracks in the cuticle) limiting their exposure to the sanitizer (Frank, 2001).

The second consideration recalls on **the estimation of the increase in biochemical contents of the sonicated F&V**. Increases in antioxidant capacity, phenolic content, or pigments can be partially explained by a better extraction of these compounds: ultrasound can be a tool to aid cell wall lysis, releasing compounds from the inside (Toma, 2001). This enhanced extraction leading to higher yields but not to higher contents may bring confusion when reporting results, so it must be cautiously interpreted (**Figure 59**A). More investigation needs to be done in this regard. Although it can be argued that ultrasound disruption of cells and vacuoles – in which these compounds are typically stored – occurs mainly to the surface of the product, the mass transfer can go deeper in the product (**Figure 59**B)(Schössler, 2012). The process, in turn, can work in the opposite direction: the pressure generated

during cavitation process of ultrasound can increase the penetration of compounds from the extracellular to the intracellular environment (Zhang, 2020). This part will be extensively discussed in Section 2.2.

2.1 Exploring sonication for its use in strawberry sanitation

In front of a technology that has revealed potential in F&V disinfection with no detrimental effects in F&V quality, we decided to give it a chance and try it for strawberry sanitation. In line with the experiments carried out in the first chapters (Chapters 1, 2, and 3), **US was proposed as an alternative technology for chlorine disinfection**. In Chapter 5, sonication was tested against artificially inoculated *L. innocua* and natural microbiota (which included TAM and Y&M). As revealed in the review (Chapter 4), sonication alone is typically not adequately effective for such purposes. For this, we decided to combine it with the same concentration of chlorine. **We wanted to know if US could enhance the effect of chlorine**. If it occurred, next experiments would be carried out in order to decrease chlorine concentration or substitute it with other chemical disinfectants, that are not as effective *per se*, but whose efficacy could be assisted by US. The experimental part proved that US was ineffective to reduce both *L. innocua* or TAM and Y&M in samples. The observed reductions were similar to those resulting from washing strawberries only with water: from this, we can infer that, similarly to Chapters 1, 2 and 3, we can reduce between 1 to 2 log CFU / g only by immersing fruit in agitated water. However, while such reductions can greatly reduce spoilage, they **are insufficient to assure safety in the event of contamination with human** (Sapers, 2005). In this regard, US were not effective for a sanitizing purpose. Moreover, the remaining populations in water after sonication were quite high (4.0 log CFU / mL in most of the cases). Contrary to what happened with PA in Chapter 1 and 2, this technology cannot be even recommended for washing water decontamination, as remaining population in it was similar to those without any treatment. If done, water recirculation would represent a cross-contamination problem, jeopardizing safety of the next washed produce. In turn, chlorine caused between 2 to 3 log reductions (99 - 99.9 % of the initial load), which was more effective than sonication alone. Chlorine reductions were marked in *L. innocua* and mold populations, and against TAM in replica 1 of the experiment. These replica differences have been already explained in Chapter 1, and are attributed to the heterogeneity of natural microbiota that accompanies the fruit. As US's efficiency in aqueous solutions can be enhanced by the addition of solids to reduce the cavitation threshold, the combination sonication/chlorination could have been meaningful especially for samples in which organic matter is present (e.g. strawberry achenes)(Blume, 1993). I**t was somewhat surprising that sonication did not enhance chlorine efficacy**. It has been suggested in the literature that such treatment would disperse bacterial flocs which leads to increased bacterial susceptibility and improved penetration of the biocide into the pathogens (Mason, 1993). However, it becomes more apparent when greater times (> 60 min) are used (Jatzwauk, 2001). Nevertheless, in the literature there are a few reports that actually perform shorter treatments (5 – 10 min) and combine US with chemical sanitizers or slightly acidic electrolyzed water, and observe an increased effect on Y&M, TAM, lactic acid bacteria, inoculated *S. enterica* and *E. coli* O157:H7 on strawberries (Lafarga, 2019).

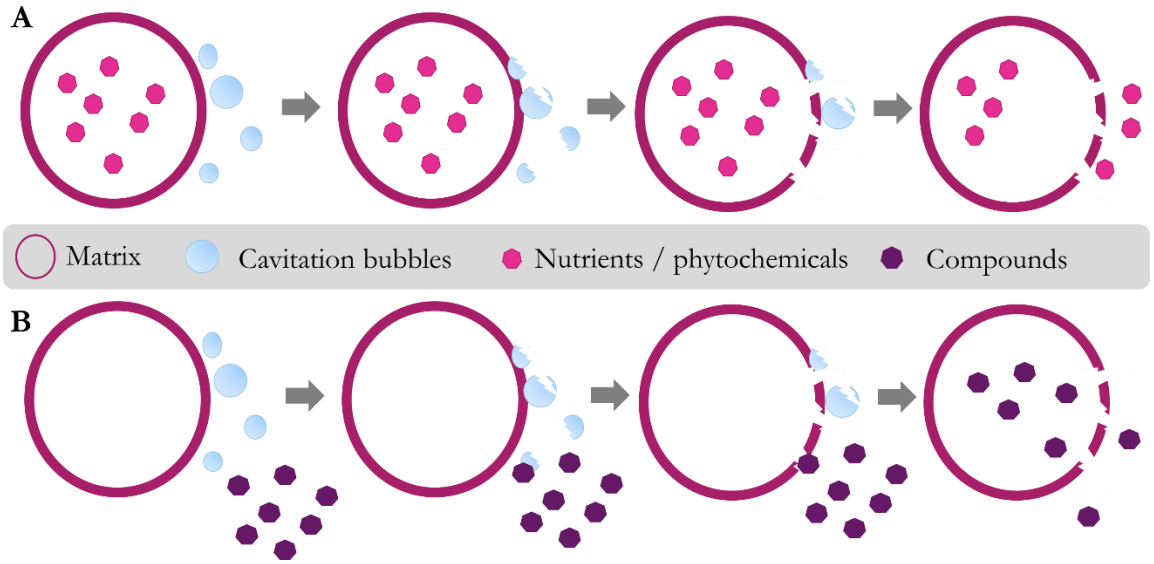


Figure 59. Sonication applications: (**A**) extraction assistance, where cavitation occurring near the membrane causes disruption and intracellular components are easily extracted, and (**B**) increase of mass transfer into the tissue after sonoporation of membrane.

We are going to take advantage of this section and focus on the strawberry quality. For the studies carried out during this thesis, strawberries were purchased during 3 seasons (2018 to 2020) to a local supermarket. The vast majority of them (89 %) came from Huelva (which is located in Andalucía), community that accounts for 97 % of Spanish strawberry production. Amongst others, the variety is typically a source of variation in characteristics of strawberries. However, they are typically commercialized as a multivarietal product; this could be advantageous for the experiments performed on them, because of the representativity it constitutes. **What do we consider quality parameters of strawberry?** When we mention quality of strawberries, primarily refer to pH, TSS, and TA, which are indicators of the acidity, soluble sugars and organic acids present in strawberries. In all the tested batches, we have not found any strange value for these parameters, which have ranged between 3.4 to 3.6 (pH), 5.9 to 6.6 °B (TSS), and 4.8 ± 9.0 mg citric acid / L (TA). These values are within the ranges reported in the literature (Ayala-Zavala, 2004; Fierascu, 2020). However, what actually gives these fruits their main characteristics are their antioxidant capacity, which is given by main biochemicals, including ascorbic acid, phenols and anthocyanins. This is the reason why the effect of the treatments applied in the present thesis (NaOCl, UV, PA, or US) has been studied also on these parameters. When the studies were carried out, batches of same season and maturity were used. The antioxidant and biochemical composition between experiments showed to be different, but we attributed this to variety, cultivar, season, or maturity stage variations (Ornelas-Paz, 2013). For this, for each of the experiments, the results were referred to a non-treated or water-treated control. In the cases the treatments behave different depending on the batch, we have presented the results separately. In agreement with the previous observations in the group, and corroborates the idea that **applied treatments efficacy are conditioned for both: treatment and matrix characteristics**. In such cases, we considered that recommendation of a determined treatment must be careful, and subjected to various considerations or pretrials. If a treatment does not exert clear or reproducible results in most of the samples, its optimal application will be difficult. For this, we would recommend to improve the conditions of the treatment or to find the requirements of the fruit matrix in which it is effective.

At the light of the results obtained from sonication on strawberries, we **decided to combine US with mild temperatures** (50 – 55 °C). The use of high temperatures in fresh and fresh-cut F&V is not an

option, as undesirable nutrient losses, and organoleptic and textural changes may occur due to the negative impact of heat on fruit and vegetable tissues (Alexandre, 2011). Thermosonication (TS) could be an alternative to such high temperatures, because when the two methods (temperature and US) are combined, their parameters needn't to be that high. Nowadays, TS is primarily used as an alternative to thermal treatments on F&V juices (Anaya-Esparza, 2017), and to F&V blanching (Alexandre, 2011). So far, there are no studies on the use of TS in fresh or fresh-cut F&V.

When studying the effect of sonication at two frequencies (35 and 130 kHz) and three temperatures (20 – as a control –, 50 and 55 °C) on the fate of *L. innocua in vitro* in Chapter 6, we observed that combining 55 °C with ultrasound at 130 kHz for 10 min led to 4 log reductions of the microorganism. We continued the experimentation *in vivo*: on artificially inoculated strawberries. A first screening was conducted in order to select the outstanding treatments and to discard those that were detrimental for strawberry quality. The results showed that, although the treatments at the higher temperatures (55 °C) and the higher times (15 min) were significantly effective (achieving reductions of *L. innocua* population about 6 to 7 log CFU / fruit), they were not a good option for our purpose, as the strawberries lost their freshness, showing a cooked aspect. It is worth saying that the sonication part in the thermosonication binome did not exert the intended effect. As the analysis of variance (ANOVA) revealed, **sonication had little impact in the outcomes**, being temperature the most significant factor. Sonication was also negligible in relation to the remaining population of *L. innocua* in water, and in relation to the TAM and Y&M reductions. The study of the effects of TS in physicochemical and biochemical properties disclosed an absence of sonication implication. Moreover, little changes were observed in this regard, proving at least that TS (< 55 °C, < 10 min) was not a harmful process for fresh strawberries, and it was able to maintain their antioxidant activities, phenolic and anthocyanin content.

Despite the fact that the performance of the mild conditions of TS was not satisfactory for a fresh product, and that sonication could be as well removed, the present results highlight the importance of testing *in vitro* and *in vivo* the new technologies for certain purposes, in order to determine their real potential and applicability. **Even that our study was focused on a fresh product**, the significant reductions observed at higher temperatures and higher times (55 °C, 15 min), although presenting a boiled aspect, represent an advantage for the product's safety. These findings, while preliminary, suggest that TS could be an alternative process for disinfection of those fruits that are going to be frozen. A deeper investigation in TS against other pathogens in strawberries, an optimization of the parameters, as well as a study on the biochemical characteristics and nutritional values is therefore suggested.

2.2 Application of US to enhance penetration of antioxidants to potatoes

Within the multiple uses of US, the extraction of compounds assisted with this technology is an effective alternative that presents enhanced yields over the traditional methods (Moreira, 2018). The implosion and cavitation break the cell walls, and this **enhances the mass transfer from solid to liquid** phase. It contributes to efficient recovery of compounds in lesser time, energy, and solvent requirements, with the advantage of lowering the temperature of extraction, for temperature-sensitive food product (Ojha, 2019). In this line, Koiwai (2007) proposed the use of US to enhance the extractions of active compounds from green tea: although extraction using hot water (> 95 %) is safe, the heat deteriorates green tea quality and catechins are lost. As it will be discussed in Section 3.2, a green tea concentrate was intended to use for its antioxidant properties in sliced potatoes as alternative treatment (Chapter 7). For the next step, the question was: **could sonication enhance the antioxidant potential of green tea, by increasing the release of the phytochemicals to the water**?

As previous studies had shown, a higher availability of its biochemicals (mainly catechins) was observed after sonication. So, we started with a screening of US and temperature conditions. Finally, we concluded that the procedure proposed by Das (2018) accomplished our purpose of increasing antioxidant capacity of the green tea concentrate.

Another reported and interesting effect of sonication is that the process also works in the opposite way, by creating microchannels within the tissues, that enhances the solution penetration into the solid matrix and increases the mass transfer (Bhargava, 2021) (**Figure 59**). So far, this method has mainly been applied to increase drug delivery through skin (Tang, 2010) or even to transfer DNA to plant cells (Y. Liu, 2006). **Little information has been found that surveyed the penetration of antioxidant solutions into potato tissue**. In Chapter 7, an approximation of the effects that US could have in penetration and efficacy of two antioxidant solutions (Natureseal ® (NS) and green tea (GT)). The experiments were designed following a factorial configuration, aiming to relate the observed effects to one of the factors (sonication (0, 35 or 130 kHz) or antioxidants (0, NS 7.5 % or GT 5%)), or both, or their interaction. The main results of this subject will be extensively discussed in Section 3, that focuses on the antioxidant solutions. The reason behind this is that **ultrasound was not a significant factor in this study**. The initial hypothesis was rejected: sonication, either at 35 or 130 kHz did not enhance the efficacy of the antioxidant solutions applied to fresh-cut potato. The application of US did not potentiate the antioxidant capacity, the PPO inhibition or the browning delay. As antioxidant capacity was significantly affected by the type of added antioxidant but not by the sonication conditions, we can deduce that US did not enhance penetration of the solutions into the tissue. Otherwise, antioxidant capacity would have been higher for those samples when compared to non-sonicated treatment.

One possible problem that we could have encountered was the damage caused by US to cell membrane. As aforementioned, ultrasound cavitation, enhanced by injected microbubbles, perturbs cell membrane structures to cause sonoporation and increases the permeability to bioactive materials. A **good approach to study cell membrane integrity** is by measuring the conductivity of a liquid containing the sample and pre-treated (in our case, boiling water for 15 min) following the indications given by Liu (2019). The conductivity assay measures ion leakage from cells, tissues, or whole plant and animal organisms whose membrane systems have been damaged (Eich, 2000). In this sense, US did not have a harmful effect on cell membranes. When cells are damaged, oxidative stress occurs. Peeling and slicing operations, as well as sonication treatments, can lead to oxidative damages, that may result in the formation of malondialdehyde. A remarkable result here was the sharp increase in MDA in control samples during the storage period at 9 °C in comparison to those that were immersed in the antioxidants. **It is well known that MDA is an important indicator of the degree of oxidative damage**, and has already been used to assess this parameter in sliced potatoes treated with plant extracts (Liu, 2019). This outcome supports the idea that the solutions stopped or delayed the production of free radicals in the potato tissue. However, the effect of these solutions will be widely discussed in Section 3.

To conclude with this section, a final remark must be done. Despite the fact that we did not find any differences in the sugar content of sonicated potatoes, a decrease in acrylamide formation in frying was observed when longer US treatments were applied (30 min)(Antunes-Rohling, 2018). For this, future work of our research group will further explore this application in ready-to-cook potatoes.

