UNIVERSITAT DE LLEIDA

Escola Tècnica Superior d'Enginyeria Agrària

OBTENCIÓN DE ZUMOS Y FRUTOS CORTADOS CON ALTO POTENCIAL ANTIOXIDANTE MEDIANTE TRATAMIENTOS NO TÉRMICOS

Memoria presentada para optar al grado de Doctor

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El éxito no se logra sólo con cualidades especiales, es sobre todo un trabajo de constancia, de método y de organización

J. P. Sergent

El presente trabajo se ha realizado en el laboratorio de Noves Tecnologies de Processat d'Aliments del Departamentde Tecnología D'Aliments de la Universitat de Lleida. Para su realización se ha contado con el apoyo del Departament d'Universitats, Recerca i Societat de la Informació de la Generalitat de Catalunya i el Fons Social Europeu.

RESUMEN

La producción y consumo de frutas y verduras mínimamente procesadas se ha incrementado en los últimos años debido a la demanda, por parte del consumidor, de alimentos naturales, de apariencia y valor nutricional semejante a los productos frescos, y listos para su consumo. Dentro de este contexto, el empleo de pulsos eléctricos de alta intensidad de campo (PEAIC) permite contrarrestar los efectos negativos producidos por el procesado térmico. Por otra parte, las atmósferas modificadas surgen con la finalidad de alargar significativamente la vida útil de los productos frescos cortados. Por tanto, el objetivo principal de esta investigación fue establecer las condiciones de procesado no térmico (pulsos eléctricos de alta intensidad de campo o cortado/envasado en atmósfera modificada) para obtener zumos y frutos cortados (tomate y fresa) con alto potencial antioxidante.

En el caso de zumos tratados mediante PEAIC, en primer lugar, se determinaron las condiciones óptimas de tratamiento por PEAIC en función de la frecuencia, anchura de pulso y polaridad para obtener zumos con alto potencial antioxidante. Seguidamente, se llevó a cabo un estudio cinético de los cambios en las propiedades antioxidantes de los zumos tratados por PEAIC a distintas intensidades de campo eléctrico. Paralelamente, se realizó un estudio comparativo de la evolución del contenido en compuestos bioactivos de los zumos pasteurización por PEAIC o por calor a lo largo de la vida útil. Tratamientos llevados a cabo con pulsos bipolares de 1 μs a 250 Hz (tomate) y 232 Hz (fresa) dieron lugar a máximas retenciones de vitamina C (90%-98% retención) y capacidad antioxidante (89%-100% retención) en ambos zumos, así como licopeno (132% retención) en zumo de tomate y antocianinas (102% retención) en zumo de fresa. Se obtuvieron modelos matemáticos secundarios que relacionaron la variación del potencial antioxidante en zumos de tomate y fresa con la intensidad de campo y tiempo de tratamiento (R^2 = 0,874-0,976). La pasteurización por PEAIC de zumo de tomate (35 kV/cm 1500 μs; con pulsos bipolares de 4 μs a 100 Hz) no solo aumentó el contenido en licopeno, β-caroteno y fitoflueno de los zumos comparado con el zumo fresco, sino que también conllevó menores perdidas de vitamina C que el tratamiento por calor. Los zumos de tomate pasteurizados por PEAIC mantuvieron mayores cantidades de carotenos (licopeno, neurosporeno y γ-carotenos), quercitina, vitamina A y C durante el almacenamiento que los tratados térmicamente. Por otra parte, la pasteurización mediante PEAIC (35 kV/cm 1700 μs; con pulsos bipolares de 4 μs a 100 Hz) afectó en menor proporción a los compuestos bioactivos (vitamina C, ácido elágico y antocianinas) presentes en el zumo de fresa que el tratamiento térmico. Del mismo modo, los zumos de fresa pasteurizados por PEAIC mostraron mayores niveles de antocianinas, ácido elágico y ácido *p*-cumárico que los procesados térmicamente durante el almacenamiento.

