Departament de Ciència Animal i dels Aliments, Facultat de Veterinària

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Improving poultry meat preservation by means of carbon dioxide atmospheres and high hydrostatic pressure

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Aida Al-Nehlawi Valverde

Dirigida per: Jordi Saldo Periago i Sònia Guri Baiget

Dr. JORDI SALDO PERIAGO,

Professor titular de Tecnologia dels Aliments del Departament de Ciència Animal i dels Aliments de la Universitat Autònoma de Barcelona;

Dra. SÒNIA GURI I BAIGET,

Technical Lead a MATGAS 2000, A.I.E. Research Center, Centre d'Excel·lència d'Agroalimentació i Aigua del Departament de R+D de l'empresa S.E. Carburos Metálicos S.A. – Grup Air Products;

FAN CONSTAR que AIDA AL-NEHLAWI VALVERDE ha realitzat, sota la seva direcció, el treball titulat "Improving poultry meat preservation by means of carbon dioxide atmospheres and high hydrostatic pressure" que es presenta per a optar al grau de Doctor.

I per a que així consti, signem el present document a Bellaterra, Cerdanyola del Vallès, el dia 18 de Juliol de 2014.

Dr. Jordi Saldo Periago | Dra. Sònia Guri Baiget

Avi, aquesta tesi és per a tu, que la vida no et va concedir prou temps per acabar la teva.

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ABSTRACT

Chicken meat is one of the most consumed meats in all over the world. In order to extend fresh poultry meat and meat products distribution, the food industry has developed preservation methods in order to prolong their shelf-life while the quality properties of the original product remain undamaged. Modified atmosphere packaging (MAP) together with refrigeration temperatures has become the most used packaging method to preserve poultry meat. It consists of packaging a food product and modifies the atmosphere that surrounds it for other gas such as nitrogen (N_2), oxygen (O_2), carbon dioxide (CO_2) or a mixture among them. Its success remains basically on the concentration of CO_2 used in the atmosphere. CO_2 has the property to dissolve in the water and fat of the product. When that happens, some intracellular alterations take place, affecting the metabolism of some of the most common spoilage microorganisms, reducing their growth, and also the action of some enzymes responsible of food degradation reactions. However, the use of high concentrations of CO_2 has been limited due to package collapse that it produces.

This Thesis was focused to improve the traditional MAP technique. The first stage of the work consisted in testing the use of high CO_2 concentrations to preserve fresh chicken meat, previously saturating the meat with pure CO_2. With this pre-treatment we expected to reduce or even avoid package collapse, presenting a chance to reduce package volumes and therefore improve package and transport efficiency.

The second stage of the project was to combine MAP with high hydrostatic pressure (HHP). HHP is one of the most promising technologies to replace heat treatments like pasteurization or even sterilization. However, high pressure levels required to achieve microbial inactivation has limited its use and implementation in food industries. The main objective of combining both technologies MAP and HHP was to reduce the pressure of the treatment without compromising microbial inactivation. Several experiments were carried, assessing the synergistic effect between both technologies and determining the optimal concentration of CO_2 and pressure level to achieve a greater inactivation of the most common spoilage and pathogenic microorganisms of poultry meat.

When fresh chicken meat was packaged with pure CO_2 before its final package with modified atmosphere, the collapse of the packages was significantly decreased in comparison with the samples directly packaged with modified atmosphere. Also, its shelf-life was increased, as a greater amount of CO_2 offered a longer bacteriostatic effect over microbial population. When saturation pre-treatments were carried out, package-product-filling volumes were successfully increased without compromising the safety of the product and the packaging collapse.

Related to the combination of CO_2 atmospheres with HHP, the synergistic effect of this combination on the reduction and inactivation of some of the most common spoilage and pathogenic bacteria of poultry products was demonstrated. After developing predictive models of the microbial reduction produced by the combination of CO_2 atmosphere

concentrations and high pressure treatments, an optimal treatment of 50% CO_2 balanced with N_2 and a pressure of 250 MPa during 10 min at room temperature was suggested for its attractive and non-extreme conditions. This was applied to fresh chicken breasts and was compared to air-pressurized and non-pressurized samples. A shelf-life of 26 days was achieved on MAP and HHP treated samples, while air-pressurized samples only achieved 15 days under acceptable microbial conditions. Although some physicochemical differences were detected in pressurized meat with respect to surface color and water holding capacity, once the meat was cooked no sensory differences were found among treatments.

The forced dissolution of the gas produced by high pressure was also studied by quantifying the gaseous embolism produced by pressurizing poultry sausages. Results suggested that when higher pressures were applied, the gas surrounding the food penetrates inside the cell at a deeper levels and its extraction with pressure release was harder, keeping it dissolved. By slowing down the pressure release speed, a greater amount of the gas remained dissolved into the product, offering a better preservation of the food when this gas was CO_2.

The experimental results obtained have demonstrated that poultry meat preservation can be improved by the use of high-concentration CO_2 atmospheres applied alone as a previous saturation treatment or in combination with high hydrostatic pressure. The work exposed on this Thesis demonstrates new ways to preserve poultry meat products, giving new prospects on meat science research and enhancing the opportunity to produce safer and long-lasting fresh meat products.

RESUM

La carn de pollastre és una de les carns més consumides a tot el món. Per tal d'ampliar els canals de distribució de la carn fresca i els seus derivats, la indústria alimentària ha desenvolupat mètodes de conservació per tal d'allargar-ne la seva vida útil i que permetin mantenir les propietats i la qualitat del producte original. L'envasament en atmosfera modificada (EAM), juntament amb les temperatures de refrigeració, s'ha convertit en el mètode d'envasament més utilitzat per conservar la carn d'aus de corral. Consisteix en envasar l'aliment i modificar-ne l'atmosfera que l'envolta per un altre gas tal com el nitrogen (N_2), l'oxigen (O_2), el diòxid de carboni (CO_2) o una mescla entre ells. El seu èxit recau essencialment sobre la concentració de CO_2 present en l'atmosfera. El CO_2 té la propietat de dissoldre's en l'aigua i en el greix dels aliments. Quan això succeeix, tenen lloc una sèrie d'alteracions a nivell intracel·lular que afecten el metabolisme d'alguns dels microorganismes alteradors més comuns, disminuint-ne el seu desenvolupament, i també l'acció d'alguns enzims responsables de les reaccions de degradació més habituals en aliments. No obstant això, l'ús d'altes concentracions de CO_2 s'ha vist limitat degut al col·lapse dels envasos que produeix.

Aquesta tesi s'ha enfocat per a millorar la tècnica d'EAM tradicional. La primera fase del treball ha consistit a provar l'ús d'altes concentracions de CO_2 per a conservar la carn de pollastre fresca, saturant prèviament la carn amb CO_2 pur. Amb aquest pretractament es pretenia reduir o fins i tot evitar el col·lapse de l'envàs, esdevenint una oportunitat per a reduir el volums dels envasos i millorar, per tant, l'eficiència en el procés d'envasament i de transport dels productes.

La segona etapa del projecte ha consistit en combinar l'EAM amb l'alta pressió hidrostàtica (APH). L'APH és una de les tecnologies més prometedores per a reemplaçar els tractaments tèrmics com la pasteurització o fins i tot l'esterilització. No obstant això, els elevats nivells de pressió necessaris per aconseguir la total inactivació de microorganismes ha limitat el seu ús i aplicació en la indústria alimentària. L'objectiu principal de la combinació de l'EAM i la APH ha estat reduir la pressió del tractament sense comprometre la inactivació microbiana. Diversos experiments s'han dut a terme per tal d'avaluar l'efecte sinèrgic entre les dues tecnologies i per a determinar la concentració òptima de CO_2 i la pressió necessària per aconseguir una major inactivació de microorganismes patògens i alteradors de la carn d'au.

Els resultats obtinguts van demostrar que quan la carn fresca de pollastre va ser envasada amb CO_2 pur abans del seu envasat definitiu amb atmosfera modificada, el col·lapse dels envasos es va reduir significativament en comparació amb les mostres directament envasades amb atmosfera modificada. La seva vida útil també va augmentar degut a que una major quantitat de CO_2 disponible a l'espai de cap ofereix millor protecció de l'aliment i un efecte bacteriostàtic més durador. Quan es van dur a terme els pre-

tractaments de saturació, els envasos es van poder omplir amb més producte sense comprometre la seguretat de l'aliment ni el col·lapse de l'envàs.

Pel que fa a la combinació d'atmosferes de CO_2 amb APH, es va poder demostrar l'existència de l'efecte sinèrgic d'aquesta combinació en la reducció i la inactivació d'alguns dels microorganismes alteradors i patògens més habituals en els productes càrnics avícoles. Un cop es van desenvolupar els models de predicció de la reducció microbiana produïda per la combinació de CO_2 i d'alta pressió, l'atmosfera de 50% CO_2 equilibrada amb N_2 i la pressió de 250 MPa durant 10 min a temperatura ambient va ser considerat el més òptim, tenint en compte condicions menys extremes i reducció microbiana produïda. Aquest tractament es va aplicar a pit de pollastre frescs i es va comparar amb mostres envasades en aire i pressuritzades i mostres no pressuritzat. La vida útil del pit de pollastre envasat amb atmosfera modificada i tractat amb pressió va ser de 26 dies, mentre que les mostres tractades amb aire i pressió només van arribar a 15 dies sota condicions microbianes acceptables. Encara que es van detectar algunes alteracions fisicoquímiques de la carn pressuritzada pel que fa al seu color superficial i a la capacitat de retenció d'aigua, un cop aquesta va ser cuita no es van trobar diferències sensorials entre els tractaments comparats.

La dissolució forçada del gas provocada per l'alta pressió també es va estudiar mitjançant la quantificació de l'embòlia gasosa produïda en salsitxes de carn d'au. Els resultats suggereixen que quan s'apliquen pressions més altes, el gas que envolta l'aliment penetra a l'interior de la cèl·lula en un nivell més profund i per això la seva extracció durant el procés de descompressió és més complicat, mantenint-se dissolt en l'aliment. Addicionalment, al reduir la velocitat de descompressió, una major quantitat del gas es va mantenir dissolt en el producte, oferint una millor preservació del l'aliment quan es tractava de CO_2.

Els resultats experimentals obtinguts han demostrat que la conservació de la carn d'au es pot millorar mitjançant l'ús d'atmosferes riques amb CO_2 aplicades individualment com un tractament de saturació previ al envasat convencional amb atmosfera modificada, o en combinació amb l'APH. Els treballs exposats en aquesta Tesi demostren noves maneres de conservar els productes càrnics avícoles donant noves perspectives en aquest camp d'investigació que donen l'oportunitat de produir productes càrnics frescos més segurs i duradors.

RESUMEN

La carne de pollo es una de las carnes más consumidas en todo el mundo. Para ampliar los canales de distribución de la carne fresca y sus derivados, la industria alimentaria ha desarrollado métodos de conservación para alargar su vida útil y que permitan mantener las propiedades y la calidad del producto original. El envasado en atmósfera modificada (EAM), junto con las temperaturas de refrigeración, se ha convertido en el método de envasado más utilizado para conservar la carne de aves de corral. Consiste en envasar el alimento y modificar la atmósfera que lo rodea por otro gas tal como el nitrógeno (N_2), el oxígeno (O_2), el dióxido de carbono (CO_2) o una mezcla entre ellos. Su éxito recae esencialmente sobre la concentración de CO_2 presente en la atmósfera. El CO_2 tiene la propiedad de disolverse en el agua y en la grasa de los alimentos. Cuando esto sucede, tienen lugar una serie de alteraciones a nivel intracelular que afectan el metabolismo de algunos de los microorganismos alteradores más comunes, disminuyendo su desarrollo, y también la acción de algunas enzimas responsables de las reacciones de degradación más habituales en alimentos. Sin embargo, el uso de altas concentraciones de CO_2 se ha visto limitado debido al colapso de los envases que produce.

Esta tesis se ha enfocado para mejorar la técnica de EAM tradicional. La primera fase del trabajo ha consistido en probar el uso de altas concentraciones de CO_2 para conservar la carne de pollo fresca, saturando previamente la carne con CO_2 puro. Con este pre-tratamiento se pretendía reducir o incluso evitar el colapso del envase, convirtiéndose en una oportunidad para reducir el volúmenes de los envases y mejorar, por lo tanto, la eficiencia en el proceso de envasado y de transporte de los productos.

La segunda etapa del proyecto ha consistido en combinar la EAM con la alta presión hidrostática (APH). La APH es una de las tecnologías más prometedoras para reemplazar los tratamientos térmicos como la pasteurización o incluso la esterilización. Sin embargo, los elevados niveles de presión necesarios para conseguir la total inactivación de microorganismos han limitado su uso y aplicación en la industria alimentaria. El objetivo principal de la combinación de la EAM y la APH ha sido reducir la presión del tratamiento sin comprometer la inactivación microbiana. Varios experimentos se han llevado a cabo para evaluar el efecto sinérgico entre las dos tecnologías y para determinar la concentración óptima de CO_2 y la presión necesaria para conseguir una mayor inactivación de microorganismos patógenos y alteradores de la carne de ave.

Los resultados obtenidos demostraron que cuando la carne fresca de pollo fue envasada con CO_2 puro antes de su envasado definitivo con atmósfera modificada, el colapso de los envases se redujo significativamente en comparación con las muestras directamente envasadas con atmósfera modificada. Su vida útil también aumentó, debido a que una mayor cantidad de CO_2 disponible en el espacio de cabeza ofrece mejor protección del alimento y un efecto bacteriostático más duradero. Cuando se llevaron a cabo los pre-

tratamientos de saturación, los envases se pudieron llenar con más producto sin comprometer la seguridad del alimento ni el colapso del envase.

En cuanto a la combinación de atmósferas de CO_2 con APH, se pudo demostrar la existencia del efecto sinérgico de esta combinación en la reducción y la inactivación de algunos de los microorganismos alteradores y patógenos más habituales en los productos cárnicos avícolas. Una vez se desarrollaron los modelos de predicción de la reducción microbiana producida por la combinación de CO_2 y de alta presión, la atmósfera de 50% CO_2 equilibrada con N_2 y la presión de 250 MPa durante 10 min a temperatura ambiente fue considerado el más óptimo, teniendo en cuenta condiciones menos extremas y reducción microbiana producida. Este tratamiento se aplicó a pechuga de pollo fresca y se comparó con muestras envasadas en aire y presurizadas y muestras no presurizadas. La vida útil de la pechuga de pollo envasada con atmósfera modificada y tratada con presión fue de 26 días, mientras que las muestras tratadas con aire y presión sólo llegaron a 15 días bajo condiciones microbianas aceptables. Aunque se detectaron algunas alteraciones fisicoquímicas de la carne presurizada en cuanto a su color superficial y a la capacidad de retención de agua, una vez ésta fue cocinada no se encontraron diferencias sensoriales entre los tratamientos comparados.

La disolución forzada del gas provocada por la alta presión también se estudió mediante la cuantificación de la embolia gaseosa producida en salchichas de carne de ave. Los resultados sugieren que cuando se aplican presiones más altas, el gas que rodea el alimento penetra en el interior de la célula a niveles más profundos y por ello su extracción durante el proceso de descompresión es más complicada, manteniéndose disuelto en el alimento. Adicionalmente, al reducir la velocidad de descompresión, una mayor cantidad del gas se mantuvo disuelto en el producto, ofreciendo una mejor preservación del alimento cuando se trataba de CO_2.

Los resultados experimentales obtenidos han demostrado que la conservación de la carne de ave se puede mejorar mediante el uso de atmósferas ricas con CO_2 aplicadas individualmente como un tratamiento de saturación previo al envasado convencional con atmósfera modificada, o en combinación con la APH. Los trabajos expuestos en esta Tesis demuestran nuevas maneras de conservar los productos cárnicos avícolas dando nuevas perspectivas en este campo de investigación que dan la oportunidad de producir productos cárnicos frescos más seguros y duraderos.

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LIST OF ABBREVIATIONS

ºHue	Hue angle
a*	Color balance between green and red
ANOVA	Analysis of Variance
Ar	Argon
atm	Atmosphera (pressure unit)
b*	Color balance between blue and yellow
C*	Chroma
cfu	Colony former units
cm	Centimeter
CO	Carbon monoxide
CO_2	Carbon dioxide
et al.	*et alter* (and others)
FD	Filling degree of the packages
g	Grams
g_v/p_v	Gas volume/Product volume ratio
h	hour
HHP	High hydrostatic pressure
kg	Kilograms
L*	Luminosity
LAB	Lactic acid bacteria
Log	Decimal logarithm
m	Meter
MAP	Modified atmosphere packaging
mL	Milliliter
MPa	Megapascals
N_2	Nitrogen
O_2	Oxygen
RH	Relative humidity
sec	Second
SGS	Soluble gas stabilization
spp.	Species
TAC	Total aerobic counts
µL	Microliter
µm	Micrometer
WHC	Water holding capacity

Chapter 1

Introduction and literature review

1. Chicken and poultry meat

1.1. Nutritional value

Following Codex Alimentarius definitions, chickens are the birds of the species *Gallus gallus*. They are specifically called broilers when they are selectively bred and reared for their meat rather than eggs.

Chicken is the most common type of consumed poultry meat in the world (FAO, 2014) and it was one of the first domesticated animals by humans (Novartis Consumer Health, 2005). Chicken is one of the main sources of meat and eggs for human consumption. It is prepared as food in a wide variety of ways, varying by region and culture. The prevalence of chickens is due to almost the entire chicken being edible, and the ease of raising them.

Depending if it is consumed with skin or not, chicken meat caloric value varies from 167 Kcal/100 g to 240 Kcal/100 g. Its low fat content (5 – 8%), which is mostly concentrated in the skin, and its fatty acids profile (1.5% polyunsaturated, 3.6% monounsaturated and 2.3% saturated) has made this meat one of the healthiest to be consumed by humans. It has high water content (73%) and almost non carbohydrates (less than 0.05%), which makes the chicken meat an important protein source (20%). It also provides important minerals, such as potassium (522 mg/100 g), phosphorus (178 mg/100 g) sodium (60 mg/100 g) and magnesium, calcium, iron and zinc among others. Vitamins are also present, especially niacin (B_3) with 5.6 mg/100 g and ascorbic acid (2.5 mg/100g) (USDA. United States Department of Agriculture, 2011).

Poultry and poultry products are a highly perishable food and their shelf-life varies between 4 and 12 days under refrigeration. Deterioration depends mainly on the microbiological quality of the poultry carcasses, as poultry meat offers the perfect environment, pH, nutrients and humidity conditions for microorganism development.

1.2. Chicken and poultry meat spoilage

In the developed world, chickens are usually subject to intensive farming methods, which require the use of a wide range of animal antibiotics. This abuse of antibiotics has originated an increasing resistance on the most common bacteria causing foodborne diseases (Nobile, Costantino, Bianco, Pileggi, & Pavia, 2013; Schwaiger, Huther, Hölzel, Kämpf, & Bauer, 2012; Yildirim, Gonulalan, Pamuk, & Ertas, 2011).

Some of the common microorganisms present in poultry meat products are responsible for the most usual food-borne intoxications. Is the case of *Campylobacter jejuni*, *Listeria monocytogenes* and *Salmonella enteritidis*.

Campylobacter is the most common human food-borne bacterial pathogen worldwide. It is responsible of campylobacteriosis in humans, a disease ranging from a self-limiting gastroenteritis to a more serious systemic infection (Young, Davis, & Dirita, 2007). Although

Campylobacter is usually considered more fragile than other bacterial pathogens and is known to be fastidious in its growth requirements, the constant increase of human foodborne infections related to *Campylobacter* suggest its great survival rate throughout the food chain, from live animals to the meat retail level (Bièche, De Lamballerie, Chevret, Federighi, & Tresse, 2012). More than 2 million people in the United States and 0.2 million in the European Union are annually infected by *Campylobacter*, being the most frequently reported food-borne illness; and the actual number of cases is believed to be around nine million each year (European Food Safety Authority, 2014).

Listeria monocytogenes is a Gram-positive rod-shaped bacterium and is an important pathogen in acidified food and other food products, such as dairy products and ready-to-eat meats and fishes. As a food borne microorganism, *L. monocytogenes* requires food processors to be particularly careful during storage because of its moderate heat resistance and the ability to grow anaerobically under refrigeration. It is a psychrotrophic organism and therefore can grow and multiply in chilled food products before consumption (Yuste, Mor-Mur, Capellas, & Pla, 1999). Cross-contamination from raw meat to other foods and survival of the microorganism in processed poultry are possible causes of its high incidence in outbreaks (Pini & Gilbert, 1988).

Salmonella is a Gram-negative facultative anaerobe mesophilic bacterium, widely distributed in nature and in the gastrointestinal tract of animals and humans. Is one of the most important pathogens associated with gastrointestinal diseases, and the majority of human cases are linked to contaminated foods. The illness caused by *Salmonella* in humans is called salmonellosis. In the European Union, over 100.000 human cases are reported each year (European Food Safety Authority, 2014). Moreover, *S. enteritidis* is one of the *Salmonella* serotypes most commonly associated with morbidity and mortality in humans (Ahmed et al., 2000). At the present moment, the control of this microorganism is a priority for the food industry and governmental organizations, especially in the case of poultry products.

About non-pathogenic microorganisms commonly present in poultry meat, *Brochothrix thermosphacta* is one of the most current spoilage organism associated with meat products stored aerobically or under modified atmospheres. The main alteration is due to the production of malodorous end-products such as acetoin and acetic, butyric, isobutyric and isovaleric acids causing off-flavors that render the affected meat unpalatable (McClure, Baranyi, Boogard, Kelly, & Roberts, 1993). As a facultative anaerobe, *B. thermosphacta* has the capability to grow on meat during both aerobic and vacuum storage, which makes it a significant meat colonizer, at times having the potential to be the dominant organism (Doulgeraki, Ercolini, Villani, & Nychas, 2012). The successful spoilage of chilled products is due mainly to its psychrotropic nature, growing between 0 °C to 30 °C. Its pH growth range (pH 5-9) is suitable for most meat products.

Other spoilage microorganisms usually present in poultry meat are strains belonging to *Leuconostoc* genus, which are Gram-positive aerotolerant lactic acid bacteria (LAB) with an economic importance in the food industry. They are related to numerous positive processes such as fermentation of foodstuff (sauerkraut, pickles, meat products, etc.), production of gas

(CO_2) in cheese presenting openness (in particular Blue-veined cheeses), production of flavour compounds in multiple dairy products and also adding potential roles in functional foods (Hemme & Foucaud-Scheunemann, 2004). On the other hand, species of *Leuconostoc* are one of the main genera associated with spoiled meat products packaged with air, vacuum or modified atmosphere (Doulgeraki et al., 2012). Good manufacturing practices and hygienic conditions during the slaughtering and processing of the meat are critical to produce a safety and durable product.

1.3. Product consumption extension

During the last decade, the consumption of chicken meat has increased rapidly all over the world and poultry production has become the fastest growing meat sector. Nowadays, chicken meat is the second most consumed meat in all over the world, with the 30% of the total meat production, just a 6% less than pig meat (FAO, 2014). As distances between processing facilities and retail distribution are increasingly longer than they used to be and because of the greater demands from retailers and consumers for individually packaged products with longer shelf-lives, interest in viable systems to extend the storage capacity or shelf-life of meat and poultry products has increased during the last two decades (Rodríguez-Calleja, Cruz-Romero, O'Sullivan, García-López, & Kerry, 2012). Currently, the most used system to preserve chicken raw meat and meat products are by means of modified atmosphere packaging (MAP) combined with refrigeration.

2. Modified atmosphere packaging

2.1. Historical background

Changes in the lifestyle of the industrialized countries have promoted the emergence of new trends in food consumption. There is currently a great interest in fresh, "natural" and healthy products, preferably with few additives and with all their original flavor and properties.

However, the rhythm of modern life has also increased significantly the demand for quick and easy preparation foods, such as ready-to-eat meals and IV and V range products. Part of this demand comes from important sectors such as hotels, restaurants and fast food chains which require large volumes of these products to ensure the availability of fresh and ready-to-serve products all the year (García-Iglesias, Gago-Cabezas, & Fernández-Nuevo, 2006). Additionally, the current economic situation has forced many producers to expand

their distribution channels, requiring longer shelf-life for their products to guarantee their good conditions to the selling point and their preservation until they are consumed.

In response to these demands, the food industry has been implementing preservation and production technologies to ensure the hygienic quality of food and extend its shelf-life with the minimum alterations of their original properties. This group of preservation technologies includes modified atmosphere packaging systems.

Modified atmosphere packaging (MAP) consists on removal and/or replacement of the atmosphere from a storage room, transportation containers or packages surrounding the product before sealing the system. MAP can be vacuum packaging, which removes most of the air before the product is enclosed in barrier materials, or forms of gas replacement, where the air is removed by vacuum or flushing and replaced with another gas mixture before packaging sealing in barrier or semi-permeable materials. The chosen atmosphere depends on the nature of the packaged product which can duplicate or triplicate its shelf-life if the gas mixture and the packaging materials are the optimal ones.

The headspace composition may change during storage in MAP, but there is no additional manipulation of the internal environment, while controlled atmosphere packaging (CAP) uses continuous monitoring and control of the environment to maintain a stable gas atmosphere and other conditions such as temperature and humidity within the package (McMillin, 2008). CAP has most often been used to control ripening and spoilage of fruits and vegetables, usually in containers larger than retail-sized packages or storage chambers.

One of the most important advantages of MAP technology is that it can be used for almost all kinds of food products, from fresh meat to vegetables, bakery or even dehydrated products or liquid foods. MAP has gained considerable popularity over the last decades as a modern non-thermal method of food preservation (Patsias, Badeka, Savvaidis, & Kontominas, 2008), basically thanks to its effectiveness and the relative low cost of investment for its implementation in the food industries.

Modified atmosphere is a relatively old process. Based on ancient writings, certain forms of modified atmosphere storage were used in China, Greece, and other early civilizations. According to some reports, fruits were sealed in clay containers together with fresh leaves and grass. The high respiration rates of leaves and grass, combined with that of the fruit, quickly modified the atmosphere in the container, creating an environment with a high concentration of carbon dioxide and low in oxygen, which helped to retard fruit ripening (Floros & Matsos, 2005).

However, it was not until 1820 that the effect of atmosphere on fruit ripening was studied (Floros, 1990). About 100 years later, the effect of carbon dioxide (CO_2) and oxygen (O_2) concentration on the germination and growth of fruit - rotting fungi at various temperatures was investigated (Brown, 1922). Similarly, in the 1930s a number of research studies were published regarding the effect of carbon dioxide and storage temperature on the inhibition of microbial growth on meat surfaces and the resulting extended product shelf life (Ooraikul & Stiles, 1995). However, it was not until the late 1950s when the first significant trials of retail size modified atmosphere packaging took place, with vacuum-

packed meat, fish and coffee (Inns, 1987). The interest in gas preservation techniques increased radically in the 1970s and 1980s, and commercial applications of MAP have progressively increased since then.

This success has been the result of several factors. Consumers required more natural, fresh-like and minimally processed foods, which became popular. Preservatives started being under suspicion and their use became increasingly restricted. As a result, the industry was in need of alternative preservation methods. At the same time, the cost of raw food products, work conditions and energy increased, which negatively affected many traditional preservation techniques such as canning and freezing. Moreover, advances in material science allowed the development of many new packaging films with various physical properties. In addition, a wide range of sophisticated packaging equipment was developed to take advantage of the improved properties of the new films. Inexpensive and easy MAP equipment appeared on the market, specially developed for foods in small and convenient retail units. As a result, many MAP studies were conducted and a large number of commercial applications were introduced (Floros & Matsos, 2005).

At the same time, lifestyle was changing and consumers were looking for convenient food products adapted to their busy lives, packaged by retail units, with a longer shelf-life than fresh products but with the same characteristics and properties. The demand of "natural" and "fresh" items and the increasing awareness of the society against chemical additives situated MAP in a great position to become the new and ideal preservation method. From back then, the use of MAP has been extended in the food industries of all over the world, becoming one of the most popular preservation methods and the most used packaging process to preserve fresh perishable food (Gould, 2000).

2.2. MAP principles

The main objective of MAP is to preserve food and extend its shelf-life, preventing or retarding any undesirable changes in their properties, safety, sensory characteristics, wholesomeness and nutritive value. To achieve that goal, MAP acts against microbial growth, reduces undesirable physiological, chemical, biochemical and physical changes and prevents product contamination (Floros & Matsos, 2005).

The description of this packaging method could not be complete without paying attention to the three main elements that make it possible: the packaging machines, the plastic films and package materials and the gases.

2.2.1. MAP machines

Depending on the food product that has to be packed and the final presentation that it would have, different packaging equipment can be used in order to obtain the desired

results. The main difference among them is the process of the introduction of the modified atmosphere, which it can be done after a vacuum process or not.

A vacuum chamber is an equipment commonly used for small productions (Figure 1.1). Vacuum times and pressures, gas pressure and sealing time can be controlled, adjusting each parameter to obtain the desired results. With this kind of equipment residual O_2 values of 0.1% can be achieved. However, the air extraction has to be taken in consideration when crusty products like bread or bakery are packed as a strong vacuum process will affect their texture. Although this equipment can pack several bags in the same cycle, it works in discontinuous cycles requiring a manual manipulation of the bags, which makes it not the best option for a food chain production but for small and retail food producers.

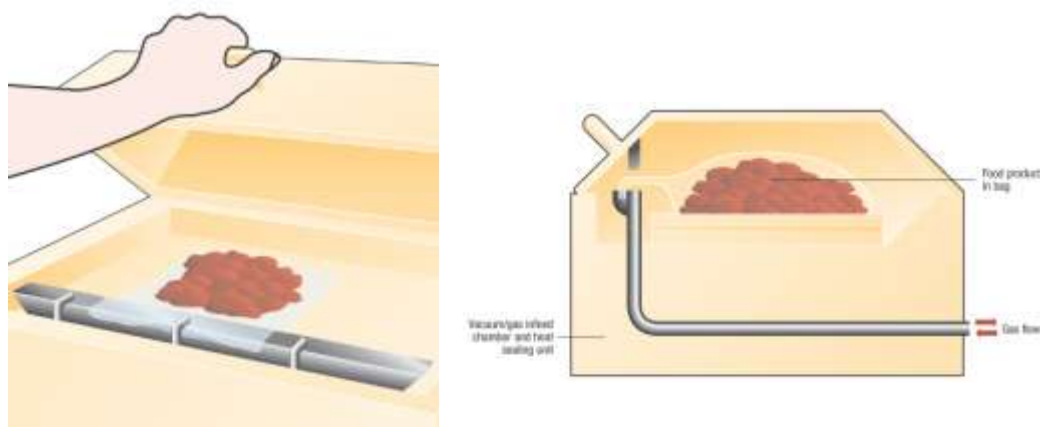

Figure 1.1. Graphical illustration of a vacuum chamber equipment
(Source: Air Products, www.airproducts.co.uk)

In the food industries, the most usual vacuum machines are the continuous tray thermo-sealers (Figure 1.2). These machines can be totally implemented in the food production chain. Once the food is placed in a tray, the air is evacuated at the sealing die and protective gas is added. Then the pack is sealed by the application of heat and pressure. Its design can be adjusted to the needs of every production process and machines are available from tabletop (manual) for the small producer, to fully automatic inline versions for larger processors. A similar version of this equipment it is also commonly used with the variation that thermoforms the trays just before the packaging (Figure 1.3). Packaging material for the base web (thermoformable film) is unwound from the reel. It is heated in the forming die and formed into pockets/trays (Air Products and Chemicals Inc., 1996)

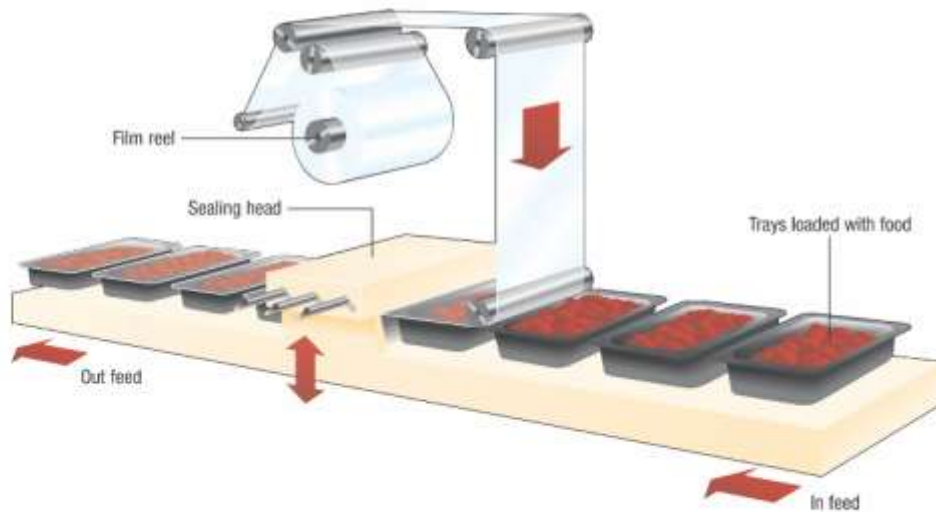

Figure 1.2. Graphical illustration of a continuous tray sealer equipment.
(Source: Air Products, www.airproducts.co.uk)

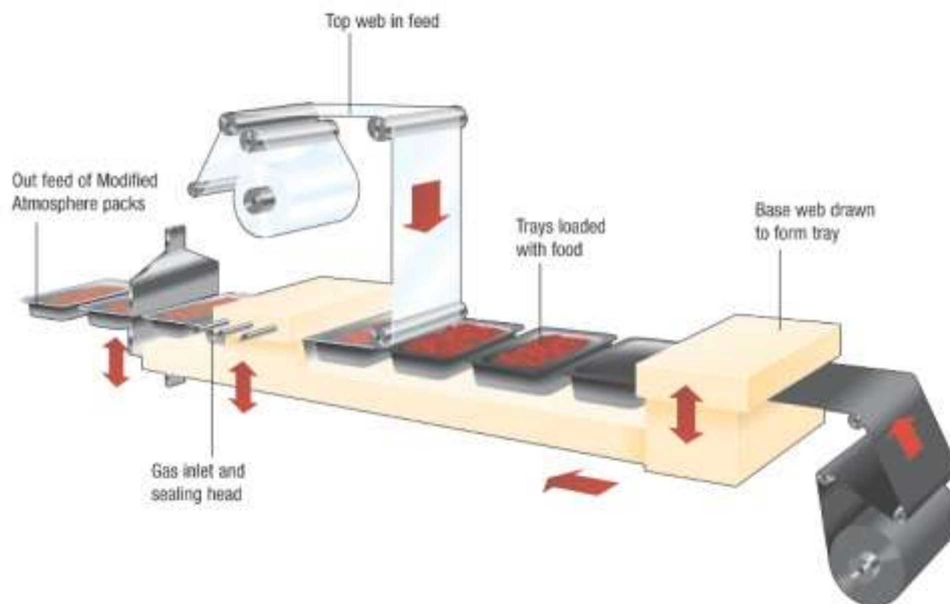

Figure 1.3. Graphical illustration of thermoform-fill-seal equipment.
(Source: Air Products, www.airproducts.co.uk)

On the other hand, when some food products are susceptible of vacuum packaging and they do not need a complete absence of oxygen to be preserved, gas-flushing-packaging equipment is the most adequate option. These so-called flow-pack machines are capable of making flexible pillow-pack pouches from only one reel of film. Horizontal form-fill-seal machines (Figure 1.4) can also overwrap a pre-filled tray of product. The air from the package is removed by a pulse of gas or continuous gas flushing. For certain very porous products (like some bakery goods), gas flushing is not capable of reducing the residual O_2 within the package to low levels. Vertical flow-pack equipment is commonly used to package vegetables, ready-to-eat salads and vegetable leaves (Air Products and Chemicals Inc., 1996).

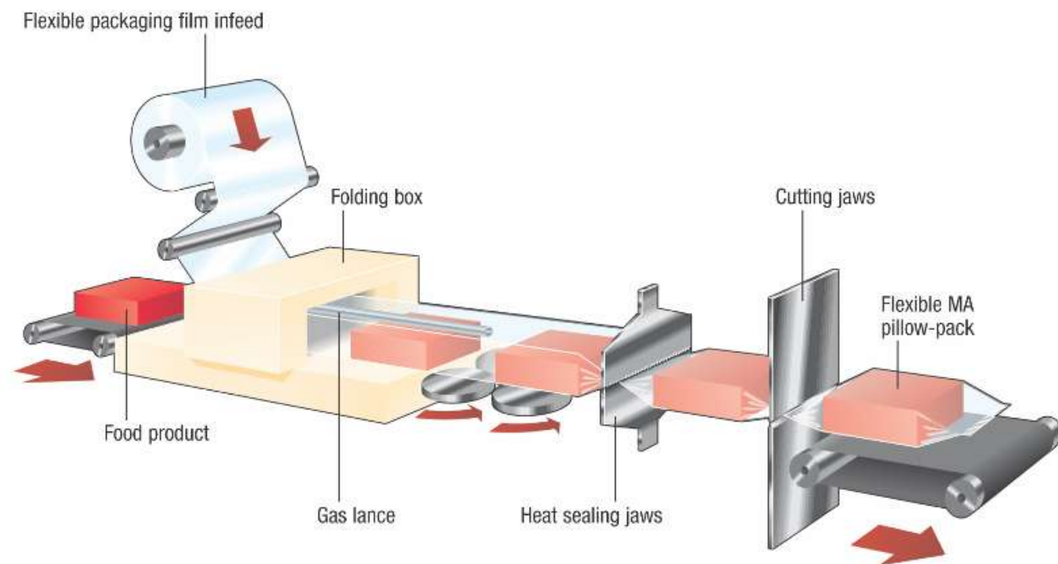

Figure 1.4. Graphical illustration of a horizontal flow-pack equipment.
(Source: Air Products, www.airproducts.co.uk)

2.2.2. Plastic materials

There are a huge variety of plastic materials used for food packaging. Their use depends mainly on the characteristics of the product and the requirements needed for its protection and presentation to the final consumer.

The most commonly used polymers for food packaging are low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), expanded polystyrene (PS-E), ethylene vinyl alcohol copolymer (EVOH) and polyvinyl alcohol (PVOH). Each type of packaging material has different advantages, disadvantages, consumer and marketing issues, environmental considerations and cost, and their use in foods is regulated by the European regulation EU 10/2011 (2011) and their posterior modifications.

A single layer or type of plastic generally does not have all of the needed properties for a food package application, therefore lamination, coating or co-extrusion is used to create layers of different plastic materials in order to obtain the desired properties. Heat sealing and barrier properties are often improved by application of coatings to the surfaces of plastic films (Jenkins & Harrington, 1991).

The plastic films used for MAP have some important properties that define them, which are specified on the technical data sheet determined by the manufacturer. The most important are barrier properties, which are usually defined by oxygen, carbon dioxide and water vapor transmission rate; technical properties, like the thickness and density of the film; mechanical properties, such as flexibility, elasticity and puncture or tear resistance; optical properties, defined by the clearness and brightness of the film; and other properties, which can be certain coatings like adhesives, anti-mist or anti-fog, antimicrobials, nanoparticles, etc.

Choosing the right plastic material is an important part to succeed on the preservation of packaged food. For example, respiration food systems like fruits and vegetables cannot be packaged on a high barrier film, because they need a constant entrance of O_2 and exit of CO_2 throughout the film to keep their respiration rate. Foods that are packaged while they are warm, like pre-cooked or ready-to-eat foods, or food that is kept under chilling conditions, need to be packed with an anti-mist film, in order to keep visible the interior and not form condensations on the interior of the top film. Moreover, all these materials have to be chemically stable in contact with food, paying special attention to the acid or alkaline foods, which can interact with them.

2.2.3. Gases

Gases are the basis of the MAP technology. There are three main gases used in MAP: nitrogen (N_2), oxygen (O_2) and carbon dioxide (CO_2). At sea level, the approximate composition of atmospheric air is 78.1% N_2, 20.9% O_2, and 0.03% CO_2. Depending on the characteristics of the product that has to be packed, one of these gases or a mixture of them will be used according to their attributes.

Nitrogen

It is an inert and tasteless gas, without any antimicrobial activity. It is not very soluble in water, and is primarily used to displace oxygen and prevent package collapse and lipid oxidation. Its use as a food additive is regulated by the European Directive nº 95/2/EC (European Union, 1995) and listed as the additive E-941. As the air atmosphere is mostly nitrogen, this is the most easy-to-obtain gas and therefore the cheapest one compared to the others. Due to its lack of interaction with the food, that could alter their properties, and the protection that can offer, the food industry uses N_2 atmospheres not only to balance the mixture with the other gases but also to swell the bags containing fragile products and to protect them against oxidation and rancidity processes.

Oxygen

As a general concept of food preservation, oxygen is an undesirable gas as it promotes the growth of aerobic microorganisms and produces degradation reactions such as oxidation and rancidity of fats and oils, rapid ripening of fruits and vegetables and color and sensory changes on the products (Floros & Matsos, 2005). However, its presence is sometimes necessary in some particular products. For example, a minimum oxygen concentration (around 5 - 10% of the atmosphere) is required by many fruits and vegetables in order to sustain their basic process of aerobic respiration and avoid anaerobic fermentations. Its presence prevents the growth of some anaerobe pathogens like *Clostridium botulinum*. In retail red meats, high O_2 concentrations (usually about 50 - 70% promote the formation of oxymyoglobin, the oxygenized form of myoglobin, which has a red bright color that makes the meat more attractive for consumers. However, it has been shown that MAP with a high

concentration of oxygen may cause quality deterioration through lipid and protein oxidation (O'Grady, Monahan, Burke, & Allen, 2000; Zakrys-Waliwander, Hogan, O'Sullivan, Allen, & Kerry, 2008) affecting the flavor of the meat (Zakrys-Waliwander, O'Sullivan, Walsh, Allen, & Kerry, 2011). It is coded as the food additive E-948.

Carbon dioxide

In perishable products packaged under modified atmospheres, the extension of their shelf-life depends directly on the concentration of CO_2 in the head space. Carbon dioxide is used basically for its antimicrobial effect on extending the lag phase and generation time of the most common spoilage microorganisms. This effect is possible because of its capacity to dissolve in the water and lipid-phase of the food. Once the equilibrium is reached, usually after 24 – 48h of packaging (Rotabakk, Wyller, Lekang, & Sivertsvik, 2008), a certain concentration of dissolved CO_2 remains dissolved in the water-phase of the product (Devlieghere, Debevere, & Van Impe, 1998), which increase the concentration of carbonic acid inside the cell, producing a lowering on the intracellular pH (Tan & Gill, 1982). Variations on the pH of the food media cause alterations in the enzyme activities and changes in membrane properties and functions, directly affecting microorganisms' growth metabolism. The negative aspect of this dissolution though, is that it results in package collapse, affecting the look of the packages. As a food additive, CO_2 is listed as E-290.

Occasionally other less-common gases can be used in combination with the ones mentioned above. For example, carbon monoxide (CO) is sometimes added to inhibit microbial growth and keep a long bright red color in red meats (Sørheim, Aune, & Nesbakken, 1997). However, CO is toxic to humans and its application is limited in some countries and prohibited in Europe. Sulfur dioxide (SO_2) may be used to prevent oxidative browning and to control the growth of bacteria and molds; although its principal application is for pest control in cereals and legumes (Riudavets et al., 2014). Ethanol has also used to retard the decay of fruits and vegetables, improve flavor, and reduce fungal activity (Lurie et al., 2006). Argon (Ar) has been used recently as a possible substitute for nitrogen, although no significant differences regarding microbial control and food shelf-life extension have been found between them (Ruiz-Capillas & Jiménez-Colmenero, 2010). This lack of advantageous results and the superior cost of argon have limited its commercial use.