2.3 The potential of US in food industry

In previous work of the group, ultrasound had shown some potential: 10 min of US at 40 kHz increased the antioxidant activity and total phenolic content of cooked *calçots* (Zudaire, 2017), treatments from 15 to 45 min of US at 40 kHz combined with mild temperatures from 55 to 75 °C

inactivated PPO and POD activities in peach cubes (Bobo, 2017), and ultrasound combined with the application of calcium on 'Conference' pears increased the effect of calcium used alone (Plaza, 2015). With these results in mind, further experiments which included the inactivation of *L. innocua in vitro* and in strawberries by sonication (Chapters 5 and 6), as well as the use of ultrasound to enhance the penetration of antioxidants into potato tissue (Chapter 7). The results of the conducted studies on the use of ultrasound did not detect any evidence of the benefits that this technology could pose for the selected F&Vs. It can be argued that the tested US conditions (only two frequencies) could be wider, that more target microorganisms could have been tested, that more combinations temperature times could have been selected, or even other antioxidant solutions could have been chosen. These are now, suggestions for future work, for more investigation on how can US increase safety and quality of F&V. However, the poor performance shown by US in the different studies carried out in the present thesis, suggests to reconsider US for such applications.

Despite this, and as Chemat (2017) highlighted, US is a green technology (saves energy and water use, and it is environmentally friendly, with a reduced carbon and water footprint when compared with traditional techniques). It has many applications in food industry beyond the ones studied here: emulsification, cutting, drying, extraction… The latter could be one of the next steps in obtaining compounds from natural sources. Taking advantage of this ability could help to increase the by-product valorization, which is another topic addressed in this thesis.

3 Plant extracts: their role in ensuring quality and safety of F&Vs

Several reasons are behind of opting for plant extracts in this thesis, as means to ensure safety and maintain quality of F&V. One of them is to **reach to the expectations and high standards consumers** have regarding this kind of products: as stated in the Introduction part, clean label, organic, or eco-friendly are some of the expected features. In this regard, plant extracts can accomplish the market requirements, once they are examined for their status as GRAS substances (FDA – CFR 184.1257) and approved for their 'addition' in food for different purposes (Faustino, 2019). The term addition has been placed between quotation marks because there is still discussion about the role some vegetable extracts are played in the food industry: are they ingredients or additives? The EU Comission refers to Reglament (CE) n° 1333/2008 which says that any substance that is typically not consumed as a food by itself or as an ingredient, and plays a functional role in the food, should be called an additive. Though, as some plant extracts are not listed in the positive additive list and do not have an E-number identification, consumers could perceive them as *not that harmful* or *ecological* or *without additives*. The second reason for their use is that some of them could be **obtained from by-products** of the food industry itself. In this regard, we want to highlight that the project **Agrimax**, one of the pillars of the present thesis, aims to give an added value to those parts of the plant that are discarded in the fruit industry. Typically, their final destination would be compost, animal feed, or even waste. However, their valorization constitutes a win-win transaction: the producing industry generates a greater income for the selling of the higher value by-products, while the buyer industry gets an alternative techno- or bio- functional additive/ingredient for its products.

3.1 The selection of the extracts

The use of plant extracts is an ancient knowledge that has arrived to our days and, in the last decades, its understanding has been amplified and deepen. Now, the compounds responsible for the main activities of plant extracts have been mostly elucidated and characterized. The antioxidant and antimicrobial characteristics of a high number of compounds has been widely reviewed in several publications (Eun, 2003; Kähkönen, 1999; Ríos, 2005). One 'must' of the extracts is the edibility: in this thesis, we have used only food grade compounds.

In relation to this, there are **commercially available** products that fulfill the requirements of food industry and have been approved for their use. For instance, organic acids and their salts (e.g. acetic, lactic, propionic, sorbic acids) are widely used as chemical antimicrobial agents because their efficacy is generally well understood and cost effective. Similarly, **other solutions are marketed as antioxidant** products, for example, the brand NatureSeal ® that sells blends focused on fresh F&V. As they promote, 'The NatureSeal line of products are precise blends of vitamins and minerals that maintain the natural texture and color of fresh-cut produce for up to 21 days, without altering the flavor. More than 30 different formulations are currently being used by over 500 processors, across 30 different countries' (www.natureseal.com/about-us, last access: 2020-12). In the prevention of browning of fresh-cut potato, sulfites have been traditionally used as antioxidants for minimally processed potatoes (Bobo-García, 2019), but they are related with health problems due to adverse reactions to it (Vally, 2012). Thus, the **research for natural anti-browning agents has been stimulated** (Parpinello, 2002). The selection of alternative antioxidants avoiding these drawbacks was considered in Chapter 7. We used **NatureSeal ® PS10** (**Figure 60**) as an alternative product, that is proven to delay browning up to 14 days in fresh-cut potatoes. Parallelly, we selected a **green tea concentrate** (**Figure 60**) because on previous studies carried out by Bobo (2014), it proved effective in inactivating potato PPO. Some studies were already published on the use of green tea in fresh-cut potatoes, for instance, Lopez (2019) impregnated them with 2.5, 3.75 and 5 % green tea assisting the process with vacuum pressure. However, they used this as a phenolic content booster for fried potatoes. In our studies, the aim of green tea was to increase the shelf-life of the fresh product.

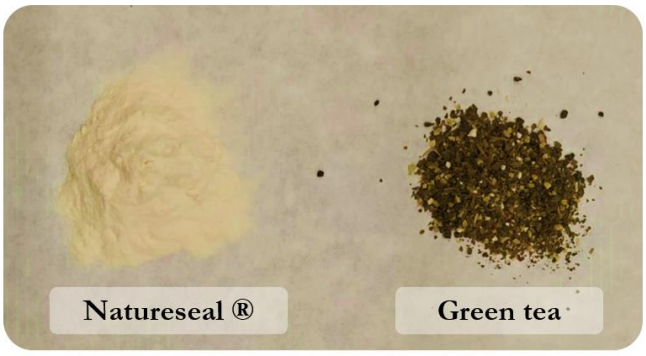


Figure 60. Antioxidant substances tested in Chapter 8: Natureseal ® and green tea.

In Chapter 8, three extracts (**Figure 61**) were characterized in order to select the most suitable to be used in F&V for safety and quality enhancement. **Ginseng** extract from *Panax ginseng* CA Meyer was prepared according the Comittee on Herbal Medicinal Products (2013) requirements, whose use is now limited to medicinal preparations and with some dose recommendations. **Fermented noni juice** is a beverage commonly prepared in the Pacific area, has been recently regulated (FAO & WHO, 2019) for its commercialization. In the study of its properties, the freeze-dried powder of this juice was used. To follow, **ferulic acid** has been widely reviewed for its health benefits and skin-care, and was first applied as a food preservative in Japan (Tsuchiya, 1975). Despite the use of ferulic acid in Europe is still not regulated, the Codex Alimentarius is considering ir to be incorporated in the additive list. However, we selected FA as part of the Agrimax project, in which the intention was to obtain a pure extract from oat by-products from the food industry. However, the process was still on its early stages, and we worked with a commercial extract from Sigma (≥99%), hoping that, in a future, the purified extract will be **validated**. For these three compounds mentioned above, a **screening on their antioxidant**

and antimicrobial properties profitable for food was carried out, that will be discussed in detail in Section 3.2.

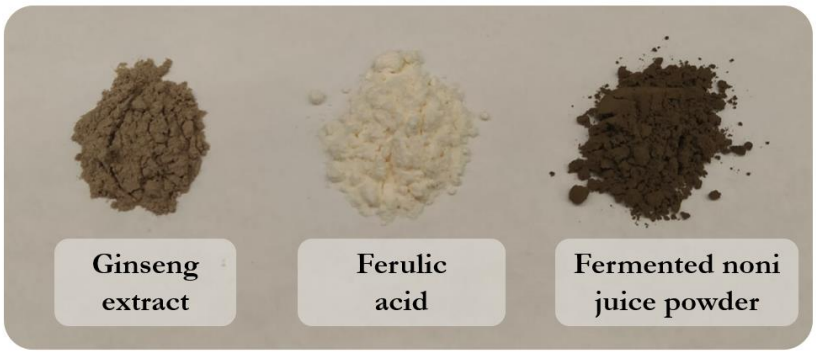


Figure 61. Evaluated compounds in Chapter 8: ginseng extract, ferulic acid and fermented noni juice powder.

3.2 Features studied and their relevance

Although a previous screening of the potential compounds to be used in this thesis was already done, based in the properties that had been already described in the literature, we selected for our experiments those mentioned in section 3.1. However, a deeper exploration or confirmation of their properties to cover their purpose was also carried out in our lab.

In the first study with extracts (Natureseal ® and green tea, Chapter 7), we studied their **ability to inhibit polyphenol oxidase**, which is the main enzyme responsible for browning in potatoes. Despite the extracts showing high antioxidant power (by using the DPPH· method) *in vitro*, the inhibition of PPO activity depends not only on the antioxidant used but also on species, cultivar or maturity of the matrices (Gul-Guven, 2017). For this, we carried out the studies using the PPO obtained from the potatoes that were going to be used in the study. In this regard, we confirmed that both of the extracts (NatureSeal ® 7 % and green tea 5 %) were capable to inhibit 95.7 and 71.5 % the PPO activity, respectively. However, and as it will be explained in Section 3.2, we could not elucidate the exact mechanism of this inhibition. As hypothesized in Soysal (2009), the polyphenols present in the green tea could be responsible from the inhibition of PPO, as they are structurally similar to the substrates it uses. On the other side, the exact composition of NatureSeal ® was not given by the provider, the only information at our disposal was that it is based on a mixture of vitamins and minerals, and literature did not reveal any relevant information.

For the next study (Chapter 8), we needed to perform a wider range of determinations, as the proposed extracts were evaluated for their use in food, but with no specific objective in mind. However, for F&Vs, the most important features to be controlled are texture, color (related with pigments or enzymes) and microorganisms of various natures (spoilage and pathogens)(Buzby, 2011). In line with this thesis, we focused in the antioxidant and antimicrobial capacities of the compounds studied (ginseng extract, GE; ferulic acid, FA; and fermented noni juice powder: FNJP). During this, we did not only learn about them, but also about the methods used.

Regarding the **antioxidant activity**, we used the index IC_{50} (concentration to reach the 50 % of the inhibition of DPPH· radical, **Figure 62**). This index is used to compare the ability of various compounds to inhibit the DPPH· oxidation. The lower the value is, the higher the antioxidant ability. There are **two main mechanisms in which antioxidant activity is played**: hydrogen atom transfer (HAT) and single electron transfer (SET). In general, they almost occur together in all samples, but in different proportions (Huang, 2005). In general, and to have an idea of the overall antioxidant capacity

of a compound, **the use of two or more methods based on different mechanisms are recommended**. However, in this case we selected this method because, despite it is always classified as a SET method, DPPH· color can be lost via either radical reaction or reduction (Prior, 2005). Moreover, other antioxidant tests carried out during this thesis (FRAP) are based in color formation and not inhibition, so IC_{50} cannot be calculated from it. The IC_{50} revealed that the antioxidant power was higher in FA, followed by FNJP and lower in GE. However, a fair comparison cannot be carried out, as FA is a 99 % purity compound, while the others are freeze-dried formats of a juice and an extract, respectively.

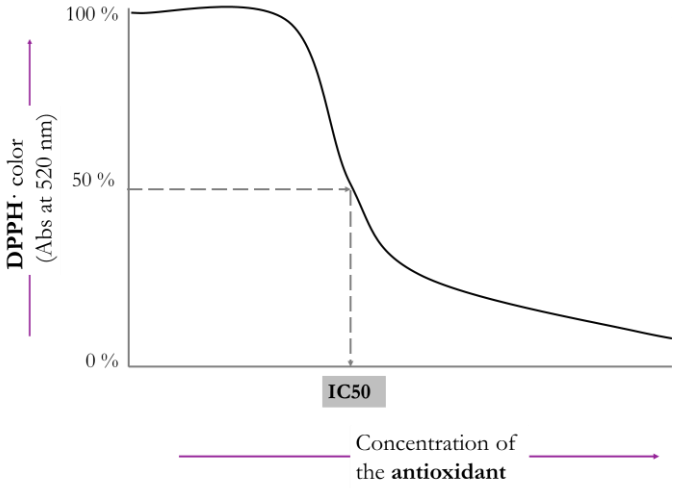


Figure 62. Calculation of IC50 in a DPPH· antioxidant assay. (1) Plotting the percentage of absorbance (520 nm) decrease in front of antioxidant concentration, (2) finding the linear regression on the linear part of the data, (3) calculating the antioxidant concentration corresponding to the 50 % of inhibition.

It is well known that **lipid oxidation** in food is a detrimental process. It deteriorates sensorial quality and nutritional value of a product. The oxidation mechanisms are basically explained by invoking free radical reactions. For this, the search of antioxidants that prevent or stop the free radical cascade is of high importance (Kolakowska, 2002). We were in front of three compounds with different antioxidant capacities, and wanted to know **whether they could may as well serve for fat peroxidation prevention**. We used the Rancimat ® method, that is an established tool for the determination of oxidation stability in fats and oils, a quicker and effective alternative for the official method of the American of Oil Chemists' Society (Markus, 1986). As it is affected by a number of factors, including the lipid profile of the studied food, we selected three matrices that could be representative of different compositions: olive oil as a stable vegetable oil, sunflower oil as a less-stable vegetable oil (Gordon, 1994), and butter, which is an animal origin fat. However, we did not see any remarkable delay in lipid peroxidation using 10 mg / mL of the compounds studied, with the exception of a 2-fold delay when using FA in olive oil. For the aim of this thesis, no further exploration of the ability to prevent fat oxidation of the extracts was carried out, as F&V typically have low fat contents. However, for other foodstuffs, such as meat or oils themselves, it would be interesting to test other matrices and concentrations.

What is relevant for F&Vs is the **activity of PPO**, especially in fresh-cut formats. When slicing and cutting operations occur, cell walls are decompartmented and get in contact with their substrates. For this, we wanted to elucidate if the three compounds studied had **at least one of the properties that inhibit PPO** activity. This can be caused by three ways: (a) inhibiting the enzyme, (b) eliminating one of the two substrates in the reaction (oxygen or polyphenols), or (c) reacting with the products of the

reaction to inhibit the formation of the colored pigments from sub steps of the nonenzymatic phase (Bobo-García, 2019) (**Figure 63**).