Por lo que respecta a frutos cortados, en primer lugar, se determinó la influencia de la variedad en las propiedades antioxidantes de tomates frescos cortados durante el almacenamiento. Seguidamente, se llevo a cabo el estudio del efecto de distintas temperaturas de conservación, así como de atmósferas de envasado en el potencial antioxidante de tomate y fresa frescos cortados. La variedad determinó significativamente el contenido en compuestos bioactivos de los tomates. El almacenamiento a 5 ºC de tomate y fresa frescos cortados contribuyó al mantenimiento del potencial antioxidante de estos productos. En fresa fresca cortada, los cambios en el contenido en vitamina C y antocianinas, así como la variación de la capacidad antioxidante a lo largo del tiempo de almacenamiento a distintas temperaturas se

predijo bien mediante el modelo de Weibull (R^2 = 0,80-0,99). Las constantes cinéticas obtenidas mediante este modelo se relacionaron adecuadamente con la temperatura de conservación a través de la ecuación de Arrhenius (E_a = 33,3-119,7 kJ/mol). La aplicación de atmósferas pobres en oxígeno fue más adecuada para prevenir la pérdida de los principales compuestos bioactivos presentes en tomate y fresa frescos cortados que las atmósferas con alta concentración de oxígeno.

Por tanto, se puede concluir que los pulsos eléctricos de alta intensidad de campo y el cortado/envasado en atmósfera modificada pueden ser buenas alternativas a los tratamientos tradicionales; ya que con estas técnicas se pueden obtener zumos y frutos cortados con alto potencial antioxidante durante un mayor tiempo.

RESUM

La producció i consum de fruites i verdures mínimament processades s'ha incrementat en el últims anys degut a la demanada, per part dels consumidors, d'aliments naturals, d'aparença i valor nutricional semblants als productes frescos, i que estiguin llestos per ser consumits. Dins d'aquest context, la utilització de polsos elèctrics d'alta intensitat de camp (PEAIC) permet contrarrestar els efectes negatius produïts pel processat tèrmic. Per una altra banda, les atmosferes modificades sorgeixen amb la finalitat d'allargar significativament la vida útil dels productes frescos tallats. Per tant, l'objectiu principal d'aquesta investigació fou establir les condicions de processat no tèrmic (polsos elèctrics d'alta intensitat de camp o tallat/envasat en atmosfera modificada) per obtenir sucs i fruits tallats (tomàquet i maduixa) amb alt potencial antioxidant.

En el cas de sucs tractats mitjançant PEAIC, en primer lloc, es van determinar les condicions òptimes de tractament per PEAIC en funció de la freqüència, amplada de pols i polaritat per obtenir suc amb alt potencial antioxidant. Seguidament, es va portar a terme un estudi cinètic dels canvis en les propietats antioxidants dels sucs tractats per PEAIC a diferents intensitats de camp elèctric. Paral·lelament, es va realitzar un estudi comparatiu de l'evolució del contingut en compostos bioactius dels sucs pasteuritzats per PEAIC o per calor durant la vida útil. Tractaments portats a terme amb polsos bipolars de 1 μs a 250 Hz (tomàquet) i 232 Hz (maduixa) van donar lloc a màximes retencions de vitamina C (90%-98% retenció) i capacitat antioxidant (89%-100% retenció) en ambdós sucs, així com de licopé (132% retenció) en el suc de tomàquet i antocianines (102% retenció) en el suc de maduixa. Es van obtenir models matemàtics secundaris que van relacionar la variació del potencial antioxidant en sucs de tomàquet i maduixa amb la intensitat de camp i temps de tractament (R^2 = 0,874-0,976). La pasteurització per PEAIC de suc de tomàquet (35 kV/cm 1500 μs; amb polsos bipolars de 4 μs a 100 Hz) no només va augmentar el contingut en licopé, β-caroté y fitoflué dels sucs en relació al suc fresc, sinó que també va comportar menors pèrdues de vitamina C que el tractament per calor. Els sucs de tomàquet pasteuritzats per PEAIC van mantenir majors quantitats de carotens (licopé, neurosporé i γ-caroté), quercitina, vitamina A y C durant l'emmagatzematge que els tractats tèrmicament. Per un altra banda, la pasteurització mitjançant PEAIC (35 kV/cm 1700 μs; amb polsos bipolars de 4 μs a 100 Hz) va afectar en menor proporció que el tractament tèrmic als compostos bioactius (vitamina C, àcid elàgic i antocianines) presents al suc de maduixa. De la mateixa manera, els sucs de maduixa pasteuritzats per PEAIC van mostrar majors nivells d'antocianines, àcid elàgic i àcid *p*-cumàric que els processats tèrmicament durant l'emmagatzematge.