The appropriate combination of gases depends on the product, the packaging material, the storage temperature and other factors, such as the initial microbiological quality or the final presentation of the product (Provincial et al., 2013).

2.3. CO_2 action mechanisms

Four possible mechanisms for CO_2 microorganisms' inhibition have been suggested (Tan & Gill, 1982): that CO_2, which can readily pass through cell membranes, forms carbonic acid within the cell with a resultant decrease in intracellular pH, which slows intracellular enzyme activities (Wolfe, 1980); that decarboxylating enzymes are specifically inhibited by a mass action effect of CO_2 (King & Nagel, 1975); that there is non-specific inhibition by CO_2 of susceptible non-decarboxylating enzymes (Ranson, Walker, & Clarke, 1960); or that dissolution of CO_2 in the cell membrane alters membrane properties and inhibits membrane functions (Sears & Eisenberg, 1961). Nevertheless, it is evident that the inhibition of the growth of microorganisms in MAP foods is significantly conditioned by the concentration of dissolved CO_2 into the product (Devlieghere & Debevere, 2000). Stiles (1991) determined that it is necessary to introduce a minimum concentration of 20 - 30% of CO_2 in the head space of packages to obtain this effect, and has been demonstrated that high concentrations of CO_2 can extend the shelf-life of many perishable products (Ahvenainen, 2003; Floros & Matsos, 2005; Sivertsvik, Jeksrud, Vågane, & Rosnes, 2004).

One of the most studied mechanisms of CO_2 to inhibit microbial growth is the formation of carbonic acid (H_2CO_3) once the gas is dissolved into the water phase of the product (Tan & Gill, 1982). Observing that CO_2 penetrates easier into the cells than other acids (such as hydrochloric and phosphoric acid), Lin, Yang & Chen (1993) suggested that a lowered pH contribute to an increase in cell permeability, which facilitates the diffusion of CO_2 into microbial cells.

Besides food composition and the concentration of CO_2 in the mixture, the dissolution of CO_2 in food depends also on the quantity of gas introduced into the package. For that reason, the ratio between the gas volume and the product volume (g_v/p_v) in the package has an important influence on the effectiveness of MAP. Therefore, when high concentrations of this gas are applied in food products with a significant content in fats and water in a flexible packaging system, the gas dissolved into the food until the equilibrium is attained. At equilibrium, the partial pressure of CO_2 within the package will be less than atmospheric; and unless CO_2 is added in excess and with a sufficient amount to saturate the meat at atmospheric pressure, the package will collapse around the meat (Gill & Penney, 1988; Gill, 1988).

The relationship between the concentration of CO_2 in the atmosphere and the amount of CO_2 dissolved in the product follows Henry's law (Schumpe, Quicker, & Deckwer, 1982) as cited by Rotabakk et al. (2010):

$$P_{CO_2}^{t=\infty} = H_{CO_2,p} \times C_{CO_2}^{t=\infty} \quad (1.5)$$

Where $P_{CO_2}^{t=\infty}$ is the partial pressure (Pa) of CO_2 that surrounds the product, $H_{CO_2,p}$ is Henry's constant for CO_2 (Pa (mg kg^{-1})$^{-1}$), and $C_{CO_2}^{t=\infty}$ is the equilibrium concentration of CO_2 (mg kg^{-1}) dissolved in the product.

When the ratio g_v/p_v and the initial partial pressure of CO_2 are known, the solubility of CO_2 into water at a given temperature could be predicted by (Sivertsvik, Jeksrud, et al., 2004):

$$C_{CO_2}^{pred} = \frac{g_v/p_v \times P_{CO_2}^{t=0} \times Mw_{CO_2}}{RT + (H_{CO_2,H_2O} \times g_v/p_v \times Mw_{CO_2})} \tag{1.6}$$

Where $C_{CO_2}^{pred}$ is the theoretical CO_2 (mg kg^{-1}) dissolved in the product, $P_{CO_2}^{t=0}$ is the initial partial pressure (Pa) of CO_2, Mw_{CO_2} is the molecular weight of CO_2 (44.01 g mol^{-1}), R is the universal gas constant (J mol^{-1} K^{-1}), T is the temperature (K) and H_{CO_2,H_2O} is Henry's constant for CO_2 in water at a given temperature (Pa(mg kg^{-1})$^{-1}$).

The solubility of CO_2 has been studied in many perishable foods (Chen & Hotchkiss, 1991; Jakobsen & Bertelsen, 2004; Rotabakk et al., 2010). The studies developed by Sivertsvik et al. to establish Henry's constant in products such as water (Sivertsvik, Jeksrud, et al., 2004), raw fish (Sivertsvik, Rosnes, & Jeksrud, 2004) and cooked meat (Sivertsvik & Jensen, 2005), have demonstrated that at least 48 hours after packaging are needed in order to assure the final equilibrium of the atmosphere inside the package.

Owing to the increase on CO_2 solubility at lower temperatures and at higher partial and total pressures, a sufficient amount of CO_2 can be dissolved into the product during some hours in pure CO_2 before the definitive packaging. This method has been called *Soluble Gas Stabilization* (SGS) (Sivertsvik, 2000, 2003), which consists in a previous saturation of the food with pure CO_2, preserving it in refrigeration temperatures for a certain time. SGS has the potential to prevent package collapse, even at high filling ratios, without compromising the quality of the packaged food, resulting in more appropriately packaged products and increased packaging efficiency (Rotabakk, Birkeland, Jeksrud, & Sivertsvik, 2006). This promising technique has been used in fish and seafood (Mendes, Pestana, & Gonçalves, 2008; Mendes, Silva, Anacleto, & Cardoso, 2011; Rotabakk, Birkeland, Lekang, & Sivertsvik, 2008; Sivertsvik & Birkeland, 2006) and meat (Rotabakk et al., 2006), and even though the results are favorable to think that shelf-life of SGS treated products can be significantly prolonged and package collapse avoided, it would be necessary to study all the possible variables, such as MAP mixtures or pack filling ratio, to find the most efficient treatment.

2.4. Packaging volume ratios

Pack to product ratio has an important influence on the preservation of MAP products, as the quantity of the gas introduced on the package depends on this ratio and therefore it is crucial for the efficacy of MAP. Theoretically, a gas volume / product volume ratio of 3:1 is the best ratio to ensure that there is enough gas to protect and preserve the packaged product. However, this ratio is undesirable because of the low packaging efficiency associated with it (Rotabakk et al., 2010). Blakistone (1999) suggested that the gas head-space in MAP must be 1.5 – 2 times the meat volume in order to prevent pack collapse. Gill and Gill (2005)

stated that the gas head-space should be no less than 2 – 3 times the meat volume. Headspace is also important in tray and lidding film packaging because product touching the film darkens more quickly (McMillin, 2008).

The potential benefits of reducing the gas head-space in meat preservation with MAP include a reduction in retail pack size which would result in lower manufacturing, transport and storage costs for the meat industry. A reduction in pack waste would also have beneficial environmental implications (Murphy, O'Grady, & Kerry, 2013).

2.5. Applications of MAP on meat products

Currently, the meat industry all over the world uses MAP to pack raw meat, obtaining longer shelf-lives and keeping the meat safe from spoilage microorganisms while preserving quality and sensory properties.

In the 1950s, more advanced forms of meat display were required by the consumers than butcher cutting and wrapping of meat in paper or waxed paper, being replaced by store cutting and display of the packages in refrigerated self-service display cases. Lighted refrigerated meat cases where consumers could handle and select among different packages required packaging that protected the contents while showing the product characteristics, primarily lean color and amounts of fat, during daily display (McMillin, 2008).

During the 1960s, investigations by Kalle, a German plastic film converter, demonstrated that fresh red meat could be preserved under refrigeration with its desirable "cherry red" oxymyoglobin color if a high carbon dioxide and high oxygen environment were present. The presence of high oxygen levels was contrary to normal practice because common belief had been that high oxygen accelerates microbial growth, respiration, and fat oxidation. Elevated carbon dioxide was shown to be sufficiently effective in retarding microbial growth, and so the adverse oxidative effects of the high oxygen could be compensated. These findings were put into practice in West Germany, where thermoform/vacuum/gas flush-seal packaging systems with high oxygen-barrier materials were employed (Brody, 2005). Subsequently, air-permeable and moisture-barrier polyvinyl chloride film that would stretch around clear or expanded polystyrene trays were developed for raw fresh meat (Brody, 2002). Consumers began to associate the bright red bloomed color of prepackaged meat in air-permeable packaging with meat freshness because this was the color of the meat first seen on display in self-service meat cases (Jenkins & Harrington, 1991).

2.5.1. MAP conditions for fresh meat and meat products

The principle reactions that affect the quality of meat and consequently limits its shelf life are color changes, growth of spoilage microorganisms, lipid oxidation and enzyme reaction. MAP can retard or even avoid some of these undesirable reactions.

For the wholesale packaging of meat, like carcasses and big pieces, avoiding the growth of spoilage and pathogenic microorganisms during the distribution to the processor plants is the main priority. Vacuum packaging is the most used method to achieve this objective. Modified atmosphere is also used, usually with a mixture composed by 40 – 50% of CO_2 and 50 – 60% of N_2. If the initial quality of the meat and its manipulation are in the right hygienic conditions, the product can achieve a shelf-life of four weeks. Avoiding the presence of oxygen is crucial to preserve these huge portions of meat, and consequently meat color will turn to brownish color while it remains in this anaerobic atmosphere.

On retail packages of meat portions and minced meat, consumer preferences are more influenced by color than any other quality factors. Even though the color of fresh packaged meat is not well correlated with its taste and eating quality, consumer still demands beef to be a bright cherry-red color, lamb a brick red color and pork and chicken an even pink color (Troy & Kerry, 2010). For that reason, mixtures with a 70 – 80% of O_2 balanced with CO_2 are used together with barrier trays of PP, PET or PS sealed with clear or printed barrier film (Belcher, 2006). In these conditions and under refrigeration temperatures, raw meat and meat products can achieve a shelf-life up to two weeks, depending on the product.

2.5.2. Effect of MAP on meat attributes

MAP protects meat products against deteriorative effects such as discoloration, off-flavor and off-odor development, nutrient loss, texture changes and other measurable factors. The properties of meat that are important in determining its shelf-life include color, flavor, microbial quality, lipid stability, water holding capacity and texture.

Color of packaged meat and its stability or discoloration are the most important quality attributes considered during its shelf-life, as consumers are more influenced by this parameter than any other. Deoxymyoglobin is the reduced form of myoglobin (Fe^{2+}) that gives purple color in the absence of O_2 when meat is first cut or has been vacuum packaged. Metmyoglobin (Fe^{3+}) is the oxidized pigment state of myoglobin, the dominant sarcoplasmic pigment in muscle, and it results in a brown or gray meat color. Oxymyoglobin is the reduced pigment form (Fe^{2+}) in which O_2 occupies the ligand position and the perceived color is bright red. The penetration depth of O_2 and the oxymyoglobin layer thickness depend upon meat temperature, O_2 partial pressure, pH, and competition of O_2 by other respiratory processes (Mancini & Hunt, 2005).

The bright red bloomed color of packaged beef is due to the predominating oxymyoglobin pigment that is easily obtained with greater than 10% of O_2 in the atmosphere. However, it has been widely demonstrated that high-oxygen atmospheres induces lipid and protein oxidation (Estévez & Cava, 2004; Y. H. Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010) causing a negative impact on meat quality, especially on its sensory attributes.

As the flavor and odor of meat are originated from lipid and peptide components (Spanier, 1992), chemical changes on lipid and fats may alter the sensory characteristics of

the meat, producing an off-flavor as a consequence to oxidative rancidity. Oxidation of lipids has been also linked to oxidation of pigments and meat discoloration (Faustman & Cassens, 1990).

Protein oxidation is another consequence of packaging meat under high oxygen concentrations and affects meat and meat products quality (Decker, Xiong, Calvert, Crum, & Blanchard, 1993). Oxidation might play a role resulting in the loss of enzyme activity, solubility and formation of protein complexes and non-enzymatic browning products (Mercier, Gatellier, & Renerre, 2004) and could be linked to meat tenderness. Zakrys-Waliwander et al. (2009) demonstrated that O_2 levels higher than 40% have a negative impact on consumer evaluated texture characteristics. However, the usual consumption of high oxygen packaged meat may have changed the sensory perception of consumers for these products, resulting in a general acceptance of oxidized flavors (Zakrys-Waliwander et al., 2008).

Variations on the proteolytic activity of meat have been suggested as the main cause for alterations on the water holding capacity (WHC) of the meat, causing the subsequent shrinkage and movement of water into extracellular spaces as exudates (Huff-Lonergan & Lonergan, 2005). Water binding or water holding capacity is a measure of the ability of meat to hold its own or added water (Hamm, 1960). It is directly related to protein degradation, and the amount of bound and lost water affects the appearance and texture of products and their economic value. Packages of meat containing fluids surrounding the meat are not desirable to consumers. These exudates are primarily composed of sarcoplasmic proteins (Savage, Warriss, & Jolley, 1990) and may be the 2 – 10% of lean weight (Offer & Knight, 1988). The increase in the drip loss of the meat is influenced by the deterioration of the proteins, but also by the atmosphere surrounding the meat. It is documented that raising the quantity of CO_2 in the head space could produce an increase in the weight losses and exudates due to pH drop, approaching to proteins isoelectric point (Stiles, 1991).

Regarding microbial populations of fresh meat, MAP can have a direct effect on the control of some of the most common spoilage and pathogenic bacteria on fresh meat. Increased levels of CO_2 inhibit microbial growth in refrigerated storage, with 20 – 40% CO_2 used in MAP (Clark & Lentz, 1969), and higher levels raise the possibility of establishing conditions where pathogenic organisms may not survive (Daniels, Krishnamuthi, & Rizvi, 1985). Gram-negative bacteria are generally more sensitive to CO_2 than Gram-positive bacteria because most Gram-positive bacteria are facultative or strict anaerobes (Tan & Gill, 1982), although some variations have been found in microorganisms of the same species. N_2 also creates anoxic atmospheres where aerobic microorganisms cannot grow.

However, it is important to clarify that MAP does not destroy microorganisms; selected atmospheres can only slow down their growth. Therefore, a great hygienic quality of the meat and good manufacturing practices are primordial to obtain good results in its preservation with MAP.

MAP efficacy on meat products preservation has been widely demonstrated (Blakistone, 1999; Hotchkiss & Langston, 1995; Lambert, Smith, & Dodds, 1991; Saucier, Gendron, &

Gariépy, 2000; Silliker, Woodruff, Lugg, Wolfe, & Brown, 1977; Vongsawasdi, Wongwichan, Khunajakr, & Dejsuk, 2008), and it has turned essential to preserve chicken meat, which offers to the microorganisms the perfect conditions for growth: neutral pH, high humidity and plenty of nutrients for almost all the spoilage and pathogenic bacteria. MAP has offered to these products the possibility to extend their shelf-life keeping the quality and the food safety standards for the consumers.

2.6. Combining MAP with other food preserving technologies

For the last two decades, combining MAP with other preserving factors has been an important object of study, especially to preserve meat products. As these alliances can have synergistic or additive effects, an intelligent combination of factors can attain an extension of shelf-life or an improvement in safety or sensory properties while maintaining meat quality.

Chilling temperatures are the most common technologies used in combination with MAP, specially to preserve fresh meat (Cayré, Garro, & Vignolo, 2005; Patsias et al., 2008; Sheridan et al., 1997; Sinelli, Limbo, Torri, Di Egidio, & Casiraghi, 2010).

Antioxidants have been widely tested in combination with high oxygen modified atmosphere to reduce lipid oxidation in red meat. Organic acids such as tannic or ascorbic acid has demonstrated a significant effect on retarding lipid oxidation while protecting red meat color, although they do not avoid protein oxidation (Lund, Hviid, & Skibsted, 2007; Maqsood & Benjakul, 2010).

Natural antimicrobial substances like essential oils have been also widely studied, either directly spread over the food or coated with the packaging material (Botsoglou, Christaki, Fletouris, Florou-Paneri, & Spais, 2002; Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007; Duan, Jiang, Cherian, & Zhao, 2010). Combined with MAP, essential oils like oregano (P. Skandamis, Tsigarida, & Nychas, 2002), thymol (Karabagias, Badeka, & Kontominas, 2011), rosemary (Keokamnerd, Acton, Han, & Dawson, 2008) and also spice extracts (Zhang, Kong, Xiong, & Sun, 2009) have demonstrated a positive effect on raw meat appearance during storage, on cooked meat flavor, slowing meat oxidation and retarding or even inhibiting the growth of spoilage and pathogenic bacteria.

Bacteriocins such as nisin have also been used as coatings for plastic materials with significant results in meat preservation (La-Storia et al., 2012). And other recent technologies such as irradiation (Grandison & Jennings, 1993) and high hydrostatic pressure (Park, Park, & Park, 2003) have also obtained promising results.

3. High hydrostatic pressure

3.1. Principles of high-pressure processing

Due to consumers demand and the advance of science and technology, food science has developed methodologies and technologies with the aim to preserve food while ensuring their quality and guaranteeing its safety for consumers. Even though thermal treatments, such as pasteurization and sterilization, are the most extensively used methods of food preservation, new processes tend to use non-thermal treatments such as magnetic or electrical fields, ionization, light pulses, high hydrostatic pressures, ultrasounds, and the application of chemical or biological products such as carbon dioxide, policationic polymers, bacteriocins and lytic enzymes (Mertens & Knorr, 1992). Some of these systems already have regulatory approval and are commonly used in the industry, while others continue to be developed and evaluated for potential commercial application (Trujillo et al., 2000).

High hydrostatic pressure (HHP), as an alternative to heat pasteurization, is one of the most promising non-thermal processing techniques for the inactivation of microorganisms as well as enzymes at low or ambient temperature, in liquid and solid food systems while preserving its nutritional and sensory attributes (Wang, Pan, Xie, Yang, & Lin, 2010).

Unlike thermal processing and other preservation technologies, HHP is an isostatic process where the pressure is transmitted uniformly and nearly instantaneously throughout the food irrespective of its geometry and equipment size. This unique property of HHP in comparison with other preservation technologies has facilitated the scale-up laboratory findings to full-scale production (Srinvasa-Rao, Chakraborty, Kaushik, Pal-Kaur, & Swami-Hulle, 2014).

The behavior of all systems under high pressure is governed by LeChâtelier's principle, which predicts that the application of pressure shifts an equilibrium towards the state that occupies a smaller volume, and accelerates processes for which the transition state has a smaller volume than the ground state (Smeller, 2002). Pressure is generated by compressing any food-grade fluid. In most current cold applications, pressure-transmitting medium is simply purified water mixed with a small percentage of soluble oil for lubrication and anticorrosion purposes. Other fluids used are aqueous solution of mono-propylene glycol for high temperature and isopropyl alcohol for low temperature treatments (Srinvasa-Rao et al., 2014).

Energy input during the high-pressure process is very small compared to thermal processes. Therefore, chemical reactions involving covalent bonds remain unaffected.

Pressurization is accompanied by a uniform temperature increase through adiabatic heating. Each material has its own specific heat of compression values, independently on the food geometry or size; in water is approximately 3 ºC/100 MPa and in fats and oils is around 6 – 8 ºC/100MPa (Balasubramanian & Balasubramaniam, 2003). For that reason, temperature increasing during the pressurization is wholly dependent on the composition of the food (Smelt, 1998; Wilson, Dabrowski, Stringer, Moezelaar, & Brocklehurst, 2008).

HHP requires airtight and flexible packages that can withstand a change in volume corresponding to the compressibility of the product (Hugas, Garriga, & Monfort, 2002). Plastic films like EVOH and PVOH are commonly used in HHP treatments, although they are not compatible with high temperatures. The use of semi-rigid trays is also possible. Vacuum-packaged products work perfectly for been treated with this technology (Srinvasa-Rao et al., 2014).

3.2. Historical background

It was first reported by Hite (1899) who treated milk at 670 MPa for 10 min and detected a 5 – 6 log-cycle reduction in total counts. Since then, its efficacy has been demonstrated several times in a wide variety of food products, not only against spoilage bacteria (Gervilla, Capellas, Ferragut, & Guamis, 1997; Rodríguez-Calleja et al., 2012; Wilson et al., 2008) but on the improvement of food properties and production processes (Juan, Trujillo, Guamis, Buffa, & Ferragut, 2007; Ma, Ledward, Zamri, Frazier, & Zhou, 2007; Oey, Lille, Van Loey, & Hendrickx, 2008; Saldo, McSweeney, Sendra, Kelly, & Guamis, 2002; Trujillo et al., 2000).

The first high-pressure-processed foods were introduced to the Japanese market in 1990 by Medidi-ya, marketing a line of jams, jellies and sauces packaged and processed without heat application (Thakur & Nelson, 1998).

At present, thanks to technological advances in equipment technology, industrial applications are widespread for a range of pressures between 100 and 1000 MPa, depending on the desired objective (Rendueles et al., 2011). Recently, HHP systems are available for treatments up to 1400 MPa, although the volume of the sample that can be treated on them is very small (up to 30 mL) (Srinvasa-Rao et al., 2014).

Currently the use of HHP embraces a wide range of foods, such as fruit juices, rice cakes, raw squid, guacamole, oysters and crustaceans. HHP is very effective for shucking raw meat from the rigid shell of crustaceans and mollusks without cooking them.

3.3. Effect of HHP on the inactivation of microorganisms

One of the principal advantages of the HHP process is the expanded shelf-life and improvement of food safety due to the inactivation of microbial population. At refrigeration, ambient or moderate heating temperature, HHP allows inactivation of pathogenic and spoilage microorganisms in foods with fewer changes in texture, color and flavor as compared to conventional technologies (Cheftel, 1995; Knorr, 1993; Velazquez, Gandhi, & Torres, 2002).

When submitted to increasing hydrostatic pressure, organisms experience the failure of several of their cellular functions: loss of membrane fluidity, which leads to reduced transmembrane transport and loss of flagellar motility; loss of protein and nucleic acid synthesis; loss of enzyme function and metabolism; and alterations in cellular architecture, etc. which eventually leads to cell death (Oger & Jebbar, 2010).

The action of HHP for microbial inactivation is based on protein denaturation, which results in enzyme inactivation (Barbosa-Canovas, Pothakamury, & Swanson, 1995), and the agglomeration of cellular proteins (Farr, 1990). After a pressure treatment, the fatty acids of the cell membrane phospholipids seems to suffer crystallization, which provokes changes on the permeability of the membrane (Cheftel & Culioli, 1997), and as a consequence, its destabilization (Hazel & Williams, 1990; Shimada et al., 1993).

Although microorganism inactivation depends primarily on the pressure applied, the holding time and the treatment temperature, external factors of the product like pH, salt content, water activity and the presence of other ingredients, such as sugars, also influence on the cell inactivation (Bajovic, Bolumar, & Heinz, 2012). A great number of factors related to the Gram type, physiological state and strain particularities also have an important influence on the microorganism inactivation (Jofré, Aymerich, Bover-Cid, & Garriga, 2010), as bacterial resistance to high pressure is highly variable even among strains of the same species (Liu, Betti, & Gänzle, 2012). The literature reports that the pressure resistance of prokaryotes exceeds that of eukaryotes; that of Gram-positive bacteria exceed that of Gram-negative bacteria; and that of cocci exceeds that of bacilli (Huang, Lung, Yang, & Wang, 2014). Pressure can also impair sublethal injury to the cell. However, spores have shown great resistance to pressure inactivation.

For most forms of vegetative bacteria, significant reductions (usually higher than 4 log units) in the population are achieved when 400 – 600 MPa at room temperature are used. However, because of the complexity of the reactions that can take place in a biological system, it is difficult to predict the effect of high pressure on any particular bacterial population (Bièche et al., 2012).

The microbial susceptibility to HHP inactivation is clearly influenced by the conditions of the environment where microorganisms are present. Therefore, the results obtained in model systems using artificial substrates, such as broths or agars, cannot be directly compared to “real” food (Claeys, Van Loey, & Hendrickx, 2003).

The chemical composition of the food is also important, since the presence of fats, proteins, minerals and sugars serves as a protector and increases microbial resistance to pressure (Black, Huppertz, Fitzgerald, & Kelly, 2007; Hauben, Bernaerts, & Michiels, 1998; Molina-Hoppner, Doster, Vogel, & Ganzle, 2004). Therefore, nutrient-rich foods, such as milk or poultry meat, can protect microorganisms from high pressure treatments (Patterson, Quinn, Simpson, & Gilmour, 1995). Food pH also influences on microbial resistance. As it has been demonstrated, susceptibility to pressure increases visibly as pH deviates from neutral values (Alpas et al., 1999), and some microorganismsseems to gain resistance to pressure if they have grown on acidic pH’s (Wouters, Glaasker, & Smelt, 1998). The efficacy of HHP

processing decreases with reduced water activity (a_w), and it is clearly noticed in foods with values below 0.9 (Black et al., 2007; Hayman, Kouassi, Anantheswaran, Floros, & Knabel, 2008; Patterson, 2005).

It has been widely reported the significant extension of shelf-life of the HHP-processed foods. Nonetheless, these foods are usually non-sterile and therefore must be refrigerated to maintain their sensory characteristics and microbiological stability. In these circumstances, evolution of survivors or recontaminants (particularly psychrotrophic pathogens) over the shelf-life period should be studied. Cells with sublethal damage, under appropriate conditions (nutrient-rich substrates, appropriate temperature and storage time), can be resuscitated (Bozoglu, Alpas, & Kaletunç, 2004) and psychrotrophs such as *Listeria monocytogenes* can constitute an important risk (Ritz, Pilet, Jugiau, Rama, & Federighi, 2006). The microbiological analyses performed to HHP foods should consider the possible presence of sublethal injured microorganisms, whose recovery requires the use of methodologies and non-selective culture media, rich in nutrients and incubated at a temperature and for sufficient time to permit the repair of damage (Sagarzazu, Cebrián, Condón, Mackey, & Mañas, 2010; Yuste, Capellas, Fung, & Mor-Mur, 2004).

A critical control point in the food industry is the handling of certain products after HHP treatment; improper handling can lead to the product becoming re-contaminated before packaging (Srinvasa-Rao et al., 2014).

3.4. Effect of HHP on enzyme activity

It is well known that high pressure has a significant impact on the activity and stability of enzymes. However, enzyme inactivation by HHP and temperature are not comparable to each other.

The mechanism of inactivation of enzymes by high pressure can be described in terms of protein denaturation (Ludikhuyze, Van-Loey, Denys, & Hendrickx, 2010). In general, the primary structure of the enzyme remains unaffected by the application of high pressure. However, tertiary and quaternary structure suffers the action of pressure, and their electrostatic and hydrophobic interactions as well as hydrogen bonding results affected. The secondary structure may be affected at higher pressures above 700 MPa. Nevertheless, a complete inactivation of specific enzymes may require thermal assistance along with high pressure application (Srinvasa-Rao et al., 2014).

3.5. HHP on meat products

High pressure processing is being increasingly used by the meat industry as a post-processing technology to extend the shelf life and to improve the safety of ready-to-eat meat

products. However, pressure can also affect some other important product qualities, such as tenderness, color and lipid oxidation (Cheftel & Culioli, 1997; Ma & Ledward, 2013), and consequently application of high pressure to raw meat has not been considered appropriate as an industrial practice (Marcos, Kerry, & Mullen, 2010). In addition, changes on the aspect of the meat may influence on the consumers, which would possibly do not recognize it as fresh meat.

Under EU regulation "fresh meat" has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere (European Parliament and the Council of the European Union, 2004). Overall, the color change induced by HHP in the meat as well as the current legal definition of fresh meat has drastically limited the use of HHP for fresh meat in the markets. Nowadays HHP is commercially used mainly as a non-thermal decontamination technology for processed (and usually cooked) and ready-to-eat meat products with high consumer acceptance, in comparison to other non-thermal decontamination technologies such as ionizing radiation. This fact is leading to an increased number of commercial installations (Bajovic et al., 2012).

HHP treatments of a moderate level of pressure (10 – 100 MPa) decreases the rate of growth and reproduction of meat microorganisms, whereas greater pressures (300 – 600 MPa) can inactivate spoilage microorganisms in or on meat. Depending upon the intensity of the pressure-heat treatment, both pasteurization and sterilization effects are possible (Nguyen et al., 2009).

As mentioned before, HHP is mainly used in a commercial environment as an effective post-packaging decontamination technology for ready-to-eat meat products and more particularly in cases where heat treatment is not possible or convenient. Pressure levels applied for the pasteurization of meats and meat products range in an area of 400 – 600 MPa with short processing times of 3 – 7 minutes and at room temperature. These treatments lead in most cases to an inactivation of more than four log units for the most common vegetative pathogenic and spoilage microorganisms resulting in an increased shelf-life and improved safety (Bajovic et al., 2012).

Besides its effect over the microbial population, high pressure also has technological advantages over conventional processes, when are used to produce further-processed products (Linton, McClemens, & Patterson, 2004). On meat products, HHP induces mechanical tissue disintegration and therefore tenderize the meat (Sun & Holley, 2010).

3.5.1. Effect of HHP on meat proteins and texture

The application of pressure on proteins affects their structure which is modified at different degrees depending on many factors such as treatment conditions and protein nature. Proteins and, in particular, multimeric protein structures, are amongst the most pressure-sensitive macromolecules in the cell. Similarly to lipids, the protein adapts to volume restrictions upon compression by changing their conformation (Oger & Jebbar, 2010).

As a general mechanism, the application of pressure induces unfolding of the protein structure and subsequent folding after pressure release. Many proteins do not recover its original tertiary structure after a pressure cycle. This can lead depending on the specific protein and conditions applied to partial or total denaturation and tuning of electrostatic interaction. Consequently the meat becomes more gel-structured and paler, losing the typical appearance of fresh meat (Bajovic et al., 2012).

Muscle proteins, including myofibrillar proteins, are unfolded up to a pressure of 300 MPa. Pressures above this level result in increased denaturation, gel formation and agglomeration of proteins. This fact can be employed in product development since enhanced gel structure and water binding capacity can be achieved by the use of certain HHP treatments (Sun & Holley, 2010).

HHP has been used successfully to improve meat tenderness. In general, low pressures (<200 MPa) can tenderize pre-rigor meat, whereas tenderization post-rigor with HHP can only be achieved combining high pressure with certain temperatures (Sun & Holley, 2010). In fresh meat, the application of low pressure levels improved the functional and rheological properties of turkey meat with low pH or pale soft exudative (PSE) meat (Chan, Omana, & Betti, 2011). Yuste et al. (1999) found that sausages made with pressure-treated poultry meat had a better texture and were juicier after cooking than those produced using untreated meat. Also Ruiz-Capillas et al. (2007) found similar results when high-pressure processing of frankfurters caused a significant decrease in hardness and chewiness in comparison with the non-pressurized samples.

Moreover the application of HHP can be used to improve the WHC of raw meat used for the production of meat products and as a result, in the development of products with reduced salt content (Chan et al., 2011). Sikes, Tobin, and Tume (2009) made use of high pressure to reduce the cook loss and to improve the texture of low-salt beef sausage batters.

3.5.2. Effect of HHP on lipid oxidation of meat

Lipid oxidation is one of the main factors affecting processed meat quality. Although the mechanisms by which HHP induces lipid oxidation on meats are not fully demonstrated, it is believed that the disruption of the membrane caused by high pressure facilitates the contact between unsaturated lipids from the membrane and enzymes, and heme and non-heme iron or other metal cations likely contributing to catalyze lipid oxidation (Bajovic et al., 2012). Lipid oxidation due to HHP has been also attributed to pressure induced protein denaturation, which leads to the release of free-radicals catalyzing oxidative processes (Cheftel & Culioli, 1997)

The type of meat that is treated also influences on the oxidation rate. In HHP treatments in a temperature range of 20 - 40 ºC, beef seems to be less stable than pork, and chicken is the most stable of those so far studied (Ma et al., 2007).

Studying the effect of high pressure and temperature on beef qualities, McArdle et al. (2010) conclude that high pressures decreased oxidative stability when meat was treated at

300 and 400 MPa, obtaining significant interactions between pressure and temperature. They also suggest that at lower pressure levels, increases in the pressurization temperature promoted lipid oxidation, while higher pressure levels induce stronger oxidation independently of the processing temperatures. When Kruk et al. (2011) studied the effect of HHP on chicken breasts they found that pressures of 300 MPa or below did not show significant differences compared to the control. However, the intensity of oxidation did increase with pressures above 300 MPa. Some authors have stated that 500 MPa is the critical pressure value that initiates lipid oxidation of chicken breasts (Orlien, Hansen, & Skibsted, 2000), although significant oxidative levels have also been found at 450 MPa (Kruk et al., 2011).

3.5.3. Effect of HHP on meat color

The color of meat depends on the optical properties of the meat surface as well as on the myoglobin content of the muscle. In contrast, the color of cured meat products is mainly created due to the presence of nitrosylmyoglobin, resulting from the reaction of nitric oxide (from sodium nitrite or sodium nitrate) with myoglobin. Color alterations produced by high pressure on fresh meat are the main reason why this technology is not widely used to preserve these types of products.

For fresh meat, poultry and derived products, pressure-induced color modifications greatly depend on treatment conditions (pressure, time and temperature), and are originated to changes in heme pigments, primarily myoglobin, such as globin denaturation, heme displacement or release, and ferrous atom oxidation (Mor-Mur & Yuste, 2003). Lighter tones are due to protein coagulation caused by pressurization. Myosin is relatively sensitive to pressure and depending on the species will denature at around 180 – 300 MPa to give an opaque appearance, similar to that seen in cooked meat (K. H. Bak, Lindahl, Karlsson, & Orlien, 2012). Generally, increasing the pressure above 300 MPa increases the color lightness.

3.6. Combining HHP with other technologies

Despite all the advantages that HHP can offer to the food industry, the economic costs of the equipment needed to reach pressures up to 600 MPa or more, which are required for an efficient bacterial inactivation, have limited the commercial breakthrough of this technology. Owing to this, the use of other technologies in combination with HHP at lower pressures has been thoroughly studied over the last two decades. Recently, to reduce the needed inactivation pressure, various effective synergistic treatments have attracted much more attention, and several combined treatments have been investigated to optimize the processes and elucidate the mechanism of HHP treatment. These combined factors include antimicrobials, pH and moderate temperature (Amanatidou et al., 2000; Garcia-Graells, Valckx, & Michiels, 2000; Somolinos, García, Pagán, & Mackey, 2008; Wang et al., 2010).

The temperature of the product and pressure fluid can also affect microbial resistance, with larger inactivation rates obtained above or below the ambient temperature (Rendueles et al., 2011). The decrease in resistance to pressure at low temperatures may be due to changes in membrane structure and fluidity through weakening of hydrophobic interactions and crystallization of phospholipids (Cheftel, 1995). Moderate heating (40 – 60 ºC) can also enhance microbial inactivation by pressure, which in some cases makes application of lower pressure an option (Carlez, Rosec, Richard, & Cheftel, 1993).

The combined use of antimicrobial compounds (bacteriocins, organic acids and essential oils among many others) together with HHP has been tested in searching for synergistic effects (Garriga, Aymerich, Costa, Monfort, & Hugas, 2002; Kalchayanand, Sikes, Dunne, & Ray, 1994; Rodriguez, Arques, Nuñez, Gaya, & Medina, 2005). Antimicrobial packaging is a practical way of applying bacteriocins to meat products. Bacteriocins are added to the packaging materials and will be released to the meat surface during storage (Begonya Marcos, Aymerich, Monfort, & Garriga, 2008). There are many bacteriocin-producing lactic acid bacteria that can be found naturally in food or intentionally added as starter cultures in fermented foods. Nisin and lysozyme have been commonly used in combination with HHP to extend shelf-life of meats (Masschalck, Van-Houdt, Van-Haver, & Michiels, 2001; Yuste, Mor-Mur, Capellas, Guamis, & Pla, 1998).

Organic acids coated with the packaging material have also been used in combination with HHP. Rodríguez-Calleja et al. (2012) went further and combined HHP with organic acids and MAP to extend shelf-life of chicken breasts fillets stored at 4 ºC. They maintained the sensory and microbiological quality of the fillets for up to 28 days, compared to control and HHP or MAP treatments.

The synergistic effect between HHP and MAP, especially with CO_2, against certain enzymes and microorganisms has been previously reported. Corwin and Shellhammer (2002) tested how combining these technologies would affect the activity of pectin methylesterase (PME) and polyphenol oxidase (PPO) and also the destruction of *Lactobacillus plantarum* and *Escherichia coli*, using pressures greater than 500 MPa for enzymes and 365 MPa and 455 MPa for microorganisms, both inoculated in orange juice. Also Park et al. (2003) studied this synergistic effect by packaging broth inoculated with *Bacillus subtilis*, *Staphylococcus aureus*, *Fusarium oxysporum* and *Fusarium sporotrichioides*. However, in both studies, the concentration of CO_2 in the packages was rather low. Wang et al. (2010) measured the amount of dissolved CO_2 in the broth treated with HHP, which was an important step, as the concentration of CO_2 limits the synergistic action of the treatment. Amanatidou et al. (2000) combined a selected atmosphere (50% O_2 / 50% CO_2) with high pressure treatment (150 MPa 10 min) to extend the shelf-life of fresh Atlantic salmon. They found significant differences by pressure treating the samples before or after the MAP, since pressurized gases showed a greater effect inactivating microbial population.

Chapter 2

Statement of the problem, objectives and working plan

1. Statement of the problem

The use of modified atmosphere packaging technique to preserve food products has been increasingly used over the last 50 years, becoming one of the most important systems to preserve fresh products. However, the present procedure is not much different from the originally developed, which it makes an opportunity to improve and extend the use of MAP.

As it has been widely demonstrated, the use of CO_2 atmospheres at higher concentrations than 20 – 30% produces a significant effect against spoilage microorganisms' growth, thanks to the intracellular changes that this gas produces when it is dissolved in the water and lipid phase of the food.

However, when this happens, packages tend to collapse, provoking an undesirable appearance of the packages and the product within, which usually change its original, fresh and bright color to darker tones when it is touching the lid film.

This problem is especially significant in fresh meat products, where high concentrations of CO_2 are not applied to avoid package collapse although it would benefit meat preservation.

In order to avoid or reduce package collapse, a previous saturation with pure CO_2 before the definitive packaging has been suggested as a possible solution. However, it has not been completely studied yet for chicken meat, where gas mixtures and pack filling ratios influence significantly on the preservation of these types of products. This could be an additional application of modified atmospheres in order to enhance the traditional technique.

Another possible way to enhance the effectiveness of MAP would be by combining it with newer technologies, such as high pressure. HHP has shown a synergistic effect that affects significantly on the extension of the food shelf-life. However, searching on the published literature, there is not much research related to this synergistic effect studied in real food. As it has been reported, the intrinsic characteristics of the food, as well as the storage conditions, usually protect microorganisms against pressure treatment and also helps in the recovery of microorganisms that have not been inactivated by the pressure treatment. Therefore, a combination of MAP and HHP could improve the shelf-life and preservation of products like fresh chicken meat; although the study of the effects of both treatments on the quality parameters of fresh meat could be necessary to have a complete evaluation.

2. Objectives

The overall goal of this research was to improve chicken meat preservation by improving the application of MAP on these types of products, combining this technique with newer proceedings or technologies.

The specific objectives that come up from the general one are:

- Study the feasibility of saturate fresh chicken meat with pure CO_2 by means of the soluble gas stabilization (SGS) pre-treatment compared to a regular MAP in order to reduce package collapse and increase its shelf-life
- Use SGS to increase the filling of the packages of chicken fresh meat with the intention to improve packaging and product-transport efficiency
- Assess the effectiveness of combining CO_2 atmospheres and high-pressure treatments on the inactivation of spoilage and pathogenic bacteria
- Evaluate the combination of using high CO_2 atmospheres together with HHP to extend the shelf life of chicken fresh meat

3. Working plan

In order to find the answer to the objectives listed above, five stages were planned and carried.