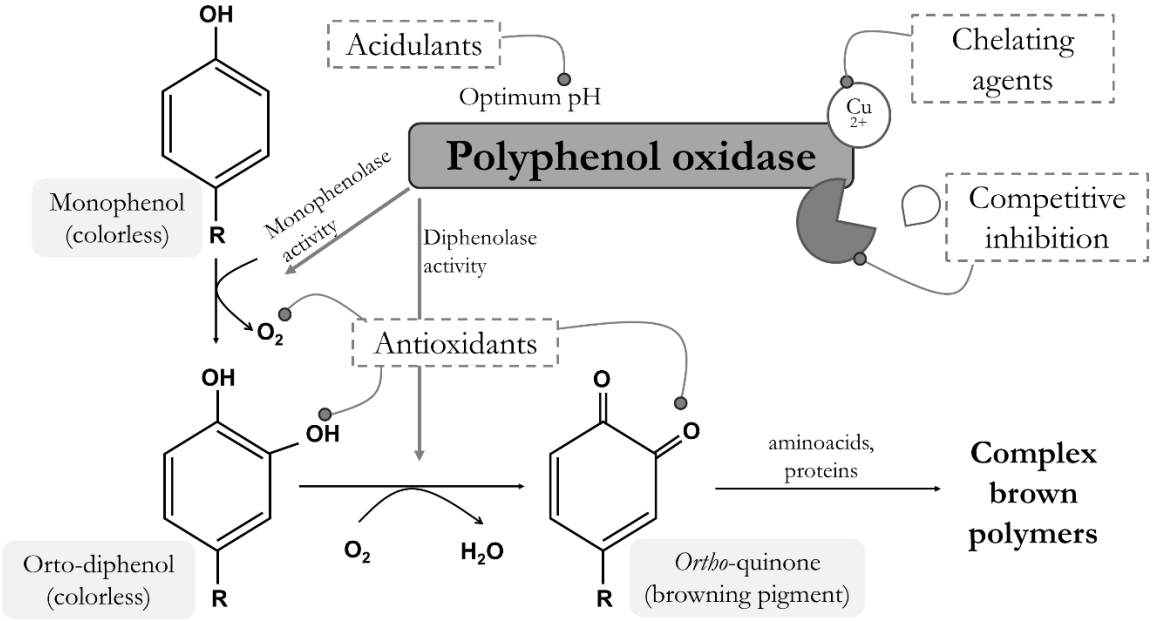


Figure 63. Simplified processes of enzymatic browning and inhibition mechanisms of anti-browning agents. In the discontinuous line boxes indicate which anti-browning agents can inhibit polyphenoloxidase and where they possibly work on the process of enzymatic browning (Adapted from (Moon, 2020).

But as commented before, **PPO is dependent on the matrix**, and it is known that is very active in apple, potato and mushroom, causing quick browning reactions in those F&Vs (Kaur, 2000). For this, we selected these three matrices as PPO sources for the following *in vitro* tests for the compounds. It was visible in the results that the effect was, as expected, dependent on the PPO source. In fact, GE was not able to inhibit potato- and mushroom- derived PPO, but achieved a 16.3 ± 1.9 % of reduction when used at 16.5 mg / mL on apple-derived PPO. If we had used FE at higher concentrations, an effect could have been observed against PPO of potato and mushroom. However, 33 mg / mL was the higher solubility of this compound in water. In contrast, FA achieved greater inhibition percentages of all three matrices and even at lower concentrations (from 2.5 to 7.5 mg / mL). FNJP was also a potential PPO inhibitor, but at high concentrations tested. All in all, and depending on the final purpose, matrix, matrix color and flavors, easiness to apply and produce, and other aspects to take into account, FA and FNJP represent a good choice for PPO inhibition and browning prevention, at least in those F&Vs. This suggests that depending on the matrix used, the antioxidant choice will vary to meet the necessary requirements to inhibit this enzyme.

The **antimicrobial activity** had already been evaluated in the literature for GE (Kachur, 2016; Pina-Pérez, 2018), FA (Ou, 2004; Pernin, 2019), or noni products (Almeida, 2019; Kang, 2019; Ulloa, 2015), but in selected microorganisms and mainly focusing on the minimum inhibitory concentration (MIC). In order to be able to select an extract depending on the purpose (in this case, the target microorganism to each food matrix) we performed a screening on a wide spectrum of food-borne pathogenic bacteria, including both Gram-positive and Gram-negative species. To do so, **one of the aspects we had to take into account was the antimicrobial test method**. One way to perform screenings is the agar diffusion method, that consists in the observance of a non-turbid circular area in an agar plate were the antimicrobial has been added. The turbidity of the media is caused by the growth of the microorganism, and the inhibition halo is the related to the absence of growth. However, this method is not appropriate

to determine the minimum inhibitory concentration (MIC), as it is impossible to quantify the amount of the antimicrobial agent diffused into the agar medium (Balouiri, 2016). In this regard, we performed this test and the microdilution test (explained below), and observed differences more sensitivity when using the latter than using the agar diffusion method. This lower sensitivity was also observed by Scorzoni (2007) in their studies: selection of the method is crucial for determining the antimicrobial activity of a compound. For this, in the present study and in the following, we preferred the microdilution method, for its higher sensitivity and for the extra information given by it.

The microdilution method is a semi-quantitative tool to elucidate which concentration (determined within a range of dillutions) gives no growth – inhibition – of a microorganism (MIC) (based typically on turbidity of the solid or liquid media). However, what happens in the non-inhibitory concentrations (NIC) is not taken into account (Lambert, 2000). We considered interesting to study the behavior when tested microorganisms grow at NIC concentrations. For this, the optical density at 600 nm (OD_{600}) was measured during the growth time. As it is a **turbidity measurement, it can be related with microbial concentration** in a suspension, but according to Stevenson (2016), they are not always synonyms. However, for the sake of our purpose, OD_{600} was a suitable parameter: detector was the same for all the microbial suspensions, all of them were bacteria with similar cell-size and increases in OD_{600} that could be attributed to cell growth in a non-specific rich media were discarded before the data analysis. The absorbance – time plot was modelized using the **Gompertz model**, which is a versatile model whose parameters explain biological behaviors, describing log-linear kinetics as well as those containing shoulder and/or tailing effects (Zwietering, 1990). With this model we were able to study the lag time (or the time the microorganisms are in lag phase, commonly known as the temporary period of non-replication seen in bacteria that are introduced to new media) the maxim growth rate (the rate of increase at the log or exponential growth phase), and the maximum OD (the population at the stationary phase, in which the number of viable cells remain the same) (Bertrand, 2019) (**Figure 64**).

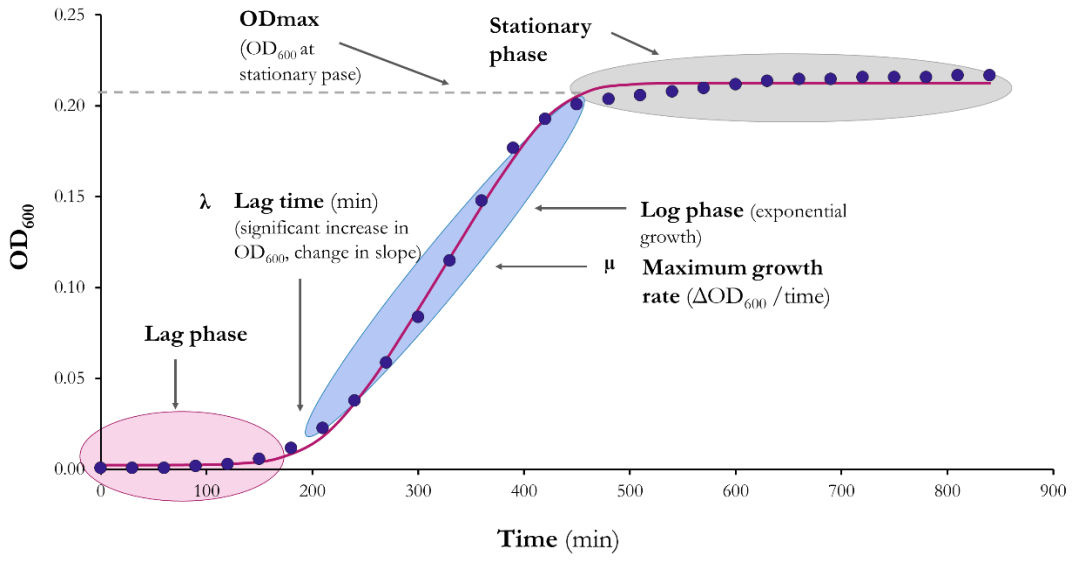


Figure 64. Modelisation of a typical bacterial growth curve using the three-parametres Gompertz model. In the figure, the increase of optical density at 600 nm (OD_{600}) during time (min). The initial part is the lag phase in which the microorganisms need to adapt to the new media. The lag time (λ) is related to the point when the lag phase finishes and the exponential growth begins. The maximum growth rate (μ) is found in this moment, and it is expressed as increase of optical density during time (ΔOD_{600} / time). Then, the viable population is stabilized in the stationary phase, which reaches the ODmax. Further increases in OD would mean increase of total cell count but not on total viable count.

Starting with a population of 2.5 × 10^5 CFU / mL, the microorganism behavior under the tested compounds and concentrations could be modelized and compared. In most of the cases, when using GE, no complete inhibition was observed even at the higher concentration tested (11 mg / mL), except for *L. monocytogenes* 1/2a. FNJP was more effective, and MIC ranged between 4.1 and 33.3 mg / mL, being *B. cereus* the most sensitive bacteria. FA proved to be a good antimicrobial agent, as relatively low concentrations (when compared to the other two) inhibited microbial growth. Only *S.* Typhimurium CECT-4549 needed more than 3.3 mg / mL of FA to be completely inhibited. Interestingly, at the higher NICs, an increase in the lag time, and a decrease in the maximum growth rate and maximum OD was observed (**Figure 65**). An implication of this is the possibility to use those extracts for different purposes, in order to delay growth of the pathogenic or spoilage microorganisms, increasing the shelf-life of the selected products.

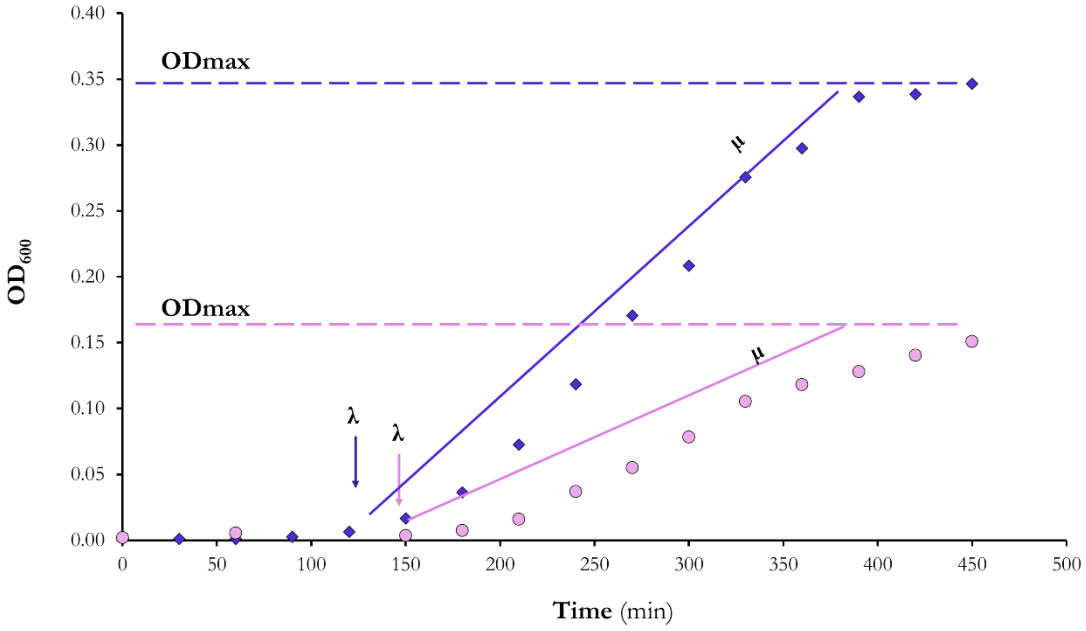


Figure 65. Example of the changes in the growth parameters in the Grompertz model on *Salmonella enterica* subsp. enterica serovar Gaminara. Comparison between control (—) and under 0.2 mg / mL FA (—).

The action modes of each antimicrobial agent have already been discussed in Chapter 8. Here, it is noteworthy to present de **different behavior between strains of the same specie**: *L. monocytogenes* (3 strains), *S. enterica* (4 strains) and *E. coli* (2 strains). Variations were observed not only in the MICs, but also in the calculated parameters describing their growth. For instance, *S.* Typhimurium was the most sensitive FNJP but was the most resistant to FA than the other *S. enterica* strains. This strain variability has been already described elsewhere (Haberbeck, 2014; Lianou, 2013), but highlights the importance to work with identified and individual strains when performing these kind of analyses. Another deep thought on the results of the microdilution experiments is that **Gram-positive and Gram-negative are not clearly distinct** in their resistance to the antimicrobials tested. Although Gram-negative bacterial pathogens are the majority of the WHO list, and are reported to be more sensitive than Gram-positive bacteria (Breijyeh, 2020), from this work it is evident that generalizations cannot be done. In fact, Wickham (2017) proposes a model in which the Gram stain groups can be generalized as being distinct in terms of intrinsic resistance, but also that the range of resistance exists as a spectrum within each group which can cause a similarity between individual organisms of different groups.

In this thesis, studies following this part have focused on the use of FA to increase shelf-life of fruits. However, although GE and FNJP have not been further explored for their application in food matrices

yet, the results observed are rather interesting. In the case of GE, its addition to F&V (in line with this thesis subject) could imply not only a decrease in the risk posed by some pathogens (as inferred by the microdilution results) but also an increase in the food value. This product is already sold in market for health purposes: increase energy levels, as an anti-inflammatory, reduce cancer risk, blood sugar diminisher… (Kim, 2017). Although there are not yet health claims regulated for this product, ts incorporation to F&V could be a good marketing if the presence of ginseng is highlighted in the packaging. Regarding FNJP, noni (*Morinda citrifolia* fruit) is gaining importance for it is related with the concept 'superfruits'. Marketing trends are focusing on a group of fruits that are quite unknown for European countries and that possess characteristic phytochemical compositions, antioxidant efficacies, and potential health benefits that can be exploited for their added value (Chang, 2018). Noni is one of them, ant the antioxidant and antimicrobial properties determined in Chapter 8 may promote its application on F&V or other foodstuffs not only to enhance their value and marketability but also to increase their shelf-life. Its application, though, must be carefully studied for its intense color and aroma, so probably application to dark F&Vs or products, or aroma encapsulation should be the next direction of investigation.