Pel que respecta als fruits tallats, en primer lloc, es va determinar la influencia de la varietat en les propietats antioxidants de tomàquets frescos tallats durant l'emmagatzematge. Seguidament, es va portar a terme l'estudi de l'efecte de diferents temperatures de conservació, així com d'atmosferes d'envasat en el potencial antioxidant de tomàquet i maduixa frescos tallats. La varietat va determinar significativament el contingut en compostos bioactius dels tomàquets. L'emmagatzematge a 5 ºC de tomàquet i maduixa frescos tallats va contribuir al manteniment del potencial antioxidant d'aquests productes. En maduixa fresca tallada, els canvis en el contingut en vitamina C i antocianines, així com la variació de la capacitat antioxidant durant el temps de conservació a diferents temperatures es va predir adequadament mitjançant el model de Weibull (R^2 = 0,80-0,99). Les constants cinètiques obtingudes

mitjançant aquest model es van relacionar adequadament amb la temperatura de conservació mitjançant la equació de Arrhenius (E_a = 33,3-119,7 kJ/mol). L'aplicació d'atmosferes pobres d'oxigen va ser més adient per prevenir la pèrdua dels principals compostos bioactius presents als tomàquets i maduixes frecs tallats que les atmosferes amb alta concentració d'oxigen.

Per tant, es pot concloure que els polsos elèctrics d'alta intensitat de camp i el tallat/envasat en atmosfera modificada poden ser bones alternatives als tractaments tradicionals; ja que amb aquestes tècniques es poden obtenir sucs i fruits tallats amb alt potencial antioxidant durant més temps.

ABSTRACT

The increase in popularity of minimally processed fruits and vegetables meets consumer's demands for healthy, nutritious and ready-to-eat food products, with a fresh-like appearance. In this way, the application of high intensity pulsed electric fields (HIPEF) is being investigated to avoid the negative effects of heat pasteurization. On the other hand, modified atmosphere packaging appears to extend the shelf-life of fresh-cut products. Therefore, the main objective of this work was to establish the non-thermal conditions (high intensity pulsed electric fields or cutting/packaging under modified atmosphere) in order to obtain juices and fresh-cut fruits (tomato and strawberry) with high antioxidant potential.

In the case of juices treated by HIPEF, firstly, the HIPEF optimal conditions in terms of pulse frequency, pulse width and polarity mode were determined in order to obtain juices with high antioxidant potential. Then, a kinetic study was carried out to relate the changes in the juices antioxidant properties to electric field strength. Parallely, a comparative study on the content of bioactive compounds in juices pasteurized by HIPEF or heat treatment through the shelf-life was conducted. Maximal vitamin C (90-98% retention) and antioxidant capacity (89%-100% retention) in both juices, as well as, lycopene (132% retention) in tomato juice and anthocyanins (102% retention) in strawberry juice were attained with HIPEF treatments carried out with bipolar pulses of 1-μs at 250 Hz (tomato) and 232 Hz (strawberry). Secondary mathematical models were obtained to relate the variation of the antioxidant potential in tomato and strawberry juices with the electric field strength and treatment time applied (R^2 = 0.874-0.976). HIPEF pasteurization (35 kV/cm 1500 μs; with bipolar pulses of 4 μs at 100 Hz) led to tomato juices not only with higher content of lycopene, β-carotene and phytofluene than fresh juices, but also with lower losses in vitamin C concentration than those thermally treated. HIPEF-treated tomato juices maintained greater carotene concentrations (lycopene, neurosporene and γ-carotene), quercetin, vitamin A and C throughout cold storage than heat-treated juices. On the other hand, HIPEF processing (35 kV/cm 1700 μs; with bipolar pulses of 4 μs at 100 Hz) had lower influence on the content of health-related compounds (vitamin C, ellagic acid and anthocyanins) of strawberry juice than thermal pasteurization. In this way, strawberry juices pasteurized by HIPEF exhibited greater levels of anthocyanins, ellagic acid and *p*-coumaric acid compared to those treated by heat.