Stage 1: Application of *Soluble Gas Stabilization* (SGS) pre-treatment on fresh chicken meat

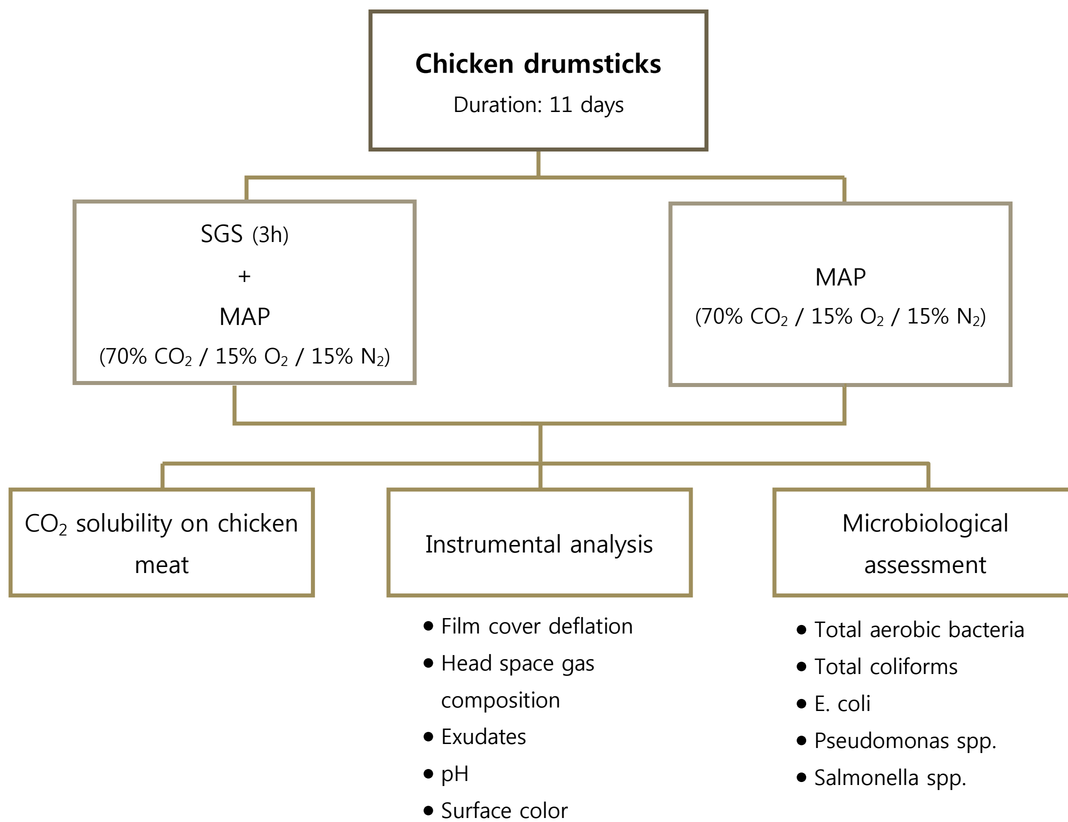

Figure 2.1. Experimental design of the experiment nº 1: Effect of high carbon dioxide atmosphere packaging and soluble gas stabilization pre-treatment on the shelf-life and quality of chicken drumsticks

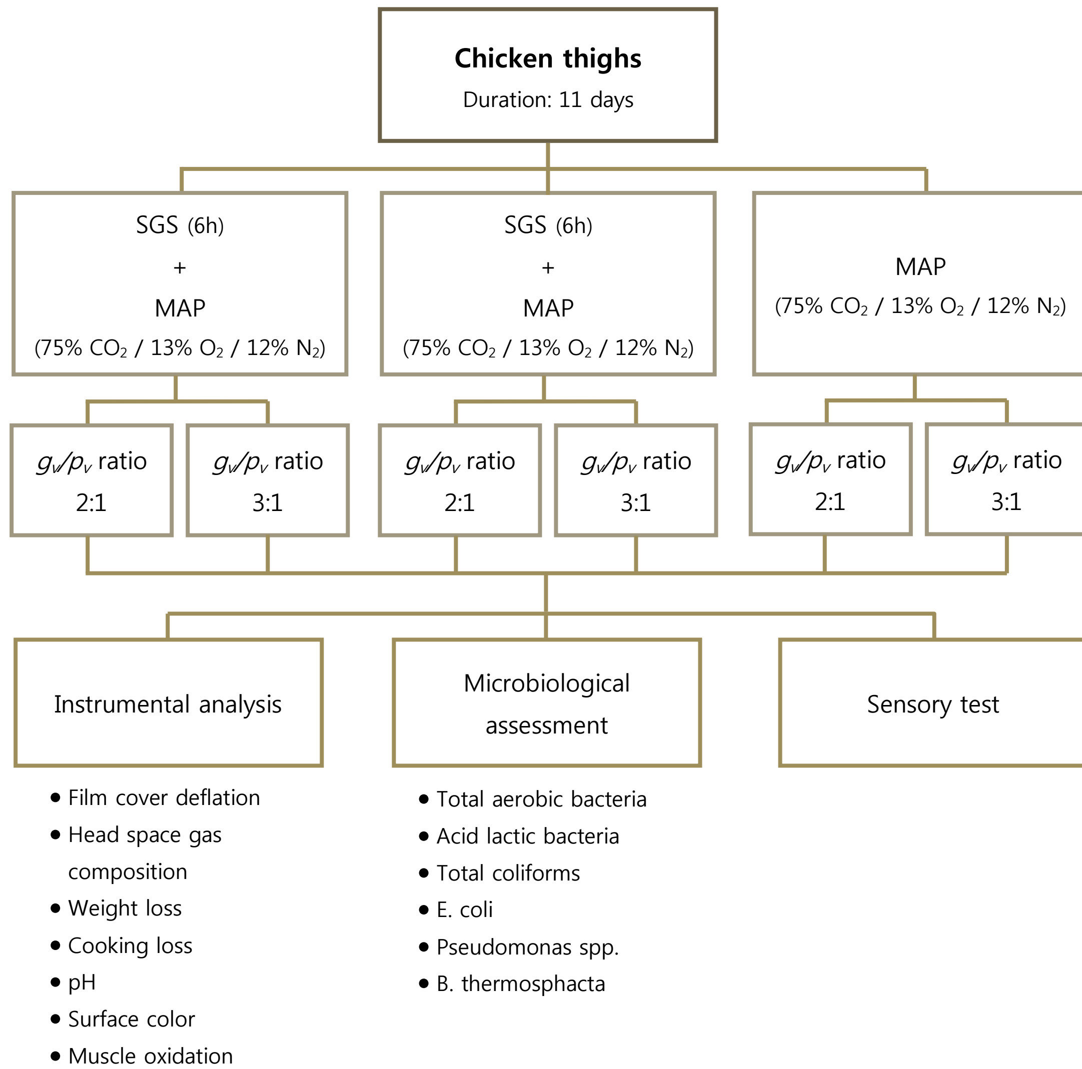

Figure 2.2. Experimental design of the experiment nº 2: Effect of combining carbon dioxide saturation treatments and pack - product filling in the chemical, microbiological and sensory properties of raw chicken thighs

Stage 2: Combination of MAP and HHP on chicken meat products

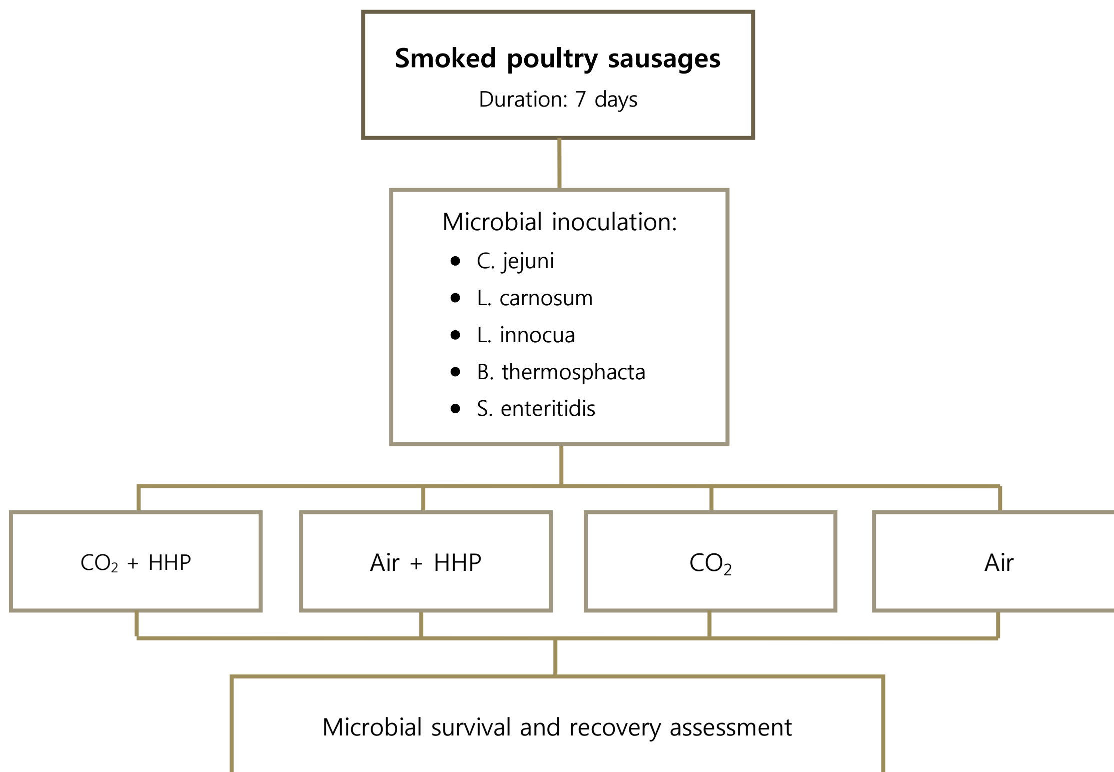

Figure 2.3. Experimental design of the experiment nº 3: Synergistic effect of carbon dioxide atmospheres and high hydrostatic pressure to reduce spoilage bacteria on poultry sausages

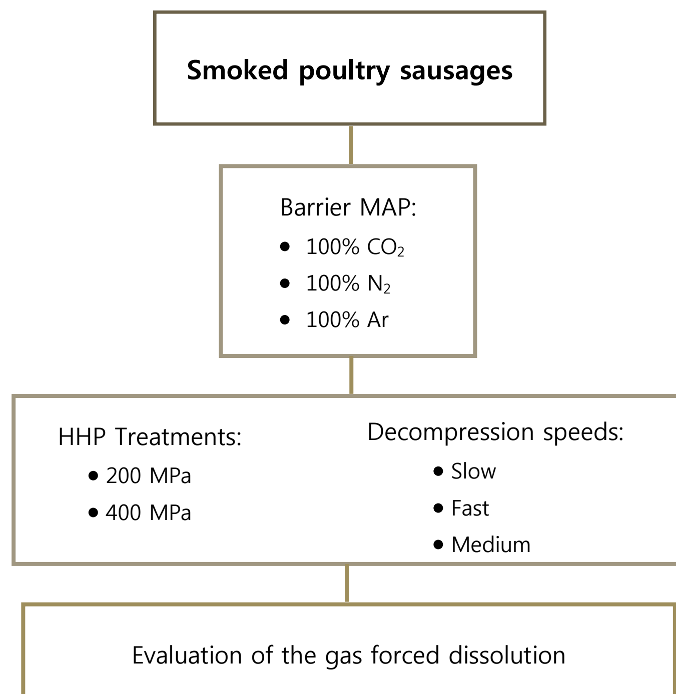

Figure 2.4. Experimental design of the experiment nº 4: Evaluation of the forced dissolution of different gases into poultry meat by high hydrostatic pressure

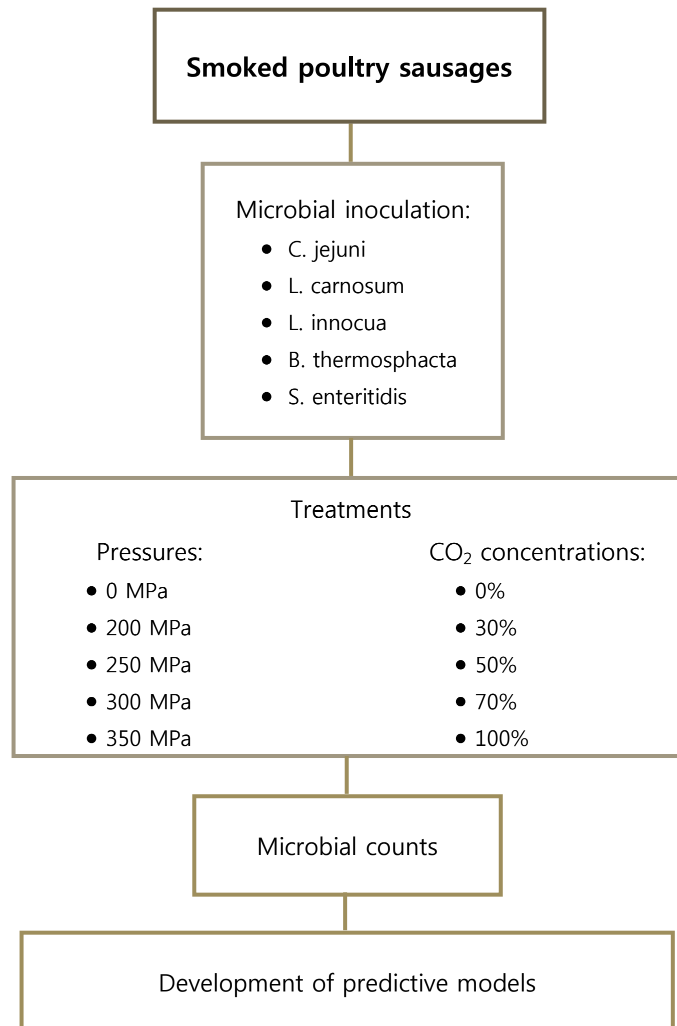

Figure 2.5. Experimental design of the experiment nº 5: Development of a response surface model to predict the synergistic effect of high hydrostatic pressure and modified atmosphere to reduce spoilage bacteria on poultry sausages

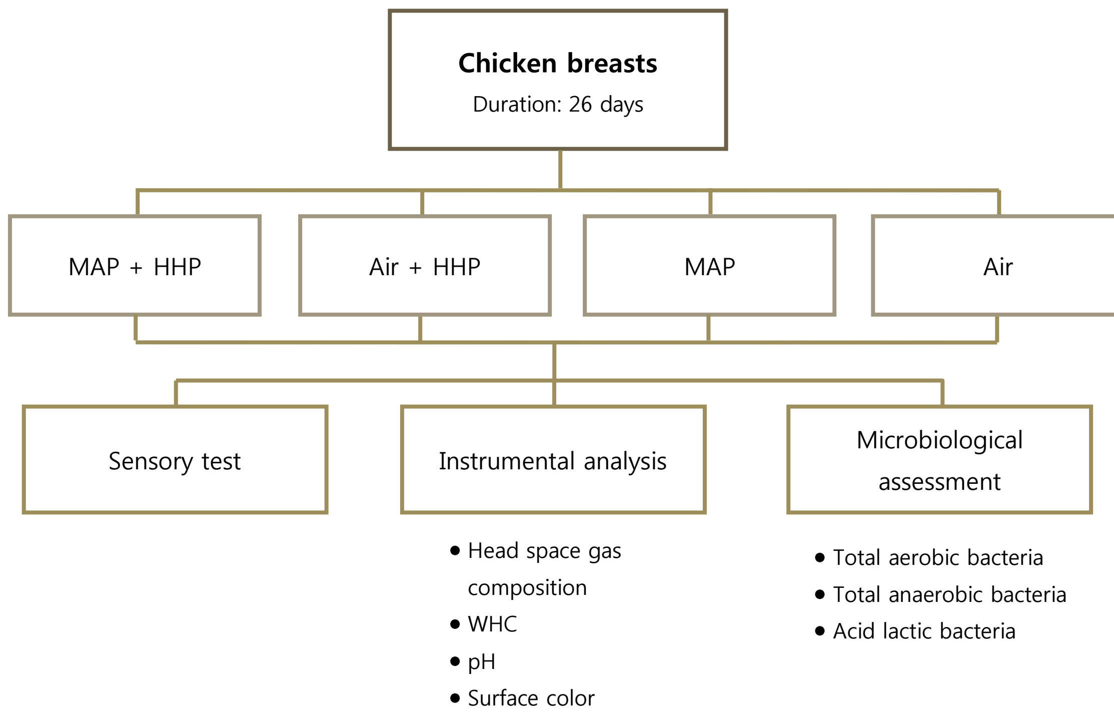

Figure 2.6. Experimental design of the experiment nº 6: Combined application of modified atmosphere packaging and high hydrostatic pressure on the shelf-life extension of fresh chicken breasts

Chapter 3

Application of Soluble Gas Stabilization pre-treatment on fresh chicken meat

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Effect of high carbon dioxide atmosphere packaging and soluble gas stabilization pre-treatment on the shelf-life and quality of chicken drumsticks

A. Al-Nehlawi [a,b], J. Saldo [b], L.F. Vega [a,c], S. Guri [a,c,*]

[a] MATGAS Research Center, Campus UAB, 08193 Bellaterra, Barcelona, Spain
[b] Animal and Food Science Department, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
[c] Carburos Metálicos, Air Products Group, C/Aragón, 300, 08009 Barcelona, Spain

Effect of high carbon dioxide atmosphere packaging and Soluble Gas Stabilization pre-treatment on the shelf-life and quality of chicken drumsticks

1. Introduction and objectives

Soluble Gas Stabilization (SGS) was first reported as an additional packaging treatment to MAP (Sivertsvik, 2000), consisting of a previous saturation of the food with pure CO_2, preserving it at refrigeration temperatures for a certain period of time. Some existing research has suggested that SGS has the potential to prevent package collapse, even at high filling ratios, without compromising the quality of the packaged food and bringing an opportunity to improve packaging efficiency. This treatment has been tested with different kinds of food products such as fish and seafood (Mendes et al., 2008, 2011; Rotabakk, Birkeland, et al., 2008; Sivertsvik & Birkeland, 2006) and also poultry (Rotabakk et al., 2006) but always using an anaerobic MAP mixture.

Going further with the SGS pre-treatment research, this work was undertaken to evaluate the effect of SGS pre-treatment on the shelf-life of raw chicken drumsticks with skin and packaged with a mixture of a high concentration of carbon dioxide and also oxygen and nitrogen. As the skin is one of the parts with higher counts of spoilage bacteria, an elevated percentage of CO_2 may help to control bacterial growth. However, the risk of package collapse increases drastically with higher concentrations of CO_2. For that reason, the second objective in the application of an SGS pre-treatment is to reduce the package collapse when the drumsticks are packaged with high concentrations of CO_2. From the results of this study, we hope to conclude that it is possible to reduce packaging volume, adapting the gas volume / product volume (*gv/pv*) ratio, reducing wastes and plastic materials without compromising the shelf-life of chicken drumsticks.

2. Material and methods

2.1. Chicken meat weight/volume relation

To determine the relationship between the volume of chicken meat and its weight, some portions of chicken, of known weight, were submerged in water, measuring the variation in liquid volume, to provide a calibration procedure for the relationship between volume and weight. To obtain this relationship, 45 portions of chicken with skin were used, and distributed in categories of drumsticks, wings, thighs and leg quarters.

2.2. Chicken packaging study

2.2.1. Samples preparation and experimental design

Fresh chicken drumsticks were obtained from a local slaughterhouse. They were quartered 20 hours after slaughtering and after the meat had achieved 4 °C after being stored in a chill chamber. The drumsticks were then transported in chilled conditions to the laboratory and stored under refrigeration at 3 ± 1 °C until packaging one hour after arrival at the laboratory. All the drumsticks were from young chickens, collected from the same batch at the plant, with all of them coming from the same farm.

Drumsticks were divided into two treatment groups, and one of them was previously treated with 100% CO_2 during 3 hours. Drumsticks were packaged in multilayer barrier bags (210 x 180 mm, 59 µm, with an oxygen transmission rate of 30 cm^3 m^{-2} 24 h^{-1} bar^{-1}, at 23 °C and 0% RH; CO_2 transmission rate of 150 cm^3 m^{-2} 24 h^{-1} bar^{-1}, at 23 °C and 0% RH; Cryovac - SealedAir, Spain), in which the air was evacuated with a compensated vacuum machine (Multivac Packaging Systems, Spain; mod. 100-A) and flushed with 100% food-grade CO_2 (S.E. Carburos Metálicos – Air Products Group). The g_v/p_v ratio inside the bags was 3:1, to ensure excess availability of CO_2. The initial atmosphere inside the bags immediately after sealing was 0.58 ± 0.3% O_2 / 98.9 ± 0.1 % CO_2 / 0.52 ± 0.2% N_2. These samples remained 3 hours under refrigeration (3 ± 1 °C).

After SGS pre-treatment, all the samples (pre-treated or not) were packaged under MAP conditions using a thermo-sealer trays machine (Belca Packaging, Spain; mod. Victoria), with polyethylene terephthalate (PET) semi-rigid trays (Linpac, Spain) and a PET barrier top web film (20 µm, with an oxygen transmission rate of 84 cm^3 m^{-2} 24 h^{-1} bar^{-1}, at 23 °C and 70% RH; and a water vapor transmission rate of 19 g m^{-2} 24 h^{-1}, at 38 °C and 90% RH; Dupont, USA). The air was evacuated and the gas mixture introduced (13.4 ± 0.1% O_2 / 69.2 ± 0.45% CO_2 / 17.4 ± 0.3% N_2), using food grade gases (S.E. Carburos Metálicos – Air Products Group) and mixed with a gas mixer (Witt-Gasetechnik GmbH & Co KG, Germany). The repackage of the SGS pre-treated samples was done in less than one minute, to prevent the CO_2 desorption. From now on, pre-

treated samples are referred as "SGS", and samples packaged directly in MAP conditions are referred as "MAP".

Each tray was stored at 3 ± 1 °C until sampling at day 1, 2, 4, 7 and 11. For each treatment and sampling day, three different trays were analyzed, each one being an independent replication. Each tray consisted of 4 or 5 drumsticks (470 – 500 g), with a g_v/p_v ratio of 3:2, a usual ratio for commercialized packages of this kind of product.

2.2.2. Film cover deflation

Usually, immediately after sealing, the film cover of a product packaged under high concentrations of CO_2 is flat or slightly convex. After packaging, CO_2 starts to dissolve into the product's water phase, resulting in either under-pressure (rigid packages) or volume contraction (flexible packages) (Sivertsvik, Jeksrud, et al., 2004). In the present case, with semi-rigid trays and a flexible film cover, the main effect was a volume contraction. To quantify this, the film cover deflation was measured at the top center of the packages using a caliper (0.01 mm accuracy) (Absolute Digmatic CD-15CPX, Mitutoyo Corporation, USA.) and reported as deflation (mm) relative to the sealing area. One measure for each tray was made, obtaining three measures for each treatment and sampling day.

2.2.3. Head space gas analysis

The head space gas composition was measured using an oxygen and carbon dioxide analyzer (MAPY 4.0, Witt-Gasetechnik GmbH & Co KG, Germany). An aliquot of the head space gas was removed using a syringe with a small diameter needle inserted into the MAP through a foam rubber septum attached to the top film, which helped prevent the mixing of air into the MAP. Oxygen and carbon dioxide values are presented as a percentage (%) of the atmosphere composition in absolute values. One measure for each tray was made, obtaining three measures for each treatment and sampling day.

2.2.4. Exudates formed

In time, chicken meat can begin to degrade causing proteins to lose their capacity to retain the water. In addition, when CO_2 is dissolved into the product, the meat pH decreases and approaches the proteins isoelectric point. When the pH reaches this point, the water retained inside the muscle is released as exudates.

The exudates formed in the trays during storage were measured gravimetrically for each replication (g), weighing all the liquid present in the package, and reported as a percentage (%) of the initial weight:

$$E = (100 \times E_w)/I_w \quad (3.1)$$

Where E are the exudates formed (%), E_w is the weight of the exudates (g) formed in each tray at sampling day and I_w is the weight of the drumsticks (g) measured at day 0.

2.2.5. pH measurement

For each replication, two measures of pH were done in different drumsticks from the same sample, using a pH-meter (Crison Basic 20+, Crison, Spain) and a penetration electrode (Crison 5232, Crison, Spain), obtaining six results for each treatment and sampling day, which were used for the statistical analysis.

2.2.6. Color assessment

The superficial color of the drumsticks was measured with a colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Japan), with illuminant D65, an aperture size of 8 millimeters and a 0º-viewing angle. In order to make representative measures of all the drumsticks, for each replication, eight measures were taken: two measures of the surface skin, which was directly in contact with the gas mixture, two measures of the skin that was at the bottom (with less contact with the atmosphere); two measures of the surface meat (without removing the skin) and two measures of the bottom meat. The parameters assessed were brightness (L^*), the balance between green and red (a^*) and the balance between blue and yellow (b^*). Chroma (C^*) is the value that measures the relative purity or saturation of a color, and was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$. The hue angle (ºHue) was calculated as ºHue = $\tan^{-1}(b^*/a^*)$. The statistical treatment was made separately for meat and skin fraction.

2.2.7. Microbiological analysis

Samples for the microbiological assessment of the product were analyzed on days 0, 4, 7 and 11. At each sampling day, three independent trays for each treatment were analyzed, performing Total Aerobic Bacteria (TAB), *Pseudomonas* spp, total coliform and *Escherichia coli*. Presence of *Salmonella* spp was investigated, also in triplicate, for each treatment at the initial control (day 0) and at the end of storage (day 11).

Immediately after opening the packages aseptically, 10 g of each sample, mixing skin and meat from the surface and the bottom in an approximate proportion of 1:1, so as to have a representative sample, were placed in stomacher bags with filter (GSI Creos, Japan). Afterward, 90 mL of 0.1% sterile peptone water was added (Oxoid, England) and was homogenized in a Pulsifier PUL 100E (Microgen Bioproducts Ltd, England) for 18 seconds at room temperature. Appropriate dilutions were carried in order to spread them on the appropriate media. For Total Aerobic Counts (TAC), 1 mL of appropriate dilutions was pour-plate inoculated in plate count agar (PCA; Oxoid, England), and incubated at 30 ºC for 48 h. *E. coli* and total coliforms were determined pour-plate spreading the appropriate dilution on a chromogenic selective media

(ChromoCult Coliform Agar; Merck KGaA, Germany) and incubating the plates at 37 °C for 24 h; *E. coli* colonies were dark-blue or dark-violet, while the rest of coliforms were red or salmon. Finally, *Pseudomonas* spp. counts were determined on a selective agar (Pseudomonas Agar Base with CFS supplement; both Oxoid, England) by spreading 0.1 mL of the dilution and incubating the plates at 25 °C and examining them at 24 h and 48 h. *Salmonella* spp. was analyzed by standard culture methods according to ISO norm 6579:2002 applied to *Salmonella* detection in food and animal feeding stuffs. Briefly, 25 g of sample diluted in peptone water were incubated at 37 °C for 18 h; afterwards 0.1 mL was inoculated in Rappaport-Vassiliadis medium with soya (Biomerieux, France), at 42 °C for 24 h, and 1 mL was inoculated in Muller-Kauffmann tetrathionate/novobiocin broth (Biomerieux, France), at 37 °C during 24 h. After that, one loop of each selective enrichment broth was streaked onto the surface of two selective solid media: XLD (Xylose lysine deoxycholate agar; Oxoid, England) and SM-ID2 (Biomerieux, France), both incubated at 37 °C for 24 h. Colonies of presumptive *Salmonella* were confirmed by biochemical test API 20E (Biomerieux, France), and identified (apiweb).

2.2.8. Statistical analysis

The experiment was conducted in triplicate (three independent packages per treatment and sampling day). Two-way analysis of variance (ANOVA) and general linear model (GLM) procedures were performed by means of SAS 9.2 (SAS Institute INC., U.S.A. – Enterprise Guide 4.2.), between the main factors "treatment" and "sampling day". When there was statistical significance ($P < 0.05$), both main factors and the interaction between them, Last Square Means (LSM) procedure was used, applying the Tukey-Kramer’s test to determine the significant differences among the averages.

2.3. CO_2 solubility in chicken meat

The amount of CO_2 dissolved per kilogram of chicken drumsticks was approximately calculated using the initial gas concentration, the CO_2 concentration at sampling time, and the trays and product volumes. The equation used was:

$$\boldsymbol{C_{CO_2}} = \frac{\left(\boldsymbol{C^i_{CO_2} \cdot V^i_{HS} - C^f_{CO_2} \cdot V^f_{HS}}\right) \cdot \boldsymbol{Mw_{CO_2}}}{\boldsymbol{W_c \cdot Mv_{CO_2}}} \tag{3.2}$$

Where C_{CO_2} is the concentration of CO_2 dissolved into the chicken meat (mg CO_2 kg^{-1} chicken meat); $C^i_{CO_2}$ is the initial concentration of CO_2 in the head space (% v/v); V^i_{HS} is the initial head space volume (mL), obtained as the difference between the volume capacity of the tray and the volume of the chicken introduced on it, calculated using equation (3.3); $C^f_{CO_2}$ is the final concentration of CO_2 in the head space (% v/v); V^f_{HS} is the final head space volume (mL)

obtained as the initial head space volume minus the collapsed volume of the film cover; Mw_{CO_2} is the molecular weight of CO_2 (g mol^{-1}); W_c is the chicken meat weight (kg) and Mv_{CO_2} is the molar volume of CO_2 (L mol^{-1}).

3. Results and discussion

3.1. Chicken meat weight/volume relation

With the experimental results obtained and applying a linear regression adjustment, the equation that relates the chicken weight with its volume is:

$$\boldsymbol{y = 0.9591x \qquad R^2 = 0.996} \tag{3.3}$$

Where y is the volume (mL) and x is the weight (g).

With this equation, the quantity of chicken needed was determined in order to obtain the desired ratio g_v/p_v inside the packages in the study of SGS (data not shown).

3.2. Chicken packaging study

3.2.1. Film cover deflation

In both treatments (MAP and SGS), the deflation of the film cover increased with increasing storage time (Figure 3.4). However, there were significant differences (P<0.05) between the treatments, showing the SGS treatment less collapse than MAP packages. The collapse of SGS samples on day 8 and 11 was significantly equivalent to the collapse of MAP samples on day 4, which is an important improvement with regard to package collapse.

These results are in agreement with those obtained by Rotabakk et al. (2006), who studied the package collapse with chicken breast fillets using different SGS application times and also comparing with a MAP treatment. They found that the deflation of the film cover significantly decreases with increasing time of SGS treatment when compared to MAP treatment.

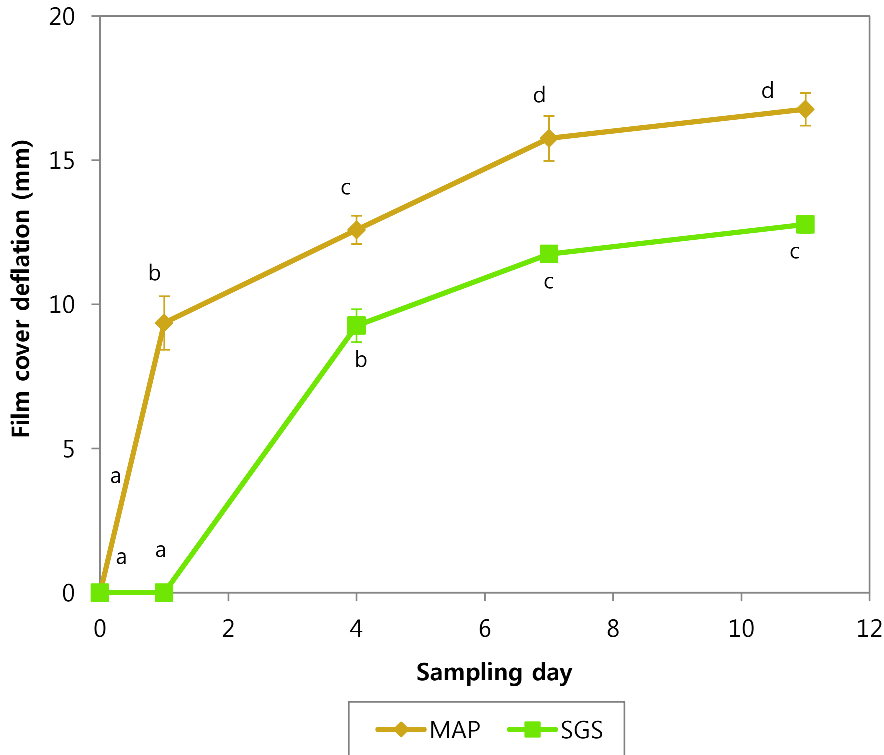


Figure 3.4. Evolution of the film cover deflation on trays with chicken drumsticks packaged under modified atmosphere conditions (70% CO_2/15% O_2/15% N_2) and pre-treated or not with SGS, stored for 11 days at 3 ± 1 ºC. Measures correspond to the average of three values ± standard error, and different superscript letters indicate significantly different values for the interaction between the treatment and the sampling day (P<0.05, Tukey's test).

3.2.2. Head space gas composition

Significantly higher CO_2 concentrations were observed in SGS drumsticks (54.8 - 58.7%) during chill storage (Figure 3.5-B), when compared with the MAP samples (49.9 - 53.2%) possibly because the drumsticks had already an amount of dissolved CO_2 in their tissue from the SGS pre-treatment. However, the decrease (almost a 13%) of the CO_2 in SGS samples during the chilled storage in MAP conditions means that the meat was not completely saturated with carbon dioxide, and therefore it is unlikely that the SGS pre-treatment time was long enough.

The most significant decrease of CO_2 in the head space was observed during the first 24 hours of storage. From that moment, the concentration of CO_2 remained almost constant until the end of the study in both MAP and SGS samples, reaching the equilibrium of the dissolved CO_2 and the atmospheric CO_2 before the first 48 h storage.

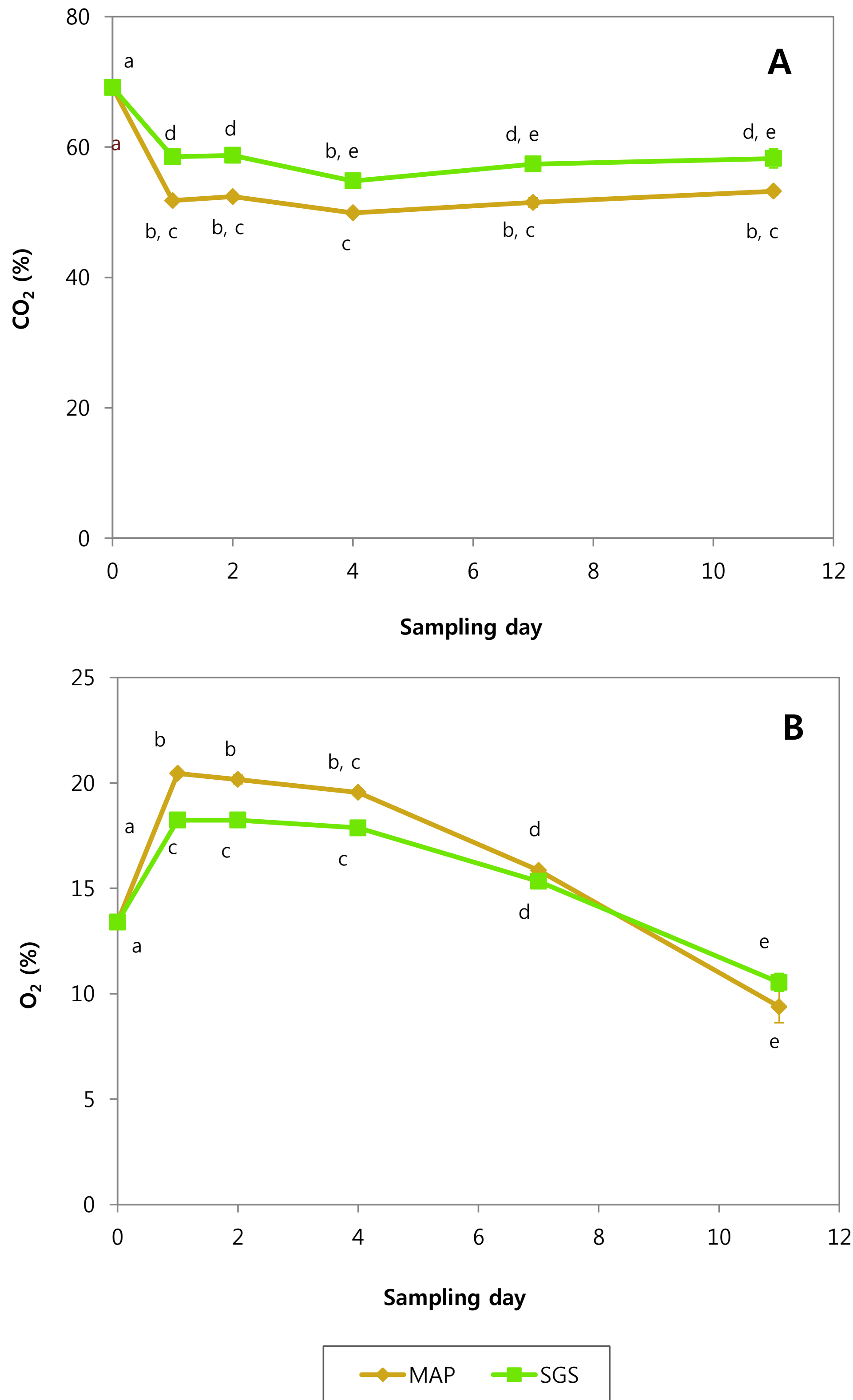


Figure 3.5. Evolution of carbon dioxide (A) and oxygen (B) percentages at the head space of trays with chicken drumsticks packaged under modified atmosphere conditions (70% CO_2/15% O_2/15% N_2) and pre-treated or not with SGS, stored for 11 days at 3 ± 1 ºC. Measures correspond to the average of three values ± standard error, and different superscript letters indicate significantly different values for the interaction between the treatment and the sampling day (P<0.05, Tukey's test).

Similar results were found by other authors; Rotabakk et al. (2006) packaged chicken breast fillets with a 60% of CO_2 balanced with N_2, obtaining higher levels of CO_2 in the head space of SGS samples than in MAP samples, after storing the SGS samples for 2 h with pure CO_2. Mendes et al. (2008) also found similar results when packaging SGS pre-treated sardine fillets with MAP (60% N_2 / 35% CO_2 / 5% O_2), where the samples presented higher values (11 – 17%) of CO_2 than samples packaged directly with MAP once the equilibrium was attained. Sivertsvik and Birkeland (2006) applied an SGS pre-treatment of 2 h to ready-to-eat shrimps, packaging then with two different concentrations of CO_2 (30% and 60%) balanced with N_2. They found that the greater the amount of CO_2 used, the faster the equilibrium is reached. With the results obtained they concluded that applying SGS together with MAP with a CO_2 concentration equal or higher than 60%, a 40 – 45% of packages volume could be reduced, with the same amount of product in it and without compromising its quality and safety. Other studies in which SGS was applied in fish (Rotabakk, Birkeland, et al., 2008) also agree with these results and confirm that the dissolution of CO_2 in the food product depends on SGS treatment time and on the partial pressure of CO_2.

With respect to oxygen, no significant differences in the amount of O_2 in the head space were observed at the end of the study between treatments (Figure 3.5-A). The increase in percentage oxygen measured at day 1 is caused by a drop in the molar concentration of CO_2 in the head space, which is relatively compensated with the increase in the percentage of O_2, but not with an increase in molar quantity. This occurs when the pressure inside the packages decreases as a consequence of the CO_2 dissolution into the drumsticks during the first 24 hours after packaging. The reduction in the amount of O_2 over the following days is likely a result of the increasing microbial activity in the meat. These results are similar to those obtained by Rotabakk et al. (2006); however, they associated the increase in the amount of O_2 to the permeation of the packaging material.

3.2.3. Exudates formed

It is documented that raising the quantity of CO_2 in the head space could produce an increase in the weight losses and exudates due to pH drop (Stiles, 1991). However, no significant differences in the exudates formed were observed among SGS pre-treated samples with respect to directly packaged MAP samples (Figure 3.6). Similar results were found in a previous study (Al-Nehlawi & Guri, 2010) where neither chicken breast fillets nor drumsticks presented significant differences on exudates formed between SGS pre-treated meat and direct packaged MAP meat. Rotabakk et al. (2006) also did not find significant differences in their results with chicken breasts.

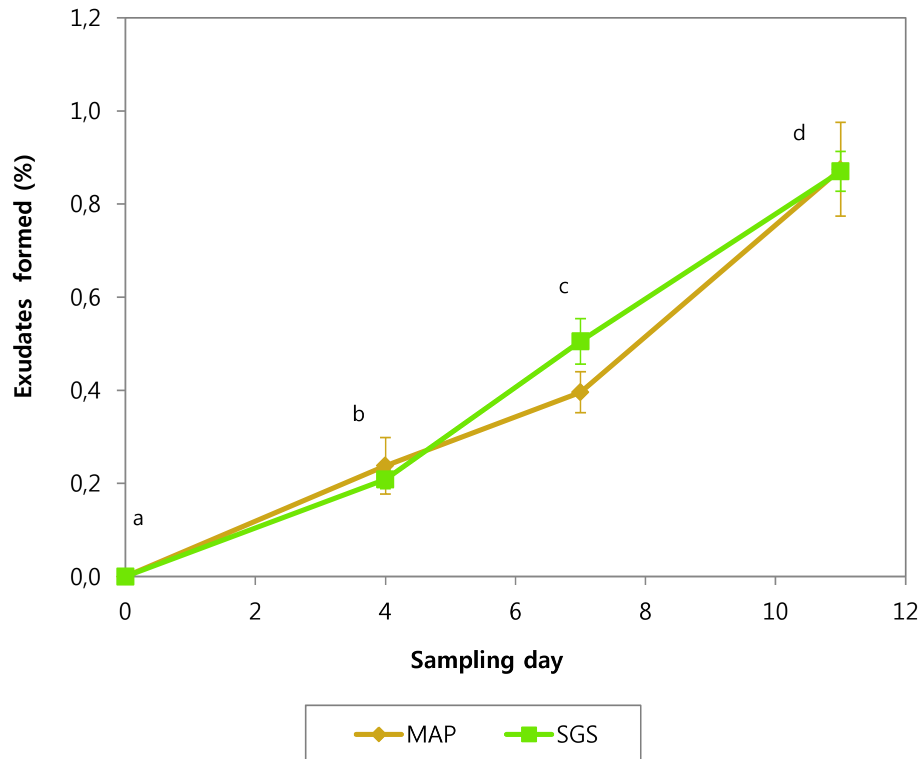


Figure 3.6. Effect of the packaging treatment on the exudates formed in samples of chicken drumsticks packaged under modified atmosphere conditions (70% CO_2/15% O_2/15% N_2) pre-treated or not with SGS, stored for 11 days at 3 ± 1 ºC. Measures correspond to the average of three values ± standard error, and different superscript letters indicate significantly different values for the interaction between the treatment and the sampling day ($P<0.05$, Tukey's test).

3.2.4. pH assessment

Drumsticks pH decreased throughout the study, being more acidic in SGS samples than MAP samples (Figure 3.7). This was probably caused by the greater amount of CO_2 dissolved, which increases the amount of carbonic acid, and consequently the amount of protons (H^+) (Bruce, Wolfe, Jones, & Price, 1996; Dixon & Kell, 1989). Statistical analysis showed significant differences between the treatments during the storage time.

The pH decrease caused by CO_2 dissolution is the main factor that contributes to the preservation of food due to its effect on the metabolism of microorganisms (Floros & Matsos, 2005). In other published studies related to preservation and shelf-life extension in meat or fish by means of modified atmosphere packaging, a decrease in pH values was also observed throughout the storage time (G. Chen & Xiong, 2008; Torrieri, Cavella, Villani, & Masi, 2006; Vongsawasdi et al., 2008). However, until now no differences have been observed between SGS and MAP treatments related to pH variations (Al-Nehlawi & Guri, 2010; Rotabakk et al., 2006; Rotabakk, Birkeland, et al., 2008). pH also can be used as an indicator of chicken meat quality deterioration, as the metabolism of some microorganisms, like lactic acid bacteria, produces proteolytic enzymes which generate basic nitrogen compounds responsible for an increase in the pH values (Mendes et al., 2011; Rodriguez-Aguilera, Oliveira, Montanez, & Mahajan, 2010).

The pH stabilization of the MAP samples can be explained considering the fact that the meat tissue has some buffering capacity (Bendall, 1972).

The pH also has an important influence on the solubility of CO_2. As Gill (1988) reported, the solubility of CO_2 in aqueous solution will increase with solution pH above 5.0, as an increasing fraction of the dissolved CO_2 will be present as HCO^{-3}. Therefore, considering the average pH of the chicken drumsticks (6.5), it is a parameter to take into consideration. Additionally, this study found that the solubility of CO_2 in beef muscle tissue at 0 ºC increased linearly with increasing tissue pH.

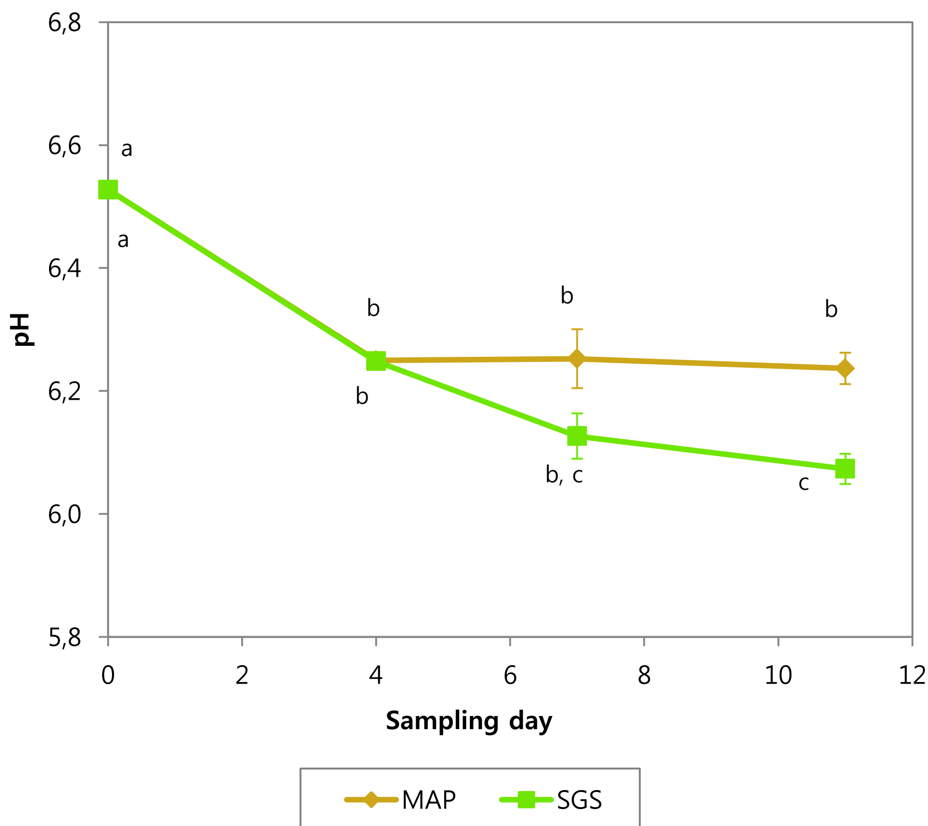


Figure 3.7. Evolution of the pH in chicken drumsticks packaged under modified atmosphere conditions (70% CO_2/15% O_2/15% N_2) and pre-treated or not with SGS, stored for 11 days at 3 ± 1 ºC. Measures correspond to the average of six values ± standard error, and different superscript letters indicate significantly different values for the interaction between the treatment and the sampling day ($P<0.05$, Tukey's test).