3.3 On the use of green tea and NatureSeal ® for sliced potato shelf-life

The main challenge of products in which PPO is quite active is typically browning reaction. It is the case of the potato, in which this surface darkening is the limiting factor of its shelf-life period (Laurila, 1998). From years, several studies have been presenting options for bisulfite substitution, but despite the effort done by researchers in the search for natural sulfite substitutes, none has been found to be as effective as sulfites in their antimicrobial action and their ability to prevent enzymatic browning (Petri, 2008). Lately, the use of acids and modified atmospheres have become important. Since browning is an oxidative reaction, it can be retarded by eliminating O_2 from the package of the vegetables. However, a part from fermentation reactions, this is not always feasible and browning will occur rapidly when oxygen is reintroduced (Limbo, 2006). The firsts questions that needed an answer for the study carried out in Chapter 7 was: **which ratio product – air space is better**? And: **hermetic package or gas exchange** allowance? And for how much? Two materials were tested: polypropylene (PP) and polylactic acid (PLA). PLA was a good option for being a bioplastic, but the provider could not offer a sealed package, that is needed for a product that is going to be sold in supermarkets. Moreover, the cap allowed the complete exchange of gases, and air composition inside was maintained at 21 % O_2 and 0.7 % CO_2. With PP and a sealed film on it, internal atmosphere changed rapidly when the product – package ratio of 1:10 (w:v), but it could be controlled by casting a 100 μm hole on it. This whole permitted a low gas exchange with the surroundings of the packaging, avoiding anaerobiosis in the package during the storage period.

For the trials, the potato cv. Monalisa was used. This variety is commercialized in Spain, and typically used for pre-cook or ready-to eat products, as it has a regular shape and adequate size. It has been the object of study of various published works in the search for anti-browning agents (Petri, 2008; Snoeck, 2011). Following the work of Bobo (2014), we used **a green tea** (GT) concentrate that proved to be effective against PPO from potato, and **was relatively unexplored in the literature**. GT was incorporated by Spanou (2013) to edible coatings in sliced potatoes, proving it has not only antioxidant effects, but also acted as a moisture barrier. Additionally, Lopez (2019) used GT to increase the phenolic content of chips, by assisting its penetration employing vacuum instead of US. As the effects of US have already been discussed in Section 2.2, this part will be focused on the effect of the selected antioxidants on potato slices. Contrary to the expectations, **GTE impregnated samples did not differ much from control treatment** in most of the parameters. It was anticipated that, due to its high antioxidant capacity and phenolic content and PPO inhibition ability observed *in vitro*, GTE would

increase the antioxidant capacity of fresh-cut potatoes, and delayed browning reactions. However, the very first problem was that the samples had acquired a darker color immediately after their immersion in GTE. We related it to natural coloration of the solution, produced by chlorophylls and flavonoids, specially quercetin (Wang, 2004). Going a step further from what was presented in the publication: the **browning index** of potatoes (which is a calculated parameter that relates L* and b* color coordinates with browning) was already higher for GT than it was in the other samples. However, its evolution was less marked than it was for CK samples. So, this 'problem' could be overcome if the potato slices were commercialized under the label of green-tea enriched, with legal health claims if this meets the regulation criteria. The present study was designed to determine whether sonication could enhance the antioxidant efficacy (by increasing the penetration of the substance on the potato tissue). Nevertheless, **NS stood out and showed a good performance in browning prevention** during the 9-day storage, by increasing the antioxidant potential of the samples. Moreover, the principal component analysis revealed that NS samples were related to lower O_2 concentrations and a lower PPO activity. It should be deeper investigated whether there is a casualty and NS blocks O_2, making it unavailable for PPO reactions. Finally, the fact that the sliced potatoes maintained the initial color during 9-days after the application of NS treatment demonstrates that this commercial product is a good choice for this kind of potato presentations.

In this sense, further studies should be conducted in order to determine the optimal concentration of green tea, or its pre-treatment, to confirm its suitability for fresh-cut potato. Also, organoleptic studies should be carried out, so as to determine if these substances affect the flavor of the product once the potatoes are cooked (in oven, fried, boiled…).

3.4 Ferulic acid incorporation in fruit matrices

The studies carried out in this section follow to the selection of FA as the most interesting compound from those studied: it showed an IC_{50} with the DPPH· method of 0.45 mg / mL, inhibited PPO from the three matrices up to 73.6 ± 4.29 % in the case of mushroom-derived PPO using 7.5 mg / mL FA, and had the lowest MIC against all the tested strains.

Several **points must be considered when applying extracts on the surface of food** for their further consumption. Beyond safety issues, the selection is carried out taking into account the organoleptic characteristics of the food product, in order to avoid rejection by the consumers due to their characteristic colors or flavors. Amongst others, they should not negatively affect color, odor or flavor; should be effective at low concentrations, compatible with the foods and have easy applications; and should be economical (Lourenço, 2019). We **selected fresh-cut apple and melon** for they have white surfaces, so FA would not be visible even at the higher concentrations. Moreover, these fruits present specific problematics FA could solve: browning (Ma, 2017) and *Salmonella* spp. and *L. monocytogenes* contaminations (CDC, www.cdc.gov > outbreaks. Last access: 2020-12-09) in apple, and both of the pathogens together with *E. coli* in pre-cut melons (CDC, www.cdc.gov > outbreaks. Last access: 2020-12-09).

Previous research has indicated that, concentration values needed to have a measurable or stable effect when applied *in vivo* may be three or four times higher than those MIC estimated *in vitro* (Gottardi, 2016). For this reason, in the first trials of Chapter 9, we considered adequate to increase the concentration from 2- to 5-fold the MIC found previously in Chapter 8. To do so, fruit pieces were immersed in solutions containing from 2.5 to 15.0 mg / mL, taking into account that the equivalence in mg/g of product could not be directly correlated to it.

In this sense, the HPLC analysis of the FA content revealed that fresh-cut apples that were immersed in a 2.5 mg / ml FA solution, contained 0.252 ± 0.04 mg FA / g FW, and melon pieces contained 1.223 ± 0.07 mg FA / g FW. With an approximate conversion from g of fruit to cm^2 (considering the apple slice as a semicylinder and the melon piece as a trapezoid with height), the apple presented approximately 0.0672 mg / cm^2 and the melon 0.736 mg / cm^2. This result supports the idea that each matrix is unique, and **each case has to be studied individually**. In the food science and technology field, the matrix is commonly defined as the part of the microstructure of foods, usually corresponding to a physical and spatial domain, that contains, interacts directly and/or gives a particular functionality to a constituent (e.g., a nutrient) or element of the food (e.g., starch granules, microorganisms)(Aguilera, 2018). As Tarko (2020) already highlighted in his work, key characteristics of a matrix, such as surface pH, porosity, and biochemical composition and availability, depend on the fruit studied. Moreover, as commented by Oliveira (2014), the growth of microorganisms on the fruit depended on the type of fruit (pear, apple, or melon), and also on the fruit presentation (fresh-cut or juice).

In Chapter 9, apple and melon were selected as examples of fruits with different pH, being 4.6 ± 0.3 and 5.7 ± 0.3 respectively. The inoculated fruit pieces were immersed in three solutions at increasing concentrations, depending on the MIC determined for each microorganism tested. Although it is unfortunate that this study did not include the HPLC analysis of FA in all of the concentrations it was immersed (low, medium, and high) and only presented the data of the immersion at 2.5 mg / mL, some interactions were observed between fruit matrix – strain – FA concentration. However, the first consideration that needs to be done is that FA did not show a **bactericidal** effect, as no immediate reductions on the pathogens studied was detected, so **its use should not be addressed for sanitizing purposes**. For this, we considered adequate to evaluate the evolution of the microbial populations after 7 days of storage. This storage was carried out at 10 °C so that the temperature was not a limiting factor for the bacteria. Otherwise, the growth after this period would be lower, and differences between FA concentrations would not be evident. From this part, results regarding fruit matrix were more evident when focusing on S. enterica populations. In melon, average reductions were 2 log CFU / g while in apple, reductions fluctuated depending on the strain. For this, the data cannot be extrapolated to other microbial strains or matrices. Another of the correlations found was that, for both the matrices, *L. monocytogenes* was more susceptible to FA, even when the lower concentrations were tested**: 2.5 and 5.0 caused a reduction of 5 log CFU / g in most of the cases** (in comparison to the non-treated control), while higher concentrations (**10.0 to 15.0 mg / mL) caused maximum reductions of 4 log CFU / g of *Salmonella enterica*** sv. Montevideo in apple. These results are hopeful when comparing them to studies carried out with other GRAS substances. For instance, Raybaudi-Massilia (2008, 2009) immersed apple var. Fuji, and pear var. 'Flor de Invierno' in malic acid (2.5 %), N-acetyl-L-cysteine (1.0 %), glutathione (1.0 %), and calcium lactate (1.0 %), which caused a > 5 log / CFU g reduction of artificially inoculated *L. monocytogenes*, *Salmonella* Enteritidis, and *E. coli* O157:H7 immediately after. On the contrary, other studies carried out by DiPersio (2003) described a lower decrease of *Salmonella* Enterica population of 0.7 and 1.1 log CFU/g after the immersion of apples in ascorbic acid (3.4 %) or citric acid (0.2 %) for 10 min. However, **this is the first study reporting a bacteriostatic effect of ferulic acid in fresh-cut fruit against pathogenic bacteria**. These findings may be advantageous to be further applied as a **preservative** in ready-to-eat products that contain apple and melon (e.g. salads or fruit salads), to assure that such counts are maintained below the safety levels stablished in the legislation (Reg. (EC) 2073/2005). It seems that FA is a promising compound to be incorporated in food. Moreover, in an investigation into its use on postharvest diseases of apple caused by *Botritys cinerea*, He (2019) reported that the FA treatment can augment the defense responses of the apple fruit, by enhancing the activities of resistance-related enzymes and boosting the accumulation of resistance-related substances against the gray mold.

A limitation of the lab scale studies is that they are focused on the effects rather on the feasibility of the process. It is a logical step, though, to verify that a substance has the expected outcome before scaling the process up. But sometimes, the adaptation to industrial scale can change parameters from the previous investigations, so validations must be done. In this sense, **we considered whether it was a better option to apply the solution by spraying it over the fruits rather than as immersing them in the FA solution** (Figure 66). If sprayed, the amount of product needed to cover all the fruits would be significantly smaller, possibly reducing, in turn, the operational and product costs. In a literature survey, results in both directions were found. While some studies reported that no significant differences in the effect of the two methods were observed (Kim, 1996), others state that immersion gives clearly better results (Esparza, 2006). In our case, immersion showed greater efficacy in inhibiting growth of pathogens, probably because of a greater impregnation of the product. However, for future research, longer spraying times could be assessed in order to improve their performance. Another way to incorporate extracts in fresh and fresh-cut fruits is by edible coatings (Rojas-Graü, 2009). This part will be discussed in Section 4.

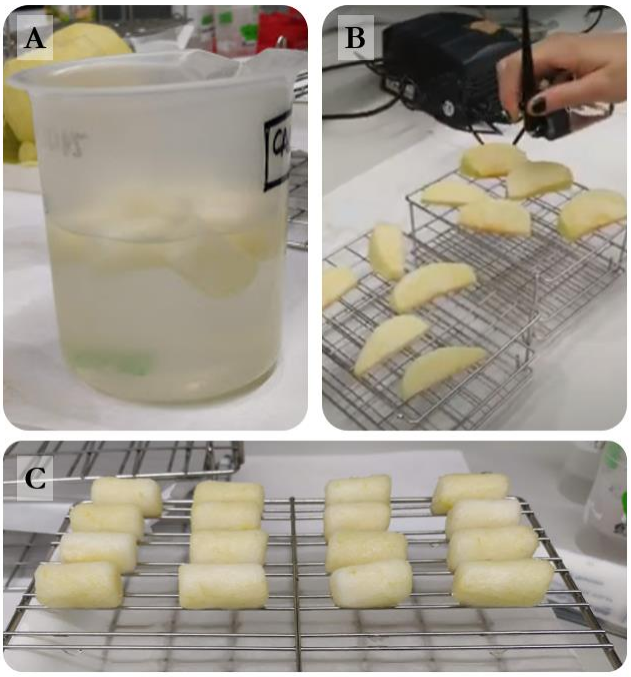


Figure 66. (A) Immersion of apple slices in FA solution, (B) spraying FA slices with FA solution, and (C) drying of melon disks after their inoculation with *Salmonella enterica* BAA-707 for FA trials.

As previously highlighted, the incorporated extracts in fresh-cut fruits aimed to ensure the safety of the products together with improve – or maintain – their quality during storage. Being browning the major problem of fresh-cut apple, we considered that a suitable control treatment should include an effective antioxidant substance. The commercial product NatureSeal ® (NS) was used for this purpose. Meanwhile, for melon pieces, water served as a control, as typically there is no need for anti-browning agents. However, this was a limitation in our study: the lack of a 'negative' control in apple samples. With that, we could have assessed the increase in shelf-life caused by FA addition. **Unexpectedly, despite the already investigated antioxidant power of FA, it did not prevent browning** as NS did, and after 5 days, apple slices with 2.5 mg / mL FA presented a browning index two times higher than those treated with NS. Similarly, in melon, no significant difference was observed in total color difference between control and FA treated samples. However, and as stated before in this discussion, matrix has a relevant effect on the behaviour of all the intrinsic or added components, so it is not

surprising that different results are observed when transferring experiments from *in vitro* to *in vivo* modes, thus the importance of scaling up gradually and considering all the steps in knowledge: from basic to applied. **On the question of how FA affected natural microbiota**, which is the responsible for a number of alterations regarding firmness and off odours, FA did not prove to be highly effective in reducing the numbers or limiting the growth of both total aerobic mesophylls or yeasts and moulds in apple and melon during storage.

In the introduction Section 1.2, it has been mentioned the **importance of the sensorial attributes** of food to reinforce the buying predisposition one they have been previously consumed the product. In this sense, the sensorial evaluation of the products that was carried out with fresh-cut apple and melon with FA was not presented for publication because it did not accomplish the adequate number of participants ($n < 30$). However, and to have an idea of what consumers would think about the product as is if eventually commercialized, the main outcomes are described next. The test consisted on the random presentation of fruit samples and the punctuation of the overall acceptability over a 9-point hedonic scale. For both fruits, the control obtained higher values (higher acceptability) than the FA treated samples. For FA samples, some of the descriptors were 'high acidity', 'poor firmness', or 'a strange off-flavor'. This highlights the idea that an integrate approach on functionality as well as on sensory properties should be carried, and in this case, some kind of improvement for FA flavor when incorporated in fruits should be done.

Having all this in mind, **future work is required for finding the best approach to apply FA in fresh-cut products**. We believe FA has great potential, especially for the control of pathogenic bacteria, (and as it has been described in Chapter 10 and discussed in Section 4.1, for its anti-browning potential) but suggest that more focus on its effects on fruit quality is carried out. One suggerence for further investigations is the combination of FA with other antioxidants to lower the application concentration. In this regard, preliminary work was done by mixing FA with NS, one as antimicrobial and the other as antioxidant. The results of this part have not been published, but showed a stimulating complementation of functions to enhance shelf-life of sliced potatoes. An interesting way to do so is by exploring the antioxidants present on the by-products of their own fruit (i.e. peel, seed, core, pulp), as properly reviewed in Ayala-Zavala (2010).