Regarding fresh-cut products, firstly, the influence of tomato cultivar in the evolution of its health-related compounds through the storage time was evaluated. Then, the effect of different storage temperatures and initial packaging atmospheres on the antioxidant potential of fresh-cut tomato and strawberry was determined. Health-related compounds varied greatly among tomato cultivar. A storage temperature of 5 ºC was adequate for maintaining health-related compounds of tomato and strawberry juice through the storage. The model based on Weibull distribution function was adequate (R^2 = 0.800-0.99) for describing changes over time in the content of vitamin C, anthocynins as well as antioxidant capacity of fresh-cut strawberries stored at different temperatures. Dependence of the rate constant of the Weibull model were adequately related to storage temperature throughout the Arrhenius relationship (E_a = 33.3-119.7 kJ/mol). Low oxygen atmospheres are more effective than superatmospheric oxygen atmospheres or air in maintaining the main health-related compounds of fresh-cut tomato and strawberry.

Therefore, it can be concluded that high intensity pulsed electric fields and cutting/packaging can be an alternative to traditional treatments to obtain juices and fresh-cut fruits with high antioxidant potential for longer periods.

ÍNDICE

Introducción

PRODUCCIÓN Y CONSUMO DE TOMATE Y FRESA

El tomate es una de las hortalizas de fruto más demandada a nivel mundial. Su importancia reside en que posee cualidades sensoriales muy apreciadas, siendo capaz de integrarse en la preparación de alimentos, ya sea cocinado o crudo en la elaboración de ensaladas. Además, el tomate es una fuente importantísima de antioxidantes debido a su alto contenido en licopeno, vitamina C y ácidos fenólicos. Dependiendo de la variedad y estación del año, la concentración de licopeno y vitamina C en el tomate es de aproximadamente unos 50 y 200 mg/kg, respectivamente (Willcox et al., 2003). En EEUU, el tomate es la primera fuente de licopeno y la tercera de vitamina C. En España, el consumo de tomate permanece estable en los últimos años (2006-2007) siendo de aproximadamente 13,5 kilos por persona y año (MAPA, 2008).

La producción Mundial de tomate fue, en 2007, de unos 126 billones de toneladas (FAOSTAT, 2008). Según datos de la ONU, los principales países productores de tomate son Estados Unidos, China, Italia, Irán, Turquía, España, Brasil, Portugal, Grecia y Chile que conjuntamente concentran el 80% de la producción mundial. En el año 2007, España fue el segundo país productor de tomate de la Unión Europea con 3.615 toneladas; de ellas, aproximadamente dos tercios se destinaron para el consumo en fresco y el resto para su transformación (FAOSTAT, 2008). En España, la producción de tomate se concentra fundamentalmente en el sur. La principal Comunidad Autónoma es Extremadura con más del 80% de la producción, a la que siguen Andalucía, Murcia y Navarra.

Por otra parte, una fruta primaveral muy popular debido a su sabor peculiar y aroma único es la fresa. Muchos estudios han demostrado que las fresas son una importante fuente de flavonoides, en concreto de antocianinas, pigmentos que proporcionan colores rojos, azules y púrpuras a las frutas. Además, son extremadamente ricas en vitamina C y ácidos fenólicos, principalmente ácido elágico (Heinonen et al., 1998).

La producción mundial de fresa se ha multiplicado por 4 en los últimos 30 años, siendo de 3.822.989 toneladas en el año 2007 (FAOSTAT, 2008). Actualmente el mayor productor de fresa del mundo es Estados Unidos, como lo indican los últimos datos emitidos por la FAO. En 2007, la producción de fresa en EEUU alcanzó la cifra de 1.115.000 toneladas; con este volumen supera en una proporción mayor al triple al segundo productor de fresa del mundo que es España. En el mismo año, España alcanzó un volumen de producción de 263.900 toneladas (MAPA, 2008). Además dentro del ámbito mundial otros países como Polonia, Japón, Italia, Republica de Corea, Turquía, Alemania y Marruecos son grandes productores de fresa.