3.2.5. Surface color assessment

Regarding the surface color of the meat portion, the ºHue value resulted significant on the interaction between the treatment and the storage day, turning from a reddish to a greenish color (Figure 3.8-A). Although the other values determined did not have any significance on the interaction of the main factors, *a** value had showed significance on the storage day, turning to a greenish color (from 3.825 at day 0 to -1.959 in SGS samples and 0.997 in MAP samples at day 11).

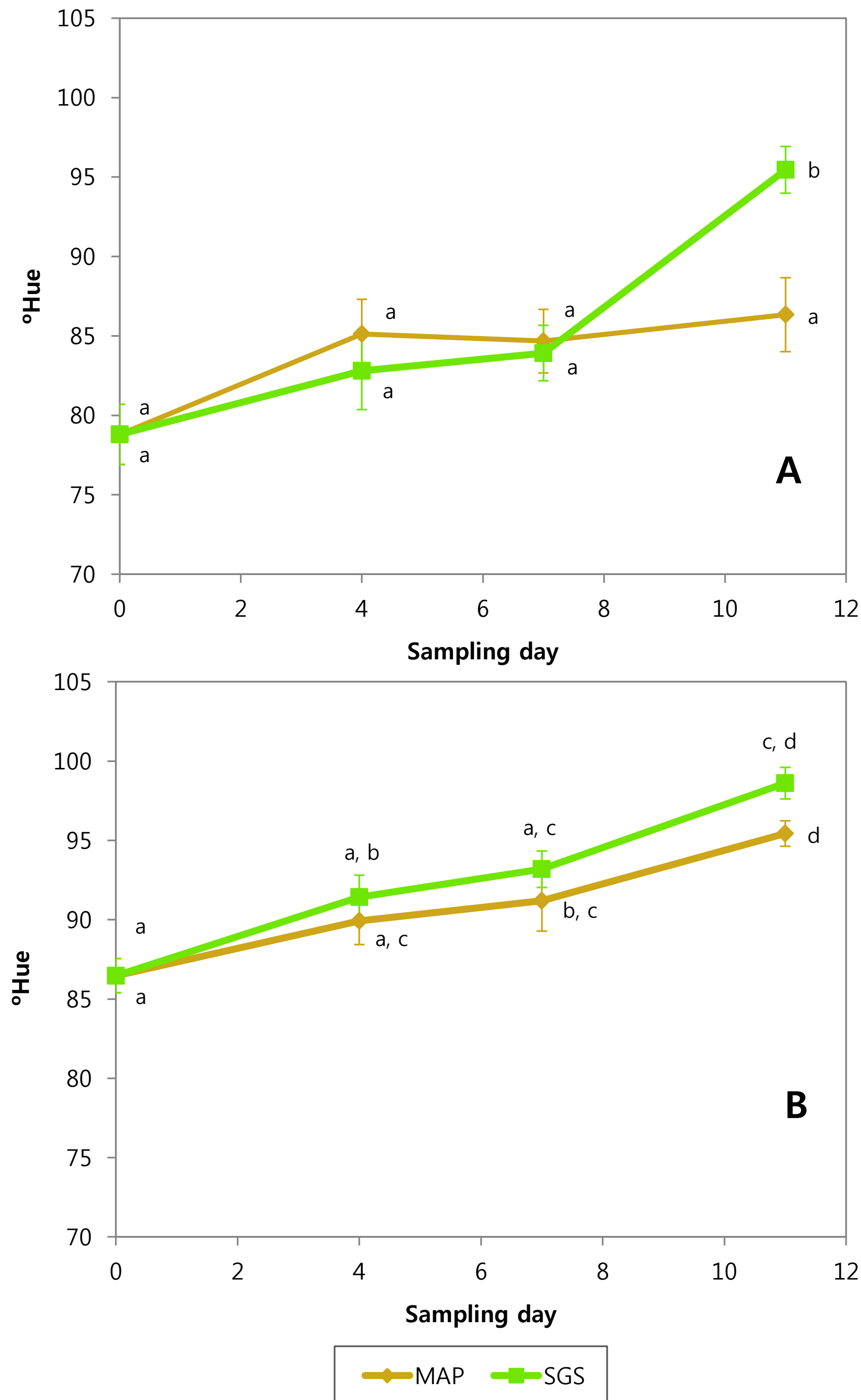


Figure 3.8. Evolution of °Hue on the surface color of meat (A) and skin (B) portion of chicken drumsticks packaged in modified atmosphere conditions (70% CO_2/15% O_2/15% N_2) pre-treated or not with SGS and stored for 11 days at 3 ± 1 ºC. Measures correspond to the average of twelve values ± standard error, and different superscript letters indicate significantly different values for the interaction between the treatment and the sampling day ($P<0.05$, Tukey's test).

Concerning the surface color of the skin (

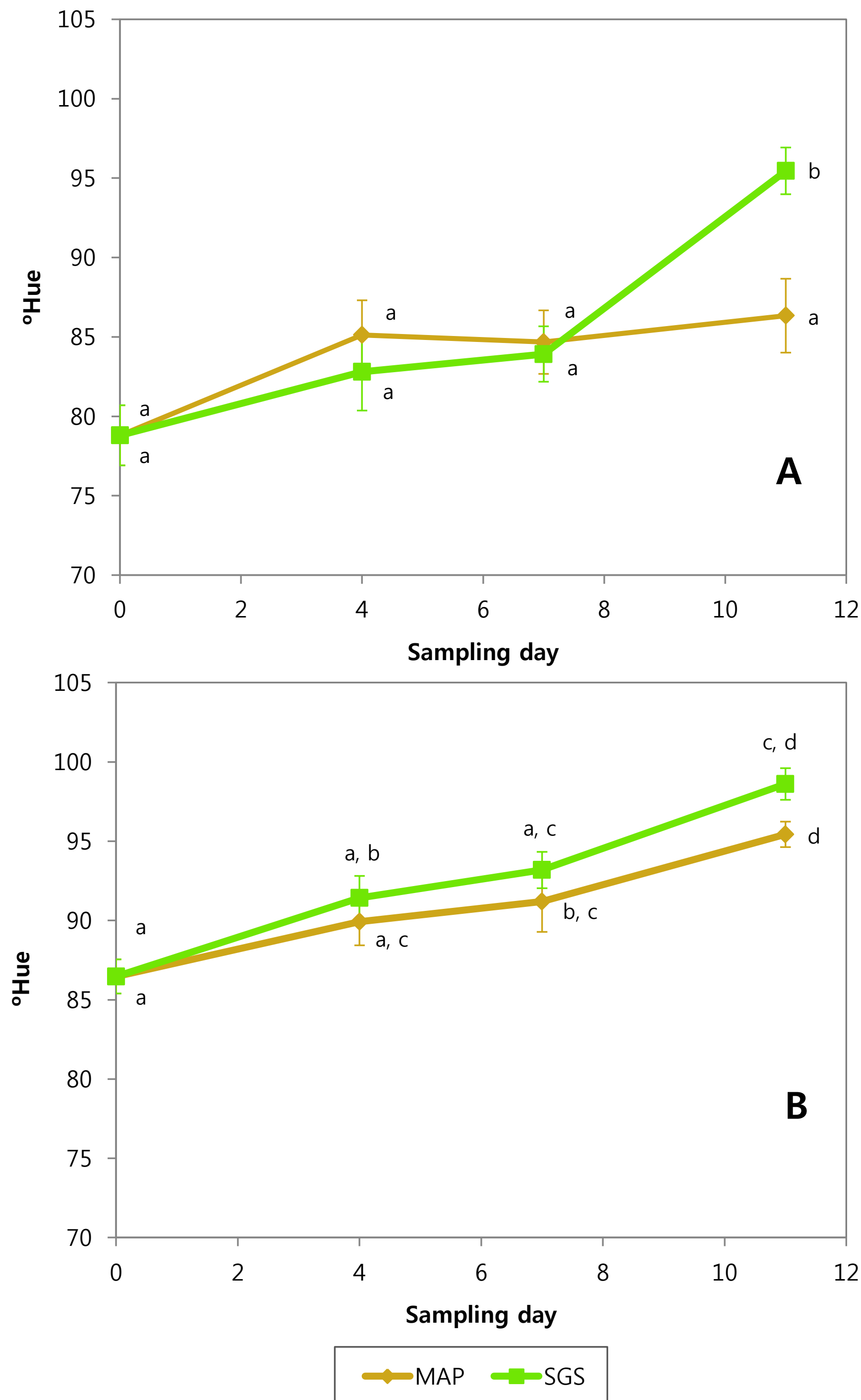


Figure 3.8-B), the interaction between the treatment and the storage time did not significantly affect its color. However, the treatment was significantly different with respect to the *L** and °Hue values on SGS samples, turning this to a brighter and greener color. Sampling day factor was significant for the other parameters, as the skin turned to a greenish and yellowish color.

Some of these results are in agreement with the ones obtained by Rotabakk et al. (2006), as they did not find either significant differences on the surface color of chicken breasts packed

under high CO_2 concentration. Also Werner et al. (2008) reported that the lightness values in poultry species had a low variation throughout the storage time whereas the standard deviations of the redness (a^*) and yellowness (b^*) values were rather high.

Nevertheless, it has to be considered that these variations in color values are hardly perceptible by the human eye. Hence, it seems that an increase in the dissolved CO_2 on the chicken meat or skin does not have a highly significant effect on the surface color of the product.

3.2.6. Microbiological analysis

Pseudomonas and TAC were the main indicators of spoilage in chicken drumsticks, showing significant differences depending on the treatment. TAC counts showed a statistical difference at day 7, when samples counts were significantly higher than those obtained with SGS samples (

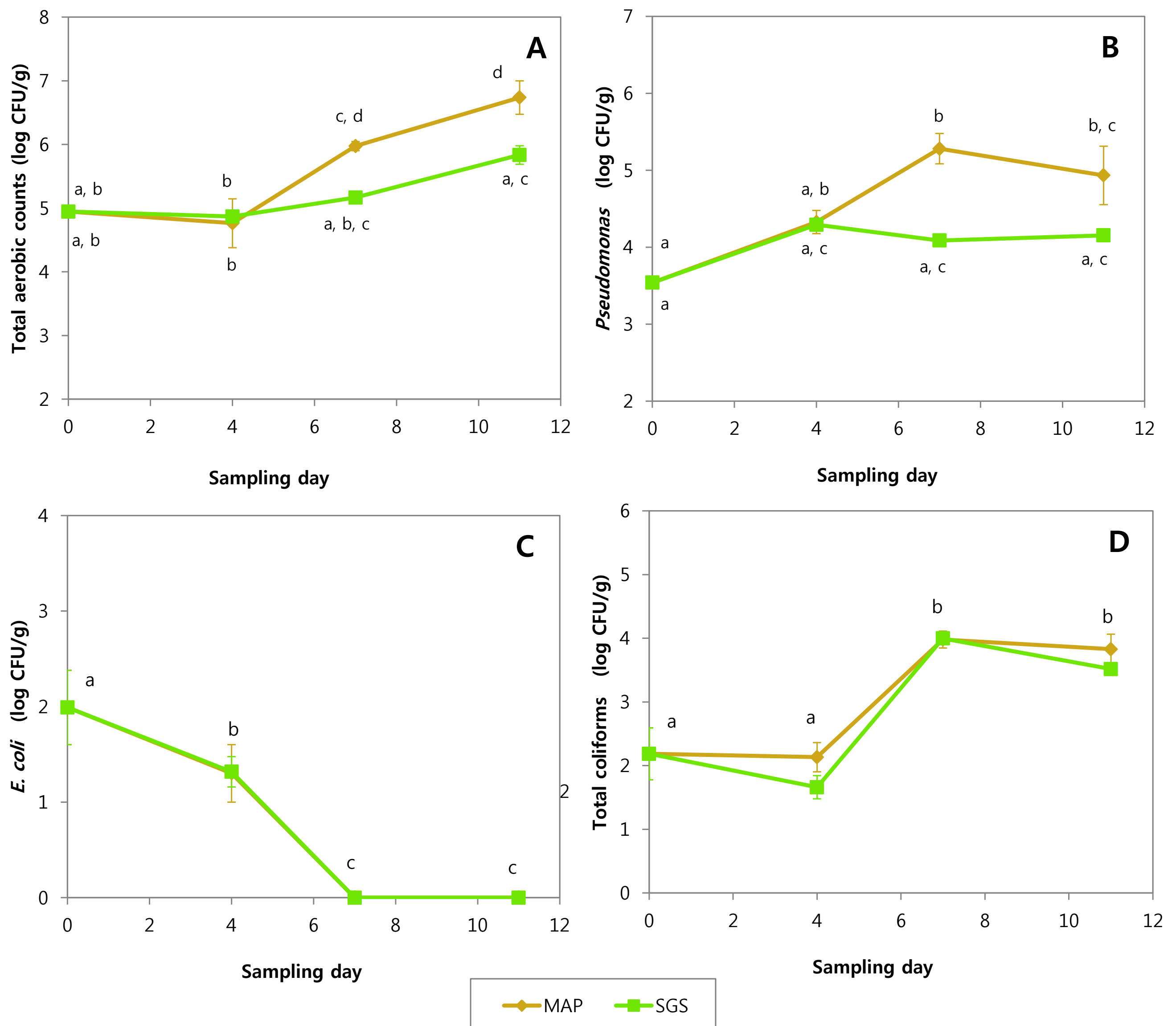


Figure 3.9-A). This increase in TAC counts explains the progressive decrease in the oxygen level in the head space. SGS treatment also had a significant effect on Pseudomonas growth, slowing down its growth and keeping it under stable at 4 log CFU/g from day 4 until the end of storage; while MAP sample counts continued increasing, reaching 5.3 log CFU/g at day 7 (

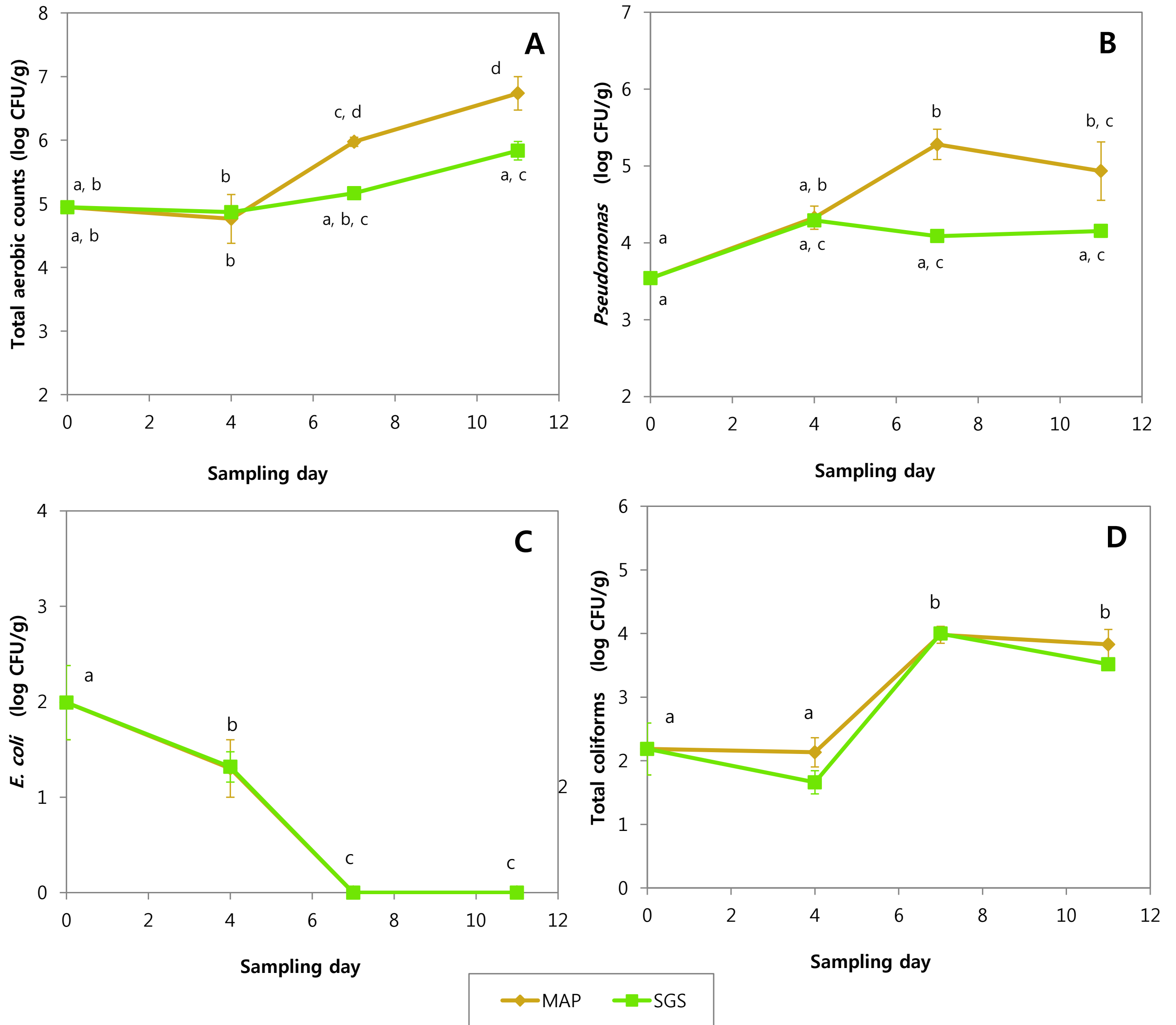


Figure 3.9-B). *E. coli* showed a special sensibility to CO_2, and the values constantly decreased from the first day for both treatments (

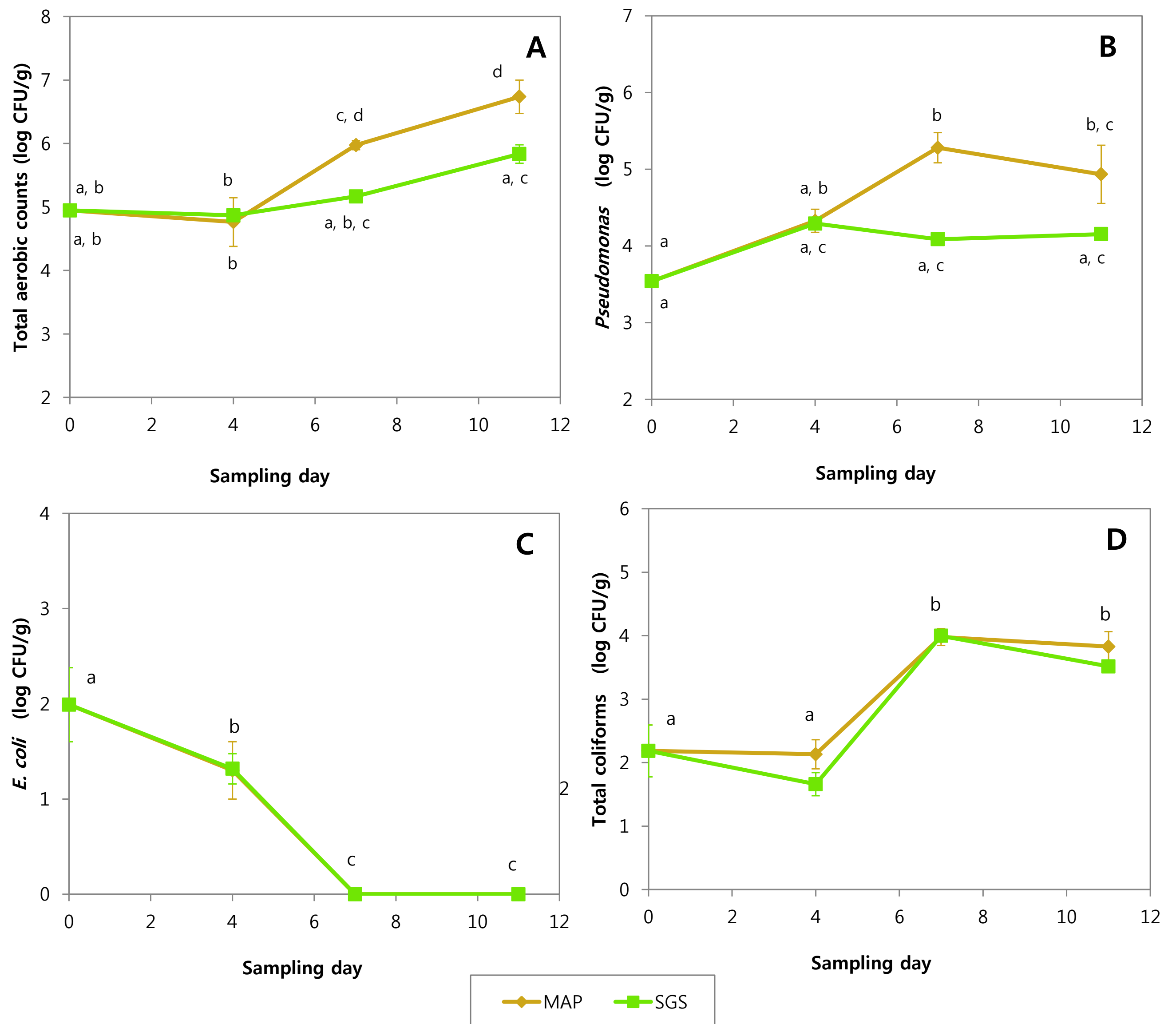


Figure 3.9-C). Regarding total coliforms, no significant differences between treatments were observed (

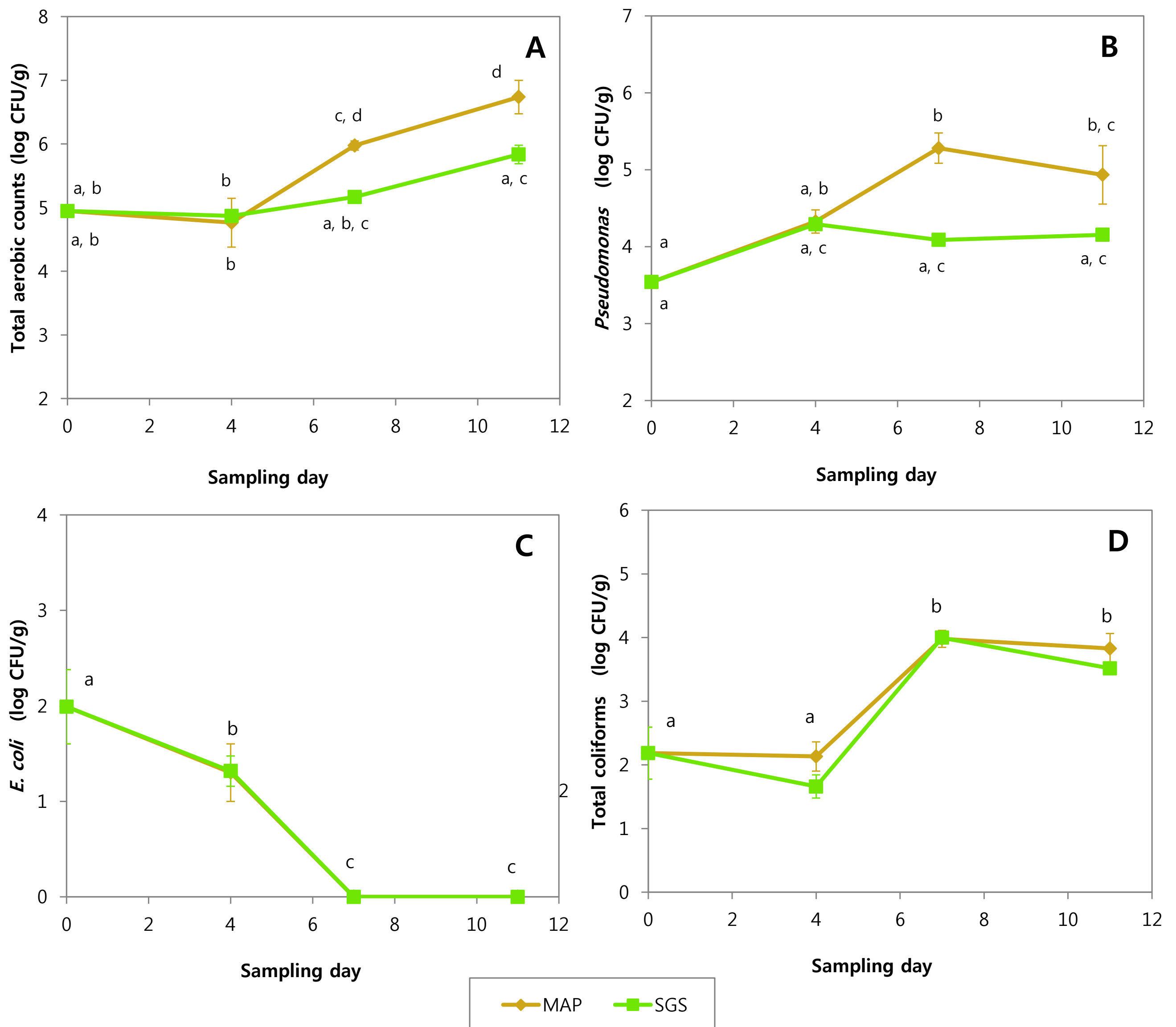


Figure 3.9-D).

About *Salmonella* spp., the initial control shows an absence of this pathogen in all the samples analyzed; however, on the last day of storage, two of the three packages of MAP samples, and one of the three packages of SGS samples were positive in the presence of *Salmonella*, all of them confirmed as *Salmonella choleraesuis arizonae*. These results are not enough to significantly relate the presence of this pathogen with the treatments applied, and also sample deviation should be considered.

Results obtained in the microbiological determinations are similar to those reported by Bennik et al. (1998), who detected an inhibitory effect of CO_2 against the growth of *Pseudomonas* and *Enterobactericiae* species, being more pronounced with the first ones. Also, Rotabakk et al. (2006) detected a decrease in the growth rate of *Pseudomonas* spp., although

they did not find a significant effect of the treatment with respect to the growth of TAC. However, applying SGS on fish, Rotabakk et al. (2008) found significant differences between SGS and traditional MAP, having a lower growth rates of TAC and psychrotrophic bacteria in SGS samples. They also observed that H_2S-producing bacteria were not affected by SGS treatment, compared to MAP. Sivertsvik and Birkeland (2006) determined that storage time, SGS treatment and the g_v/p_v ratio affect significantly the microbial growth of ready-to-eat shrimp, presenting lower counts of TAC and psychrotrophic bacteria in samples treated previously with SGS.

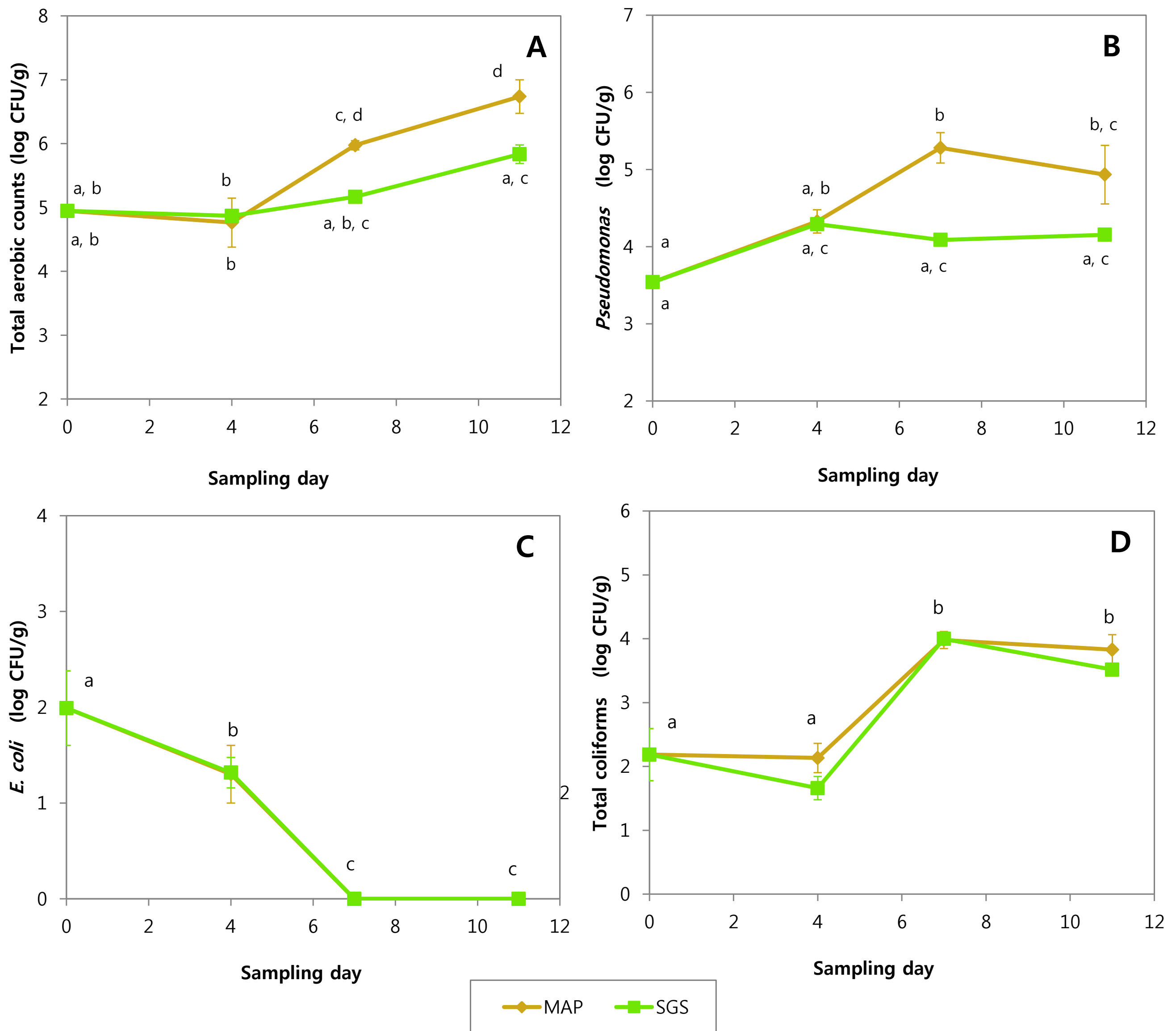


Figure 3.9. Effect of the packaging treatment on the growth of TAC (A), *Pseudomonas* spp. (B), *E. coli* (C) and total coliforms (D) in chicken drumsticks packaged under modified atmosphere conditions (70% CO_2/15% O_2/15% N_2) and pre-treated or not with SGS, stored for 11 days at 3 ± 1 ºC. Measures correspond to the average of three values ± standard error, and different superscript letters indicate significantly different values on the interaction between the treatment and the sampling day (A and B) or on the storage time (C and D) (P<0.05, Tukey's test).

3.3. Determination of the CO_2 solubility on chicken meat

With the results obtained previously, the quantity of CO_2 that dissolves into the chicken drumsticks packaged directly with MAP was determined using the equation described in the section 2.3. Considering the results, the most significant dissolution is produced within the first 24 hours of packaging (Figure 3.5-B). The average of CO_2 dissolved was 567 mg CO_2 kg^{-1} of chicken. These results are similar to the ones reported by Rotabakk et al. (2010), when they calculated the absorption and desorption of CO_2 in chicken breast fillets. They found that samples packaged with pure CO_2 for 2 hours had a concentration of CO_2 in the meat about 450 mg CO_2 kg^{-1} meat. The slightly greater values obtained in chicken drumsticks are probably caused by the presence of the skin, which due to the high fat percentage of its composition, increases the dissolution of CO_2 on it.

During the SGS pre-treatment, no quantification of the dissolved CO_2 was made. Despite this, during the MAP storage time, in SGS pre-treated samples 361 mg CO_2 kg^{-1} of chicken were dissolved. The difference in the amount of CO_2 dissolved between treatments may be caused because of the amount of CO_2 previously dissolved during the SGS pre-treatment. Mendes et al. (2008) reported that the levels of CO_2 in the head space of packaged sardine fillets pre-treated with SGS and then packaged in air conditions could be equivalent to the ones obtained packaging directly with MAP using a mixture of 5% O_2 / 35% CO_2 / 60% N_2. They concluded that SGS treatment has high advantages, because the product then needs much lower g_v/p_v ratios in packages or can even be vacuum packaged, and therefore obtaining the same amount of dissolved CO_2 with equivalent levels of microorganisms' growth inhibition as with traditional MAP, but with a smaller pack size.

Therefore, knowing the amount of CO_2 that dissolves into chicken meat, it is possible to find the tightest g_v/p_v ratio; and together with SGS, a higher filling degree of the packages is possible, without compromising microbial and sensory quality of the product and avoiding package collapse and chemical changes.

4. Conclusions

The application of SGS pre-treatment (100% CO_2, 3h) to raw chicken drumsticks improves the microbiological quality of the meat compared with traditional MAP, increasing its shelf-life. Package collapse can be reduced using SGS pre-treatment, without changing the sensory properties of chicken. A higher filling degree of the packages would be also possible to attain using an SGS pre-treatment.

It could be necessary to increase the SGS pre-treatment time to assure a higher amount of dissolved CO_2 into drumsticks. However, it would be required to find out if extending the SGS pre-treatment, so as to saturate the meat almost completely, would be compatible with the industrial time-procedures, looking for a feasible application in the food industry.

Effect of combining carbon dioxide saturation treatments and pack - product filling on the chemical, microbiological and sensory properties of raw chicken thighs

A. Al-Nehlawi [a], J. Saldo [a], S. Guri [b,c], M.G. O'Sullivan [d], J.P. Kerry* [d]

[a] *Animal and Food Science Department, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
[b] *MATGAS Research Center, Campus UAB, 08193 Bellaterra, Barcelona, Spain*
[c] *S.E. Carburos Metálicos – Air Products Group, C/ Aragón 300, 08009 Barcelona, Spain*
[d] *Food Packaging Group, School of Food & Nutritional Sciences, University College Cork, Cork, Ireland*

Food Control Journal (submitted)

Effect of combining carbon dioxide saturation treatments and pack – product filling on the chemical, microbiological and sensory properties of raw chicken thighs

1. Introduction and objectives

Currently the meat industry all over the world uses modified atmosphere packaging (MAP) to pack raw meat. Owing to the use of this technology the shelf-life of raw meat can be extended, keeping the meat safe from spoilage microorganisms and preserving quality and sensory properties. Pack to product ratio has an important influence on the preservation of MAP products, as the quantity of the gas introduced on the package will depend on this ratio. The potential benefits of reducing the gas head-space to meat ratios in MAP include a reduction in retail pack size which would result in lower manufacturing, transport and storage costs for the meat industry.

As it has been previously demonstrated, Soluble Gas Stabilization (SGS) pre-treatment has the potential to significantly reduce package collapse, even at high filling ratios, without compromising the quality of the packaged food, therefore increasing packaging efficiency. Although this treatment has been applied to study chicken preservation using different pre-treatment times and pressures (Al-Nehlawi, Saldo, Vega, & Guri, 2013; Rotabakk et al., 2006), the effect of CO_2 saturation on the sensory properties of the meat perceived for the consumers has not been determined yet. It has been demonstrated in red meat that CO_2 flavour can be detected by consumers (O'Sullivan, Cruz-Romero, & Kerry, 2011). Additionally, the possibility to reduce, or even avoid, package collapse would be a matter of interest for the meat industry, in order to reduce package size, reduce packaging waste and improve packaging efficiency.

Considering the possibilities of SGS pre-treatment, the first objective of this study was to investigate the effects of this procedure together with a high CO_2 atmosphere on the sensory properties of chicken thighs and on the package collapse. The second objective was to determine if SGS pre-treatment had an impact on the quality of fresh chicken thighs packaged using different filling ratios while assessing different saturation times in an attempt to prove that SGS pre-treatment can optimize the packaging of chicken meat by maximizing the filling of the meat trays based on pack to product ratio.

2. Material and methods

Fresh chicken thighs, with skin and bone, were obtained from a local poultry meat supplier and transported to the laboratory under chilled conditions. Upon arrival, chicken fillets were stored at 2 ºC and processed within 24 hours. All the thighs were from young chickens, collected from the same batch at the plant, with all of them coming from the same farm.

Three different SGS pre-treatment times (0h, 6h, 9h) and two different pack to product ratios (2:1 and 3:1) were the variables chosen for consideration in this study. Consequently, chicken thighs were divided into six groups, each one assigned to a different treatment (Table 3.1).

To adjust for appropriate product weights (Al-Nehlawi et al., 2013), 480g of chicken thighs were packaged in order to obtain a gas volume and product volume ratio (g_v /p_v) of 2:1, and 360g of thighs to obtain a ratio of 3:1.

Table 3.1. Experimental design and variables analyzed in the trial of chicken thighs.

MAP mixture:	75% CO_2 / 13% O_2 / 12% N_2
Sampling days:	0, 3, 4, 6, 7, 10, 11
Physical and chemical analysis:	Film deflation, head space gas composition, pH, weight loss, cooking loss, surface color, muscle oxidation
Microbiological analysis:	*Brochothrix thermosphacta, Escherichia coli*, Lactic acid bacteria, *Pseudomonas* spp., Total aerobic counts, total Coliforms
Sensory evaluation:	Appearance, liking of flavor, juiciness, tenderness, acidity, sourness, oxidation flavor, off-flavor and overall acceptability.

Treatment	**SGS time**	**Filling volume degree (FD) (g_v:p_v)**
A	0	2:1
B	0	3:1
C	6	2:1
D	6	3:1
E	9	2:1
F	9	3:1

Samples with SGS pre-treatment (samples C, D, E and F) were packaged under 100% CO_2 atmosphere using a thermo-sealer tray machine Gustav Mueller vs 100 (Gustav Müller and Co., Zum Wingert 5, 6380 Bad Homburg 6, Germany) using high gas barrier polystyrene/ EVOH/ polyethylene trays (0.5 mm, CO_2 transmission rate <4 cm^3 m^{-2} 24 h^{-1} bar^{-1} and O_2 transmission

rate <1 cm^{-3} m^{-2} 24 h^{-1} bar^{-1}, both determined by the manufacturer at 23 ºC and 0% RH) and sealed with a laminated barrier film (43 µm, CO_2 transmission rate <4 cm^3 m^{-2} 24 h^{-1} bar^{-1} and O_2 transmission rate <1 cm^3 m^{-2} 24 h^{-1} bar^{-1}, both determined by the manufacturer at 4 ºC and 80% RH; Cryobac Sealed Air, England). The g_v /p_v ratio inside the trays was high enough to ensure excess availability of CO_2. Gas composition of the SGS treatment was 99.99 ± 0.01% CO_2, and the internal pressure was equal to the atmospheric pressure. Samples C and D remained 6 h under SGS conditions and samples E and F did it for 9 h, both under refrigeration (3 ± 1 ºC). After that time, samples were immediately packaged under MAP conditions described below.

For MAP treatment, samples previously weighted were packaged under a gas mixture of 74.6 ± 0.1% CO_2 / 13.6 ± 0.01% O_2 / 11.8 ± 0.1 % N_2 using the same equipment and materials as described for the SGS pre-treatment. Samples C, D, E and F were repackaged in less than one minute to avoid CO_2 desorption.

Each tray was stored at 3 ± 1 ºC until sampling at day 3, 4, 6, 7, 10 and 11. For each treatment and sampling day, three different trays were analyzed, each one being an independent replication. Different trays were also used for microbiological, sensory and physical and chemical analyses.

2.1. Film cover deflation

To quantify the visual impact of the CO_2 dissolution on the packages, the film cover deflation was measured at the top center of the packages using a caliper (0.01 mm accuracy) (Absolute Digmatic CD-15CPX, Mitutoyo Corporation, USA) and reported as deflation (mm) relative to the sealing area. One measure for each tray was made on days 3, 4, 6, 7, 10 and 11 after packaging the meat, obtaining three measures for each treatment and sampling day.

2.2. Head space gas composition

Gas analyses were performed using a PBI- Dansensor check Mate 9900 (DK-4100, Ringsted, Denmark). The sensor needle was pierced through the laminated film and a specially fitted air impermeable septum to check the gas concentration inside the packages before opening.

O_2 and CO_2 values are presented as a percentage (%) of the atmosphere composition in absolute values. One measurement for each tray was made on days 3, 4, 6, 7, 10 and 11, obtaining three measures for each treatment and sampling day.

2.3. Weight loss and cooking loss

The weight loss and cooking loss of thighs were measured gravimetrically using a College B 502 Mettler Toledo scale (Zurich, Switzerland) and reported as a percentage of the raw meat.

Weight loss was measured at days 3, 6 and 10, and was calculated as:

$$\boldsymbol{W_l = \frac{(I_w - D_w) \times 100}{Iw}} \qquad (3.10)$$

Where W_l is the weight loss of the raw meat (%), I_w is the initial weight of the raw meat (g) and D_w is the weight of the raw meat on test day (g).

Cooking loss was measured at days 0, 3, 6 and 10 of the study, and was calculated as:

$$C_l = \frac{(I_w - C_w) \times 100}{Iw} \qquad (3.11)$$

Where C_l is the cooking loss of the raw meat (%), I_w is the initial weight of the raw meat (g) and C_w is the weight of the cooked meat on test day (g). Samples were cooked using an industrial oven (Zanussi mod. FCF 10 2, Pordenone, Italy), at 200 ºC for 25 minutes with forced convection, ensuring an internal temperature of the meat of 72 ºC.

2.4. Measurement of muscle pH

The pH of the muscle was recorded using a portable pH meter (Mettler Toledo, MP 125, Switzerland). As the heterogeneity of the chicken thighs can alter the measures, pH was always measured on the white muscle portion of the thigh, by making a small hole and inserting a glass electrode approximately 0.5 cm into the muscle. A total of two measures were taken per each tray on days 0, 4, 7 and 11, obtaining six results for every treatment and sampling day.

2.5. Color determination

The surface color of the thighs was measured at days 0, 3, 6 and 10, just after opening the packages. Samples were measured according to CIE L^* a^* b^* color system using Minolta CR 300 colorimeter (Minolta Camera Co. Ltd., Osaka, Japan). The chroma meter was calibrated on the Hunterlab color space system using a white tile (L^* = 97.4, a^* = − 0.07, b^* = 1.70, Minolta calibration plate).

In order to make representative measures of all the portions, for each replication, eight measures were taken: four measures of the skin surface which was directly in contact with the

gas mixture, and four measures of the surface of the white muscle (removing the skin when that was necessary). The statistical treatment was made separately for meat and skin fraction.

2.6. Measurement of muscle oxidation (TBARs)

Lipid oxidation was measured at days 0, 3, 6 and 10 of storage, using the 2-thiobarbituric acid assay of Siu and Draper (1978). The malondialdehyde (MDA) content was calculated using an extinction coefficient of 1.56 × 105 M− 1 cm− 1. Results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde/kg chicken meat.