4 Edible coatings as strategies to increase shelf-life of fresh-cut products

4.1 Overall view

The current level of consumption in modern societies is forcing the arrival of politics in order to reduce the huge amount of waste (e.g. food and its packaging) that is generated. Some regulations have appeared to boost circular economy, which aim to give a use to those previously called 'waste' or 'sub-products' and now called 'by-products', reintroducing them in the production chain while adding value to those products. The legislation is included in the 'Circular Economy Pack', which includes the packaging and their residue handling (Dir. (UE) 2018/852), the residue management (Dir. (UE) 2018/851), and the reduction of one-use plastic (Dir. (UE) n° 2019/904). The reduction in plastic packaging, the promotion of giving alternative uses to by-products, together with the aim to increase shelf-life of F&Vs, in order to decrease the amount of waste, make edible coatings a worth exploring option.

One approach to incorporate functional substances on a fruit matrix is by integrating them in an edible coating. The types and advantages of the use of edible coatings have already been highlighted in the Introduction. **The delay of deterioration of the fruit** (i.e. lower respiration rate, retarded senescence,

decrease in weight loss) **given by the coatings together with the possibility to add the ferulic acid on it fostered us to explore this option**. On a quick bibliometric analysis in the database Scopus, using the key words: *edible coating, fruit* OR vegetable** we saw that this topic is exponentially increasing. Among them, when adding the key word *Aloe vera* to the search, we found a sharp increase on the use of this gel as an edible coating since 2010 (**Figure 67**).

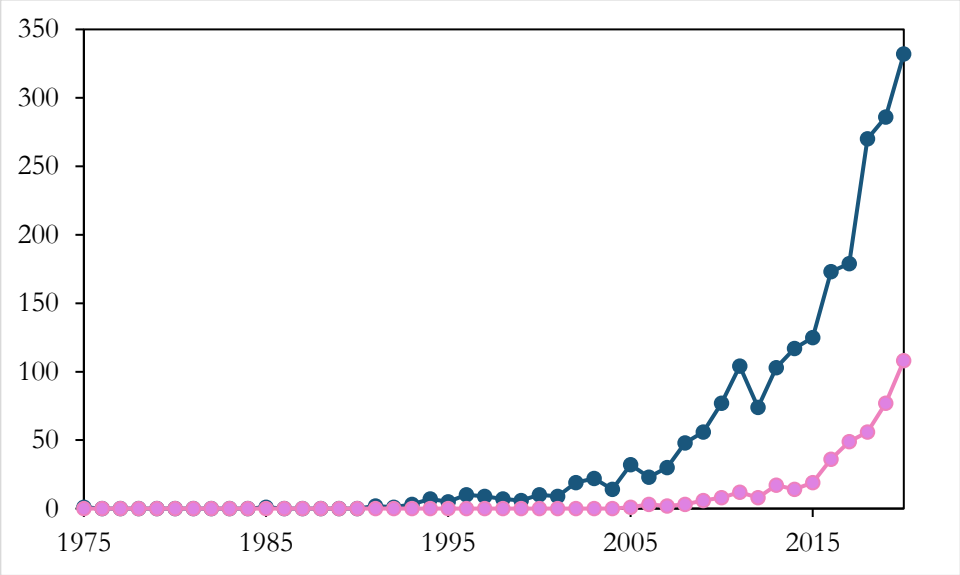


Figure 67. Time evolution on the number of publications per year in Scopus (bibliometric analysis using *edible coating, fruit* OR vegetable** as keywords).

There was one review by Misir (2014) that described the main outcomes in fruit quality when using AVG as an edible coating. After 6 years, more than a hundred reports on this topic had been published, and we considered appropriate to update the review. In Chapter 11 we presented the new advances on the use of AVG (**Figure 70**) on fresh and fresh-cut F&V. **One thing that captivated our attention was the fact that**, due to the natural components of AVG (namely aloin and aloe emodin), this gel **has shown antioxidant and antimicrobial properties *per se***. The addition of this coating on F&Vs could represent solely a great added value of the products, as revealed in the review and highlighted but other authors who focus not on the effect this gel exerts to the food but to the beneficial impact on human health (Eshun, 2004). For the sake of this thesis This could be advantageous if we wanted to use this gel in apples: the antioxidant activity could inhibit or delay browning, and the antimicrobial capacity increase de safety and decrease the deterioration of fruit due to natural microbiota.

4.2 Incorporating ferulic acid in edible coatings for fresh-cut apples

Considering the results obtained in Chapter 9, we wanted an alternative way to incorporate ferulic acid in fresh-cut apple. The main problem this product presented was browning, and the study revealed that immersion of the apple slices in a 2.5 mg / mL solution was not enough to prevent it. The first thought was that FA was not well absorbed by the fruit surface: when compared to the same immersion treatment in melon, apples showed lower FA retention. We considered that one solution was to suspend FA in a solution that acted also as an edible coating. This way, **we could increase the ratio FA:product surface (and increase then, its anti-browning effects) while having the advantages of incorporating an edible coating in a fresh-cut fruit**. In Chapter 10 we investigated two edible coatings: sodium alginate casted with calcium lactate and aloe vera gel (**Figure 68**).

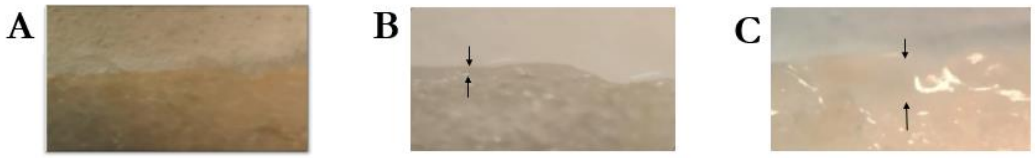


Figure 68. Stereomicroscope image of apple sections with different coating treatments: (**A**) control, (**B**) *Aloe vera* gel and (**C**) alginate coating.

This experience was carried out in Fraunhofer Institute IVV in Freising, Germany, in a four-months stay. In their research group, Parreidt (2019) **developed an improvement on the alginate-based coating** process: instead of one calcium dipping step, two dipping steps (one before and the other after the dipping in alginate) were more suitable for hydrophilic cut surfaces, especially on porous ones. They did their studies on melon and strawberry, and observed that this process was more suitable for surfaces similar to melon. This additional immersion at the beginning of the coating process could promote gel formation on the fruit surface and the uniformity of the coating. As this process is reported to improve the adhesion of the coating to the surface, it could be a good option for incorporation of FA on fresh-cut apple surface to increase the FA retention on it. In fact, a number of studies have recognized the potential of alginate-based edible coatings for quality preservation of F&V (Dong, 2013; Nair, 2020; Parreidt, 2018). Except for the study carried out by Alves (2017), in which FA was incorporated with a soy protein coating in apples, the **addition of FA in polysaccharide-based coatings on fresh-cut apples was still unexplored**.

The first challenge was the **coating incorporation to the fruit matrix**. We needed a system that allowed the immersion of the fruit piece to the coating solution, the manipulation of the product to distribute the coating uniformly and get rid of the excess of coating, and then its drying. Finally, we came up with a system that permitted the manipulation of five apple disks at once, consisting on 5 needles that perforate the apple disk and allow its subjection. As the basis was flat, it also allowed the drying in the laminar flow cabinet for the necessary time (**Figure 69**).

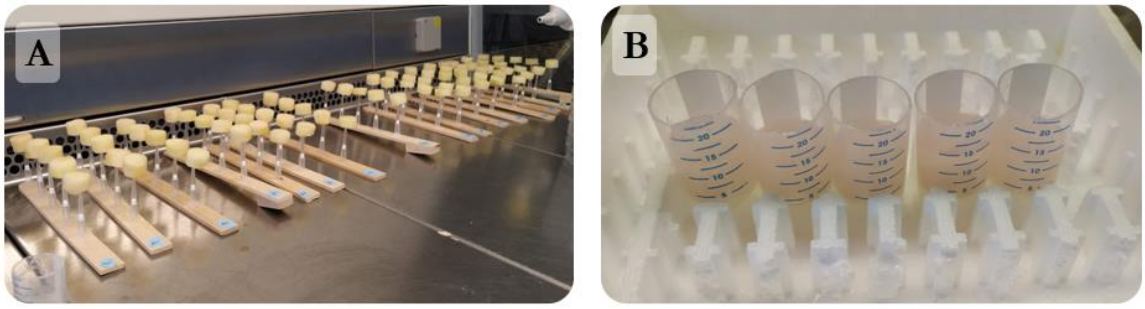


Figure 69. Coating system for apples: (A) apple pieces drying in a cabinet, standing on the needle and (B) immersion tanks for individual apple discs.

The second challenge was to **adequately formulate the AVG** coating, as the group had not worked with Aloe vera. The review in Chapter 11 includes a section on AVG preparation, which was the starting point for ours (**Figure 70**). It is important to note here a decrease of the pH of the AVG is needed to avoid browning and other deterioration reactions in the product so, in most cases, citric or ascorbic acid are added to it. This option was discarded because we wanted to know the effect of AVG alone, as we considered that these compounds could act as antioxidants masking the effect of FA. Eventually, the best option was the pasteurization of the gel at 80 °C for 5 min to prevent microbial spoilage and

further fermentations. As the product did not have antioxidants, the AVG was prepared immediately before the trials and stored a maximum of 5 days at 5 °C.

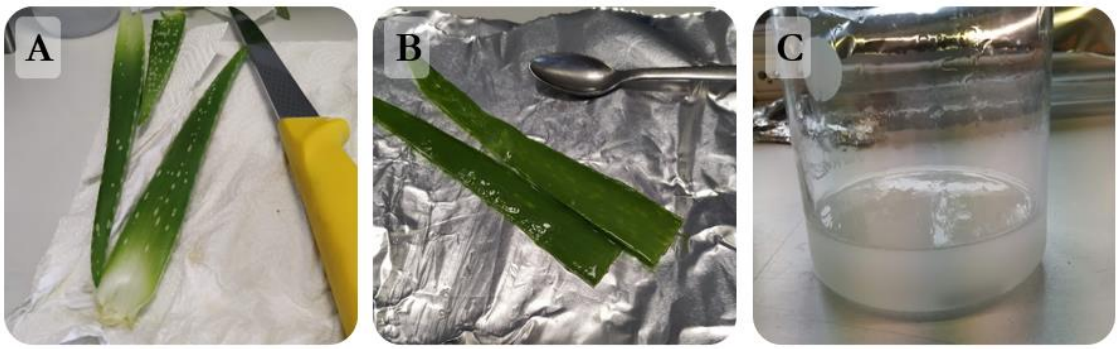


Figure 70. *Aloe vera* gel preparation. (**A**) Clean leaves from *Aloe vera*. (**B**) Longitudinal cut of Aloe vera leaves. Inside, the gel. (**C**) The gel after pasteurization at 80 °C for 5 min and homogeneisation.

The study was focused on solving or **preventing three problems** that are characteristic of fresh-cut apples: (i) weight loss (ii) browning and (iii) microbial growth (pathogenic and spoilage microbiota). The first two problems are related with the visual aspect of the product. It has already been highlighted in the Introduction and in most of the Chapters that this quality feature is one of the most important ones in consumers' acceptance of the product. For this, the reduction or delay of these issues is crucial when considering the aptitude of a proposed coating. In this regard, the AVG and the calcium-alginate (CA) based coatings with FA stood out, as they could both significantly reduce weight loss (from 11 % in non-treated control to 9 % in AVG-FA and 8 % CA-FA treatments) and delay browning (browning index was already higher than 10 in non-treated samples after 30 min, while it did not reach 6 points even after 7 days in AVG-FA treatments or the 10 points after 6 days in CA-FA treatments). Moreover, the findings also revealed that AVG-FA and CA-FA reduced 2.5 and 3.5 log CFU / g, respectively, the population of artificially inoculated *Listeria monocytogenes* on fresh-cut apples.

But, **was the addition of the coating necessary**? Except for the weight loss, which was indeed improved by the addition of a coating, especially the alginate-based, most of the other parameters were mainly affected by the addition of FA more than the coating. In this study, **two improvements** from what was done in Chapter 9 were added: an increase in FA concentration and the combination with two edible coatings. Contrary to what happened in Chapter 9 with the immersion of samples in 2.5 mg FA/ mL, the use of 10 mg / mL prevented the increase of browning index, which did not reach values of 10 even after. This already marked a difference with the lower concentration, which was not able to prevent browning even at the first days of storage. When talking about the color of the samples, FA was also the major factor affecting the browning index. The anti-browning effect of FA was not increased by its incorporation in an edible coating (similar browning index evolution was found between the coated and non-coated samples with FA). Moreover, AVG alone was able to decrease browning to the same extent, regardless the incorporation of FA or not. If only was the only shelf-life limiting of these products, **AVG could well accomplish the anti-browning effects by itself** due to its mentioned antioxidant properties. This property, in fact, was already reported in sweet cherry coated with AVG 100 % (Serrano, 2017) or in white button mushroom coated with AVG 25, 50 or 75 % (Mirshekari, 2019).

Antioxidant solutions, such as ascorbic acid and its derivatives and sulfites, have been traditionally employed since they were found to be the most effective in controlling browning (Dias, 2020). However, regardless of their efficacy, there is an increasing consumer demand for synthetic

compounds' replacement by natural and more sustainable compounds as food ingredients. The findings of our study have determined the suitability of FA as a substitute option for the synthetic antioxidants, as it can well be obtained from by-products of the agri-food industry (Kumar, 2014). **One question that remained opened** in the study was about the relationship between the apple phenols and the paper of the polyphenol oxidase in browning. Browning, a physiological disorder that results mainly from the oxidation of natural phenolic compounds by polyphenoloxidase (PPO) and peroxidase (POX) leading to the formation of brown pigments, could not be related with PPO activity of the samples. Although Shannon (1967) proposed that FA may act as a competitive inhibitor of apple PPO, preventing the binding between substrate and enzyme by occupying the latter's active sites, we could not find a clear correlation. Despite we found that concentrations of 7.5 % of FA inhibited 41 % the PPO from apple (Chapter 8), the results *in vivo* were the opposite, and PPO activity had increased after the storage period. As the activity of PPO was not the reason underlying the browning prevention, other mechanisms must have been implicated. Similar to what happened in Chapter 7 with sliced potatoes and the low correlation found between potato PPO and browning reactions, further studies are proposed for understanding the browning mechanisms in some F&V and the exact relation with browning enzymes and substrates.