2.7. Microbiological analysis

For the microbiological assessment of the product, samples were analyzed on days 0, 4, 7 and 11. At each sampling day, three independent trays from each treatment were analyzed for total aerobic counts (TAC), *Pseudomonas* spp., total coliform, *Escherichia coli*, lactic acid bacteria (LAB) and *Brochothrix thermosphacta*.

The packages were opened and 25 g of thighs, mixing meat portion and skin portion in an approximately relation of 50/50, were taken aseptically and homogenized with 225 mL of sterile peptone water (CM1049; Oxoid, England) in stomacher bags during 90 seconds at room temperature. Cell counts were determined by plating serial dilutions of the meat homogenate.

For TAC, 1 mL of the appropriate dilutions were inoculated by the pour-plate method in plate count agar (CM0325; Oxoid, England), and incubated at 30 ºC for 48 h. *E. coli* and total coliforms were determined on a chromogenic selective media (CM1046; Oxoid, England) by the spread-plate method and incubated at 37 ºC for 24 h; *E. coli* colonies were purple, while the rest of coliform colonies were pink. *Pseudomonas* spp. was determined on a selective agar (Pseudomonas Agar Base CM0559 with CFS supplement SR0103; both Oxoid, England) by the spread-plate method, incubated at 30 ºC for 48 h. LAB were determined using pour-plate method and MRS agar (CM0361; Oxoid, England), applying an extra lay of medium to ensure microaerofilic conditions, incubated at 30 ºC and examined at 48 h and 72 h. *B. thermosphacta* counts were determined on STAA agar (CM0881 with selective supplement SR0151E; both from Oxoid, England) using spread-plate method and incubating the plates at 25 ºC for 48 h.

2.8. Sensory evaluation

A 10-member sensory panel (5 males and 5 females), within the age bracket of 20 – 35 years was recruited in University College Cork, Ireland. Selection criteria for panelists were that they had to be habitual chicken consumers and be available and motivated to participate in all

testing days throughout the experiment. All panelists had previously participated in oxidized meat and warmed-over-flavor (WOF) sensory evaluation studies and were familiar with these sensory phenomena.

Three independent trays for each treatment were used for sensory test on days 3 and 7. Samples were taken immediately after open the packages and cooked using an industrial oven (Zanussi mod. FCF 10 2, Pordenone, Italy), at 200 ºC for 25 minutes with forced air. Then, the white muscle portion of each thigh was extracted and cut into pieces of approximately 1 cm^3. Two replicates of each treatment and two from the control (fresh meat) were presented to panelists for evaluation and they were required to rinse with water before tasting each sample. Panelists were asked to indicate their score on a 10 cm continuous line scale ranging from 0 (extremely dislike) to 10 (extremely like). They were asked to evaluate the chicken meat using hedonic descriptors: liking of appearance, liking of flavor and overall acceptability, and the intensity descriptors: juiciness, tenderness, acidity, sourness, oxidation flavor and off-flavor. The off-flavor term was explained to the panelists as a rancid, cardboard or linseed oil-like flavor (Tobin, O'Sullivan, Hamill, & Kerry, 2012). Sensory analysis was undertaken in the panel booths at the university sensory laboratory that conforms to international standard ISO 8589 (Standardization, 1988). The sample presentation order was randomized to prevent any flavor carryover effects.

2.9. Statistical analysis

The entire experiment was conducted in triplicate (three independent packages per treatment and sampling day). Three-way analysis of variance (ANOVA) and general linear model (GLM) procedures were performed by means of JMP Software (SAS Institute INC., U.S.A.), between the main factors "SGS time", "Filling volume" and "sampling day". When there was statistical significance ($P < 0.05$), Least Square Means (LSM) procedure was used, applying Student's T test to determine the significant differences among the averages.

3. Results and discussion

3.1. Chemical and physical evolution

During the SGS treatment the packages suffered a visible collapse as a consequence of the decrease of the CO_2 partial pressure within the packages, due to the dissolution of the gas in the water and fat tissue of the chicken meat. When the meat was packaged with conventional modified atmosphere, the evolution of package collapse was significantly different. These differences became more evident throughout the storage time (Figure 3.12). Statistical

analysis showed that at the end of the storage (day 11) there were no differences between samples A and B with respect to pack collapse (Figure 3.13). On samples with a 6 h SGS pre-treatment, samples with a low volume product regime (treatment D) had the same pack collapse at day 7 of storage as high volume product regime samples (treatment C) had on day 3. This finding clearly shows the influence that product filling volume in packs has on pack collapse. Comparing SGS pre-treatments, at day 6 of storage, samples with an SGS of 6 hours and a low filling product volume ratio (treatment D) demonstrated greater pack collapse than samples with an SGS of 9 hours and the same product filling volume (treatment F), which shows that the longer the SGS pre-treatment time the lower the package collapse that is produced. When comparing these samples with those with no SGS pre-treatment, the benefits of pre-treatment on pack collapse are evident. Similar results were found in previous studies (Al-Nehlawi & Guri, 2010; Al-Nehlawi et al., 2013; Rotabakk, Wyller, et al., 2008) where the difference in package collapse between conventional MAP and SGS were also significant. However, in these studies the influence of product filling volume was not considered. Rotabakk et al. (2006) did study the influence of the filling volume, working with approximate g_v/p_v ratios of 2:1 and 1:1; but they could not obtain accurate measurements due to the early contact between the meat and the cover film.

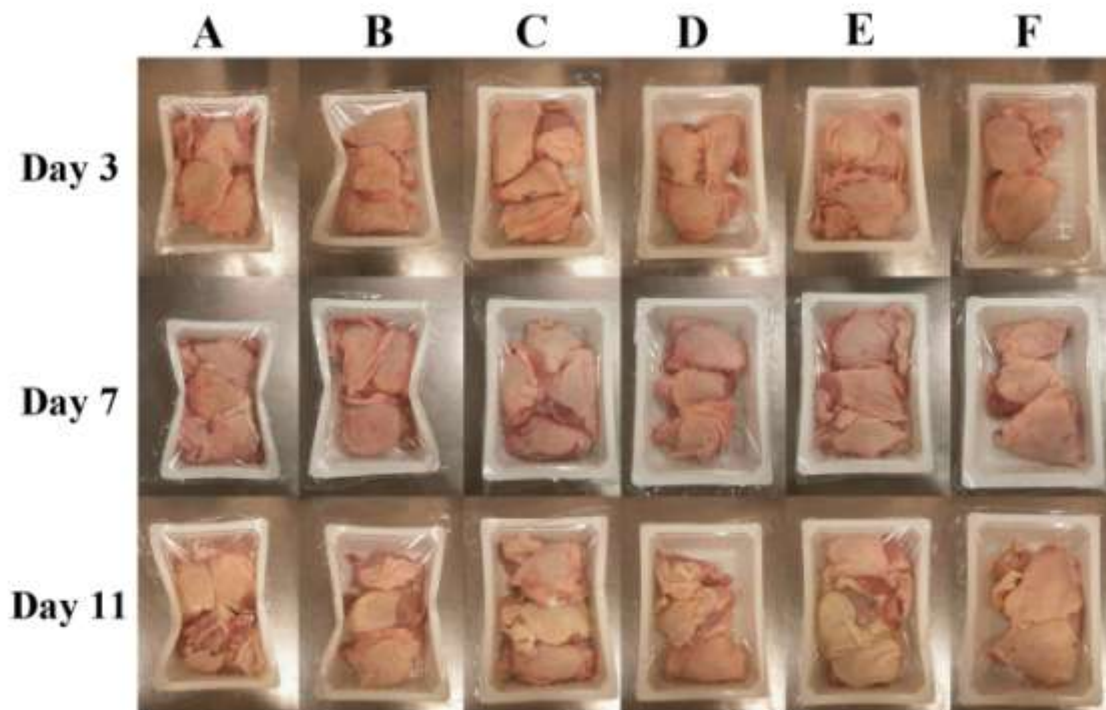


Figure 3.12. Pictures of the evolution of the package collapse on chicken thighs packaged under modified atmosphere (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h, E, F=9h) and different product filling volumes (A, C, E g_v/p_v = 2:1; B, D, F g_v/p_v = 3:1), stored for 11 days at 3 ± 1 ºC.

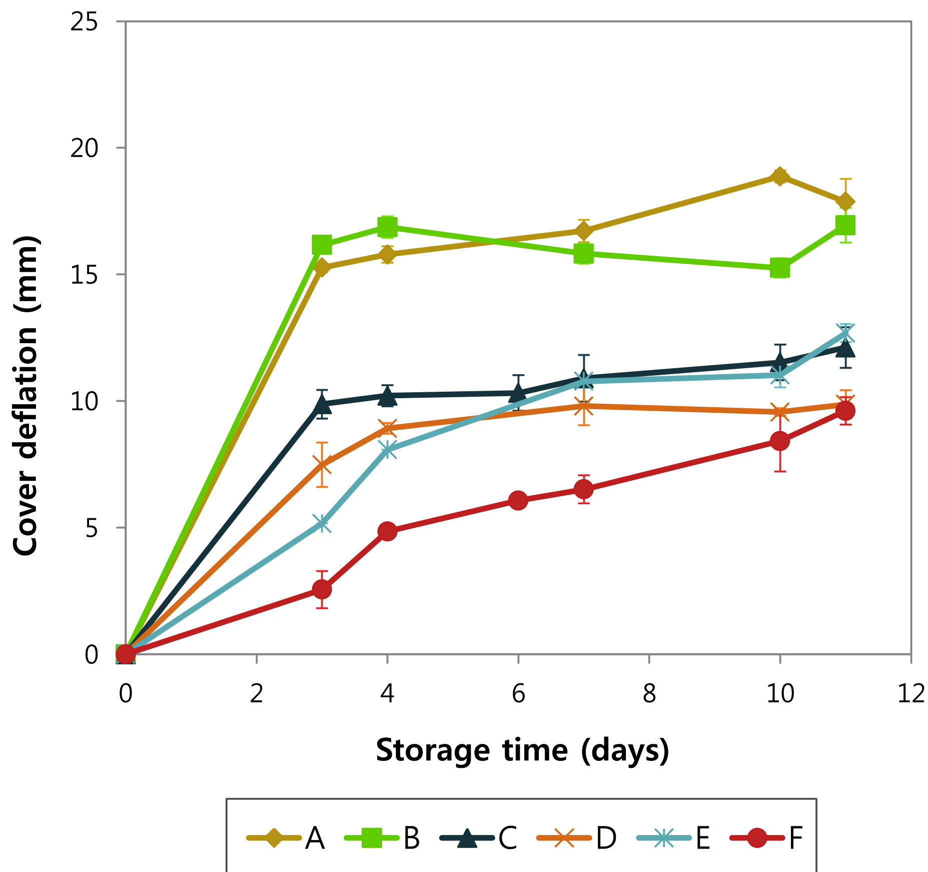


Figure 3.13. Evolution of the cover film deflation (mm) on trays with chicken thighs packaged under modified atmosphere conditions (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h, E, F=9h) and different filling volumes (A, C, E g_v/p_v = 2:1; B, D, F g_v/p_v = 3:1). Samples were stored for 11 days at 3 ± 1 ºC. Values correspond to the average of three measures ± standard error.

With respect to changes in packaging atmospheres, the concentration of CO_2 decreased significantly during the initial stages of storage, arriving at the equilibrium in less than 48 h (Figure 3.14-A). The gaseous environment in MAP systems can change as a result of the permeability of packaging material, respiration or absorption of gas by the food product and microbial growth (Esmer, Irkin, Degirmencioglu, & Degirmencioglu, 2011). However, considering the results reported previously by Rotabakk et al. (2006) and Al-Nehlawi et al. (2013), when chicken is packaged with a high CO_2 modified atmosphere, the principal reason for the decrease of the concentration of this gas in the head-space is due to its solubility in the water and lipid phase of the meat and its associated skin.

When treatments are compared, the greatest and most significant differences occurred between SGS pre-treatment samples and those non-pre-treated samples. Pre-treated samples reached the atmosphere equilibrium at higher CO_2 concentrations than non-pre-treated samples, due to the important amount of CO_2 dissolved into the meat during the pre-treatment. Similar results were found in previous studies (Mendes et al., 2008; Rotabakk, Birkeland, et al., 2008) when the concentration of CO_2 in the head-space of SGS samples was significantly higher

than in conventional MAP samples. However, no differences (P>0.05) were observed between the different SGS times, obtaining similar concentrations of CO_2 in all the pre-treated samples.

On the other hand, significant differences regarding the filling degree (FD) ratio were present, showing that packs containing a low product filling volume (treatments B, D and F) reached equilibrium at higher concentrations of CO_2 than packs containing a higher product filling volume (treatments A, C and E). It seems that instead of the duration time of SGS pre-treatment, the filling volume of the packages has a more significant influence on the final amount of CO_2 available in the pack head-space. Sivertsvik and Birkeland (2006) also investigated the effects of using two different MAP gas fills, but they did not find differences between them when gas pack equilibrium was reached.

The percentage of O_2 also decreased as a consequence of biochemical and microbiological activities along the storage time (Figure 3.14-B). The increase in O_2 measured at day 3 on samples A and B was caused by a drop in the molar concentration of CO_2 in the pack head-space, which is relatively compensated with through an increase in the percentage of O_2, but not with an increase in molar quantity (Al-Nehlawi et al., 2013). On a point of difference with other related studies and coinciding with CO_2 percentage, it seems that the product filling volume within packages also has a significant effect on O_2 percentage, more so than the effect of pre-treatment time.

No significant differences were observed between packaging treatments with respect to pH values in chicken thigh meat (Figure 3.15). However, samples not pre-treated with CO_2 had slightly higher pH values (around 6.1 units) than samples which had received a SGS treatment (pH values between 5.8 and 5.9). This difference can be attributed to CO_2 being absorbed into chicken meat to a higher degree following SGS pre-treatment. It is commonly known that the dissolution of CO_2 in food products produces a fall in pH due to the formation of carbonic acid (HCO_3^-), despite the buffering capacity of the chicken meat tissue (Bendall, 1972; Gill, 1988). Similar results were reported in shelf-life studies when using chicken products packaged under different MAP conditions (Patsias et al., 2008; Patsias, Chouliara, Badeka, Savvaidis, & Kontominas, 2006; Vongsawasdi et al., 2008), where the differences in pH values between samples packaged with high and low CO_2 were not significant. Moreover, in other SGS treatments applied to chicken products (Al-Nehlawi & Guri, 2010; Rotabakk et al., 2006), no differences were observed between MAP and SGS treatments with respect to pH values.

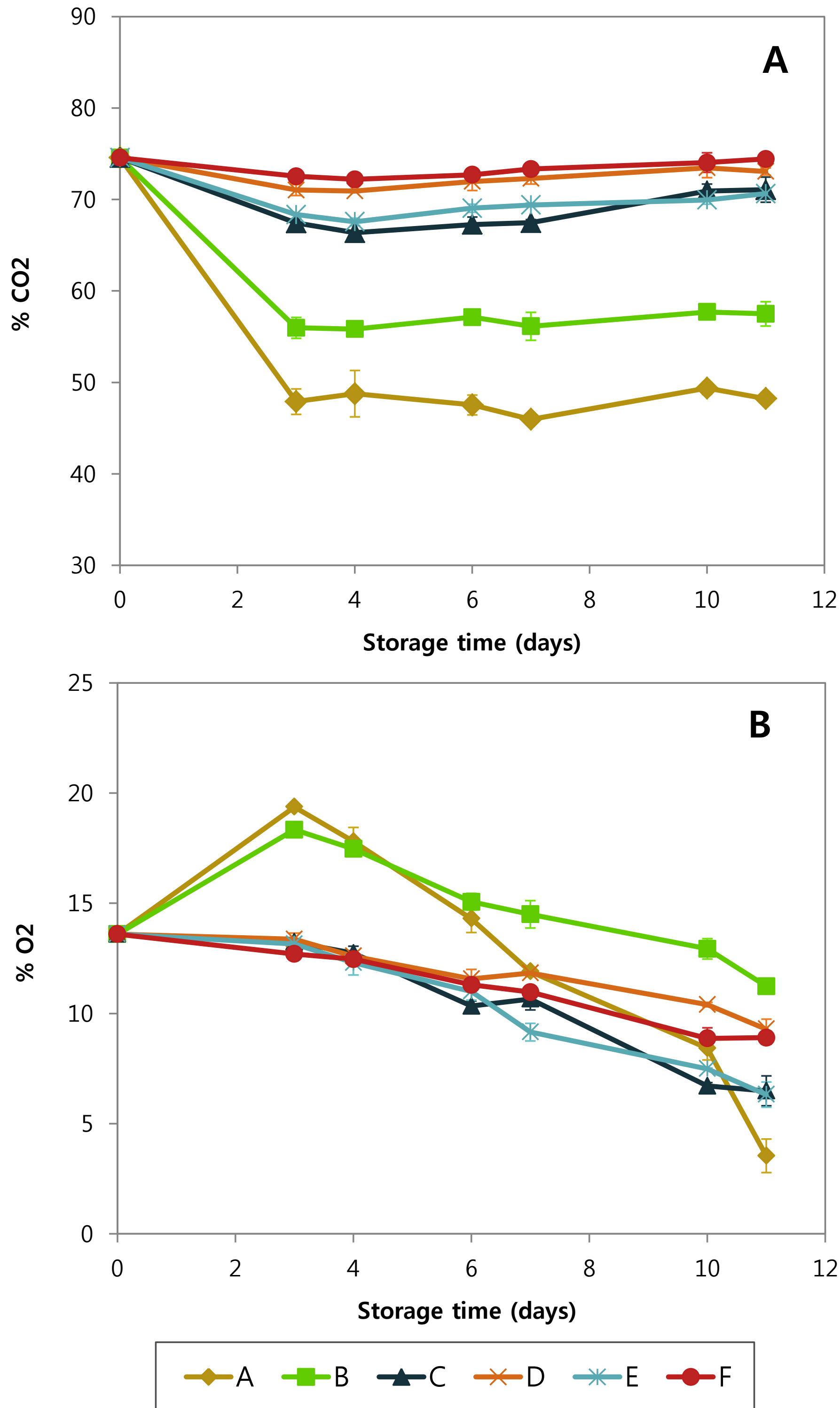


Figure 3.14. Evolution of carbon dioxide (A) and oxygen (B) percentages at the head space of trays with chicken thighs packaged under modified atmosphere (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h, E, F=9h) and different filling volumes (A, C, E g_v/p_v = 2:1; B, D, F g_v/p_v = 3:1). Samples were stored for 11 days at 3 ± 1 ºC. Values correspond to the average of three measures ± standard error.

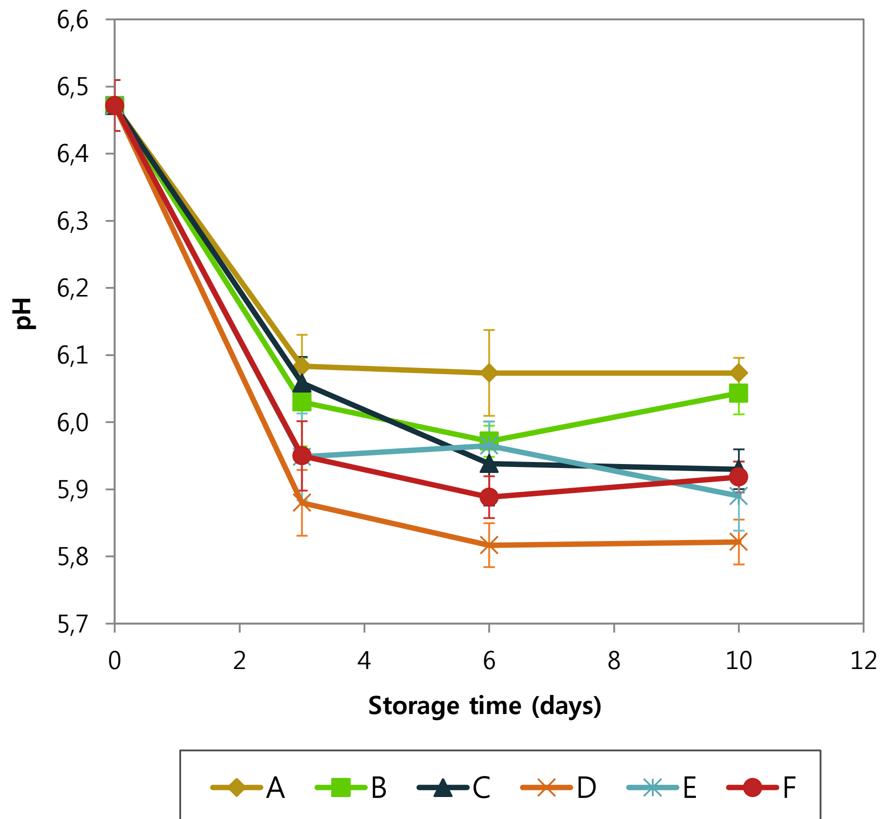


Figure 3.15. pH evolution of chicken thighs packaged under modified atmosphere (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h, E, F=9h) and different filling volumes (A, C, E g_v/p_v = 2:1; B, D, F g_v/p_v = 3:1). Samples were stored for 11 days at 3 ± 1 ºC. Values correspond to the average of three measures ± standard error.

During product storage, chicken thigh surface colour changed from a reddish tone to a yellowish tone in both skin and meat portions. Although no statistical differences were detected between packaging treatments, data showed a greater increase of ºHue values in SGS pre-treated samples at the end of the study, for both skin and meat (Figure 3.16). These changes may be explained by the growth and proliferation of yeasts or moulds, which can cause yellowing of the meat. Additionally, as myoglobin is more saturated with CO_2 in pre-treatment packs, more O_2 is available in such packs to interface and interact with polyunsaturated fatty acids, which can result in the promotion of yellow ºHues in meat. Bekhit and Faustman (2005) cited that for chicken, turkey and pork, grayish-pink color is considered to be normal. Nevertheless, as it has been reported by some other authors (Patsias et al., 2008; Pettersen, Nissen, Eie, & Nilsson, 2004), these changes in chicken surface colour are not significant and hardly perceptible by the human eye.

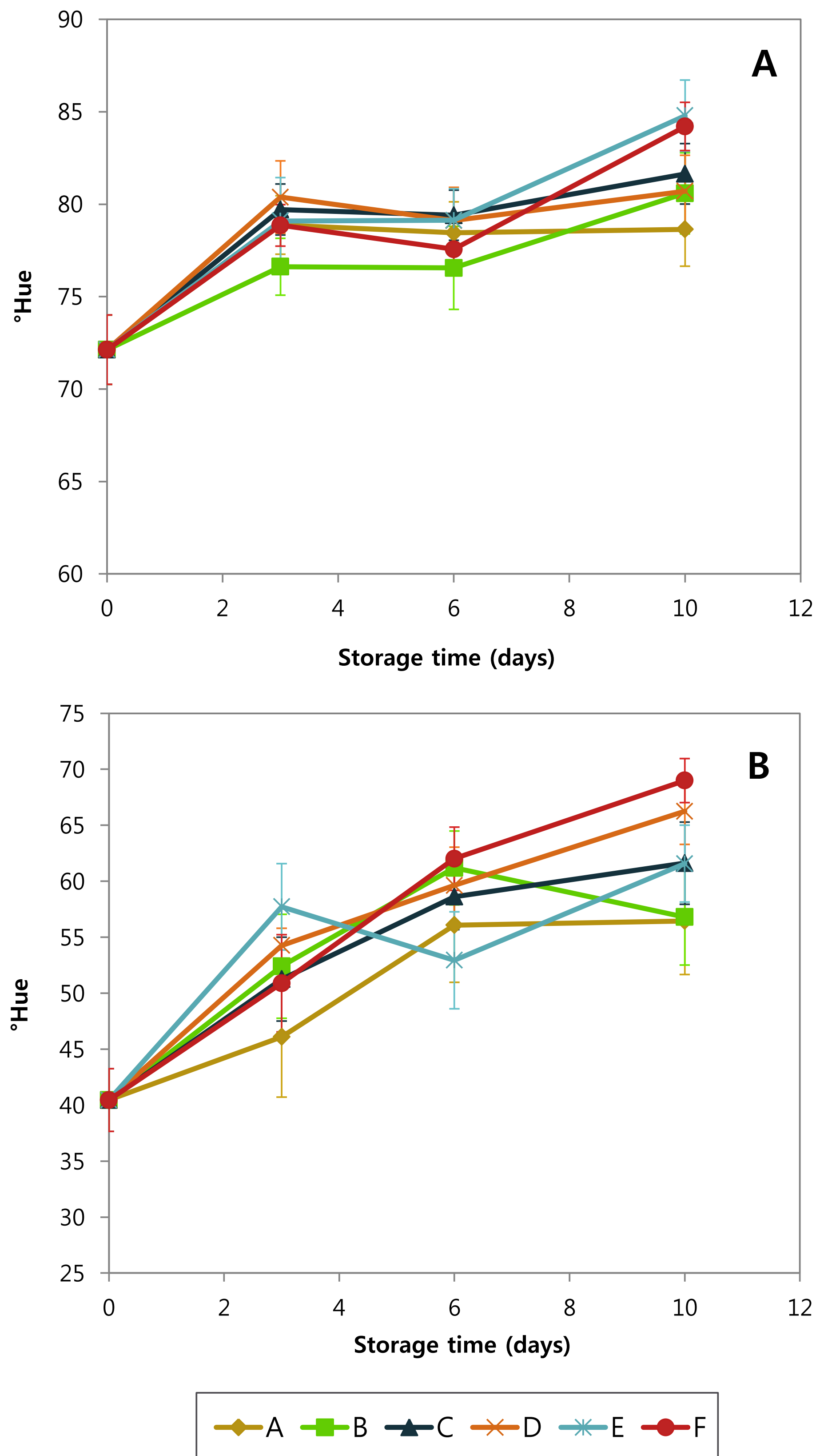


Figure 3.16. Evolution of the ºHue angle of the surface colour of skin (A) and meat portion (B) of chicken thighs packaged under modified atmosphere (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h, E, F=9h) and different filling volumes (A, C, E *gv/pv* = 2:1; B, D, F *gv/pv* = 3:1). Samples were stored for 11 days at 3 ± 1 ºC. Values correspond to the average of twelve measures ± standard error.

When chicken meat starts to deteriorate, one of the first and most visual consequences is the reduction of the water holding capacity of muscle proteins, causing the movement of water into extracellular spaces (Huff-Lonergan & Lonergan, 2005). In addition, when CO_2 is dissolved into the product, the pH of the meat decreases due to the carbonic acid formed, thereby approaching the isoelectric point of the proteins. When the pH reaches this point, the water retained in the muscle is released as exudates, and consequently the weight of the meat decreases.

As high concentrations of CO_2 and pure CO_2 pre-treatments were used in this study, we anticipated significant weight losses in chicken meat owing to the absorptive nature of CO_2 in water and fat. Unexpectedly, the greater (P<0.05) weight loss (1.2%) occurred in chicken samples which did not receive SGS pre-treatment and which had the highest product filling volume (2:1). No further significant differences were observed between the other treatments, which reached 0.5 – 0.7% of weight loss (Figure 3.17).

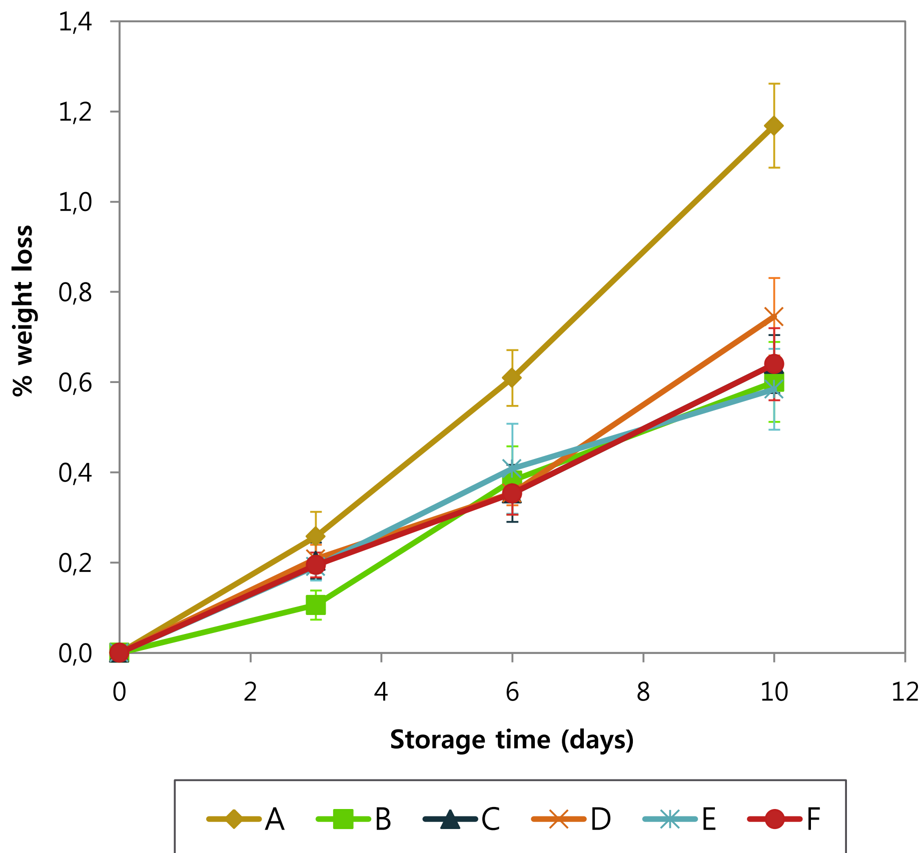


Figure 3.17. Evolution of weight loses of chicken thighs packaged under modified atmosphere (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h, E, F=9h) and different filling volumes (A, C, E g_v/p_v = 2:1; B, D, F g_v/p_v = 3:1). Samples were stored for 11 days at 3 ± 1 ºC. Values correspond to the average of three measures ± standard error.

This finding might be explained if we consider the pressures present inside non-flexible MAP formats. Although all samples were packaged at 1 atmosphere (atm), the dissolution of the CO_2 from the head-space alters the partial pressure within the packages. When packs collapse, an under-pressure situation is produced within the pack, which might affect the product. In this experiment, the samples that suffered the greatest package collapse also had the highest weight losses. It will be interesting in future work to measure actual pressures inside packages to demonstrate this theory.

Regarding cooking losses, samples that lost the least weight during storage had more water to lose during the cooking process. Consequently, packaging treatment had a significant ($P<0.05$) influence on cooking loss, and pre-treated samples lost more weight than non-pre-treated ones. Additionally, sampling day had a significant ($P<0.05$) effect on the results, indicating that all samples produced drip and cooking loses over the course of the study. Similar results were reported by O'Sullivan et al. (2011) when they packaged beef under different atmospheres, including 100% CO_2.

TBA values were not significantly different until day 6 of storage. From that day, TBA values significantly increased for pre-treated samples while values of non-pre-treated samples remained more or less constant (Table 3.2). Considering these results it seems that samples that had a longer exposure to CO_2, during the pre-treatment or because they have a low product filling volume, suffered a higher rate of lipid oxidation. It might be possible that because the chicken meat in pre-treatment packs was saturated with CO_2, this meant that the O_2 present in the final MAP gas flush was completely free to interact with the polyunsaturated fatty acids present on the acidified surface of the chicken meat over time. It is generally reported that the longer the exposure of meat to O_2, the higher the oxidation level determined in meat (O'Grady et al., 2000; Zakrys-Waliwander et al., 2008, 2009). In other studies involving seafood and where SGS pre-treatments were applied, similar results were found. Packing sardine fillets, Mendes et al. (2008) found that despite the absence of O_2 in some packages, significant quantities of TBA reactive substances were produced. The same authors found similar results when they applied SGS pre-treatments to octopus (Mendes et al., 2011), where pre-treated samples had higher MDA values than control samples. It has been suggested that MDA production depends not only on the amount of O_2 in the package (Goulas & Kontominas, 2007), but also on other factors such as the type of microbial flora present (Ruiz-Capillas & Moral, 2001), the probable inactivation of antioxidative enzymes as a result of the carbonic acid formation inside the meat in CO_2 enriched atmospheres (Masniyom, Benjakul, & Visessanguan, 2002), as well as the CO_2 dissolved in the tissue which would favor autoxidation of polyunsaturated fatty acids (L. S. Bak, Andersen, Andersen, & Bertelsen, 1999; Ruiz-Capillas & Moral, 2001). Overall, the lipid oxidation levels determined in this study are not high enough to produce rancid odors or taste, as it was confirmed in the sensory test.

Table 3.2. Changes in thiobarbituric acid reactive species (TBARS in mg malondialdehyde (MDA)/kg white muscle) of chicken thighs packaged under modified atmosphere (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h; E, F=9h) and different filling ratios (A, C, E g_v/p_v = 2:1; B, D, F g_v/p_v = 3:1). Samples were stored for 11 days at 3 ± 1 ºC. Values correspond to the average of three measures ± standard error.

SGS		Day			Treatment
	FD	0	6	10	
0	2:1	0.130 ± 0.01	0.315 ± 0.02	0.304 ± 0.06	A
	3:1	0.130 ± 0.01	0.289 ± 0.03	0.334 ± 0.02	B
6	2:1	0.130 ± 0.01	0.466 ± 0.11	0.536 ± 0.05	C
	3:1	0.130 ± 0.01	0.430 ± 0.05	0.811 ± 0.06	D
9	2:1	0.130 ± 0.01	0.376 ± 0.03	0.715 ± 0.05	E
	3:1	0.130 ± 0.01	0.356 ± 0.01	0.713 ± 0.02	F

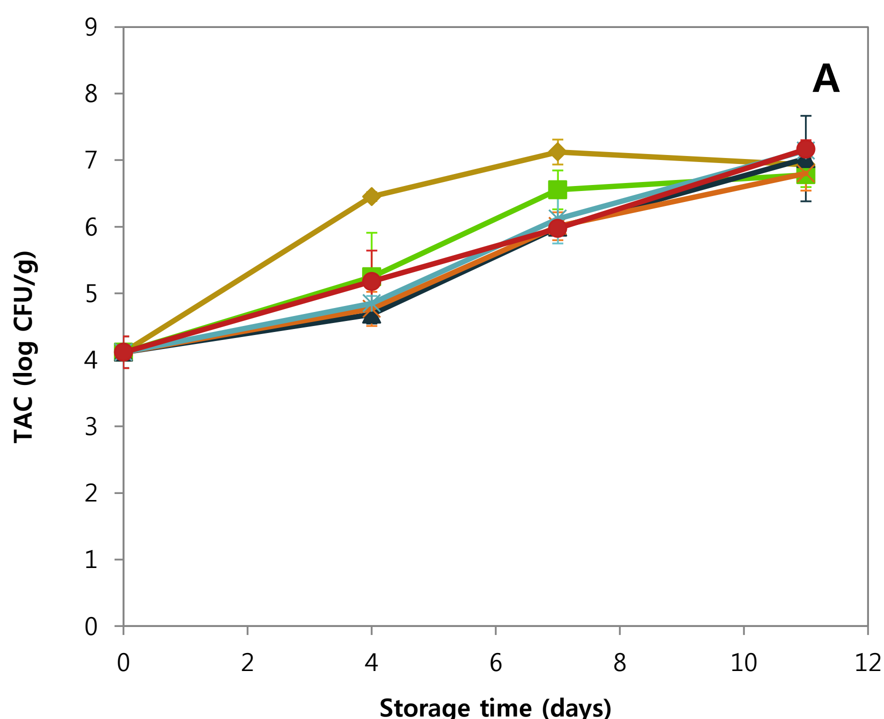
3.2. Microbiological results

Results obtained showed that the interaction among factors and the significance of the data were relatively variable depending on the microorganisms studied (Table 3.3). Giving a generic assessment about the microbiological analysis, it seems that there was no significance (P>0.05) in the triple interaction between treatment, product filling volume and sampling day. However, some positive significances were found for some variables. Regarding total aerobic counts (TAB), a statistical significance was found between treatment-type and sampling day, and samples without SGS pre-treatment presented higher values along with storage time. However, final counts at day 11 of storage were not significantly different between treatments (Figure 3.18-A). The growth of *B. thermosphacta* on non-pre-treated samples and packaged with a g_v/p_v ratio of 2:1 had significantly (P<0.05) higher counts than other treatments (Figure 3.18-B). It seems that the growth of *B. thermosphacta* and also coliforms and *E. coli* are significantly affected by product filling volume (Table 3.3). However, TAB, LAB and *Pseudomonas* growth were not affected by product filling volume, but by storage time. It seems that neither treatment, nor product filling volume, significantly affected the growth of *Pseudomonas* spp., although samples packaged without SGS and with a g_v/g_p 2:1 had higher counts than observed for other samples (Figure 3.18-C). These results are slightly different to those obtained by Rotabakk et al. (2006) who showed that pre-treatment and filling volume had some effect on *Pseudomonas* numbers. Patsias et al. (2008, 2006) also found that packaging with high CO_2 atmospheres had a positive correlation with microbial counts, obtaining lower values when gas mixtures with a percentage of CO_2 higher than 60% were used. Additionally, Gill et al. (1990) demonstrated that carcasses packaged under a pure CO_2 atmosphere improved their microbiological quality compared to vacuum packaged equivalents.

It was noticed in this study that initial microbial counts were rather high, possibly due to the manipulation of the meat during the cutting and transport process. Compared with previous work (Al-Nehlawi & Guri, 2010; Al-Nehlawi et al., 2013) where the initial microbial contamination was lower, the differences between SGS and MAP treatments on microbial assessment were more evident and statistically different than those determined in the current study.

Table 3.3. Significance (*P* value*, ANOVA, Student's T Test; α=0.05) of the storage day (0, 4, 7 and 11 days), SGS treatment (0, 6 and 9 h) and product filling volume (g_v/p_v 2:1, 3:1) on the microbial counts (log CFU/g) of total aerobic bacteria (TAC), *Brochothrix thermosphacta* (BT), Lactic acid bacteria (LAB), *Escherichia coli* (EC), total coliforms (TC) and *Pseudomonas* spp. (PS) in chicken thighs packaged under modified atmosphere conditions (75% CO_2 / 13% O_2 / 12% N_2) and stored for 11 days at 3 ± 1 ºC.

	TAC	BT	LAB	EC	TC	PS
Storage day	**<0.001**	**<0.001**	**0.0009**	0.1352	0.2942	**<0.001**
SGS treatment	**0.0037**	**<0.001**	**0.0004**	0.3044	0.6749	0.2039
FD	0.1792	**0.0007**	0.4993	**0.0003**	**0.0013**	0.0902
Day*SGS*FD	0.4488	0.7529	0.3019	0.7808	0.4147	0.7239
Day*SGS	**0.0118**	0.0774	0.1319	0.0733	0.2979	0.3569
SGS*FD	0.1243	0.3435	0.0626	0.2955	0.2191	0.3589
Day*FD	0.8330	0.0949	0.8594	0.1557	0.2557	0.3774



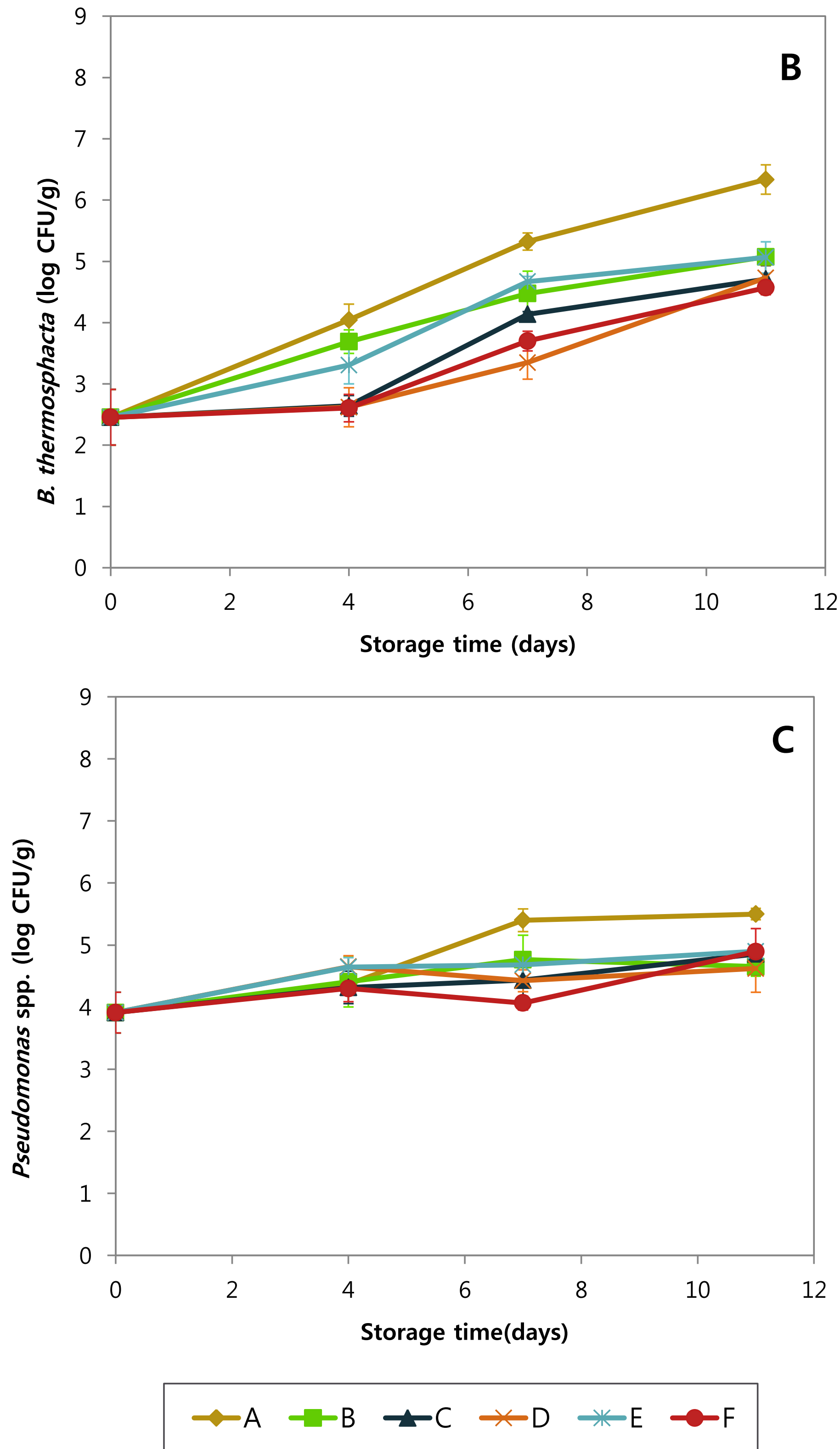
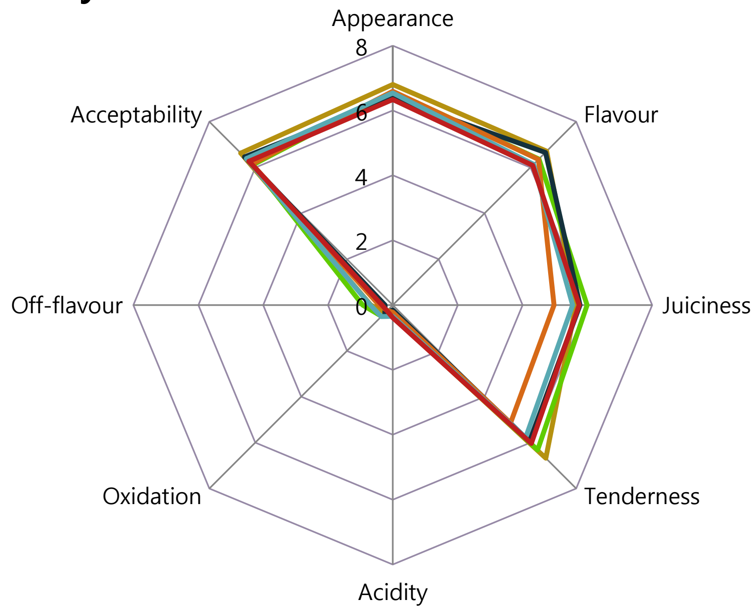


Figure 3.18. Evolution of microbiological growth of total aerobic counts (A), *Brochothrix thermosphacta* (B) and *Pseudomonas* spp. (C) in chicken thighs packaged under modified atmosphere (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h; E, F=9h) and different filling ratios (A, C, E g_v/p_v = 2:1; B, D, F g_v/p_v = 3:1). Samples were stored for 11 days at 3 ± 1 ºC. Values correspond to the average of three measures ± standard error.

3.3. Sensory analysis

Once the chicken thighs were cooked, neither SGS pre-treatment nor product filling volume had any significant effect on the sensory parameters evaluated (Figure 3.19). Nonetheless, some differences were detected for some factors on the tests carried on days 3 and 7 of the study.



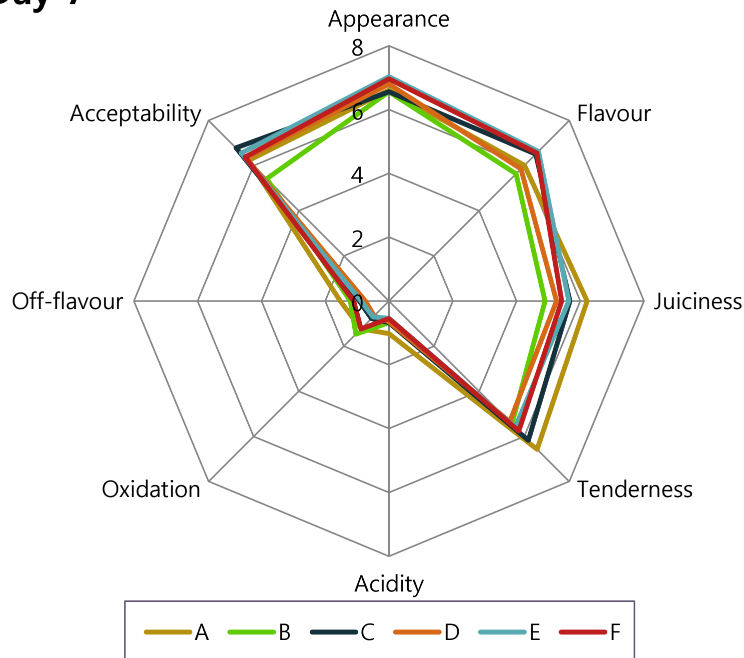


Figure 3.19. Assessment of the sensory parameters of chicken thighs packaged under modified atmosphere (75% CO_2 / 13% O_2 / 12% N_2) at different SGS pre-treatment times (A, B= 0h; C, D=6h; E, F=9h) and different filling ratios (A, C, E g_v/p_v = 2:1; B, D, F g_v/p_v = 3:1). Samples were evaluated by a 10 member panel in two different days (A= day 3; B= day 7) along the storage time.

In the first evaluation, only the samples with an SGS pre-treatment of 6 hours and an FD of 3:1 had different scores on the parameters "Tenderness" and "Juiciness", obtaining a lower score compared to the rest of the treatments. However, all treatments had similar scores when their "Overall Acceptability" was evaluated. On the second test day (day 7 of storage), the "Flavour-Liking" hedonic sensory descriptor of samples with an SGS pre-treatment of 9 hours obtained the highest score. Samples without SGS and the lowest product filling volume had the lowest punctuation on the descriptors "Overall Acceptability", "Juiciness" and "Flavour-Liking". Even though the results were not significantly different between them, samples with SGS pre-treatments and highest product filling volume reached the highest scores for the "Overall Acceptability" hedonic descriptor. Rotabakk et al. (2006) reported similar results when they analyzed the off-odor of raw meat until day 17 of their experiment. They found that trays with high product filling volume had worse off-odor development when compared with mean odor scores from meat trays containing a normal product filling volume. When Patsias et al. (2006) compared the sensory acceptability of air and MAP-packaged chicken, they found that up to day 12 air-packaged samples had better scores than MAP samples, whereas after this time, significant differences were observed and samples packaged with the highest percentage of CO_2 had the best acceptability until the end of the study. No significant differences in sensory properties were found when SGS pre-treatment was applied to sardine fillets (Mendes et al., 2008) or octopus (Mendes et al., 2011). Therefore, it can be concluded that SGS pre-treatment does not produce alterations in the sensory properties of food once is cooked, irrespective of the treatment applied.

4. Conclusions

This study shows the advantages of using SGS pre-treatment on the shelf-life of raw chicken meat before final packaging under typical MAP conditions. Previous dissolution of CO_2 into the meat during pre-treatment can prevent package collapse even when a high product filling volume is used. Considering the ratio between the gas volume and the product volume commercially used nowadays for chicken thighs, the use of a 6 hour SGS treatment may reduce this ratio considerably, and hence increasing packaging efficiency and reducing packaging waste.

The CO_2 saturation treatment does not affect the sensory properties of the meat once it is cooked. As the growth of spoilage bacteria is negatively affected by the amount of CO_2 dissolved into the meat, by using this SGS pre-treatment a better meat quality can be obtained, with lower counts and with the same physical, chemical and sensory properties as conventional packaged meat.

SGS pre-treatment is feasible to be applied to the chicken meat industry prior to the packaging of chicken carcasses and related products.

Chapter 4

Combination of MAP and HHP on poultry meat preservation

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Synergistic effect of carbon dioxide atmospheres and high hydrostatic pressure to reduce spoilage bacteria on poultry sausages

A. Al-Nehlawi [a,*], S. Guri [b,c], B. Guamis [a], J. Saldo [a]

[a] Departament de Ciència Animal i dels Aliments, Centre Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA), XaRTA-XIT, Facultat de Veterinària, Universitat Autònoma de Barcelona, Edifici V, Campus UAB, 08193 Bellaterra, Barcelona, Spain
[b] MATGAS Research Center, Campus UAB, 08193 Bellaterra, Barcelona, Spain
[c] S.E. Carburos Metálicos, Air Products Group, C/Aragón 300, 08009 Barcelona, Spain

Synergistic effect of carbon dioxide atmospheres and high hydrostatic pressure to reduce spoilage bacteria on poultry sausages

1. Introduction and objectives

The synergistic effect between HHP and CO_2 against certain enzymes and microorganisms has been previously reported (Corwin & Shellhammer, 2002; Park et al., 2003; Wang et al., 2010). However, these studies have been carried mainly in broths and model foods and there is not much results on real food and none referred to meat or meat products. As it has been previously demonstrated, pressurization is generally more effective in inoculated suspensions (buffers or microbiological media) than in real foods (Mor-Mur & Yuste, 2005; Solomon & Hoover, 2004), as the intrinsic characteristics of the food, as well as the storage conditions, greatly influence survival or death of the microorganisms that have not been inactivated by the treatment.

HHP has a huge potential in reducing microbiological growth, and its combination with cheaper technologies such as MAP could be an innovative way to improve and expand the commercial use of this technology. For that reason, in the present study the synergistic effect between both technologies has been studied on poultry sausages inoculated with several spoilage and pathogenic microorganisms commonly present on this kind of product.

2. Material and methods

2.1 Bacterial culture preparation

Considering the usual microorganisms present in the spoilage of chicken meat, *Leuconostoc carnosum* (CECT 4024), *Brochothrix thermosphacta* (CECT 847), *Salmonella enteritidis* (CECT 4300), *Campylobacter jejuni* (CECT 7572) and *Listeria innocua* (CECT 910T) as a non-pathogenic indicator microorganism for *L. monocytogenes*, were selected to be inoculated in chicken sausages.

Strains were recovered from the lyophilized form following the instructions of Spanish Type Culture Collection (CECT, University of Valencia, Spain), and were kept at -20 °C in cryobeads (Nalgene System 100, Microkit Iberica S.L., Madrid, Spain).

For each microorganism, the first bacterial culture was obtained by inoculating a cryobead in 20 mL of nutritious media (Table 4.1) and incubated at required conditions. After the incubation time, 0.1 mL of this first culture was transferred to 20 mL of the same nutritious media and incubated again at the same conditions to ensure the stationary phase of the culture, when the microorganisms are generally more resistant (Linton, McClements, & Patterson, 2001; Rendueles et al., 2011). The final concentrations of the inoculums were 10^7-10^9 cfu/mL for each microorganism.

Table 4.1. Conditions and media used for the preparation of the inoculums and enumeration of the strains used with chicken sausages.

Microorganism	Incubation temperature (°C)	Incubation time (h)*	Recovering media	Growth media	Spread method
Brochothrix thermosphacta	25	48	BHI Broth	STAA+spl	Spread plate
Campylobacter jejuni	37**	48	Col.Blood Agar	CCDA+spl	Spread plate
Leuconostoc carnosum	28	48	MRS Broth	MRS Agar	Pour plate
Listeria innocua	37	24	TSB	ALOA	Spread plate
Salmonella enteritidis	37	24	TSB	SMID2	Spread plate

ALOA: ALOA Agar Chromogenic selective media (Biomerieux, France)
BHI Broth: Brain Heart Infusion Broth (CM1135, Oxoid, England)
CCDA + spl: Campylobacter Blood-Free Selective Medium + CCDA Selective supplement (CM0739 + SR0155, Oxoid, England)
Col.Blood Agar: Columbia 5% Blood Agar Plates (Biomerieux, France)
MRS Broth: De Man, Rogosa, Sharpe Broth (CM0359, Oxoid, England)
MRS Agar: De Man, Rogosa, Sharpe Agar (CM0361, Oxoid, England)
STAA + spl: STAA Agar + STAA selective supplement CM0881 + SR0151, Oxoid, England)
SMID2: ASAP Chromogenic selective media (Biomerieux, France)
TSB: Tryptone Soya Broth (CM0876, Oxoid, England)
*Samples treated with HHP were kept 24 h more at incubation due to the small size of the colonies (see point 3)
** *Campylobacter jejuni* incubation was done at microaerofilic conditions (5% O_2, 10% CO_2) using gas generators (CN0025 CampyGen, Oxoid, England) and anaerobic jar (AG0025, Oxoid, England)

2.2 The matrix

Smoked vacuum packed poultry sausages from the same batch were used as the matrix to inoculate the microorganisms used in this study. The product was purchased from a local supermarket and was kept under refrigeration until its usage.

Total aerobic counts and total anaerobic counts were determined in order to know the initial contamination of the product (results not shown). Due to the nature of the studied microorganisms and the selective media used in its growth, the initial contamination of the samples was not considered significant.

2.3 Samples inoculation

For each treatment and sampling day, three sausages were inoculated for each microorganism separately. Inside the laminar flow cabin with a sterile environment, 0.1 mL of the agitated inoculum was spread on the surface of the sausage. After 15 minutes of drying, sausages were placed in gas barrier bags (50 µm, O_2 transmission rate <5 cm^3 m^{-2} $24h^{-1}$ bar^{-1} and CO_2 transmission rate <25 cm^3 m^2 24 h^{-1} bar^{-1}, both determined by the manufacturer at 23 ºC and 0% RH; Linpac, Spain). The final concentrations of the inoculums on the sausages went from 10^6 to 10^8 cfu/mL, depending on the microorganism. Once were inoculated, sausages were treated and kept under refrigeration during the study.

2.4 Packaging and HHP treatment

Immediately after placing the sausages into the bags, four groups of samples were made and different treatments were carried: 1. Sausages packaged with air (Air); 2. Sausages packaged with pure CO_2 (CO2); 3. Sausages packaged with pure CO_2 and treated with HHP (CO2+HHP); and 4. Sausages packaged with air and treated with HHP (Air+HHP).

The air inside the bags of HHP+CO2 and CO2 samples was removed and flushed with food grade pure CO_2 (SE Carburos Metálicos, Air Products, Spain) during 20 seconds to ensure the desired atmosphere (98.7 ± 0.4% CO_2). Air+HHP and Air samples were flushed with compressed air to reach the same bag volume as samples packaged with CO_2. The gas volume/ product volume ratio (g_v/p_v) was 4:1 in all the bags.

CO2+HHP and Air+HHP samples were pressurized in a discontinuous high hydrostatic pressure equipment (GEC Alsthom ACB, Nantes, France) with a pressure chamber of a 2 L capacity. Samples were submerged in water, which acted as the hydrostatic fluid medium. Sausage samples were pressurized at 350 MPa for 10 minutes. Pressurization and depressurization times were 120 seconds approximately. The treatment was carried at room temperature (20 ± 2ºC) and the temperature of the water was measured at the beginning and at the end of the treatment.

2.5 Microbiological analyses

Microbiological analyses were performed 20 ± 2h after the treatment and repeated on day 7 for all the microorganisms, keeping the samples at 2 ± 1 ºC during that time.

Immediately after opening the packages aseptically, the sausage contained in each package was placed in a stomacher bag with filter (GSI Creos, Japan). Afterward, the inside of the original bag was washed with 180 ml of 0.1% sterile peptone water (CM1049, Oxoid, England) in order to sweep all the microorganisms that could have remained in the film, and then it was poured out to the stomacher bag together with the sausage. The sample was homogenized in a Pulsifier PUL 100E (Microgen Bioproducts Ltd, England) for 90 seconds at room temperature. Appropriate dilutions were spread on the following media, with the given incubation time and temperature (Table 4.1).

2.6 Statistical analysis

This experiment was run 2 times with triplicate analyses (three independent bags) for all the treatments and sampling days. Two-way analysis of variance (ANOVA) and general linear model (GLM) procedures were performed by means of JMP Software (SAS Institute INC., U.S.A.), between the main factors "Treatment" and "Sampling day". When there was statistical significance ($P<0.05$), Last Square Means (LSM) procedure was used, applying the Tukey-Kramer's test to determinate significant differences among the averages.

3. Results and discussion

After incubating the plates, it was observed that in those samples pressure treated, the size of the survivor colonies was smaller when compared with unpressurized samples. The same effect was reported by Corwin and Shellhammer (2002) when they combine carbon dioxide and high pressure, exemplifying the injuring effect of the HHP treatment. These samples were incubated 24h more in order to ensure its complete growing.

3.1 Survival and recovery of the strains

3.1.1 Brochothrix thermosphacta

After the pressure treatment, sausages packaged with air suffered a 3 log cfu/g reduction, dropping to 4 log at day 7 of storage. However, samples packaged with CO_2 had a reduction of more than 5 log cfu/g after the treatment, and survivor cells did not recover throughout the time, resulting with the inactivation of *B. thermosphacta* after 7 days of storage. Samples not treated with pressure were not significantly different from each other, not showing any counts

reduction or increase during the storage (Figure 4.1). These results demonstrate the synergistic effect of combining HHP and CO_2 atmosphere over the survival of *B. thermosphacta*. Vercammen et al. (2011) inactivated *B. thermosphacta* inoculated in cooked ham packaged under MAP (70% N_2 and 30% CO_2) treating it at different pressures and temperatures. At 25 °C, they only obtained a reduction of 1 log cfu/g when the pressure applied was 300 MPa, and more than 4 log cfu/g when they increased the pressure up to 400 MPa. However, they did not consider any interaction between the pressure and the atmosphere. Esmer et al. (2011) studied the response of *B. thermosphacta* at modified atmospheres with CO_2, and they reported that with high concentrations of carbon dioxide such as 50% and 70%, the growth of *B. thermosphacta* could be inhibited, something that other authors also observed (Ercolini, Russo, Torrieri, Masi, & Villani, 2006; Koutsoumanis, Stamatiou, Drosinos, & Nychas, 2008; Sheridan et al., 1997). However, in other research works an important growth of *B. thermosphacta* was noticed when CO_2 atmospheres were used (Berruga, Vergara, & Gallego, 2005; Gill & Harrison, 1989; Patsias et al., 2008; P. N. Skandamis & Nychas, 2002), which suggest that besides the atmosphere, there are other factors that influence on the growth of this bacteria, such as another competitor microorganisms or the strain used.

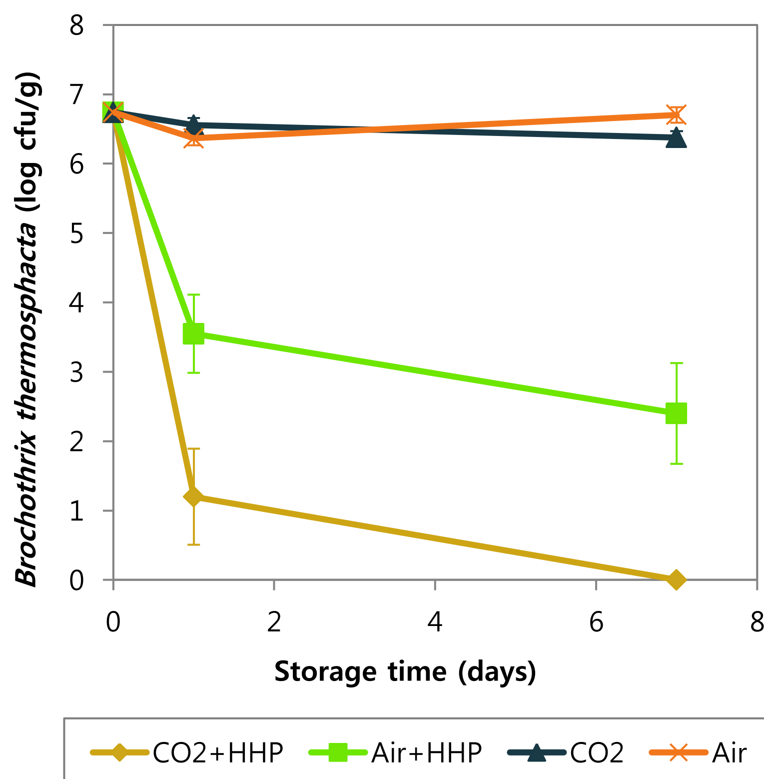


Figure 4.1. Effect of high hydrostatic pressure (350 MPa 10 min at room temperature) and carbon dioxide atmosphere on *Brochothrix thermosphacta* inoculated on poultry sausages stored at 2 ± 1 °C. Measures correspond to the average of three measurements ± standard error.

3.1.2 Campylobacter jejuni

In pressure treated samples, colony counts of *C. jejuni* dropped hastily, resulting in its inactivation and non-recovery after 7 days of storage (Figure 4.2). Samples non-pressure-treated also exhibited a reduction of more than 2 log units on the first control, and colonies were completely inactivated at day 7, probably due to unfavorable atmospheric conditions. These results are similar to the ones obtained by Solomon and Hoover (2004) when they treated inoculated *C. jejuni* with different pressures (0 – 400 MPa). *C. jejuni* was completely inactivated at 325 MPa when it was inoculated into Bolton broth or phosphate buffer and at 375 – 400 MPa when it was inoculated in food. It seems that food systems offer a much greater pressure-protective effect of the cells than the broth or the buffer. Furthermore, these authors found that storage temperature after the pressure treatment has an important influence on the recovery of the cells, which shows a great effect at lower temperatures. Examining the response of *C. jejuni* to high pressure shock, Bièche et al. (2012) found that the behavior of this bacteria is different from other Gram negative species, developing poor protection systems compared to others, which could explain its sensibility to pressure treatments. After exposing *C. jejuni* to a different sublethal stresses, Sagarzazu et al. (2010) concluded that no protective responses to HHP were observed, indicating that this technology could be combined with other stressing agents without the possible risk of inducing resistance if incorrectly applied.

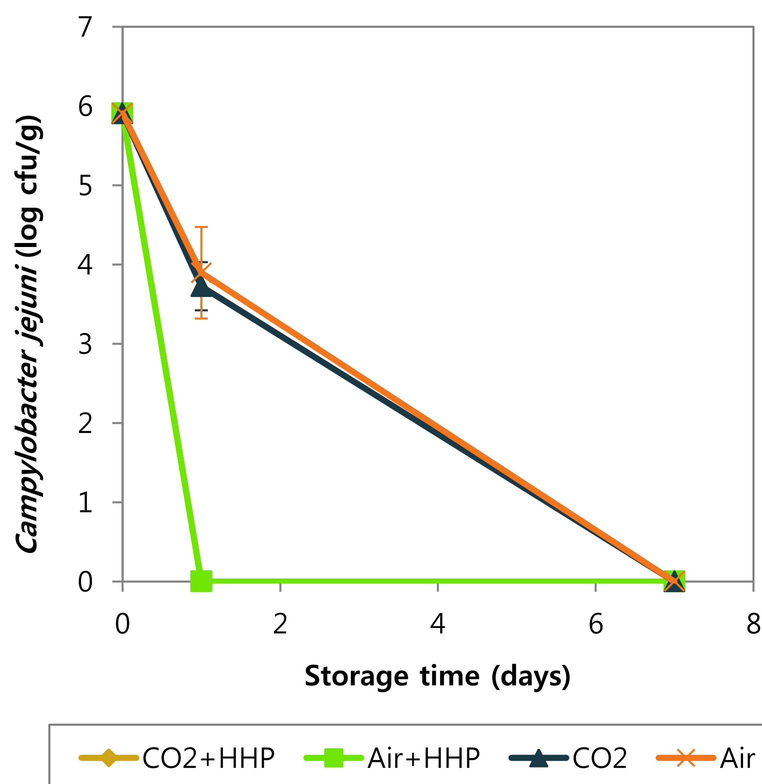


Figure 4.2. Effect of high hydrostatic pressure (350 MPa 10 min at room temperature) and carbon dioxide atmosphere on *Campylobacter jejuni* inoculated on poultry sausages stored at 2 ± 1 ºC. Measures correspond to the average of three measurements ± standard error.

3.1.3 Leuconostoc carnosum

L. carnosum counts of sausages packaged with pure CO_2 and treated with HHP had a reduction of almost 4 log cfu/g, remaining constant until the end of the storage without cell recovery; while in sausages packaged with air, the high pressure treatment only produced a reduction of less than 1 log cfu/g (Figure 4.3). Without high pressure treatment, samples packaged with air had higher counts at day 7 than samples packaged with CO_2, although the differences were not statistically significant.

The response of LAB to high pressure treatments has been already reported (Patterson, McKay, Connolly, & Linton, 2010; Yuste, Pla, Capellas, Ponce, & Mor-Mur, 2000). Vercammen et al. (2011) treated cooked ham up to 500 MPa at 25 °C during 10 min to inactivate *L. carnosum*, and with 400 MPa they had a 5 log reduction and 4 log when the treatment temperature was 5 °C. Amanatidou et al. (2000) combined HHP (150 MPa during 10 minutes) and a 50% O_2 / 50% CO_2 atmosphere to preserve fresh salmon, and they found that samples previously packaged with MAP and then treated with pressure achieved a microbiological shelf-life of 18 days, 12 days just with the MAP and 8 days when the salmon was only vacuum-packed. Considering this research, it is evident that the addition of pressurized CO_2 reduces significantly the growth of spoilage bacteria according also to our results. Corwin and Shellhammer (2002) also found that combining pressure and CO_2 was significantly effective for inactivating other lactic bacteria, while no significant effect was observed when carbon dioxide was applied to unpressurized samples.

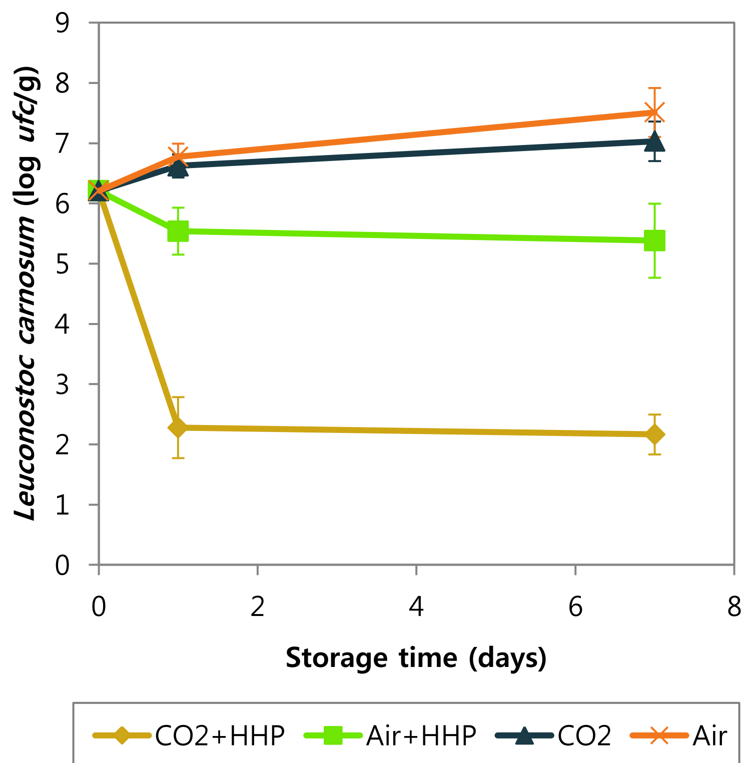


Figure 4.3. Effect of high hydrostatic pressure (350 MPa 10 min at room temperature) and carbon dioxide atmosphere on *Leuconostoc carnosum* inoculated on poultry sausages stored at 2 ± 1 °C. Measures correspond to the average of three measurements ± standard error.

3.1.4 Listeria innocua

In the present study, *L. innocua* was the microorganism that showed the greatest resistance to the treatments applied, with less than 3 log cfu/g reduction at the end of the storage in high pressure and CO_2 packaged samples. Sausages treated with pressure and packaged with air had only a 0.5 log cfu/g reduction at day 1, but showed a recovery of the cell counts at day 7, ending with the same counts than samples not treated by pressure (Figure 4.4).

Carlez et al. (1993) and Patterson et al. (1995), observed a significant reduction of *Listeria* counts of about 6 log units when inoculated minced meat was treated at 350 MPa for 20 min at 4 °C and at 375 MPa for 15 min at 20 °C, respectively, under air atmosphere. However, working with the same strain of *L. innocua* used in this study, Yuste et al. (1999) only had 1 log unit reduction when inoculated mechanically recovered poultry meat was treated at 350 MPa during 10 min at 20 °C. When Gervilla et al. (1997) studied the effect of different HHP treatment conditions on the destruction of *L. innocua* inoculated on ewe's milk, they found that *Listeria* was less sensitive to pressure when the treatments were carried out at 25 °C than at 2 °C, 10 °C or 50 °C, reducing only 1 log cfu/g at 350 MPa at that temperature. They also concluded that the maximum survival rate at 350 MPa is near 23 °C. This may explain why *L. innocua* resisted the combination of HHP and CO_2 atmosphere more than the other microorganisms studied.

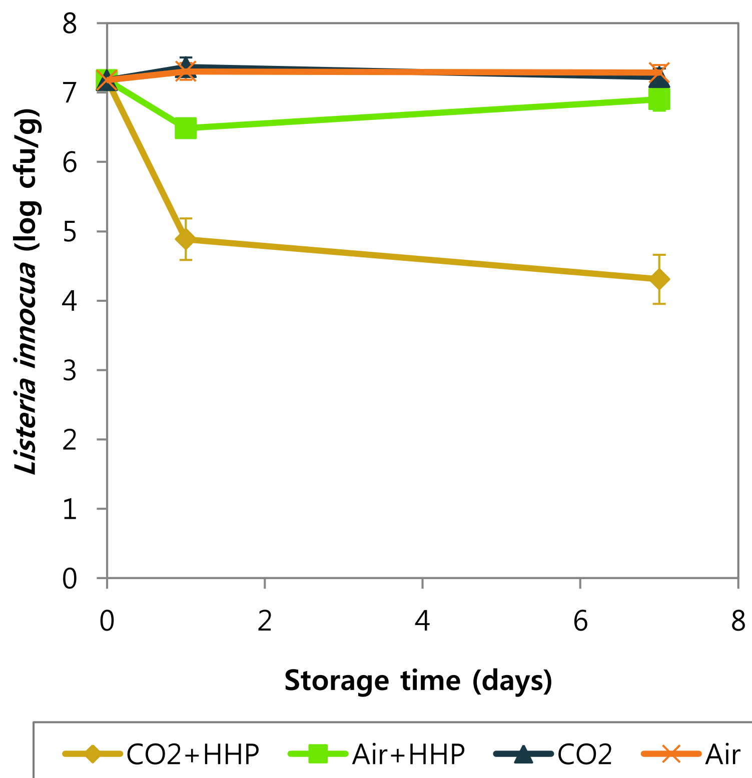


Figure 4.4. Effect of high hydrostatic pressure (350 MPa 10 min at room temperature) and carbon dioxide atmosphere on *Listeria innocua* inoculated on poultry sausages stored at 2 ± 1 °C. Measures correspond to the average of three measurements ± standard error.

3.1.5 Salmonella enteritidis

The effectiveness of HHP on the growth of *Salmonella* species has been well reported (Shigehisa, Ohmori, Saito, Taji, & Hayashi, 1990; Yuste et al., 2004; Yuste, Pla, & Mor-Mur, 2000). In contrast, combining this technology with pure CO_2 atmosphere did not show the expected results (Figure 4.5). Sausages treated with pressure and packaged with air had a reduction of almost 4 log units after the treatment and 7 log cfu/g after 7 days. However, under CO_2 atmosphere, only 2 log cfu/g were reduced, reaching 2.5 log units at day 7. Nychas and Tassou (1996) studied the growth and survival of *S. enteritidis* on fresh poultry meat packaged under different atmospheres, and they found that *this microorganism* survived and also grew in packages with 100% CO_2. Similar behavior was detected by Stepanović et al. (2003) when they studied the influence of the atmosphere composition on the *Salmonella* spp. biofilm production. These authors found that *Salmonella* spp. produced the highest quantity of biofilm under a CO_2-rich atmosphere, while at aerobic conditions the amount of biofilm produced was lower. All these conclusions induce to think that CO_2 has a lower bacteriostatic effect against *Salmonella* when compared to other microorganisms.

On chicken sausages not treated with pressure, air packaged samples showed statistically (P<0.05) lower counts than sausages packaged with CO_2, which confirms the hypothesis mentioned before.

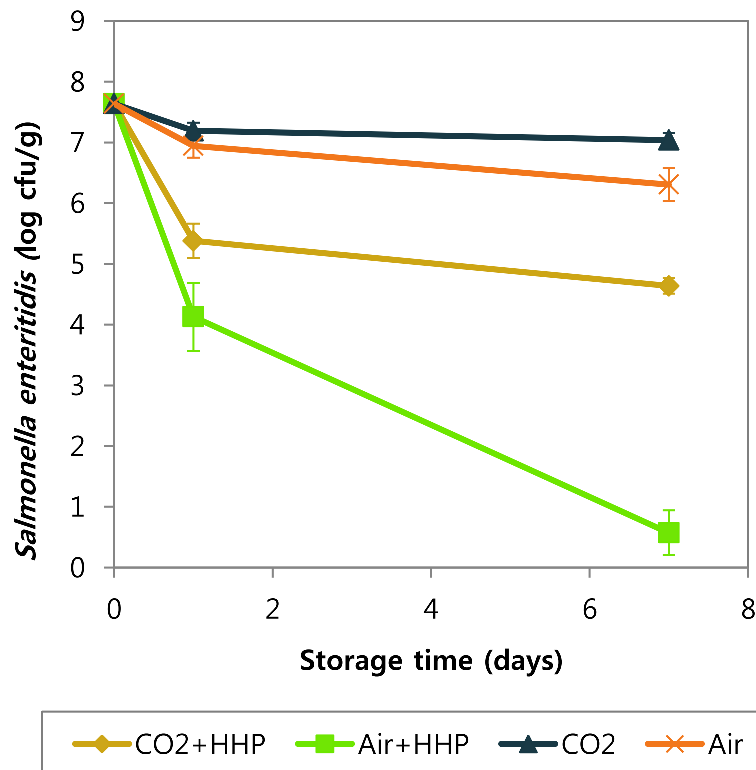


Figure 4.5. Effect of high hydrostatic pressure (350 MPa 10 min at room temperature) and carbon dioxide atmosphere on *Salmonella enteritidis* inoculated on poultry sausages stored at 2 ± 1 ºC. Measures correspond to the average of three measurements ± standard error.

3.2 General mechanism of bactericidal synergistic action of CO_2 and HHP

It is well known that carbon dioxide can dissolve into food products, especially in meat, particularly into its water and fat content (Carroll, Slupsky, & Mather, 1991; Jakobsen & Bertelsen, 2006; Rotabakk, Lekang, & Sivertsvik, 2007; Sivertsvik, Jeksrud, et al., 2004). One of the most studied mechanisms of CO_2 to inhibit microorganism growth is the formation of carbonic acid (H_2CO_3) once it dissolves into the water phase of the product (Tan & Gill, 1982). Observing that CO_2 penetrates easier into the cells than other acids (such as hydrochloric and phosphoric acid), Lin, Yang & Chen (1993) suggested that a low pH contribute to an increase in cell permeability, which facilitates the diffusion of CO_2 into microbial cells. In addition, after a high pressure treatment, the microorganisms' cell membrane has their properties and structure damaged, which better facilitates the penetration of carbon dioxide into the cell. This could explain why samples packaged with CO_2 and treated with high pressure obtained higher reductions on microbial counts than those not treated with HHP either preserved with CO_2. The same conclusions were found in similar works, when the combination of carbon dioxide and high pressure was studied (Park et al., 2003; Wang et al., 2010).

When high carbon dioxide atmospheres are used for preserving meat products, package collapse is produced as a consequence of the difference between atmospheric pressure and the CO_2 partial pressure within the package (Gill, 1988). Based on these facts, one of the initial hypotheses of this experiment was that high pressure would force the dissolution of CO_2 inside the sausage, which might be noticed by an immediate reduction of the bags volume. However, no significant volume reduction was detected after the pressure treatment. Nevertheless, some interesting differences were detected on the overall aspect of the sausages treated. Thanks to the external skin that sausages have, an important amount of bubbles were formed on their surfaces upon pressure release, showing important differences depending on the atmosphere (air or CO_2) present inside the bags. Samples packaged with air had a higher amount of bubbles than samples packaged with pure CO_2 (Figure 4.6). Although there are not references that relate this fact to high pressure, the logical explanation for this is that high pressure causes a forced penetration of the gas (either carbon dioxide or air) into the sausages during the compression time. Once the dwell pressure is reached, the gas remains in the product and is when CO_2 dissolves in the water phase, forming carbonic acid and acting changing the pH of the cells. At normal atmospheric conditions, the concentration of dissolved CO_2 can be related to the external partial pressure of CO_2 and Henry's law constant. However, under pressurized CO_2 conditions, these assumptions are not necessarily valid, and the equilibrium that dissolved CO_2 reaches is therefore pressure and temperature dependent (Garcia-Gonzalez et al., 2007). After 10 minutes, the depressurization phase starts, and gas that was in the product comes out abruptly. This gas extraction is responsible for the bubbles formed on the surface of the sausages, which are quite evident on air–packed samples (Figure 2B). However, in CO_2 packed samples an important amount of this gas remains dissolved into the sausage, slowly being released and improving its bacteriostatic properties and only slight bubbles are formed (Figure 2A).

It would be interesting to study this phenomenon in further studies, and possibly comparing with other atmospheres, such as nitrogen or oxygen.

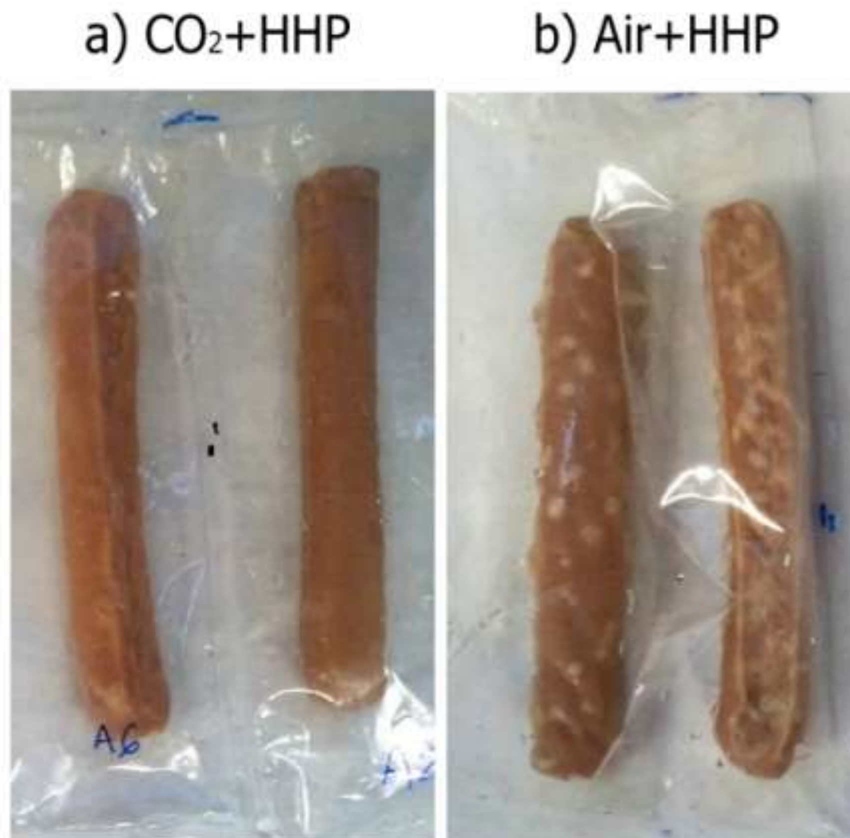


Figure 4.6. Pictures of the aspect of the sausages packaged with pure CO_2 (A) and air (B) after the HHP treatment at 350 MPa during 10 min at room temperature (20 ± 2 ºC).

4. Conclusions

In this experiment, the synergistic effect of combining CO_2 atmospheres and HHP to reduce or even inactivate five of the most common spoilage and pathogen bacteria of poultry meat products has been studied and effectively demonstrated for *B. thermosphacta*, *L. carnosum* and *L. innocua*. The results obtained suggest that when high pressure is applied, the cell membrane is damaged and consequently a larger amount of CO_2 can penetrate into the cell, causing a greater lethal effect on microorganisms. In the presence of carbon dioxide, much lower pressures are needed to achieve the desired bacterial inactivation, which give the chance to reduce the costs of this pressure technology, making it more attractive for the food industry.

Evaluation of the forced dissolution of different gases into poultry sausages by means of high hydrostatic pressure

1. Introduction and objectives

It has been reported that the HHP pressure treatment of CO_2-packaged samples has a synergistic effect on the inactivation of microorganisms. Previous studies (Al-Nehlawi, Guri, Guamis, & Saldo, 2014) have suggested that the cell damage produced by high pressure facilitates the penetration of carbon dioxide into the microorganisms' cells, affecting their metabolism and consequently their growth. When products with artificial skin like sausages are packaged with gas and high-pressure treated, it is possible to observe the formation of certain bubbles trapped between the meat and the skin after pressure release. This phenomenon is usually missed, but it is noticeable in sausages as the bubbles distort the aspect of the sausage peel. From the different parameters considered relevant for this gaseous embolism, the most important is the gas used for MAP and the pressure release rate.

There are not many works related to the interaction between MAP and HHP. The lack of interest in these studies is the low industrial interest in using a large part of the available space in the pressure chamber to fill it with no-product (gas space), which forces an increase in the pumping power needed to fill the space occupied by the gas. This affects the effectiveness of the process and increases its cost.

However, with the aim to go further in the study of the synergistic effect detected on the previous research with poultry sausages packaged with pure CO_2 and treated with HHP (Al-Nehlawi et al., 2014), this study has wanted to evaluate the possible forced dissolution of gas produced by HHP by means of a quantification of the gaseous embolism produced on poultry sausages.

2. Material and methods

2.1 Packaging

Smoked vacuum packed poultry sausages from the same batch were individually packaged in gas barrier bags (50 μm, O_2 transmission rate <5 cm^3 m^{-2} $24h^{-1}$ bar^{-1} and CO_2 transmission rate <25 cm^3 m^{-2} 24 h^{-1} bar^{-1}, both determined by the manufacturer at 23 ºC and 0% RH; Linpac, Spain). Food-grade nitrogen (N_2), argon (Ar) and carbon dioxide (CO_2) (SE Carburos Metálicos, Air Products, Spain) were used to package the sausages.

CO_2 is the most used gas for MAP as it has the highest preservative effect on food products, thanks to its ability to dissolve into the fat and water content, forming carbonic acids. N_2 is commonly used as an inert gas to fill packages and avoid package collapse. Ar was included as another level in this study due to it is increasingly use as an inert gas on MAP systems and also because it is used on high pressure situations, such as deep diving on gas mixes that exclude N_2.

Each gas was flushed during 20 seconds inside the bags to ensure a 100% concentration of each gas in the head space of the packages. The gas volume and product volume (g_v/p_v) ratio was 4:1 to ensure the excess availability of the gas.

The composition of the atmosphere inside the bags was checked by using an oxygen and carbon dioxide analyzer (OxyBaby, Witt-Gasetechnik GmbH & Co KG, Germany), taking an aliquot of the gas with a syringe through a foam rubber to avoid the introduction of the external atmosphere.

2.2 HHP treatment

The pressure treatment was done at room temperature (22 ± 2 ºC) for 10 min at pressure levels of 200 or 400 MPa with Stansted HP-8000 equipment (Model ISO-Lab FPG11500; Stansted Fluid Power Ltd., UK). After holding time, pressure was released at three different rates: slow (100 MPa/min), medium (500 MPa/min), or fast (1000 MPa/min).

2.3 Experimental design and bubbles evaluation

Two pressure levels, three different gases and three released speeds were compared. For each treatment (gas, pressure and released speed), three replicates were used, meaning each replicate an independent bag with one sausage on it.

After the treatment, packages were open and the images of all the sausages surfaces were digitally recorded. The parameter chosen as a response variable was the number of bubbles occurred by surface unit.

2.4 Statistical analysis

Analysis of variance (ANOVA) and means separation were performed to evaluate the bubbles quantification at different conditions of pressure, gas and pressure releasing speed. The statistical data analysis was conducted by performing a Tukey test (P<0.05). The software program used was Statistica, version 7.0 (StatSoft Inc., USA).

3. Results and discussion

The synergistic effect between CO_2 and HHP on the shelf-life of food products has been reported before (Al-Nehlawi et al., 2014; Amanatidou et al., 2000; Basak, Ramaswamy, & Piette, 2002). When a high-pressure treatment is performed, the cell damage produced by pressure facilitates the penetration of the gas into the sausages during the compression time. Once the dwell pressure is reached, the gas remains in the product and it is when CO_2 dissolves in the water phase, forming carbonic acid and acting by lowering the pH of the cells. After 10 minutes, the depressurization phase starts, and the gas that was dissolved in the product comes out abruptly. In products with peel or skin like sausages, this gas extraction is the responsible of the bubbles formed on the surface of the product, as a consequence of the gas being trapped between the meat and the peel.

In the present study, after high-pressure treatment, the bubbles formed on the sausages surface were quite evident on N_2 and Ar samples in contrast with CO_2 packed samples, where the alterations on the sausages skin were less evident (Figure 4.7).

CO_2 was the gas that produced less bubbles at any pressure and release speed, followed by Ar and N_2. By varying the pressure release speed it was observed that the slower pressure release rate, the lower amounts of bubbles in the sausages were produced (Figure 4.8).