The effect of the increase in FA concentration alone was also noticeable in the microorganisms studied. While the use of 2.5 mg / mL was not able to control TAM populations in the fruit samples, when it was increased to 10 mg / mL, a significant effect attributed FA (regardless the coating treatment) was observed in the inhibition of TAM and *L. monocytogenes* growth. the TAM populations after 7 days were significantly lower than they were in their non-FA counterparts. However, those effects were not replicated when studying the effect on artificially inoculated *Saccharomyces cerevisiae* alone. The differences shown by TAM and this yeast strain are attributed to the resistance heterogeneity of the TAM population in apple (which includes both bacteria – Proteobacteria (80 %), Bacteroidetes (9 %) Actinobacteria (5 %) and Firmicutes (3 %) – and yeasts – *Aureobasidium* spp., *Hanseniaspora* spp., *Rhodotorula* spp, *Saccharomyces* spp., and *Candida* spp. –)(Wassermann, 2019; Wei, 2017) compared to that of one single and artificially inoculated strain, similarly to what happened in Chapters 1 and 2 with strawberries. One unexpected outcome was that the coat casted with alginate and calcium lactate promote the growth of *S. cerevisiae* after 7 days. The population of this yeast had not increase in the control samples, while only in the CA coated apple discs, populations increased 1 log units. Contrarily, the same treatment prevented the growth of *L. monocytogenes* to the same extent that all the treatments containing FA (2.5 log units, approximately).

Another feature that was already highlighted in the Introduction part and we have monitored in most of the assays with fruit or potato pieces has been the respiration rate (RR). It is generally a good indicator of the metabolic rate of F&V, hereafter its control can be an effective means of regulating general metabolism and extending postharvest storage life of these commodities (Mathooko, 1996). The quantification of this parameter hence, gives an idea on how fast will a product ripen and eventually deteriorate. The faster it respires, the faster its metabolism goes, exhausting nutrients and increasing the degradation products. In the present thesis, **two set-ups for determining the respiration** rate were employed. In Chapters 3 and 7, a plastic cup with a lid was used, which was hermetically closed with Parafilm (**Figure 71**A). In this set-up, the gas-meter (CheckMate 3, DanSensor) takes 1 mL of the gas inside the cup with a needle, that goes through a septum that covers the orifice (to avoid further exchange of gas) (**Figure 71**B). This leads to a decrease in the available gas amount in the head space. Moreover, the headspace in this system is measured by difference of the calculated volume of the cup minus the approximated volume of the fruit inside (which is calculated by establishing that 1 g of fruit equals to 1 mL of volume). This tentative measurement may not be the most appropriate if we want to

have an exact and precise value. Meanwhile, in Chapter 10, a cell that uses Vaseline and screws for a hermetic closing was used (**Figure 71**C). The first advantage of this set-up is that the gas used to measure O_2 is returned to the cell without altering the head-space volume. The second advantage is that the head-space volume is measured with exactitude by pumping an inert gas (N_2) inside the cell containing the fruit pieces and calculating how much air has been moved, making the calculations more accurate. However, one drawback of this method is that CO_2 cannot be determined by the instrument (Oxi 340i meter, WMW) (**Figure 71**D). The lower accuracy of the first set-up is compensated with the CO_2 measurement, which also gives the confirmation that the O_2 decrease can be attributed to respiration and not to other processes such oxygen scavenging or trapping by added compounds to the fruit. Despite all of this, both are hermetic methods that are the a non-destructive and relatively simple option to measure respiration of F&V that have low respiration rates (for high respiration rates, the flow methodology is used, in which, every time the gas measurement is done, new air is introduced to compensate the low oxygen levels and high carbon dioxide levels after a short assay period. In our case, enough O_2 was left in the headspace during all the assay so there was no need for gas replacement) (Fonseca, 2002).

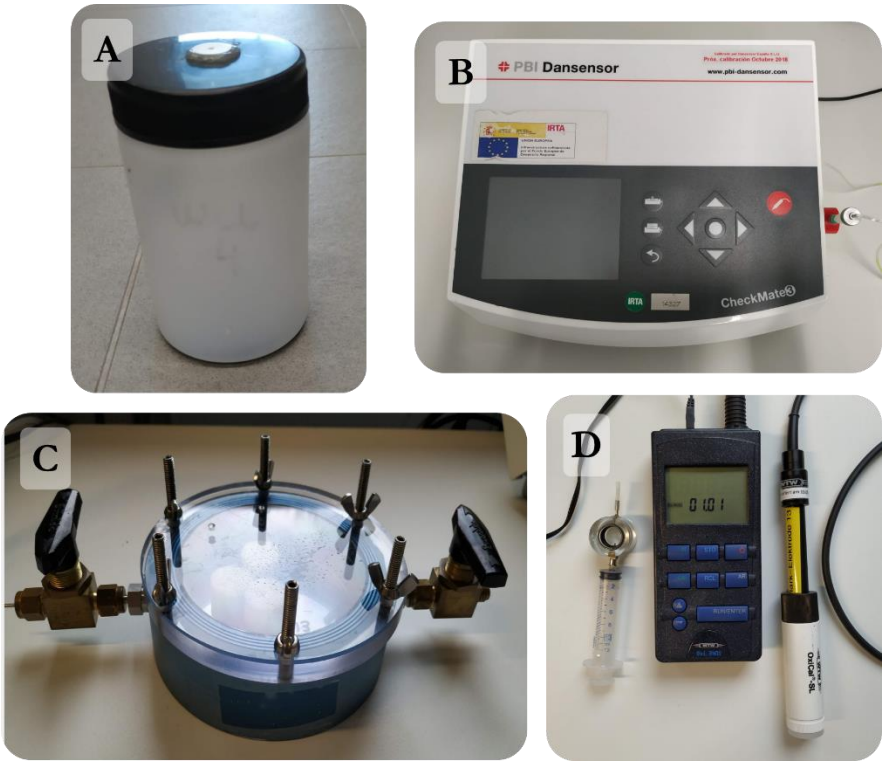


Figure 71. Respiration rate measurement equipment. (**A**) is CheckMate Dansensor, measurement of O_2 and CO_2, (**B**) the hermetic pot with a septum, (**C**) is Oxi 34i Meter for measuring O_2, and (**D**) hermetic cell with two valves for gas inlet and outlet.

In Chapter 10, the **results on respiration rate were intriguing at first**. Our initial hypothesis on the outcomes in this parameter was based on the reported effect of the edible coatings (both AVG and CA) on creating a barrier for gas exchanges thus decreasing respiration rates (Olivas, 2005). However, the factor that significatively affected RR was the incorporation of FA to the apple pieces. Eventually, a strong correlation was found between the epiphytic microbiota. It is well true that this is a factor, along with temperature or stress, can affect RR (Balla, 2005). Microorganisms consume the oxygen on their metabolic processes, which also include nutrient intake from the fruit, the release of residue substances, and the degradation of the cell walls. This leads to firmness loss, off-flavors and changes

in color (Lorenzo, 2018). The results obtained are encouraging for promoting the use of FA in fresh-cut apples, as the growth limitation of the natural microbiota

The last point on discussion in this topic relates with the **sensorial screening of the product**. Although results were not presented for publication, a visual analysis on the samples was performed during all the storage period at 5 ± 0.5 °C. The relative punctuations given in a non-scored scale of 10 points (cm) by the evaluators revealed that, as expected, acceptance was indirectly related with the browning index of the samples. The results corroborate what literature already reports: browning is a defect that causes rejection in consumers. A taste evaluation was also conducted on samples, in this case, only at the beginning of storage. The most underrated treatment was the alginate-based coating, with or without ferulic acid. The participants described a strange mouth-feel that was not related with fresh apple, and some of them could even feel the coating as a peel separating from the product. The results were rather unsatisfactory, but point out the need of improving the product presentation.

All in all, the study on the combination of two edible coatings (*Aloe vera* gel or alginate casted with calcium) with ferulic acid has led to some considerations. First, the need to incorporate FA with coatings. In fact, with the only increase of FA concentration in the immersion step, better results (especially in browning prevention) were observed when compared to Chapter 7. Moreover, FA alone also led to a decrease in TAM and *L. monocytogenes* populations after 7 days of storage when compared to the non-FA treatments, questioning the need to use an edible coating for it. The Aloe vera gel has shown a great potential in inhibiting browning during storage. Despite this, AVG or CA did not remarkably pose a great benefit on weight loss (from 11 to 8-9 %) and on water vapor transmission rate. However, results cannot be extrapolated to other situations, as in the literature there are already reported benefits on the use of those kind of films. The main outcome then of this study was the potential of FA on the quality and safety preservation of the fresh-cut apples. Moreover, FA has been used for decades for skin-care and it has shown aptitudes as an antioxidant, antiinflammatory, antimicrobial, antiallergic, hepatoprotective, anticarcinogenic, antithrombotic, increase sperm viability, antiviral and vasodilatory actions, metal chelation, modulation of enzyme activity, activation of transcriptional factors, gene expression and signal transduction (Kumar, 2014). An implication of this possibility is that the products using FA could, eventually (after a long process demonstrating its effects and the EFSA acceptance), be regulated to include health claims on their label, increasing their attractiveness to the consumers'. Meanwhile, the use of FA in food is only regulated in Japan, but it is interesting that more and more studies on this topic are carried out, as the more information available, the higher basis for sustaining a decision.

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Conclusions

CONCLUSIONS

The objective of this thesis was to find solutions to some specific problems in the fresh and fresh-cut fruit and vegetable industry. The results obtained in the studies carried out during the course of the thesis lead to the following conclusions:

STRAWBERRY SANITATION PROCESSES AND THEIR EFFECT IN FRUIT QUALITY

1 The combination of **water assisted ultraviolet-C light and peracetic acid at 40 ppm for 2 min** (WUV+PA) proved to be a suitable **alternative for chlorine sanitation** of whole fresh strawberries.

This general conclusion is supported by the following findings:

1.1 The decreases of artificially inoculated ***Listeria innocua*** **in strawberries** were ca. 4 log units after disinfection with peracetic acid at 40 or 80 ppm, and ca. 6 log units after WUV+PA sanitation.

1.2 The decreases of artificially inoculated ***Salmonella enterica*** **in strawberries** were ca. 5 log units after WUV+PA sanitation.

1.3 Remaining population of ***Listeria innocua*** **in washing water** after peracetic acid at 40 and 80 ppm significantly decreased when compared to water control. Remaining populations in washing water after both treatments were 1.5 log CFU / mL.

1.4 Sanitation with WUV+PA proved to be more effective than ultraviolet or peracetic acid treatments alone. It resulted in remaining populations of ***Listeria innocua*** **and** ***Salmonella enterica*** **in washing water** lower than 0.6 log CFU / mL, evidencing the suitability of this treatment to recirculate water in the F&V industry.

1.5 The observed effect of sanitation treatments (peracetic acid, ultraviolet light, and their combination) in **naturally occurring microorganisms** in strawberries was lower when compared to artificially inoculated microbiota. This was attributed to the higher attachment and heterogeneity that defines epiphytic microbiota.

2 The proposed sanitation method was not detrimental for **strawberry quality and nutritional parameters**, studied immediately after the washing treatments or during storage (fresh and fresh-cut strawberries for 11 days at 4 °C, and frozen strawberries for 12 months at – 20 °C).

This general conclusion is supported by the following findings:

2.1 The **physicochemical parameters** pH, total soluble solids, and titratable acidity did not overcome major changes after the treatments or during storage.

2.2 **Total aerobic mesophilic** bacteria and **yeasts and molds populations** did not grow during storage in any of the presentations.

2.3 The major **color** change occurred after washing strawberries with peracetic acid at 80 ppm, in which total color difference was higher than 3.5 points. Color was maintained during storage in any of the presentations, except for a decrease in a* values in fresh-cut strawberries at the end of the 11-day storage period.

2.4 **Firmness** was maintained immediately after all the treatments, and during storage at 4 °C of fresh and fresh-cut fruits. However, firmness decreased by 65 % in frozen-thawed strawberries, regardless the sampling time within storage at -20 °C.

2.5 No significant changes were observed in **antioxidant values, total phenolic content or total anthocyanin** content after the sanitation treatments nor during storage. Phenolic profile and organic acid contents were evaluated but no clear pattern could be elucidated.

2.6 **Vitamin C** was maintained immediately after the washing treatments (peracetic acid, ultraviolet light, and their combination) and during all shelf-life of frozen strawberries. However, a decrease of ca. 18 to 30 % was detected in fresh and fresh-cut formats at the end of the shelf-life.

3 Efficacy of **sonication** treatment, alone or in combination with mild temperatures (50 and 55 °C), was negligible in the sanitation of strawberries, and it was not detrimental for their nutritional and biochemical quality.

This general conclusion is supported by the following findings:

3.1 ***In vitro*** **survival curves of** ***Listeria innocua*** revealed a decrease in such populations only after 10 min and especially when combined with mild temperatures. Maximum reduction (3.8 log units) was observed after 15 min of sonication at 130 kHz and 55 °C.

3.2 Treatments of sonication alone, combined or not with chlorine, did not enhance the decrease of artificially inoculated **of** ***Listeria innocua*** **in strawberries**.

3.3 **Temperature was the main factor** affecting *L. innocua* population in strawberries. Main reductions (ca. 3 log units) were achieved after 5 min at 55 °C or after 10 min at 50 °C. Longer treatments led to a cooked aspect of the product.

3.4 Thermosonication at 130 kHz up to 5 min at 55 °C or 10 min after 50 °C was not detrimental for **biochemical parameters** (antioxidant capacity, total phenolic content, total anthocyanin content). However, fruit **quality parameters** were affected: a purplish color and a softer firmness was observed for the long times studied. It is therefore suggested that the fate of thermosonicated strawberries is to be destined to frozen products.

SLICED POTATO SHELF-LIFE: THE USE OF ANTIOXIDANTS

4 **Sonication** (35 – 130 kHz, max. 250 W) did not enhance the penetration of the selected antioxidants into the potato tissue.

4.1 Although the **literature review** published in the mark of this thesis had revealed a synergy between the use of sonication and other physical or chemical technologies in the sanitation and preservation of fresh and fresh-cut fruits and vegetables, these effects could not be depicted in the present studies.

5 **Natureseal** ® 7.5 % could be a good option for increasing shelf-life of sliced potato (up to 9 days at 4 °C), better than the use of **green tea extract** 5 %.

This general conclusion is supported by the following findings:

5.1 **Natureseal** ® prevented **browning** in sliced potatoes during the storage period. Potatoes with this product showed a higher **antioxidant** capacity than samples without antioxidant or with green tea extract.

5.2 Despite the *in vitro* potential of **green tea** in inhibiting **potato polyphenol oxidase**, the given coloration to the sliced potatoes decreased its suitability to be applied as an anti-browning solution.

5.3 Moreover, no direct relationship was found between the observed further **browning** and polyphenol oxidase activity *in vivo*.

5.4 **Other parameters**, such as reducing sugars, membrane integrity, malondialdehyde content or dry matter of sliced potatoes were not significantly affected by sonication or antioxidant solutions.

ANTIOXIDANT AND ANTIMICROBIAL COMPOUNDS, AND THEIR POTENTIAL TO BE APPLIED IN FRESH-CUT FRUITS (apple and melon)

6 The three evaluated compounds (**ginseng extract, ferulic acid and fermented noni juice powder**) presented **antioxidant** capacity and showed **antimicrobial** activities against 13 strains of food-borne pathogens. From them, **ferulic acid** (99 %) was the most effective at lower concentrations.