The interaction between the pressure applied and the gas being pressurized was statistically significant (P<0.05). Counterintuitively, samples treated at 400 MPa had less bubbles than samples treated at 200 MPa (Figure 4.8). To understand this fact, we should take in consideration the significance that environmental pressure and gas partial pressure have on CO_2 dissolution (Jakobsen & Bertelsen, 2004; Jakobsen & Risbo, 2009; Rotabakk et al., 2007). The effect of pressurized gases on microbial populations has been studied before (Debs-Louka, Louka, Abraham, Chabot, & Allaf, 1999). When Wiebe and Gaddy (1939) studied the effect of pressure (up to 70 MPa) and temperature (50, 75 and 100 °C) on de solubility of CO_2 in water they found an increase on the solubility at increasing pressures and decreasing temperatures. García-Gonzalez et al. (2007) described the mechanism of dissolved CO_2 in seven different levels of action: (1) solubilization of pressurized CO_2 in the external liquid phase, (2) cell membrane modification, (3) intracellular pH (pH_i) decrease, (4) key enzyme inactivation/cellular metabolism inhibition due to pH_i lowering, (5) direct (inhibitory) effect of molecular CO_2 and HCO_3^- on

metabolism, (6) disordering of the intracellular electrolyte balance, and (7) removal of vital constituents from cells and cell membranes. They also stated that pressure controls both the solubilization rate of CO_2 and its total solubility in a suspending medium. Therefore, a higher pressure enhances CO_2 solubilization to facilitate both acidification of the external medium (Step 1 of the mechanism) as well as its contact with the cells (which facilitates Step 2 of the mechanism, and indirectly also Steps 3–6). In addition, CO_2 at higher pressures in general exhibits a higher solvating power, thus also facilitating Step 7 in the mechanism. This might explain why CO_2 packaged samples had less bubbles when pressure was higher, as CO_2 dissolves in a deeper levels and its extraction with pressure release is harder, keeping dissolved in the product. Then less gas is extracted and consequently fewer bubbles are formed. As N_2 is 60 times less soluble than CO_2 (Mitz, 1979), we might expect these results. The release rate depends basically on the combination of solubility rate and the chemical reaction rates for the formation of carbonic acids and bicarbonates; therefore CO_2 release rate is much lower than N_2 release rate. This difference is also used on some British ale pressurized with a high N_2 mix to prevent the product to be too fizzy.

Pressure releasing speed was also significant on the bubbles formation ($P<0.05$), as well as the triple interaction between the main factors. In general, faster releasing speed produces more bubbles in all the gases and pressures studied (Figure 4.8). Lin et al. (1994; 1992) observed that, at high pressure, supercritical carbon dioxide penetrates cells and ruptures them when it was suddenly released. They also improved the rate of disruption by repeatedly releasing the CO_2 pressure. Fraser (1951) observed that the best microbial destruction results were obtained when the CO_2 pressure was released as rapidly as possible. Some researchers (Fraser, 1951; Lin, Chan, Chen, & Chen, 1991) have insisted that rapid decompression is a parameter that enhances the microbial inactivation process. The explanation that Debs-Louka (1999) suggested was that as those authors worked under CO_2-supercritical conditions (pressure and temperature), in which the fluid penetrates more easily into the cells, a rapid decompression may provoke cell rupture as a result of the expansion of CO_2 within the cells. The great influence of the pressurized gas on the microbial population inactivation compared to unpressurized gas has been previously reported (Amanatidou et al., 2000; Corwin & Shellhammer, 2002; Garcia-Gonzalez et al., 2007; Wiebe & Gaddy, 1939). By using high-pressure treatments and regulating the decompression speed, greater amounts of CO_2 can remain dissolved into the sausage, improving its microbiological preservation.

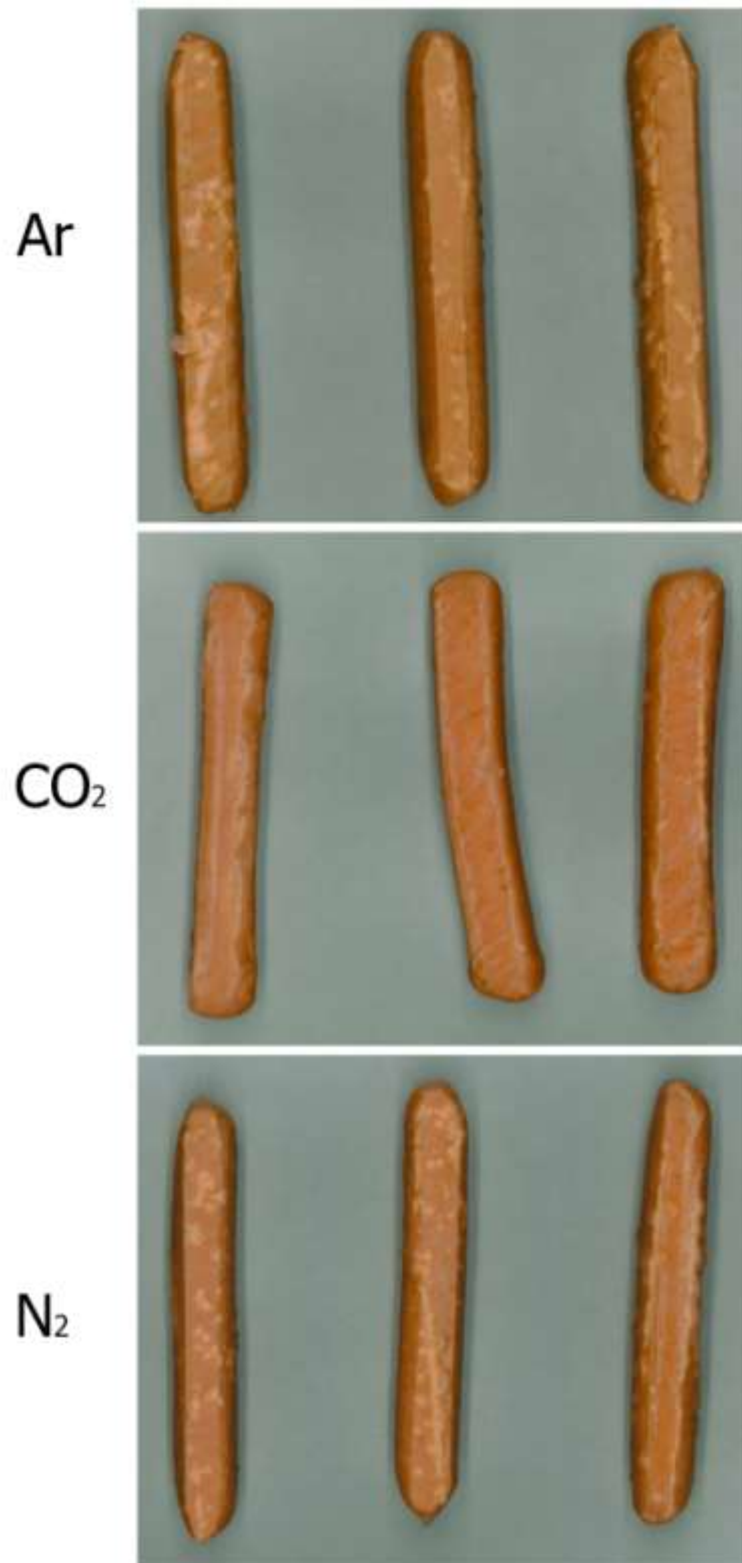


Figure 4.7. Pictures of the aspect of the sausages packaged with pure argon (Ar) carbon dioxide (CO_2) and nitrogen (N_2) after being high-pressure treated (200 MPa during 10 min at room temperature) and decompressed at 1000MPa/sec.

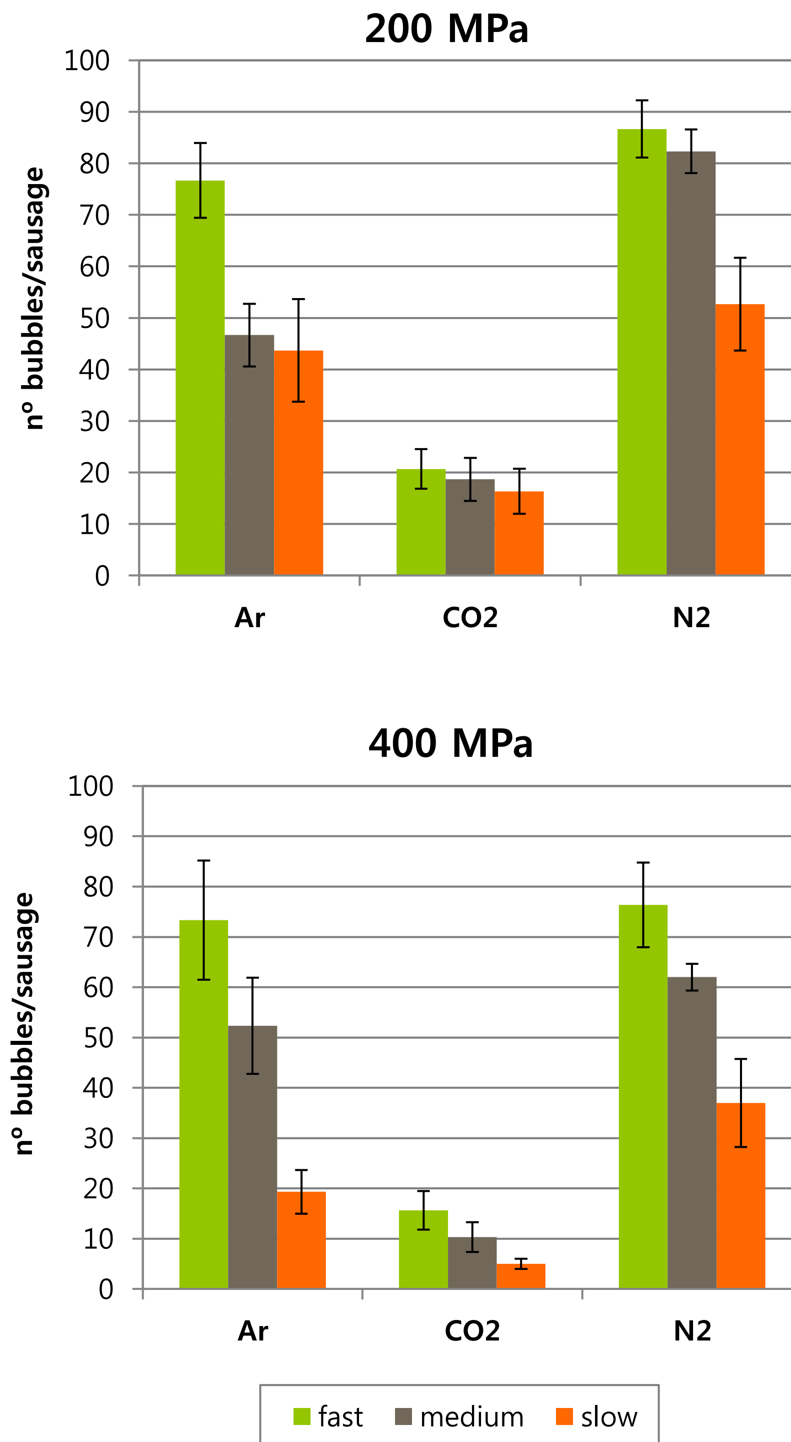


Figure 4.8. Amount of bubbles counted on the sausages surface after being packaged with modified atmosphere (Ar, CO_2 or N_2) and treated with high pressure (200 or 400 MPa for 10 min at room temperature) applying three different speeds of pressure release. Results show the average of three replicates ± standard error.

4. Conclusions

The results obtained suggest that when HHP is applied to a MAP product, the cell membrane is damaged because of the pressure applied and consequently a higher amount of gas can penetrate into the cells. When the pressurized gas is carbon dioxide, its penetration into the cells is increased because of the cell membrane damage, and a greater amount of the gas dissolves. At higher pressures this forced dissolution increases. In addition, slowing down the pressure release speed, a greater amount of the gas remains dissolved in the product.

In this experiment the amount of gas dissolved into the meat has been quantified by means of the enumeration of the bubbles formed between the meat and the peel of the sausages during the pressure release of the HHP treatment, demonstrating differences between soluble gases (CO_2) with less soluble gases (N_2 and Ar).

Development of a response surface model to predict the synergistic effect of high hydrostatic pressure and modified atmosphere packaging to reduce spoilage bacteria on poultry sausages

1. Introduction and objectives

The study of the synergistic effect between HHP and a pure CO_2 atmosphere on poultry sausages (Al-Nehlawi, Guri, Guamis & Saldo, 2014) showed the positive combination between these two technologies against the most common spoilage and pathogenic microorganisms of this food product. However, it might be necessary to test different atmospheres and different pressures in order to find the optimum synergistic treatment at lower CO_2 concentrations and inferior pressure levels, with the aim of developing an attractive treatment from an economical and productive point of view.

The effect of compressed carbon dioxide on different types of microorganisms has been studied by several researchers (Ballestra, Abreu-Da-Silva, & Cuq, 1996; Debs-Louka et al., 1999), and it has been demonstrated that increasing the partial pressure of carbon dioxide added to the packaging atmosphere prolonged the shelf life of packaged products (Jakobsen & Risbo, 2009; Sivertsvik, Jeksrud, et al., 2004). HHP can be applied at lower levels to pressurize CO_2 obtaining the same reductions in microbial population than higher pressures or greater CO_2 atmospheres applied separately (Amanatidou et al., 2000; Park et al., 2003; Wang et al., 2010).

With the aim to continue the research on the synergistic effect between MAP and HHP, on this study the response of combining lower pressures and different CO_2 concentrations on the reduction of pathogens and spoilage bacteria frequently present in poultry meat has been studied. The objective was to generate a response surface model that correlates the amount of CO_2 and the pressure with the reduction of microorganism counts in order to predict this reduction on real poultry products before running the treatment.

2. Material and methods

2.1. Experimental design

In order to include a wide range of pressures and CO_2 atmospheres, five different pressures and five different atmospheres were combined. As it was observed in some previous experiments (results not shown here) up to 350 MPa the microorganisms studied were inactivated, either packaged with CO_2 or air, and consequently no conclusions referred to the synergistic effect could have made. For that reason pressures studied were lower than 400 MPa. Atmospheres used were air and 30, 50, 70 and 100% CO_2 balanced with N_2 when needed.

For the experimental design, a response surface methodology was applied. Two factors, CO_2 concentration (CO2) and high pressure treatment (HHP) were studied at five levels. Response surface is a statistical methodology which consists of two distinct parts, the experiment design and the analysis of the data. The response for each treatment level is the response variable, which in this study was the rate of inactivation. Response-surface methodology is used to study the relationship between the response variables and the factors; it also allows a mathematical model to be developed that can predict the value of the response variable for given levels of pressure and CO_2 concentration. This randomized design yielded 12 experiments (Table 4.2), all of them carried randomly on the same machine batch, reducing this way the risk of an equipment variation error.

For each treatment and microorganism two replicates were used, meaning each replicate an independent bag with one sausage on it. Samples were analyzed overnight after the treatment (about 15 ± 2 h after).

Table 4.2. Treatments carried out combining different pressures (HHP) and atmospheres (MAP), following a central composite design where the two factors (high-pressure level and CO_2 concentration) were studied at five levels.

Treatment nº	MAP	HHP
1	$50CO_2$ $50N_2$	0
2	Air	200
3	$30CO_2$ $70N_2$	200
4	$70CO_2$ $30N_2$	200
5	Air	250
6	$50CO_2$ $50N_2$	250
7	100 CO_2	250
8	$30CO_2$ $70N_2$	300
9	$70CO_2$ $30N_2$	300
10	$50CO_2$ $50N_2$	350
11	100 CO_2	350
12	Air	350

2.2. Bacterial culture preparation and samples inoculation

Common microorganisms of poultry meat spoilage were selected in order to be inoculated in the samples. Strains of *Leuconostoc carnosum* (CECT 4024), *Brochothrix thermosphacta* (CECT 847), *Salmonella enteritidis* (CECT 4300), *Campylobacter jejuni* (CECT 7572) and *Listeria innocua* (CECT 910T) as a non-pathogenic indicator microorganism for *L. monocytogenes*, were recovered from the lyophilized form following the instructions of Spanish Type Culture Collection (CECT, University of Valencia, Spain), and were kept at -20 ºC in cryobeads (Nalgene System 100, Microkit Iberica S.L., Madrid, Spain).

For each microorganism, the first bacterial culture was obtained by inoculating a cryobead in 20 mL of nutritious media and incubated at required conditions (Table 4.1, page 88). After the incubation time, 0.1 mL of this first culture was transferred to 20 mL of the same nutritious media and incubated again to ensure the stationary phase of the culture, which is generally when the microorganisms are more resistant (Huang et al., 2014; Patterson, 1999). The concentrations of the final inoculums were 10^6-10^8 cfu/mL for each microorganism.

2.3. The matrix

Smoked vacuum packed poultry sausages from the same batch were used as substrates to inoculate the microorganisms used in this experiment. The product was purchased from a local supermarket and kept under refrigeration until its usage.

Total aerobic counts and total anaerobic counts were previously determined in order to know the initial contamination of the product (results not shown); although due to the nature of the studied microorganisms and the selective media used in its growth, the initial contamination of the samples was not considered significant.

2.4. Sample inoculation

For each treatment and sampling day, two sausages were inoculated for each microorganism separately. Inside the laminar flow cabin with a sterile environment, 0.1 mL of the agitated inoculum was spread on the surface of the sausage. After 15 minutes of drying, sausages were placed in gas barrier bags (50 µm, O_2 transmission rate < 5 cm^3 m^{-2} $24h^{-1}$ bar^{-1} and CO_2 transmission rate < 25 cm^3 m^{-2} 24 h^{-1} bar^{-1}, both determined by the manufacturer at 23 ºC and 0% RH; Linpac, Spain). The final concentrations of the inoculums on the sausages ranged from 10^6 to 10^8 cfu/mL, depending on the microorganism. Once were inoculated, sausages were packaged and pressure treated (see point 2.5).

2.5. Packaging and HHP treatment

Immediately after placing the sausages into the bags, they were packaged replacing the air inside the bags by the appropriate food-grade gas mixture (SE Carburos Metálicos, Air Products Group, Spain). Air samples were flushed with compressed air to reach the same volume as the other samples. At the end of the filling, the gas volume/ product volume ratio (g_v/p_v) was 4:1 in all the bags.

Right after the packaging, pressure treatments were carried out in a discontinuous high hydrostatic pressure equipment (GEC Alsthom ACB, Nantes, France) with a pressure chamber of 2 L capacity. The duration of all the treatments was 10 min at room temperature (22 ± 2 ºC).

2.6. Microbiological analyses

After keeping the samples at 2 ± 1 ºC during night, microbiological analyses were performed for all the microorganisms and treatments. Immediately after opening the packages aseptically, the sausage was placed in a stomacher bag with filter (GSI Creos, Japan). The inside of the original bag was washed with 180 ml of 0.1% sterile peptone water (CM1049, Oxoid, England) in order to sweep all the microorganisms that could have remained in the film, and then it was poured out to the stomacher bag together with the sausage. The sample was homogenized in a Pulsifier PUL 100E (Microgen Bioproducts Ltd, England) for 90 seconds at room temperature. Appropriate dilutions were spread on the following media, with the given incubation time and temperature (Table 4.1).

2.7. Statistical analysis of the data

Two-way analysis of variance (ANOVA) and a central composite design were performed between the main factors “HHP” and “CO_2 concentration”. This set of designs consists of a full or fractional two-level experiment, designed to value all of the linear and interaction terms, and of center points and star points used to estimate the quadratic terms. When there was statistical significance ($P<0.05$) a response surface model was carried out, considering the linear and quadratic full factorial of both main factors. Response-surface contours and other statistical analyses were obtained by means of the software Statistica, version 7.0 (StatSoft. Inc., USA) and.

3. Results and discussion

The models obtained by means of the software showed the possible behavior that the microorganisms studied would have when they would be exposed to a certain concentration of CO_2 and treated at a certain pressure.

In general, the models predict that the inactivation of microorganisms caused by pressure is more significant than the reduction caused by carbon dioxide (Table 4.3). When samples were treated at 350 MPa, higher reductions of *B. thermosphacta* (Figure 4.9), *L. carnosum* and *L. innocua* counts were detected at greater concentrations of CO_2. This did not happen with *S. enteritidis*, which seemed to gain resistance with CO_2 atmospheres (Figure 4.12). As it happened in previous studies (Al-Nehlawi et al., 2014), *C. jejuni* is too sensitive to pressure and to other atmospheres that differ from its growth optimal one (5% O_2, 10% CO_2, 85% N_2), and due to the unfavorable circumstances, its inactivation was completed at almost all the treatments carried, which made impossible to generate a model predicting the behavior of this microorganism.

Nevertheless, what it seems evident is that the results are not the same if the MAP is done after than before the HHP treatment. When samples are packaged with carbon dioxide and then treated with pressure, the cell damage produced by the HHP increases the penetration of compressed CO_2 into the cell, producing a greater dissolution of this gas in the aqueous phase of the meat. When the pressure releases and the gas expands upon sudden, greater cell damage is caused and disruption of intracellular enzymes is also produced (Al-Nehlawi et al., 2014; Amanatidou et al., 2000; Wang et al., 2010).

Table 4.3. *P*-values (α=0.05) of the main factors and their interaction on the reduction of studied microorganisms inoculated on poultry sausages packaged under different CO_2 concentrations and treated at different high pressures.

Factor	*B. thermosphacta*	*C. jejuni*	*L. carnosum*	*L. innocua*	*S. enteritidis*
% CO_2	0.0015	0.5354	0.0002	0.0000	0.0035
HHP	0.0000	0.0000	0.0000	0.0000	0.0000
%CO_2*HHP	0.0057	0.7707	0.0050	0.0002	0.0982

3.1. *B. thermosphacta*

Following the model predictions, inactivation of *B. thermosphacta* can be achieved at 350 MPa and 100% CO_2 (Figure 4.9). This results match with the ones obtained experimentally in previous studies (Al-Nehlawi et al., 2014) where the counts at these conditions were reduced in 6 log cfu/g on *B. thermosphacta* after the pressure treatments, being completely inactivated after 7 days of storage. Vercammen et al. (2011) inactivated *B. thermosphacta* inoculated in cooked ham packaged under MAP (30% CO_2/70% N_2) treating it at different pressures and

temperatures. At 25 ºC and 400 MPa of pressure these authors found a reduction of 4.5 log cfu/g, and in our model, at 350 MPa and a 30% of CO_2 we can predict a reduction of 4 log cfu/g. However, at 300 MPa and the same atmosphere conditions they just obtained 1 log cfu/g reduction, while our model predicts a reduction of 2.5 log at the same conditions. Rodríguez-Calleja et al. (2012) also combined high pressure with other preservation techniques to prolong the shelf life of chicken breast fillets, first treating the meat at 300 MPa during 5 minutes and after that, packaging the fillets with 30% CO_2 / 70% N_2 gas mixture. After the treatment, counts of *B. thermosphacta*, which were found to be under the detection limits, apparently survived the HHP treatment since they proliferated during storage.

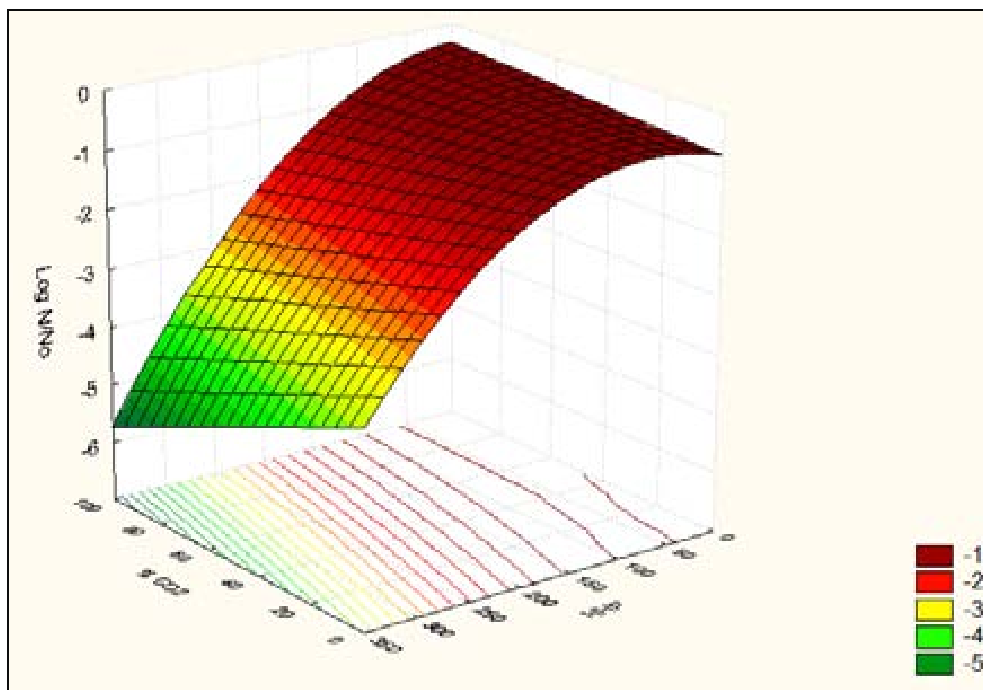


Figure 4.9. Response surface model of the effect of HHP and CO_2 atmospheres on the reduction of *B. thermosphacta* inoculated in poultry sausages. The model was developed by experimental results obtained using a central composite design.

3.2. *L. carnosum*

In the model of *L. carnosum* (Figure 4.10), the most effective atmosphere for the reduction of this lactic bacteria seems to change depending on the pressure of the treatment: at pressure under 300 MPa the most effective atmosphere is the one with 50% of CO_2, and at pressures above 300 MPa the atmosphere with 70% CO_2 was the one that produced a greater count reduction. Even though the tendency of the model seems that at pressures higher than 350 MPa there would not be differences between atmosphere composition, under 350MPa both the pressure treatment and the atmosphere composition has a statistically significant effect on the reduction of *L. carnosum* (Table 4.3). *Leuconostoc* is one of the genera more often detected

in meat MAP products (Nieminen et al., 2011). Its capacity to develop at both anaerobic and aerobic atmospheres at low temperatures and acid pH values makes this lactic acid bacterium (LAB) a great spoilage microorganism. The synergistic effect of HHP and CO_2 on the reduction of *L. carnosum* was demonstrated in inoculated poultry sausages (Al-Nehlawi et al., 2014), reducing more than 4 log units when the atmosphere was 100% CO_2 and the pressure treatment was 350 MPa during 10 min at room temperature, which matches with the model (Figure 4.10). Basak et al. (2002) demonstrated that the destruction of *Leuconostoc mesenteroides*, one of the most resistant strains of *Leuconostoc* gender, depends both on the pressure level and the treatment time. At 200 MPa during 10 minutes they had a reduction of less than 1 log in inoculated orange juice packaged under air conditions; and when the pressure was 350 MPa the reduction did not increase significantly, achieving 1 log cfu/g. Both results match with the model, which predicts 1 log reduction of *L. carnosum* when there is no CO_2 in the atmosphere and whatever the pressure is used.

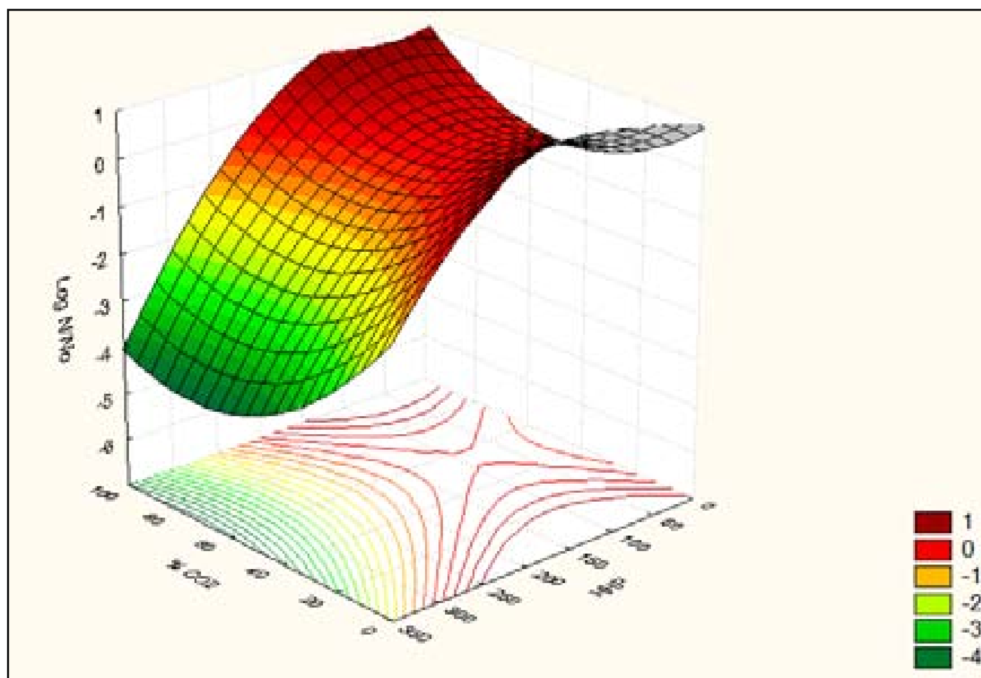


Figure 4.10. Response surface model of the effect of HHP and CO_2 atmospheres on the reduction of *L. carnosum* inoculated in poultry sausages. The model was developed by experimental results obtained using a central composite design.

3.3. *L. innocua*

L. innocua model is similar than the other Gram positive microorganisms (Figure 4.11). Although its high resistance to high pressure (Carlez et al., 1993; Gervilla et al., 1997), its reduction is positively correlated with the amount of CO_2 present in the atmosphere (Table 4.3). Following the model, without carbon dioxide in the atmosphere, a pressure of 350 MPa is

needed to obtain a reduction of 1 log cfu. This matches with the results obtained by López-Pedemonte et al. (2007), who obtained less than 1 log reduction when 300 MPa were applied to model cheeses at 20 °C during 10 min. Also Gervilla et al. (1997) obtained similar results treating ewe's milk at 200, 300 and 350 MPa during 10 min at 25 °C. It seems that the temperature of the treatment, its duration and the nature of the food substrate has a strong influence on the reduction of *Listeria* spp. (Simpson & Gilmour, 1997; Yuste et al., 1999), and it has been previously reported that at room temperature (20 - 25 °C) pressures up to 300 MPa are needed to start reducing *Listeria* spp. (Carlez et al., 1993; Gervilla et al., 1997), which is confirmed by the model. However, when pressure was combined with a pure CO_2 atmosphere, less than 250 MPa are needed to obtain the same colonies reduction. The effectiveness of CO_2 on *Listeria* spp. has been widely reported (Marshall, Andrews, Wells, & Farr, 1992; Nissen, Alvseike, Bredholt, Holck, & Nesbakken, 2000; Provincial et al., 2013; Rutherford et al., 2007), demonstrating that higher amounts of CO_2 in the atmosphere slow the growth rate of this microorganism. Amanatidou et al. (2000) inoculated *L. monocytogenes* on fresh Atlantic salmon and observed a 2.5 log cfu/g reduction in samples packaged under 50% CO_2 and 50% O_2 atmosphere and only a reduction of 1 log cfu/g in vacuum-packed samples when both were treated at 150 MPa during 15 minutes. However, when pressure treatment lasted 5 min, no significant differences between atmospheres were found and only a reduction of 0.5 log cfu/g was achieved. This demonstrates that the longer the exposure time of the product to pressurized CO_2, the greater the synergistic effect is produced.

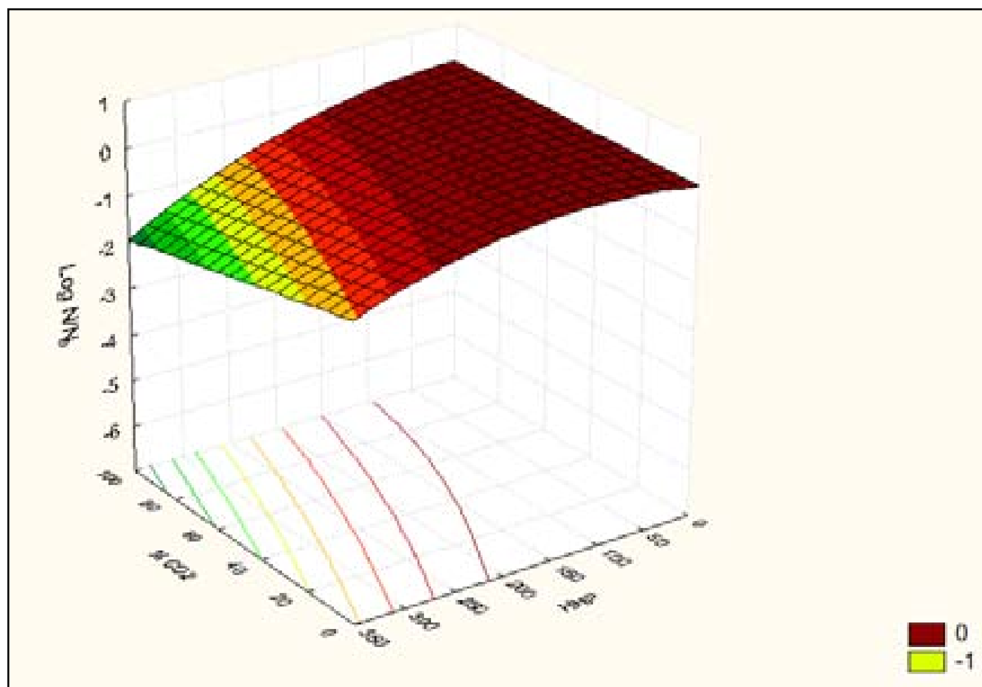


Figure 4.11. Response surface model of the effect of HHP and CO_2 atmospheres on the reduction of *L. innocua* inoculated in poultry sausages. The model was developed by experimental results obtained using a central composite design.

3.4. *S. enteritidis*

Regarding *S. enteritidis*, the model shows that the behavior of this microorganism in the presence of CO_2 and HHP was the opposite than the other microorganisms studied (Figure 4.12). Although higher pressures produce a great reduction of *S. enteritidis* counts, the presence of CO_2 seems to protect *S. enteritidis*. For instance, with an atmosphere of 100% CO_2 a pressure of 350 MPa is needed to reduce 2 log units of this microorganism; while if the atmosphere is air (practically 0% CO_2) less than 300 MPa are needed to obtain the same reduction. At pressures below 250 MPa less than 1 log of *S. enteritidis* is reduced. Shigehisa et al. (1990) also determined the inactivation *Salmonella typhimurium* treating pork slurries at 100, 200 and 300 MPa for 10 min at 25 ºC, obtaining a reduction of 0, 3 and 6 log units/g respectively. In our predictive model the maximum reduction predicted is 3 log unit/g when the pressure treatment is 350 MPa and the atmosphere is air. These differences are probably because of the nature of the inoculated substrate, as microorganisms possess strong environmental adaptability and parameters like the pH, substrate composition or even the presence of another competitor microorganism can affect the growth and survival of them. The resistance of *Salmonella* to high concentrations of CO_2 has been reported before. Provincial et al. (2013) inoculated *S. enteritidis* on sea bream and observed a continuous increase in counts through the storage time, even in packages with 100% CO_2. The same ability to survive in the presence of CO_2 was also observed by Skandamis et al. (2002) when beef fillets where packaged under 40% CO_2 atmosphere and compared with air packaged fillets. Amanatidou et at. (2000) inoculated *S. typhimurium* in salmon and after packaging with an atmosphere 50% CO_2 / 50% O_2 and pressure treating the fillets at 150 MPa they obtained a reduction of 0.5 log cfu/g when the pressure treatment lasted 5 min and less than 1.5 when the duration was 15 min. Even the strain and the treatment temperature were not the same, these values are similar to the ones obtained in our model, when at this pressure and with a treatment duration of 10 min the reduction of *S. enteritidis* was less than 1 log.

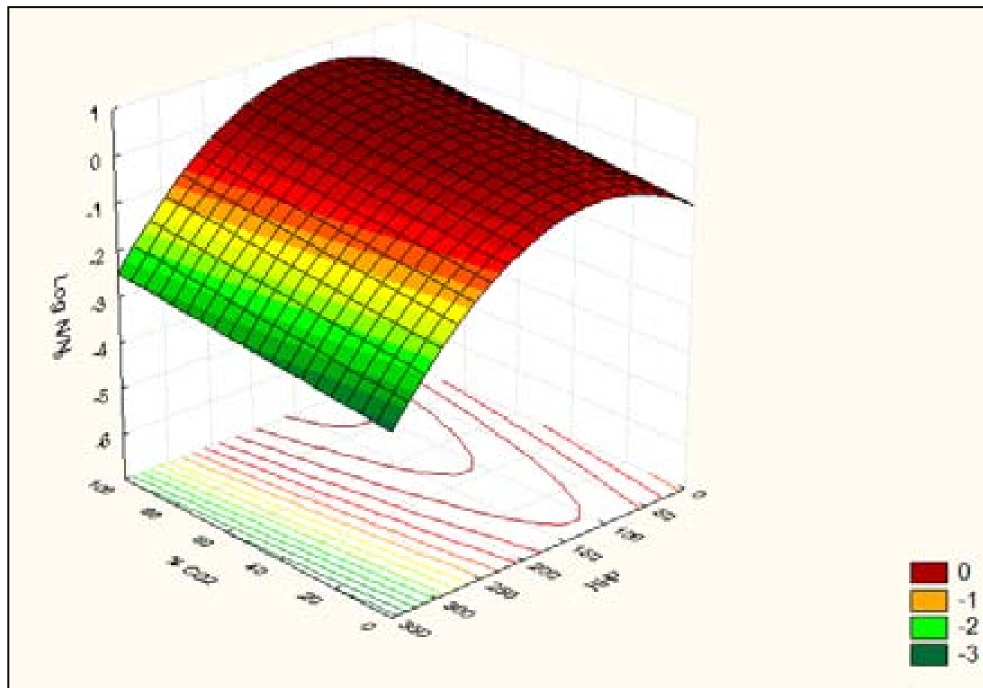


Figure 4.12. Response surface model of the effect of HHP and CO_2 atmospheres on the reduction of *S. enteritidis* inoculated in poultry sausages. The model was developed by experimental results obtained using a central composite design.

4. Conclusions

In this study, the synergistic effect of the combination of high hydrostatic pressure and CO_2 atmospheres has been modeled at different pressures and different CO_2 concentrations to predict the reduction of *Brochothrix thermosphacta*, *Campylobacter jejuni*, *Leuconostoc carnosum*, *Listeria innocua* and *Salmonella enteritidis* on poultry sausages. The model predictions have been compared with the data present on the bibliography.

In general, even though the capability of some microorganism to grow in the presence of CO_2, it seems that when this gas is combined with high pressure, the bacterial development is significantly and negatively affected. The effect of pressurized CO_2 is greater than the non-pressurized gas, as the treatment with pressure causes damage to the cell membrane, increasing the penetration of the gas inside the cells, which produces changes on the intracellular pH that affects their metabolism negatively.

It was observed that each microorganism has a different response to the treatments. A high pressure and pure CO_2 atmospheres are not always the most effective treatment, as in the case of *Salmonella enteritidis*, which becomes more resistant to high pressure when a CO_2 atmosphere is involved.

After comparing the models with the present literature, it can be settled that they can predict the behavior on the reduction of the most common poultry spoilage bacteria when

poultry sausages are treated with high pressures up to 350 MPa and packaged with different CO_2 atmosphere concentrations. This gives the chance to design a more effective treatment with the attractiveness that the pressure applied can be reduced without compromising the inactivation of spoilage bacteria. However, as food composition significantly affects the microorganisms' response to high-pressure treatments, different models should be developed for other poultry products or raw meats to be more accurate with the predictions.

Combined application of modified atmosphere packaging and high hydrostatic pressure on the shelf-life extension of fresh chicken breasts

1. Introduction and objectives

Modified atmosphere packaging (MAP) has become one of the most used systems to preserve raw meat and meat products, together with refrigeration. For the last two decades, with the aim to improve MAP output, several authors has reported the combining application of MAP with other preserving techniques, including high hydrostatic pressure (HHP) (Al-Nehlawi et al., 2014; Amanatidou et al., 2000; Park et al., 2003). HHP can alter some of the typical characteristics of fresh meat like texture and especially color, which can be remarkably modified (Bajovic et al., 2012).

HHP has been increasingly used during the last decade in the meat industry as a post-processing technology to extend the shelf life and to improve the safety of ready-to-eat meat products. However, its application to raw meat does not convince to the consumers, as the color and texture alterations derived from pressurization are not accepted by the consumers (B Marcos et al., 2010).

The use of combined MAP and HHP has been tested before in inoculated orange juice (Corwin & Shellhammer, 2002), Atlantic salmon (Amanatidou et al., 2000) and smoked poultry sausages (Al-Nehlawi et al., 2014), reporting a synergistic effect when the product is packaged with modified atmosphere with a certain concentration of CO_2 before being treated with high pressure. However, no registered bibliography has been found related to chicken or fresh meat.

With the aim to go further with these potential technologies, the objective of this study was to evaluate the effect of combining MAP and HHP on the shelf - life of fresh chicken meat, adjusting the parameters (pressure and CO_2 concentration) to obtain an efficient and attractive treatment for the food industry.

2. Material and methods

2.1. Sample preparation

Fresh chicken breasts were obtained from a local slaughterhouse and transported to the laboratory under chilled conditions. All the breasts were from commercial broilers, collected from the same batch at the plant, all of them coming from the same farm. Upon arrival, chicken fillets were processed inside a cold chamber at 2 ± 1 ºC, being cut in small portions of approximately 20 g each. A total amount of 100 g of breast portions were placed in gas barrier bags (50 µm, O_2 transmission rate < 5 cm^3 m^{-2} 24 h^{-1} bar^{-1} and CO_2 transmission rate < 25 cm^3 m^{-2} 24 h^{-1} bar^{-1}, both determined by the manufacturer at 23 ºC and 0% RH; Linpac, Spain).

2.2. Packaging and HHP treatment

Immediately after placing the breast portions into the bags, four groups of samples were made and different treatments were carried: 1. Chicken packaged with air (Air); 2. Chicken packaged with MAP (MAP); 3. Chicken packaged with air and treated with HHP (Air+HHP); 4. Chicken packaged with MAP and treated with HHP (MAP+HHP).

The air inside the bags of MAP+HHP and MAP samples was removed and flushed with a gas mixture of 50% food-grade CO_2 and 50% food-grade N_2 (S.E. Carburos Metálicos S.A., Air Products Group, Spain) during 20 seconds to ensure the desired atmosphere inside the bags (50.9 ± 0.7 % CO_2 / 48.8 ± 1.2% N_2). Air+HHP and Air samples were flushed with compressed food-grade air to reach the same bag volume as samples packaged with MAP. The final gas volume/ product volume ratio (g_v/p_v) was 3:1 in all the bags.

MAP+HHP and Air+HHP samples were pressurized at 250 MPa for 10 minutes in HHP equipment (Stansted HP-8000, Model ISO-Lab FPG11500; Stansted Fluid Power Ltd., UK) with a pressure chamber of 5 L capacity. Samples were submerged in water, which acted as the hydrostatic fluid medium. Pressurization and depressurization times were 90 seconds approximately. The treatment was carried out at room temperature (21 ± 2 ºC) and the temperature of the water was controlled during all the process, reaching a maximum temperature of 29 ± 2 ºC during the pressurization.

The gas mixture and pressure used were chosen as a result of previous experiments in where several concentrations of CO_2 combined with different pressures were evaluated (Al-Nehlawi et al., 2014), considering this combination the most attractive because of the low pressure and CO_2 concentration that showed a great effect against inoculated bacteria.

After the treatment and during all the study, samples were kept under refrigeration (3 ± 1 ºC).

2.3. Experimental design and determinations

Three samples were analyzed for each treatment and sampling day, understanding each sample as an independent bag with approximately 100 g of chicken each, all of them previously weighted. The sampling plan was done considering all the literature and previous work regarding chicken shelf-life, conducting the analysis of the samples until day 26 for MAP+HHP treatment, day 20 for Air+HHP treatment, day 15 for MAP samples and day 7 for Air packaged samples.

2.4. Head space analysis

Before the bags were opened, the head space gas composition was measured in all the samples using an oxygen and carbon dioxide analyzer (OxyBaby, Witt-Gasetechnik GmbH & Co KG, Germany). An aliquot of the head space gas was removed using a syringe with a small diameter needle inserted into the bag through a foam rubber septum attached to the top film, which helped prevent the mixing with air. Oxygen and carbon dioxide values are presented as a percentage of the atmosphere composition in absolute values. One measure for each bag was made, obtaining three measures for each treatment and sampling day.

2.5. Microbiological assessment

Right after testing the atmosphere composition, the packages were aseptically opened and immediately after, a portion of chicken was placed in a sterile stomacher bag with filter (GSI Creos, Japan). Afterward, 180 mL of 0.1% sterile peptone water was added (CM1049, Oxoid, England) and the bag content was homogenized in a Pulsifier PUL 100E (Microgen Bioproducts Ltd, England) for 90 seconds at room temperature. Appropriate dilutions were carried out in order to spread them on the appropriate media. For total aerobic counts (TAC) and total anaerobes, 1 mL of appropriate dilutions were pour-plate inoculated in plate count agar (CM0325; Oxoid, England) and incubated at 30 ºC for 48 h, using an anaerobic atmosphere (AN0025 sachets and AnaeroJar, Oxoid, England) for the growth of anaerobes. Lactic acid bacteria (LAB) were determined using pour-plate spreading and MRS agar (CM0361; Oxoid, England), applying an extra lay of medium to ensure microaerofilic conditions, incubating the plates at 30 ºC and examining them at 48 h and 72 h. Each microorganism was determined for each sample, obtaining three counts for each microorganism, treatment and sampling day.

2.6. Water holding capacity

During storage time, chicken meat begins to degrade, and one of the first signs of this degradation is that proteins lose their water holding capacity (WHC). In addition, when CO_2 is dissolved into the product, the meat pH decreases and it approaches to the proteins isoelectric point, which stimulates the releasing of muscle water. Furthermore, high pressure also produces protein denaturation, changing their WHC properties.

WHC was determined adapting the filter paper press method. A known amount of chicken meat between 1.5 - 2.5 g was placed between four filter papers of known weight, two on each side of the sample as this type of meat has a high water content; everything was placed between two cover glasses under a pressure of 1 kg for 10 min. After that, the filter paper was removed and weighted, obtaining the water absorbed. Samples moisture was also measured gravimetrically. WHC was calculated following the next equation:

$$\%WHC = \frac{Wc - Wp}{Wc} \cdot 100 \qquad (4.13)$$

Where Wc is the water in chicken (g), calculated using the moisture determined for each sample, and Wp is the water that remained in the filter paper (g), calculated gravimetrically as the difference of the papers weight before and after the test.

2.7. pH measurement

For each sample pH was analyzed twice using a pH-meter (Crison Basic 20+, Crison, Spain) and a penetration electrode (Crison 5232, Crison, Spain), obtaining six results for each treatment and sampling day.

2.8. Color assessment

The superficial color of the breasts was measured with a colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Japan), with illuminant D65, an aperture size of 8 millimeters and a 0°-viewing angle. For each sample eight measures were taken, having a total of 24 values for each treatment and sampling day in order to have a representative measurement of the meat. The parameters assessed were brightness (L^*), the balance between green and red (a^*) and the balance between blue and yellow (b^*).

2.9. Sensory analysis

Sensory assessment was carried out by means of triangle tests at day 4 after the treatment. Twenty habitual chicken consumers within the age bracket of 25 – 55 years were recruited for the sensory tests. All panelists had previously participated in sensory evaluation of meat studies and were familiar with this kind of tests. Samples were cooked immediately after open the bags using an industrial convection oven (Sveba-Dahlen AB, Sweden), at 200 ºC for 20 minutes with forced air. Every breast portion was cut into pieces of approximately 1 cm^3. Four triangle tests were carried out, according to Stone and Sidel (1993) procedure: 1. MAP vs. MMAP+HHP; 2. MAP vs. Air+HHP; 3. Air vs. MAP+HHP; 4. Air vs. Air+HHP. The order of presenting the tests was randomized to prevent any flavor carryover effects and panelists were required to rinse with water before tasting each sample. For each test they were required to answer the difficulty to find the different sample, choosing among the options "very easy", "easy", "difficult", "very difficult" and "almost impossible", and they also had to choose which of the three samples they preferred. As color of breast portions was not different among treatments once the samples were cooked, neither by instrumental analysis nor with the naked eye (data not shown), sensory evaluation analysis were performed in the regular light.

The objective of the sensory test was to evaluate the effect of the pressure and MAP on chicken breasts taste compared with non-treated samples.

2.10. Statistical analysis

All data were statistically analyzed by the General Linear Model (GLM) and the significance of differences ($P<0.05$) among samples at each sampling day was determined by a two-way analysis of variance (ANOVA) both procedures performed by means of STATISTICA 7.0 (StatSoft Inc., USA). Mean values and standard errors are reported in figures and tables.

3. Results and discussion

3.1. Instrumental measurements and atmosphere evolution

When a high concentration of carbon dioxide is used, the usual evolution of this atmosphere is a significant decrease of the amount of CO_2 in the head space as a consequence of its dissolution in the water phase of the product and its diffusion through the packaging film (Jakobsen & Risbo, 2009; Rotabakk et al., 2007). In the present study, significant differences were found between MAP packaged samples. On the first sampling 24 h after the treatment, MAP+HHP samples showed an important decrease of CO_2 percentage compared to MAP

samples. However, on the following control at day 5, no significant differences were found between MAP treatments, achieving both a near-equilibrium of 37 - 39% of CO_2 in the head space until day 9 (Figure 4.14). From that day, CO_2 percentage on MAP+HHP samples continued decreasing, while on MAP samples started to increase, coinciding with an increase on the TAC and LAB counts (Table 4.4). This continuous dissolution of CO_2 on the meat of pressurized samples may be due to the cell damage produced by pressure, which allowed a better access of the gas to the interior of the cell. The possibility of a forced gas dissolution has been suggested previously (Al-Nehlawi et al., 2014) when packaging poultry sausages with pure CO_2 and treating them with HHP. Compared to air packaged ones, significant changes on the surface skin of the sausages evidenced that a forced dissolution of the gas, either air or modified atmosphere, followed by a sudden gas release was produced. Consequently, as the HHP increase the cell membrane permeability (Wang et al., 2010), a higher amount of CO_2 can penetrate inside the cells.

Samples packaged with air, treated or not with pressure, and packaged with MAP but not treated with pressure, showed an increase of the $\%CO_2$ at the end of their storage time, as a consequence of the metabolic activity of the microorganisms present on chicken.

Although atmosphere equilibrium is usually reached within the first 48 h after packaging, the concentration of CO_2 on MAP+HHP samples continued decreasing along the storage time.

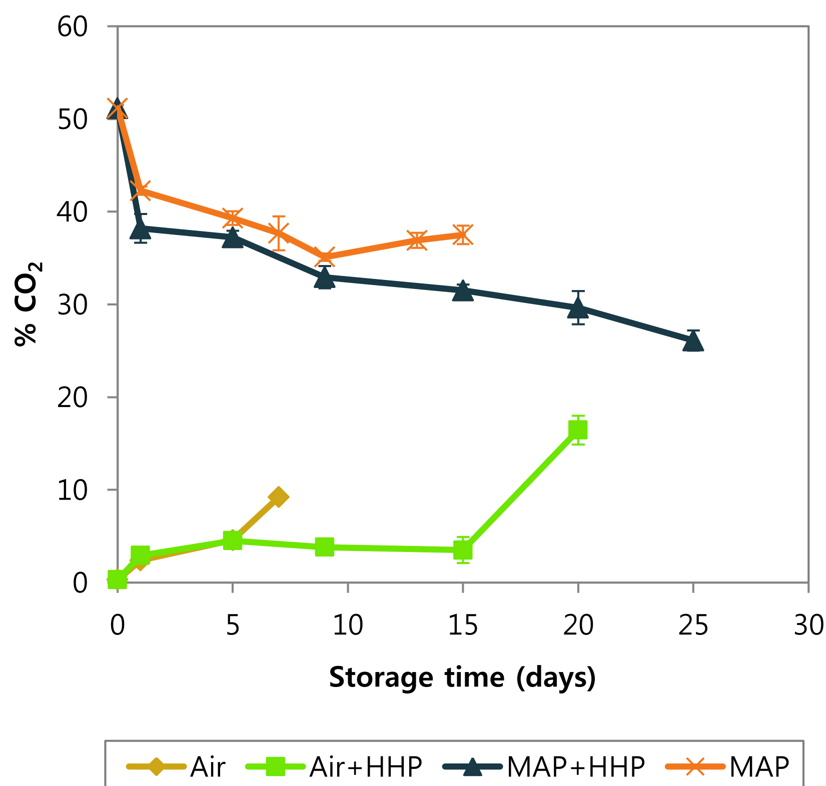


Figure 4.14. Evolution of carbon dioxide concentration (%) measured in the head space of each bag containing chicken breast portions, packaged under modified atmosphere (50% CO_2 / 50% N_2) or air before being treated or not with high pressure (250 MPa during 10 min at room temperature). Samples were kept at 3 ± 1 ºC during the storage. Values correspond to the average of three measures ± standard error.

Regarding WHC, the composition of the atmosphere did not affect significantly the WHC of the meat, but pressure treatment had an effect (P<0.05) on water retention, showing significant lower values on pressure treated samples. The WHC value of fresh chicken meat was found to be 73.0 ± 1.5%. At day 5, pressurized samples showed values of 63.1 ± 3.0% and 63.2 ± 2.6% in MAP+HHP and Air+HHP respectively, keeping around these values until the end of the storage; while unpressurized samples did not have significant variations during the study (Figure 4.15). No significant changes were detected on meat moisture, maintaining the initial values of 75.7 ± 1.5% variation throughout the storage time in all the treatments carried out (Figure 4.15). Lakshmanan, Parkinson and Piggott (2007) neither found differences on the assessment of moisture when fresh Atlantic salmon was treated with pressures up to 200 MPa. However, it has been found that at pressures upper than 300 MPa, the moisture content of the samples increase significantly while increasing the pressure (Cruz-Romero, Smiddy, Hill, Kerry, & Kelly, 2004; Kruk et al., 2011). Lean muscles consist of approximately 75% water of which majority is held within the structure of the muscle and muscle cells (Huff-Lonergan & Lonergan, 2005). Therefore, any treatment affecting structural changes in the muscle can cause the release of water entrapped within the muscle structures and HHP has been shown to influence strongly the structure and function of muscle proteins (Gross & Jaenicke, 1994; Mozhaev, Heremans, Frank, Masson, & Balny, 1996). These statements are in agreement with the results found in the present study, where pressures of 250 MPa did not affect the moisture content but WHC.

Studying the effect that high pressure has on lipid degradation and water holding capacity of fish, Wada and Ogawa (1996) correlated the decrease of WHC to a greater lipid oxidation. Moreover, precipitation of sarcoplasmic proteins on the myofibrils has also been suggested as the possible cause of WHC loss in meats with altered water retention properties (Lopez-Bote & Warriss, 1988; Monin & Laborde, 1985). Marcos et al. (2010) suggested that pressure induced denaturation of sarcoplasmic proteins could influence on the loss of WHC in pressurized meats, which may explain the losses of WHC on the MAP+HHP and Air+HHP samples.

Changes in pH during the study were found to be significant, showing variations among treatments (Figure 4.16). MAP samples had a significant decrease in pH values. This variation can be explained when the solubility of CO_2 in the water phase of the meat is considered, which forms carbonic acid, resulting in a decrease of the intracellular pH (Al-Nehlawi et al., 2013; Tan & Gill, 1982). Air packaged samples also shown a slight decrease on the pH, probably due to the growth of LAB. However, pressure treated samples had a different conduct, showing a significant increase in pH values throughout the study, regardless of the atmosphere used. Similar results have been found by other authors on meat, reporting increases on pH values when pressures above 200 MPa were applied to beef (McArdle et al., 2010), lamb (McArdle, Marcos, Mullen, & Kerry, 2013) and chicken (Rodríguez-Calleja et al., 2012). Increase in pH after HHP has been attributed to a decrease in available acidic groups in the meat as a result of conformational changes associated with protein denaturation (Mandava, Dilber, & Fernansez, 1995). The increase of muscle pH induced by HHP has also been attributed to the redistribution

of ions, which is facilitated by the increased ionization that occurs at elevated pressures (Macfarlane, McKenzie, Turner, & Jones, 1981).

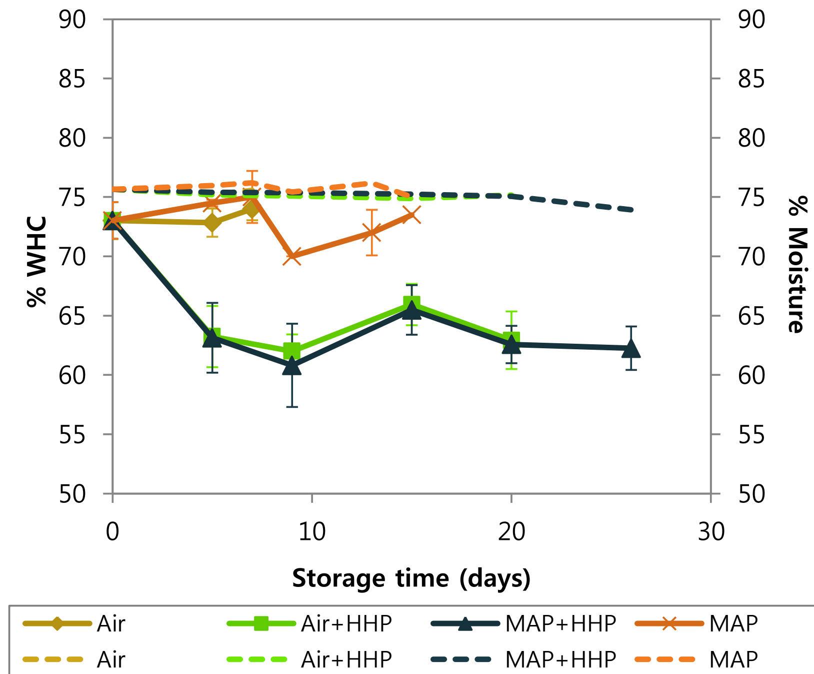


Figure 4.15. Evolution of water holding capacity (solid line) and moisture (dashed line) of chicken breast portions packaged under modified atmosphere (50% CO_2 / 50% N_2) or air before being treated or not with high pressure (250 MPa during 10 min at room temperature). Samples were kept at 3 ± 1 ºC during the storage. Values correspond to the average of three measures ± standard error.

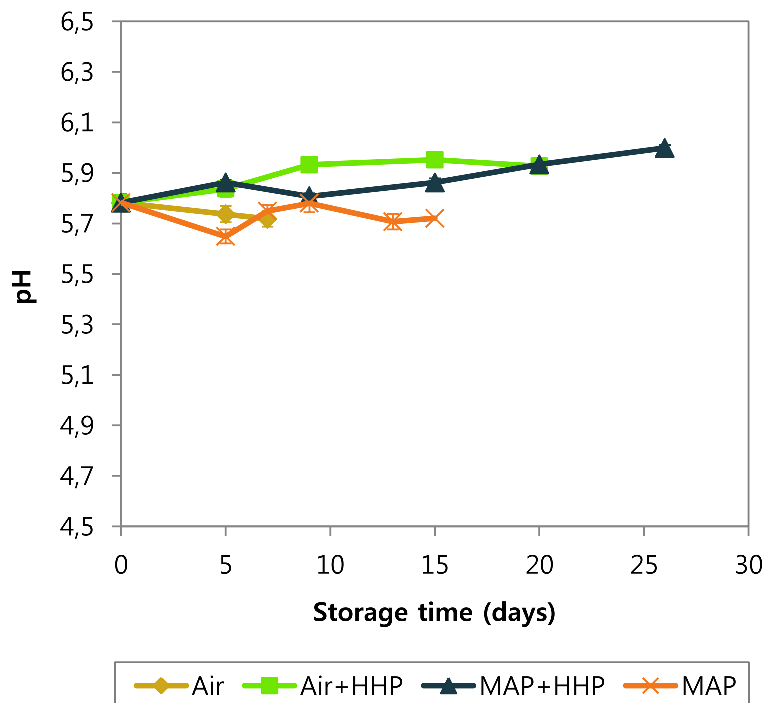
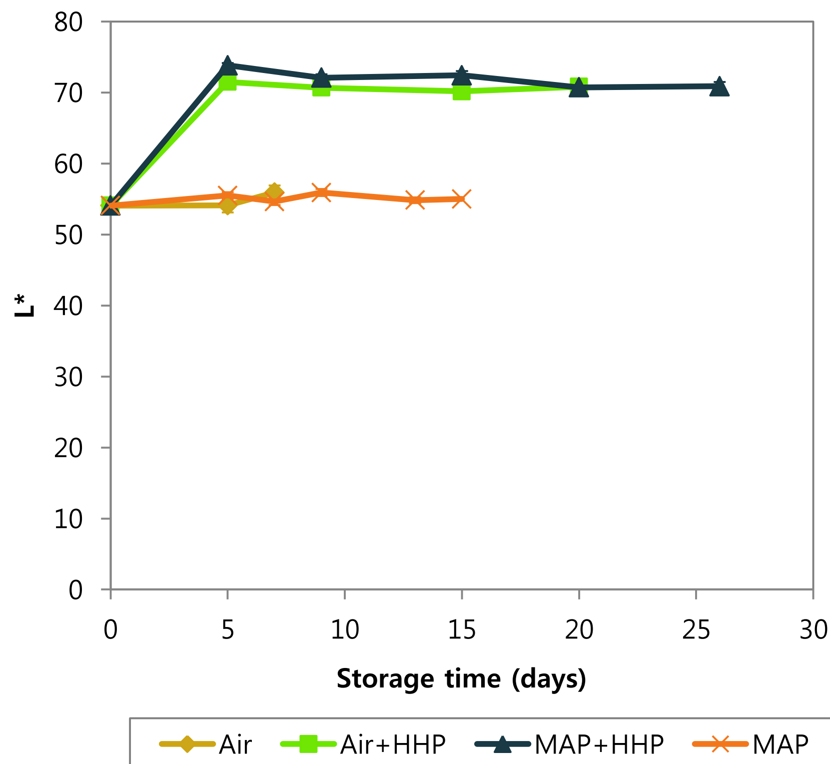


Figure 4.16. Evolution of pH on chicken breast portions, packaged under modified atmosphere (50% CO_2 / 50% N_2) or air before being treated or not with high pressure (250 MPa during 10 min at room temperature). Samples were kept at 3 ± 1 ºC. Values correspond to the average of six measures ± standard error.

Some significances of pressure on chicken breasts color characteristics were observed, although the atmosphere composition did not influence on that parameter (Figure 4.17). After the treatment, *L** values of pressure treated samples increased significantly (P<0.05), remaining stable throughout the study. Non pressurized samples did not have significant variations along the storage time in what *L** value concerns. Regarding *a** and *b** values, pressurized samples showed a decrease on both parameters on the first sampling day, although no significant differences were found in *b** values at the end of the storage. Carlez, Veciana-Nogues, and Cheftel (1995) suggested that fresh meat discoloration during HHP is due to a "whitening" effect (increase in *L** values) at 200 – 350 MPa caused by globin denaturation, heme release or displacement, or by oxidation of ferrous myoglobin to ferric metmyoglobin at or above 400 MPa. Rodríguez-Calleja et al. (2012) also obtained increases on *L** values by treating chicken breasts with pressure at 300 MPa, although they obtained also increases on yellowness parameter. Kruk et al. (2011) also detected an increase on the brightness and redness of the chicken breasts color after treating the meat with different pressures. They reported that the higher the pressure the greater the *L** value. However, in agreement with our results, other authors have reported reduced *a** values after treating the meat with pressure (Y. I. Kim, Lee, Lee, Kim, & Yamamoto, 2007; Mariutti, Orlien, Bragagnolo, & Skibsted, 2007). Regarding *b** values, literature reports that this color parameter is not always affected by the high pressure treatment, and factors like other additives or treatment temperatures have some influence on its variation (Kruk et al., 2011; B Marcos, Aymerich, & Garriga, 2005; Mariutti et al., 2007).



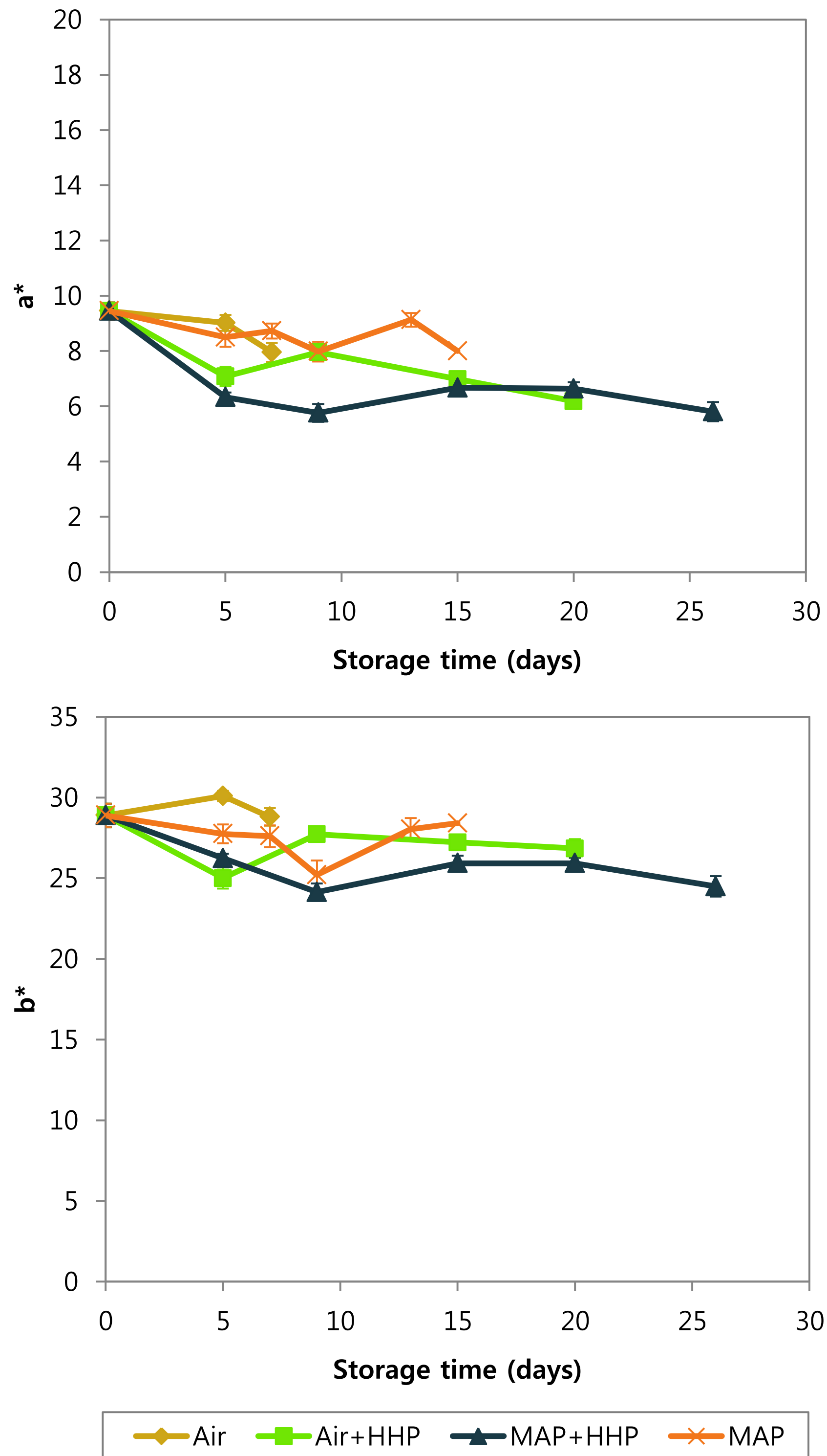


Figure 4.17. Evolution of surface color (*L**= lightness; *a**= redness; *b**= yellowness) on fresh chicken breast portions, packaged under modified atmosphere (50% CO_2 / 50% N_2) or air before being treated or not with high pressure (250 MPa during 10 min at room temperature). Samples were kept at 3 ± 1 ºC. Values correspond to the average of 24 measures ± standard error.

3.2. Microbiological assessment

Regarding the microbiological evolution of the samples, pressure treatment did not inactivate completely the bacterial population present on meat. However, significant differences ($P<0.05$) were found considering the atmospheres: samples packaged under modified atmosphere had lower counts on the first control day (day 5) than their respective packaged under air (Table 4.4). TAC and LAB counts of air packaged breasts increased constantly from the first day, exceeding 10^6 and 10^4 cfu/g respectively by day 7, making this the end of their shelf life. In contrast, anaerobes continuously decreased due to the aerobic atmosphere conditions. In MAP samples TAC and LAB counts remained with the initial values until day 7 and anaerobes did not show any growing until day 9. From that moment counts increased until the end of the study, obtaining similar microbial populations of TAC and LAB at day 15 than air samples had at day 7. These differences were also noticeable in pressurized samples. While Air+HHP samples exceed 10^7 cfu TAC/g at day 20, HHP+MAP samples did not achieve 6 log cfu TAC/g when the study finished at day 26. There were not differences between pressurized samples on LAB counts, probably due to the ability of these bacteria to grow in CO_2 atmospheres. Significant differences on anaerobes populations of pressurized samples were detected, exceeding 5 log cfu/g at day 20 on MAP+HHP samples, almost a week later than Air+HHP samples (Table 4.4). This demonstrates the synergistic effect of MAP and HHP on chicken meat, obtaining an acceptable fresh chicken with a 26 days shelf-life, 20 days longer in respect to non-treated breasts. Previous studies have reported the effectiveness of these technologies when they are combined, demonstrating their success against the most common food spoilage and pathogen microorganisms (Al-Nehlawi et al., 2014; Corwin & Shellhammer, 2002). Amanatidou et al. (2000) went further demonstrating how important is the order of applying the treatments, showing a significant lower effect on microbial counts of fresh salmon when MAP was applied after pressure treatment. This fact demonstrates that is not only the presence of carbon dioxide what influences on the reduction of microbial counts but the pressurized CO_2. When food is packaged under CO_2 atmosphere and then pressurized, the damage and the alterations produced by high pressure on the cell membrane facilitates the penetration of a greater amount of CO_2 inside the cell at relatively low pressures, producing a stronger effect against microorganisms than applying MAP alone or only pressure (Al-Nehlawi et al., 2014). For most forms of vegetative bacteria, significant reductions (usually higher than 4 log units) in the population are achieved when 400 – 600 MPa at room temperature are used (Rendueles et al., 2011). Furthermore, combining HHP with previous packaging with carbon dioxide atmospheres has demonstrated to produce a strong synergistic effect against spoilage microorganisms, obtaining the same reductions at lower pressures (Al-Nehlawi et al., 2014; Amanatidou et al., 2000; Corwin & Shellhammer, 2002; Park et al., 2003; Wang et al., 2010).

Table 4.4. Microbial evolution (log cfu/g of meat) of fresh chicken breast portions after different treatments stored at 3 ºC. Values correspond to the average of 3 measures ± standard error.

	Air	MAP	Air+HHP	MAP+HHP
TAC				
0	3.07 ± 0.11	3.07 ± 0.11	3.07 ± 0.11	3.07 ± 0.11
5	5.23 ± 0.12	2.96 ± 0.24	2.11 ± 0.26	1.77 ± 0.05
7	6.14 ± 0.25	3.01 ± 0.12	–	–
9	–	4.09 ± 0.08	2.76 ± 0.30	2.23 ± 0.07
13	–	5.10 ± 0.01	–	–
15	–	6.20 ± 0.10	5.04 ± 0.27	2.87 ± 0.14
20	–	–	7.37 ± 0.02	4.44 ± 0.46
26	–	–	–	5.96 ± 0.10
LAB				
0	2.79 ± 0.13	2.79 ± 0.13	2.79 ± 0.13	2.79 ± 0.13
5	3.42 ± 0.23	2.60 ± 0.07	2.25 ± 0.10	1.82 ± 0.13
7	4.10 ± 0.17	2.74 ± 0.27	–	–
9	–	3.28 ± 0.16	2.16 ± 0.04	1.90 ± 0.12
13	–	3.84 ± 0.12	–	–
15	–	4.20 ± 0.10	2.29 ± 0.22	2.19 ± 0.09
20	–	–	3.27 ± 0.03	3.56 ± 0.12
26	–	–	–	4.31 ± 0.06
Anaerobes				
0	3.09 ± 0.11	3.09 ± 0.11	3.09 ± 0.11	3.09 ± 0.11
5	2.74 ± 0.03	2.66 ± 0.07	2.96 ± 0.16	2.49 ± 0.07
7	2.29 ± 0.27	2.58 ± 0.08	–	–
9	–	2.60 ± 0.08	2.21 ± 0.09	2.21 ± 0.25
13	–	4.09 ± 0.02	–	–
15	–	5.20 ± 0.10	4.24 ± 0.18	2.79 ± 0.08
20	–	–	5.10 ± 0.53	4.24 ± 0.18
26	–	–	–	5.42 ± 0.11

TAC: total aerobic counts; LAB: lactic acid bacteria

Air: samples packaged with air; MAP: modified atmosphere packaged samples (50% CO_2 / 50% N_2); Air+HHP: air packaged samples pressurized at 250 MPa during 10 min at room temperature; MAP+HHP: 50% CO_2 / 50% N_2 packaged samples, pressurized at 250 MPa during 10 min at room temperature.

3.3. Sensory analysis

Sensory assessment of the different treatments carried out on chicken breasts was not found to be significant (P>0.05) (Stone & Sidel, 1993) as three of the four triangle tests run did not have enough correct judgments for being considered conclusive (Table 4.5). Since the small molecules involved in cooked meat flavor are unlikely to be affected by pressure, it could be anticipated that any effects on them would have been minimal (Ma et al., 2007). However, some interesting statements can be deduced observing the results obtained. Between MAP and MAP+HHP samples, 66.7% (P>0.05) of the right consumers preferred MAP breasts than pressurized ones. This option was also preferred for the 60% (P>0.05) of the right consumers when MAP and HHP+Air samples were compared. It seems that when MAP breasts are compared to pressurized ones, consumers prefer the non-pressurized samples. However, when non-pressurized samples are packaged with air, consumers' preference change. When Air and MAP+HHP samples were compared, the 66.7% (P>0.05) of the right consumers preferred pressurized samples. Similar results were found when Air and Air+HHP samples were tested, where the 58.3% (P<0.05) preferred also the pressurized samples (Table 4.5).

When Mor-Mur and Yuste (2003) compared pressurized vacuum-packed sausages with heat-treated ones by means of triangle tests, they found that consumers preferred also pressurized samples. Working also with fresh chicken breasts, Rodríguez-Calleja et al. (2012) also found that the consumers overall acceptance had higher scores in pressurized samples; although breasts were packaged under modified atmosphere conditions (30% CO_2/70% N_2) the packaging was done after the HHP treatment, which would explain the disparities with our results and remarking how important is the treatments applying order.

Table 4.5. Results of the sensory assessment of fresh chicken breast portions after different treatments of MAP and HHP, at day 4 of storage at 3 ºC.

Triangle test[1]	Correct judgments	Subject preferences[2]	Difficulty of the test[2]
MAP *vs.* MAP+HHP	9[a]	MAP = 6 MAP+Air = 3	Very easy = 0 Easy = 4 Difficult = 3 Very difficult = 2 Almost impossible = 0
MAP *vs.* Air+HHP	5[a]	MAP = 3 Air+HHP = 2	Very easy = 0 Easy = 3 Difficult = 1 Very difficult = 1 Almost impossible = 0
Air *vs.* MAP+HHP	9[a]	Air = 3 MAP+HHP = 6	Very easy = 0 Easy = 2 Difficult = 5 Very difficult = 2 Almost impossible = 0
Air *vs.* Air+HHP	12[b]	Air = 5 Air+HHP= 7	Very easy = 0 Easy = 1 Difficult = 8 Very difficult = 1 Almost impossible = 2

[1] *n* = 20 subjects / test

[2] Only the right subject opinions were considered (*n* = number of correct judgments)

[a] Not significant ($P>0.05$)

[b] Significant ($P<0.05$)

Air: samples packaged with air; MAP: modified atmosphere packaged samples (50% CO_2 / 50% N_2); Air+HHP: air packaged samples pressurized at 250 MPa during 10 min at room temperature; MAP+HHP: 50% CO_2 / 50% N_2 packaged samples, pressurized at 250 MPa during 10 min at room temperature.

4. Conclusions

This study has demonstrated how the synergistic effect existing by combining high CO_2 atmospheres with HHP can extend the shelf-life of skinless fresh chicken breasts up to 26 days. Combined with MAP, pressure levels can be reduced significantly without compromising the destruction rate of most of the common food spoilage microorganisms, obtaining an efficient and attractive treatment for the meat industry. Even though high pressure alters the fresh look of chicken to a cooked appearance, this could be masked in marinated and spiced products, which usually have a shorten shelf-life.

In general, consumers found difficult to determinate the different sample and due to the high number of incorrect judgments, it can be presumed that sensory differences produced by the different treatments are not significant. Nevertheless, another study with more tests and more panelists would be necessary to conclude that.

Chapter 5

Concluding summary

Concluding summary

The research work exposed in this Thesis goes deeply in the study of high carbon dioxide atmospheres to preserve fresh chicken meat. In addition, the synergistic effect of modified atmosphere and high hydrostatic pressure has been widely evaluated in order to improve and innovate on chicken meat preservation.

Even though CO_2 has a bacteriostatic effect over the most common food-spoilage bacteria, its use in high concentrations in MAP products has been limited due to the package collapse. When CO_2 dissolves into the water and lipid phase of the food, an under-pressure situation inside the package is produced, and consequently it collapses. In order to reduce or avoid this, a pre-treatment carried out by packaging the meat with pure CO_2 during a certain time before its final packaging under modified atmosphere, has been tested.

In the first study, this pre-treatment, known as soluble gas stabilization (SGS), applied during 3 h on fresh chicken drumsticks, showed differences on the package collapse, which was significantly reduced in SGS samples. Measures of the head-space gas composition showed that once the internal atmosphere arrived at the equilibrium, pre-treated samples had significantly higher concentrations of CO_2 compared to MAP samples, which ensures a better preservation of the product. Regarding the physicochemical evolution of the samples, no major differences in the exudates formed were observed among SGS pre-treated samples with respect to directly packaged MAP samples. As the dissolved CO_2 turns into carbonic acid when it contacts with the water phase of the food, pH drop is expected to occur. As SGS samples had greater concentrations of dissolved CO_2, its pH was significantly lower compared to MAP samples. An increase of the dissolved CO_2 on the chicken meat or skin did not have a highly effect on the surface color of the product. On the other hand, microbial results were determinant on this study, as *Pseudomonas* and total aerobic counts, which are the main indicators of spoilage in chicken products, shown significant differences depending on the treatment. The greater amount of CO_2 dissolved during the SGS pre-treatment produced the greater bacteriostatic effect on drumsticks microbial population was produced, maintaining the counts under acceptable limits until the end of the storage (day 11), while MAP samples overpass those limits on day 7.

With the results obtained in the instrumental measures, the amount of CO_2 that dissolves into the chicken drumsticks packaged directly with MAP was determined, using an equation developed for this experiment. The data shown that during the MAP process, lower quantities of CO_2 were dissolved in SGS pre-treated samples. This difference is believed to be caused due to the amount of CO_2 previously dissolved during the pre-treatment, confirming that chicken fresh-meat can be totally or partially saturated with this gas.

With the aim to go further on this research, the sensory impact of the SGS pre-treatment on chicken meat was investigated. The effect that different saturation pre-treatments and different package-fillings may have on the package collapse was also investigated in the same study. Both factors (g_v/p_v ratio and SGS pre-treatment time) had a great significant effect on the collapse of the packages. While trays without pre-treatment were totally collapsed on day 3 of the experiment, in pre-treated samples collapse were reduced and even avoided in some of the treatments run. Concerning to the sensory analysis, once the chicken thighs were cooked, no significant differences were found among the treatments. Regarding the physicochemical and microbial assessment of the samples, the results were similar as the ones reported in the previous study. The filling of the packages seemed to have a greater influence over the evolution of the head-space atmosphere, keeping greater values of CO_2 in the head-space on samples with a 3:1 ratio. SGS pre-treatment also affected the lipid oxidation of the white muscle part of the thighs. It seems that the CO_2 dissolved in the tissue may favor autoxidation of polyunsaturated fatty acids. However, as the oxidation values were low, no off-flavors or rancidity tastes were detected by the panelists.

In order to extend the use of CO_2-atmospheres on poultry meat, the combination of these atmospheres with HHP was investigated. Poultry sausages were inoculated with spoilage and pathogen strains before being packaged with 100% CO_2 and HHP-treated at 350 MPa for 10 min. Treatments with air-packaged sausages were also run in order to compare the results and determinate the synergistic effect between CO_2 atmospheres and high pressure. Strains of *B. thermosphacta*, *L. carnosum* and *L. innocua* showed an important reduction when CO_2 and pressure were involved, significantly higher than in samples that were just treated with pressure. No cell-recovery was detected after 7 days of the pressure treatment on CO_2 packaged samples, meaning that the gas also controlled the recovery of sublethal injured microorganisms. It was deducted that because of the cell membrane damage that high pressure produces, a larger amount of CO_2 can penetrate into the cell, causing more damage on the metabolism of the microorganisms. However, *S. enteritidis* showed an increase on its resistance to pressure when it was surrounded by CO_2.

After running the pressure treatment with poultry sausages, some noticeable changes were detected on the surface of the sausages, were a significant amount of gas bubbles were formed between the meat and the skin of the product. These bubbles were strongly visible on sausages packaged with air, while sausages under CO_2 atmospheres showed considerably less bubbles on its surface. Going further with this research, a new experiment was designed with the aim to evaluate the possible forced dissolution of gas produced by HHP by means of a quantification of the gaseous embolism produced on poultry sausages. Sausages were packaged under pure CO_2, N_2 and Ar atmospheres, in order to compare soluble and non-soluble gases. Pressure treatments were conducted at two pressure levels and three different pressure-release speeds. Results, measured as the number of bubbles formed on the sausages'

surface, demonstrate that when pressure treatment is produced, the gas that surrounds the product is forced to be dissolved in the product, where it remains during the pressurization. When the decompression is produced, the gas comes out abruptly, keeping trapped on the sausage peel in bubbles form. However, in CO_2-packaged sausages, significantly fewer bubbles were formed, making evident that an important part of the gas remains dissolved in the product. The pressure applied (200 MPa and 400 MPa) was also significant, and less bubbles were quantified when pressure treatment was stronger. It seems that at higher pressures CO_2 dissolves in a deeper levels and its extraction with pressure release is harder, keeping dissolved in the product and, therefore, forming less bubbles on the sausages' surface. Pressure-releasing speed also showed to be significant. Faster releasing speed produces more bubbles in all the gases and pressures studied. With this experiment, it can be concluded that by using high-pressure treatments and regulating the decompression speed, greater amounts of CO_2 can remain dissolved into the meat, improving its microbiological preservation.

With the objective to continue the research on the synergistic effect between MAP and HHP, a response surface model that correlates the amount of CO_2 and the high-pressure with the reduction of microorganisms' counts was developed in order to predict this reduction on real poultry products before running the treatment. In general, the models predict that the inactivation of microorganisms caused by pressure was more significant than the reduction caused by CO_2 atmosphere. Moreover, higher concentrations of CO_2 produced greater reductions of microorganisms. However, as it was observed in previous experiments, *S. enteritidis* gained resistance at higher concentrations of CO_2. For *L. carnosum* the most effective atmosphere for its reduction depended on the treatment pressure, being more effective at 50 – 70% CO_2. After validating the models with the existing bibliography it can be settled that they can predict the behavior on the reduction of some of the most common poultry spoilage bacteria in poultry sausages treated with high pressures up to 350 MPa and packaged with different CO_2 atmosphere concentrations.

With the aim to confirm that this synergistic effect was also valid on fresh meat, the combined use of MAP (50% CO_2 / 50% N_2) and HHP (250 MPa, 10 min) on fresh chicken breasts was assessed. As done previously, MAP-pressurized samples were compared to non-pressurized and air-pressurized ones, analyzing physicochemical, microbial and sensory parameters to determine the shelf-life of the product. The experiment demonstrates that fresh skinless chicken meat can achieve a shelf-life of 26 days in acceptable microbial counts for its safety consume. Samples only pressure-treated and not packaged with modified atmosphere only achieved a shelf-life of 15 days under the microbial limits, while MAP and non-pressurized samples had a shelf-life of 13 days. Compared to the control (air packaged meat), it is possible to obtain fresh chicken meat with a three-week longer shelf-life. Changes on the water holding capacity of pressurized samples were significant as HHP treatments affect the internal structure of proteins. The color of the meat was also affected, having the pressurized samples a whiteness

color that gives to the meat a certain cooked aspect. This color could be masked in marinated and spiced products. Nevertheless, once the meat was cooked no differences were found by the consumers on the sensory triangle test.

Finally, this research work has demonstrated that poultry meat preservation can be improved by the use of high-concentration CO_2 atmospheres applied alone as a previous saturation treatment or in combination with high hydrostatic pressure. The work exposed on this Thesis gives new prospects on meat science research and enhance the opportunity to produce safer and long-lasting fresh meat products.

Chapter 6

Final conclusions

Final conclusions

The conclusions obtained in this Thesis are numerated below:

1. By using SGS pre-treatment, fresh chicken meat shelf-life was extended, keeping its microbial counts under acceptable limits during more days and maintaining similar physicochemical and sensory properties as conventional packaged meat.

2. Package collapse was reduced and even avoided when SGS pre-treatment was applied in fresh chicken meat with skin and bone, even at greater fillings of the packages. This gives the option to reduce package volumes or increase product filling in order to improve packaging and product-transport efficiency without compromising the quality and safety of raw chicken meat.

3. Calculating the amount of dissolved CO_2 during the shelf-life of MAP chicken it was observed that an important quantity of carbon dioxide was dissolved during the SGS pre-treatment. When these pre-treated samples were immediately packaged under normal MAP conditions, a greater concentration of CO_2 was retained in the head-space during its shelf-life, which gave it a longer protection and control over the microbial population of the meat during its storage.

4. The synergistic effect of combining CO_2 atmospheres and HHP to reduce or even inactivate poultry meat spoilage and pathogenic bacteria was effectively demonstrated for *B. thermosphacta*, *L. carnosum* and *L. innocua*. On the other hand, *S. enteritidis* showed to gain resistance when CO_2 atmospheres were involved and *C. jejuni* was too sensitive to resist non-favorable atmospheres or pressure treatments.

5. Fresh skinless chicken meat packaged under 50% CO_2 / 50% N_2 and pressurized at 250 MPa during 10 min at room temperature achieved a shelf-life of 26 days with acceptable microbial levels for its safety consumption, 10 days longer than just pressure-treated samples.

6. The differences detected on the surface color and WHC of pressurized meat were not noticeable once the meat was cooked, and generally consumers did not find differences in the sensory test among treatments.

Chapter 7

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