This general conclusion is supported by the following findings:

6.1 **Antioxidant activity** expressed by half inhibitory concentration (IC_{50}) of the ginseng extract, ferulic acid and fermented noni juice powder was 29.9, 0.4 and 3.8 mg / mL, respectively.

6.2 Concentrations of 7.5 mg / mL ferulic acid inhibited ca. 38, 41 and 74 % potato-, apple-, and mushroom- **polyphenol oxidase**. Concentrations of 100 mg / mL of fermented noni juice powder inhibited 86, 72 and 90 % the activity of the mentioned polyphenol oxidases, respectively.

6.3 Minimum inhibitory concentration (**MIC**) values against food-borne pathogens, including *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Bacillus cereus* or *Salmonella enterica* were lower for ferulic acid (1.7 to 3.3 mg / mL) than they were for ginseng extract (> 11.0 mg / mL) and fermented noni juice powder (4.1 to > 33.0 mg / mL).

6.4 Microbial growth **modelisation** indicated that lag phase was extended and maximum growth rate was decreased when microorganisms were submitted to non-inhibitory concentrations.

7 Immersion of **fresh-cut apple and melon in ferulic acid (≤ 2.5 mg / mL)** resulted in a decreased growth of artificially inoculated **pathogens**, but showed no significant impact in fruit **quality** during the storage period.

This general conclusion is supported by the following findings:

7.1 Reductions in artificially inoculated *Listeria monocytogenes* (three strain cocktail) in fresh-cut apple and melon after 7 days of storage at 10 °C were ca. 2.5 higher in **immersion** treatments than they were by spray application of ferulic acid. Reductions in *Salmonella enterica* (four strain cocktail) were similar regardless the application method.

7.2 Although no bactericidal effect was observed, **reductions in pathogenic populations after 7 days** of storage at 10 °C ranged from 2 to 5 log units in *Listeria monocytogenes*, and from 0.5 to 3 log units in *Salmonella enterica* when compared to the non-ferulic acid samples.

7.3 The incorporation of ferulic acid did not significantly reduce the **browning** in fresh-cut apples during storage, and did not pose any significant difference in total color difference values of fresh-cut melon after storage at 4 °C.

7.4 In general, ferulic acid incorporation was not able to control **natural microbiota** evolution in the samples during the storage period at 4 °C.

8 The incorporation of **ferulic acid (10 mg / mL) in fresh-cut apple** proved to be advantageous to delay browning reactions, increase antioxidant values and reduce artificially inoculated *Listeria monocytogenes*. The use of **edible coatings** based on sodium alginate casted with calcium lactate or on *Aloe vera* gel, alone or in combination with ferulic acid, permitted to increase shelf-life of fresh-cut apple during storage at 4 °C for 7 days.

This general conclusion is supported by the following findings:

8.1 The addition of ferulic acid was the main factor in maintaining the **color** in fresh-cut fruits. Moreover, the application of *Aloe vera* gel alone also helped in delaying the increase of browning index of fresh-cut fruits. However, no direct relationship between browning reactions and polyphenol oxidase activity was detected.

8.2 The **literature review** published on the mark of the present thesis on the use of *Aloe vera* gel as an edible coating for fresh and fresh-cut fruits and vegetables had already revealed the antioxidant and antimicrobial potential of its use.

8.3 Edible coatings helped in decreasing **weight loss** of the samples during storage. Other parameters such as pH, firmness or water activity were not affected by coating.

8.4 Ferulic acid controlled the **growth of artificially inoculated *Listeria monocytogenes***. By the end of the 7-day storage, pathogenic populations were ca. 2 log units lower than they were samples without ferulic acid. Additionally, the use of calcium alginate-based coating alone had similar effects.

8.5 Although no remarkable effect was observed against **artificially inoculated *Saccharomyces cerevisiae***, ferulic acid reduced the growth of **naturally occurring microbiota** during storage.

CONCLUSIONES

El objetivo de la presente tesis fue encontrar soluciones a problemas específicos encontrados en la industria de la fruta y hortaliza fresca y mínimamente procesada. Los resultados obtenidos en los estudios durante el transcurso de la tesis dieron lugar a las siguientes conclusiones:

PROCESOS PARA LA DESINFECCIÓN DE FRESAS Y SU EFECTO EN LA CALIDAD DE LAS MISMAS

1 La combinación de los tratamientos **por luz ultravioleta C asistida por agua con ácido peracético durante dos minutos** (WUV+PA) demostraron ser una buena **alternativa para la desinfección con hipoclorito** para fresas enteras.

Esta conclusión general está fundamentada en los siguientes resultados:

1.1 La población de ***Listeria innocua*** inoculada artificialmente **en fresas** se redujo alrededor de 4 unidades logarítmicas tras la desinfección de la fruta con ácido peracético a 40 u 80 ppm, y alrededor de 6 unidades logarítmicas tras la desinfección con WUV+PA.

1.2 La población de ***Salmonella enterica*** inoculada artificialmente **en fresas** fue reducida alrededor de 5 unidades logarítmicas tras la desinfección con WUV+PA.

1.3 La población de ***Listeria innocua*** **que permaneció en el agua de lavado** tras los tratamientos con ácido peracético a 40 u 80 ppm se redujo significativamente comparada con el agua control. Las poblaciones remanentes fueron 1.5 log UFC / mL.

1.4 La desinfección con WUV+PA demostró ser más eficaz que los tratamientos por separado con ultravioleta o ácido peracético. Las poblaciones **de *Listeria innocua* y *Salmonella enterica* que quedaron en el agua de lavado** tras el tratamiento WUV+PA fueron menores que 0.6 log UFC / mL, manifestando la adecuación de este tratamiento para recircular agua en la industria de la fruta y hortalizas.

1.5 Los tratamientos desinfectantes (ácido peracético, luz ultravioleta, y su combinación) fueron menos efectivos frente a la **microbiota natural** presente en las fresas que frente a los patógenos artificialmente inoculados. Esto fue atribuido a la mayor fijación a la superficie de la fresa, así como a la heterogeneidad que define a la microbiota natural.

2 Los métodos desinfectantes propuestos no fueron perjudiciales para **la calidad y los parámetros nutricionales de las fresas**, tanto inmediatamente después de los tratamientos como durante el almacenamiento de la fruta (fresas frescas enteras y cortadas durante 11 días a 4 °C, y fresas congeladas durante 12 meses a – 20 °C).

Esta conclusión general está fundamentada en los siguientes resultados:

2.1 No hubo cambios remarcables en los **parámetros fisicoquímicos** pH, sólidos solubles totales y la acidez titulable, tanto inmediatamente después de los tratamientos como durante el almacenamiento de la fruta.

2.2 Las poblaciones de **microorganismos aerobios totales** y de **levaduras y mohos** se mantuvieron durante el almacenamiento en todas las presentaciones.

2.3 El mayor cambio de **color** detectado ocurrió tras el lavado de fresas con ácido peracético a 80 ppm (la diferencia total de color fue mayor de 3.5 puntos). El color se mantuvo durante el almacenamiento en todas las presentaciones, con la excepción de los valores a* en fresas cortadas al final del almacenamiento de 11 días a 4 °C.

2.4 La **firmeza** de las fresas fue mantenida inmediatamente tras los tratamientos y durante el almacenamiento a 4 °C en fresas enteras y cortadas. Sin embargo, decreció hasta un 65 % en las fresas descongeladas, independientemente del tiempo de muestreo durante el almacenamiento a – 20 °C.

2.5 No se observaron cambios significativos en **los valores antioxidantes, en el contenido total de fenoles o en el contenido total de antocianinas**, tras los tratamientos desinfectantes o durante el almacenado. El perfil fenólico y los ácidos orgánicos fueron evaluados, pero no se pudo destacar ningún patrón relacionado.

2.6 La **vitamina C** se mantuvo inmediatamente tras los tratamientos desinfectantes (ácido peracético, luz ultravioleta, y su combinación) y durante la vida útil de las fresas congeladas. Sin embargo, se detectó una disminución de entre el 18 y 30 % en los formatos de fresa entera y cortada al final de su vida útil.

3 El efecto de los tratamientos de **sonicación**, combinados o no con temperaturas media-altas (50 y 55 °C), fue negligible en la desinfección de fresas, y no fue perjudicial para su calidad nutricional y bioquímica.

Esta conclusión general está fundamentada en los siguientes resultados:

3.1 Las **curvas de supervivencia de *Listeria innocua in vitro*** revelaron un descenso de la población a partir de los 10 minutos de tratamiento especialmente cuando la sonicación se combinó con temperaturas media-altas. Las máximas reducciones observadas (3.8 unidades logarítmicas) se observaron tras 15 minutos de sonicación a 130 kHz y 55 ºC.

3.2 Los tratamientos de sonicación, combinados o no con hipoclorito, no produjeron un descenso de la población ***Listeria innocua*** **inoculada artificialmente en fresas**.

3.3 **La temperatura fue el factor fundamental** en el efecto de los tratamientos frente a *Listeria innocua* en fresa. Las mayores reducciones (alrededor de 3 unidades logarítmicas) se produjeron tras 5 minutos a 55 ºC o tras 10 minutos a 50 ºC. Tratamientos más extensos otorgaban un aspecto cocido del producto.

3.4 La termosonicación a 130 kHz durante 5 minutos a 55 ºC o 10 minutos a 50 ºC no fue perjudicial para los **parámetros bioquímicos** (capacidad antioxidante, contenido total de fenoles, contenido total de antocianinas). No obstante, los **parámetros de calidad** sí que fueron afectados: se observó un color más violáceo y un descenso de la firmeza tras los tratamientos más extensos. Por lo tanto, se sugiere que las fresas termosonicadas se destinen a producto congelado.

VIDA ÚTIL DE PATATA CORTADA: EL USO DE ANTIOXIDANTES

4 La **sonicación** (35 – 130 kHz, máx. 250 W) no incrementó la penetración de los antioxidantes seleccionados en el tejido de patata.

4.1 A pesar que la **revisión literaria** publicada en el marco de esta tesis había revelado una sinergia entre el uso de la sonicación y otras tecnologías físicas o químicas para desinfectar y preservar frutas y hortalizas, estos efectos no se pudieron observar en los estudios de esta tesis.

5 El **Natureseal** ® 7.5 % podría ser una buena opción para incrementar la vida útil de la patata cortada (hasta 9 días a 4 ºC), mejor que el uso de extracto de té verde al 5 %.

Esta conclusión general está fundamentada en los siguientes resultados:

5.1 El **Natureseal** ® previno el **pardeamiento** en patatas cortadas durante su almacenamiento. Las patatas con este producto mostraron una mayor capacidad **antioxidante** que las patatas sin antioxidante o con extracto de té verde.

5.2 A pesar del potencial del **té verde** para inhibir la **polifenol oxidasa** observado *in vitro,* la coloración que otorgaba a las patatas disminuía su viabilidad para ser aplicado como solución antioxidante.

5.3 Además, no se encontró ninguna relación directa entre el **pardeamiento** y la polifenol oxidasa de las patatas *in vivo*.

5.4 **Otros parámetros**, como los azúcares reductores, la integridad de la membrana, el malonil dialdehído o la materia seca de las patatas cortadas no fueron significativamente afectados por la sonicación o por soluciones antioxidantes.

COMPUESTOS ANTIOXIDANTES Y ANTIMICROBIANOS, Y SU POTENCIAL PARA SER APLICADOS EN FRUTAS MÍNIMAMENTE PROCESADAS (manzana y melón)

6 Los tres compuestos evaluados (**extracto de ginseng, ácido ferúlico y zumo de noni fermentado y liofilizado**) presentaron capacidad **antioxidante** y mostraron actividad **antimicrobiana** frente a las 13 cepas estudiadas de patógenos de transmisión alimentaria. De ellos, el **ácido ferúlico** (99 %) destacó por ser el más efectivo a concentraciones menores.

Esta conclusión general está fundamentada en los siguientes resultados:

6.1 La **actividad antioxidante** expresada como la concentración media inhibitoria (IC_{50}) del extracto de ginseng, ácido ferúlico y zumo de noni fermentado y liofilizado fue 20.9, 0.4 y 3.8 mg / mL, respectivamente.

6.2 Concentraciones de 7.5 mg / mL de ácido ferúlico inhibieron la **polifenol oxidasa** de patata, manzana y champiñón al 38, 41 y 74 %. Concentraciones de 100 mg / mL de zumo de noni fermentado y liofilizado inhibieron el 86, 72 y 90 % de la actividad de dichas polifenol oxidasas, respectivamente.

6.3 Los valores de capacidad inhibitoria mínima (**MIC** por sus siglas en inglés) frente a varios patógenos de transmisión alimentaria, incluyendo *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Bacillus cereus* o *Salmonella enterica* fueron menores en ácido ferúlico (1.7 a 3.3 mg / mL) que en extracto de ginseng (> 11.0 mg / mL) o que en el zumo de noni fermentado y liofilizado (4.1 a >33.0 mg / mL).

6.4 La **modelización** del crecimiento microbiano indicó que la fase de latencia se alargaba y la velocidad de crecimiento decrecía cuando los microorganismos eran sometidos a concentraciones no inhibitorias.

7 La inmersión de **cortes de manzana y melón en ácido ferúlico (≤ 2.5 mg / mL)** resultó en un descenso de los **patógenos** inoculados artificialmente, pero no mostró ningún impacto en la **calidad** de la fruta durante su almacenamiento.

Esta conclusión general está fundamentada en los siguientes resultados:

7.1 Tras 7 días de almacenamiento a 10 ºC, las reducciones de *Listeria monocytogenes* (cóctel de 3 cepas) artificialmente inoculada en manzana y melón mínimamente procesados fueron 2.5 veces mayores en los tratamientos por **inmersión** que en la aplicación por *spray* del ácido ferúlico. Las reducciones de *Salmonella enterica* (cóctel de 4 cepas) fueron similares independientemente del método de aplicación empleado.

7.2 A pesar de que no se observó efecto bactericida, las **reducciones en las poblaciones de patógenos tras 7** días a 10 ºC fueron de entre 2 a 5 unidades logarítmicas para *Listeria monocytogenes* y de entre 0.5 a 3 unidades logarítmicas para *Salmonella enterica*.

7.3 La incorporación de ácido ferúlico no redujo significativamente el **pardeamiento** en los gajos de manzana durante el almacenamiento, y no supuso ninguna diferencia en el color de los gajos de melón tras el almacenamiento a 4 ºC.

7.4 En general, la incorporación de ácido ferúlico no fue capaz de controlar el crecimiento de la **microbiota natural** en las muestras durante el almacenamiento a 4 ºC.

8 La incorporación de **ácido ferúlico (10 mg / mL) en manzana cortada** demostró ser útil para retrasar las reacciones de pardeamiento, incrementar la capacidad antioxidante y reducir la población de *Listeria monocytogenes* artificialmente inoculada. El uso de **cubiertas comestibles** basadas en alginato sodio con lactato cálcico o en gel de *Aloe vera*, combinadas o no con ácido ferúlico, permitió incrementar la vida útil de la manzana mínimamente procesada durante el almacenamiento durante 7 días a 4 ºC.

Esta conclusión general está fundamentada en los siguientes resultados:

8.1 La adición de ácido ferúlico fue el factor más importante en el mantenimiento del **color** de la fruta cortada. Además, la aplicación del gel de *Aloe vera* también retrasó el incremento del índice de pardeamiento de las muestras. No obstante, no se encontró relación directa entre las reacciones de pardeamiento y la actividad de la polifenol oxidasa.

8.2 La **revisión literaria** publicada en el marco de esta tesis sobre el uso del gel de *Aloe vera* como cubierta comestible para fruta y hortalizas frescas y mínimamente procesadas ya había puesto en evidencia su potencial uso antioxidante y antimicrobiano.

8.3 Las cubiertas comestibles retrasaron la **pérdida de peso** de las muestras durante el almacenamiento. Otros parámetros, como el pH, la firmeza, o la actividad de agua, no fueron afectados por la cubierta comestible.

8.4 El ácido ferúlico controlo el **crecimiento de *Listeria monocytogenes***. Al final del período de almacenamiento de 7 días, las poblaciones de patógenos eran 2 unidades logarítmicas menores en la fruta con ácido ferúlico comparada con la fruta sin ácido ferúlico. Además, el uso de la cubierta comestible de alginato cálcico tuvo efectos similares.

8.5 A pesar de que no se observó ningún efecto destacable en la población de ***Saccharomyces cerevisiae* inoculada artificialmente** en la manzana cortada, el ácido ferúlico sí que fue capaz de reducir el crecimiento de la **microbiota natural** durante el almacenamiento de la misma.

CONCLUSIONS

L'objectiu de la present tesi va ésser aportar solucions a problemes específics de la indústria de la fruita i hortalissa fresca i mínimament processada. Els resultats obtinguts en els estudis durant el transcurs de la tesi van donar lloc a les següents conclusions:

PROCESSOS PER A LA DESINFECCIÓ DE MADUIXES I EL SEU EFECTE EN LA QUALITAT DE LES MATEIXES

1 La combinació dels tractaments **per llum ultraviolada C assistida per aigua amb àcid peracètic durant dos minuts** (WUV + PA) van demostrar ser una bona **alternativa per a la desinfecció amb hipoclorit** per maduixes senceres.

Aquesta conclusió general està fonamentada en els següents resultats:

1.1 La població de ***Listeria innocua*** inoculada artificialment **en maduixes** es va reduir al voltant de 4 unitats logarítmiques després de la desinfecció de la fruita amb àcid peracètic a 40 o 80 ppm, i al voltant de 6 unitats logarítmiques després de la desinfecció amb WUV + PA.

1.2 La població de **Salmonella enterica** inoculada artificialment **en maduixes** va ser reduïda al voltant de 5 unitats logarítmiques després de la desinfecció amb WUV + PA.

1.3 La població de ***Listeria innocua*** **que va romandre en l'aigua de rentat** després dels tractaments amb àcid peracètic a 40 o 80 ppm es va reduir significativament comparada amb l'aigua control. Les poblacions romanents van ser 1.5 log UFC / mL.

1.4 La desinfecció amb WUV + PA va demostrar ser més eficaç que els tractaments per separat amb ultraviolada o àcid peracètic. Les poblacions de ***Listeria innocua*** **i** ***Salmonella enterica*** **que van quedar en l'aigua de rentat** després del tractament WUV + PA van ser menors que 0.6 log UFC / mL, manifestant el potencial d'aquest tractament per recircular aigua a la indústria de la fruita i hortalisses.

1.5 Els tractaments desinfectants (àcid peracètic, llum ultraviolada, i la seva combinació) van ser menys efectius enfront de la **microbiota natural** present en les maduixes que enfront dels patògens artificialment inoculats. Això va ser atribuït a la major fixació a la superfície de la maduixa, així com a la heterogeneïtat que defineix a la microbiota natural.

2 Els mètodes desinfectants proposats no van ser perjudicials per a **la qualitat i els paràmetres nutricionals de les maduixes**, estudiats tant immediatament després dels tractaments com durant l'emmagatzematge de la fruita (maduixes fresques senceres i tallades durant 11 dies a 4 °C, i maduixes congelades durant 12 mesos a - 20 °C).

Aquesta conclusió general està fonamentada en els següents resultats:

2.1 No hi va haver canvis remarcables en els **paràmetres fisicoquímics** pH, sòlids solubles totals i l'acidesa titulable, tant immediatament després dels tractaments com durant l'emmagatzematge de la fruita.

2.2 Les poblacions de **microorganismes aerobis totals** i de **llevats i floridures** es van mantenir durant l'emmagatzematge en totes les presentacions.

2.3 El major canvi de color va ser detectat després del rentat de maduixes amb àcid peracètic a 80 ppm (la diferència total de color va ser major de 3.5 punts). El color es va mantenir durant l'emmagatzematge en totes les presentacions, amb l'excepció dels valors a * en maduixes tallades a la fi de l'emmagatzematge de 11 dies a 4 °C.

2.4 La **fermesa** de les maduixes es va mantenir immediatament després dels tractaments i durant l'emmagatzematge a 4 ° C en maduixes senceres i tallades. No obstant això, va decréixer fins a un 65% en les maduixes descongelades, sense importar el temps de mostreig durant l'emmagatzematge a - 20 °C.

2.5 No es van observar canvis significatius en els **valors antioxidants, en el contingut total de fenols o en el contingut total de antocianines**, després dels tractaments desinfectants o durant l'emmagatzematge. El perfil fenòlic i els àcids orgànics van ser avaluats, però no es va poder destacar cap patró relacionat.

2.6 La **vitamina C** es va mantenir immediatament després dels tractaments desinfectants (àcid peracètic, llum ultraviolada, i la seva combinació) i durant la vida útil de les maduixes congelades. No obstant això, es va detectar una disminució d'entre el 18 i 30% en els formats de maduixa sencera i tallada a la fi de la seva vida útil.

3 L'efecte dels tractaments de **sonicació**, combinats o no amb temperatures mitjana-altes (50 i 55 °C), va ser negligible en la desinfecció de maduixes, i no va ser perjudicial per a la seva qualitat nutricional i bioquímica.

Aquesta conclusió general està fonamentada en els següents resultats:

3.1 Les **corbes de supervivència de *Listeria innocua in vitro*** van revelar un descens de la població a partir dels 10 minuts de tractament especialment quan la sonicació es va combinar amb temperatures mitjana-altes. Les màximes reduccions observades (3.8 unitats logarítmiques) es van observar després de 15 minuts de sonicació a 130 kHz i 55 ºC.

3.2 Els tractaments de sonicació, combinats o no amb hipoclorit, no van produir un descens de la població ***Listeria innocua*** **inoculada artificialment en maduixes**.

3.3 **La temperatura va ser el factor fonamental** en l'efecte dels tractaments enfront de *Listeria innocua* a maduixa. Les majors reduccions (al voltant de 3 unitats logarítmiques) es van produir després de 5 minuts a 55 °C o després de 10 minuts a 50 ºC. Tractaments més extensos atorgaven un aspecte cuit al producte.

3.4 La termosonicació a 130 kHz durant 5 minuts a 55 °C o 10 minuts a 50 ºC no va ser perjudicial per als **paràmetres bioquímics** de les maduixes (capacitat antioxidant, contingut total de fenols, contingut total de antocianines). No obstant això, els **paràmetres de qualitat** sí que van ser afectats: es va observar un color més violaci i un descens de la fermesa després dels tractaments més extensos. Per tant, es suggereix que les maduixes termosonicades es destinin a producte congelat.

VIDA ÚTIL DE PATATA TALLADA: L'ÚS DE ANTIOXIDANTS

4 La **sonicació** (35 - 130 kHz, màx. 250 W) no va incrementar la penetració dels antioxidants seleccionats en el teixit de patata.

4.1 Malgrat que la **revisió literària** publicada en el marc d'aquesta tesi havia revelat una sinergia entre l'ús de la sonicació i altres tecnologies físiques o químiques per desinfectar i preservar fruites i hortalisses, aquests efectes no es van poder observar en els estudis d'aquesta tesi.

5 El **Natureseal** ® 7.5 % podria ser una bona opció per incrementar la vida útil de la patata tallada (fins a 9 dies a 4 ºC), millor que l'ús d'extracte de te verd a el 5 %.

Aquesta conclusió general està fonamentada en els següents resultats:

5.1 El **Natureseal** ® va prevenir **l'enfosquiment** en patates tallades durant el seu emmagatzematge. Les patates amb aquest producte van mostrar una major capacitat **antioxidant** que les patates sense antioxidant o amb extracte de te verd.

5.2 Tot i el potencial de el **te verd** per inhibir la **polifenol oxidasa** observat in vitro, la coloració que atorgava a les patates disminuïa la seva viabilitat per a ser aplicat com a solució antioxidant.

5.3 A més, no es va trobar cap relació directa entre **l'enfosquiment** i la polifenol oxidasa de les patates *in vivo*.

5.4 **Altres paràmetres**, com els sucres reductors, la integritat de la membrana, el malonil dialdehid o la matèria seca de les patates tallades no van ser significativament afectats per la sonicació o per solucions antioxidants.

COMPOSTOS ANTIOXIDANTS I ANTIMICROBIANS, I EL SEU POTENCIAL PER A SER APLICATS A FRUITES MÍNIMAMENT PROCESSADES (poma i meló)

6 Els tres compostos avaluats (**extracte de ginseng, àcid ferúlic i suc de noni fermentat i liofilitzat**) van presentar capacitat antioxidant i van mostrar activitat antimicrobiana enfront de les 13 soques estudiades de patògens de transmissió alimentària. D'ells, l'àcid ferúlic (99%) va destacar per ser el més efectiu a concentracions menors.

Aquesta conclusió general està fonamentada en els següents resultats:

6.1 **L'activitat antioxidant** expressada com la concentració mitjana inhibitòria (IC_{50}) de l'extracte de ginseng, àcid ferúlic i suc de noni fermentat i liofilitzat va ser 20.9, 0.4 i 3.8 mg / mL, respectivament.

6.2 Concentracions de 7.5 mg / ml d'àcid ferúlic van inhibir la **polifenol oxidasa** de patata, poma i xampinyó a l'38, 41 i 74 %. Concentracions de 100 mg / mL de suc de noni fermentat i liofilitzat van inhibir el 86, 72 i 90% de l'activitat d'aquestes polifenol oxidases, respectivament.

6.3 Els valors de capacitat inhibitòria mínima (**MIC** per les seves iniciales en anglès) enfront de diversos patògens de transmissió alimentària, incloent *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Bacillus cereus o Salmonella enterica* van ser menors en àcid ferúlic (1.7 a 3.3 mg / mL) que en extracte de ginseng (> 11.0 mg / mL) o que en el suc de noni fermentat i liofilitzat (4.1 a > 33.0 mg / mL).

6.4 La **modelització** de el creixement microbià va indicar que la fase de latència s'allargava i la velocitat de creixement decreixia quan els microorganismes eren sotmesos a concentracions no inhibitòries.

7 La immersió de **talls de poma i meló en àcid ferúlic (≤ 2.5 mg / ml)** va resultar en un descens dels **patògens** inoculats artificialment, però no va mostrar cap impacte en la **qualitat** de la fruita durant el seu emmagatzematge.

Aquesta conclusió general està fonamentada en els següents resultats:

7.1 Després de 7 dies d'emmagatzematge a 10 ºC, les reduccions de *Listeria monocytogenes* (còctel de 3 soques) artificialment inoculada en poma i meló mínimament processats van ser 2.5 vegades més grans en els tractaments per **immersió** que en l'aplicació per esprai de l'àcid ferúlic. Les reduccions de *Salmonella enterica* (còctel de 4 soques) van ser similars independentment de el mètode d'aplicació empleat.

7.2 Tot i que no es va observar efecte bactericida, les **reduccions en les poblacions de patògens després de 7** dies a 10 ºC van ser d'entre 2 a 5 unitats logarítmiques per *Listeria monocytogenes* i de entre 0.5 a 3 unitats logarítmiques per *Salmonella enterica*.

7.3 La incorporació d'àcid ferúlic no va reduir significativament **l'enfosquiment** en els grills de poma durant l'emmagatzematge, i no va suposar cap diferència en el color dels grills de meló després de l'emmagatzematge a 4 ºC.

7.4 En general, la incorporació d'àcid ferúlic no va ser capaç de controlar el creixement de la **microbiota natural** a les mostres durant l'emmagatzematge a 4 ºC.

8 La incorporació **d'àcid ferúlic (10 mg / ml) en poma tallada** demostrar ser útil per retardar les reaccions d'enfosquiment, incrementar la capacitat antioxidant i reduir la població de *Listeria monocytogenes* artificialment inoculada. L'ús de **cobertes comestibles** basades en alginat sodi amb lactat càlcic o en gel d'*Aloe vera*, combinades o no amb àcid ferúlic, va permetre incrementar la vida útil de la poma mínimament processada durant l'emmagatzematge durant 7 dies a 4 ºC.

Aquesta conclusió general està fonamentada en els següents resultats:

8.1 L'addició d'àcid ferúlic va ser el factor més important en el manteniment del **color** de la fruita tallada. A més, l'aplicació de el gel d'*Aloe vera* també va retardar l'increment de l'índex de enfosquiment de les mostres. No obstant això, no es va trobar relació directa entre les reaccions d'enfosquiment i l'activitat de la polifenol oxidasa.

8.2 La **revisió literària** publicada en el marc d'aquesta tesi sobre l'ús del gel d'*Aloe vera* com coberta comestible per fruita i hortalisses fresques i mínimament processades ja havia posat en evidència el seu potencial ús antioxidant i antimicrobià.

8.3 Les cobertes comestibles van retardar la **pèrdua de pes** de les mostres durant l'emmagatzematge. Altres paràmetres, com el pH, la fermesa, o l'activitat d'aigua, no van ser afectats per la coberta comestible.

8.4 L'àcid ferúlic va controlar el **creixement de *Listeria monocytogenes***. A la fi del període d'emmagatzematge de 7 dies, les poblacions de patògens eren 2 unitats logarítmiques menors en la fruita amb àcid ferúlic comparada amb la fruita sense àcid ferúlic. A més, l'ús de la coberta comestible d'alginat càlcic va tenir efectes similars.

8.5 Tot i que no es va observar cap efecte destacable en la població **de *Saccharomyces cerevisiae* inoculada artificialment** en la poma tallada, l'àcid ferúlic sí que va ser capaç de reduir el creixement de la **microbiota natural** durant l'emmagatzematge de la mateixa.

Improvement of the quality and safety of vegetable products through innovative technologies.

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