Dentro del mercado español, Andalucía produce el 96% de la fresa de España, en concreto la provincia de Huelva produjo 245.000 toneladas en 2007. La segunda Comunidad Autónoma productora de fresas es Cataluña con 3.600 toneladas (MAPA, 2008). Actualmente, el 98% de la superficie cultivada de fresa es de la variedad "Camarosa". Esta variedad parece ser demasiado grande y oscura para la población europea; no obstante esas características son muy apreciadas por los consumidores españoles.

España, en la actualidad, es el primer país del mundo exportador de fresas con 216.641 toneladas, en el año 2005, lo cual representa el 65% de la producción total; es decir, que de la producción de fresa de España, solamente el 35% es para consumo interno y el resto se exporta. Esta cantidad de toneladas exportadas por España supera en un proporción bastante

considerable al segundo mayor exportador del mundo que es EEUU, y que en 2005 alcanzó la cifra de 97.383 toneladas (FAOSTAT, 2008).

El consumo en España de frutas y hortalizas en fresco y transformadas se incrementó un 4,9% y 1,1%, respectivamente, entre los años 2006 y 2007 (MAPA, 2008). No obstante, a pesar de que España es uno de los países con mayor consumo de Europa en este tipo de productos, el consumo de frutas y hortalizas aún está por debajo de las recomendaciones de las guías alimentarias. Por tanto, es fundamental conseguir aumentar la ingesta de fruta tanto en fresco como transformada, ya que las frutas son alimentos indispensables en nuestra alimentación. En la actualidad existen asociaciones sin ánimo de lucro como '5 al día' que fomentan el consumo diario de frutas y hortalizas. Esta organización cuenta con el apoyo de organismos internacionales como la FAO y organizaciones que promueven la importancia de una dieta equilibrada como factor determinante en la prevención de las principales enfermedades. Su nombre se basa en la ración mínima de consumo diario de frutas y hortalizas frescas recomendada por la comunidad científica y médica en una dieta saludable.

COMPUESTOS BIOACTIVOS Y CAPACIDAD ANTIOXIDANTE DE TOMATE Y FRESA

En los últimos años se han publicado un gran número de estudios epidemiológicos que relacionan el consumo de una dieta rica en frutas y hortalizas con una menor predisposición a padecer ciertas enfermedades como cáncer y enfermedades cardiovasculares (Ames et al., 1993). Aunque estos efectos beneficiosos han sido inicialmente relacionados con un mayor consumo de fibra dietética (Periago et al., 1993), en la actualidad hay un mayor número de constituyentes de los productos vegetales que se están asociando con este efecto protector y se les denomina, en general, compuestos bioactivos. Los compuestos bioactivos se definen como sustancias no nutritivas que se encuentran en concentraciones muy bajas en los alimentos, intervienen en el metabolismo secundario de los vegetales, y pueden tener un impacto significativo en la salud humana (Kris-Etherton et al., 2002). Si bien, recientemente algunos nutrientes han sido incluidos como compuestos bioactivos por presentar, además de su función esencial en el organismo, un efecto beneficioso para la salud (Jeffery et al., 2003). Estas pequeñas moléculas que no pueden ser sintetizadas en el cuerpo y, por tanto, deben ser obtenidas de la dieta, van a contribuir al sistema de defensa antioxidante. Por otra parte, estos compuestos se pueden agrupar según su estructura química en vitaminas antioxidantes, compuestos fenólicos, derivados de los terpenos, compuestos azufrados, fitoestrógenos, péptidos y aminoácidos, minerales, ácidos grasos poliinsaturados, fibra dietética, bacterias lácticas y ácido fítico. En general, la mayoría de compuestos bioactivos se caracterizan por poseer una marcada actividad antioxidante. Esta actividad se pone de manifiesto en su capacidad para atrapar radicales de oxígeno, de nitrógeno y radicales orgánicos.

Los tomates y las fresas se caracterizan por presentar altos contenidos en vitamina C, compuestos fenólicos y fibra. Además el tomate es un alimento rico en compuestos carotenoides, algunos de ellos con actividad de provitamina A.

Dentro del grupo de **vitaminas antioxidantes,** la vitamina C se encuentra presente en fresas y tomates en concentraciones que oscilan entre 40-90 mg/100g y 20-30 mg/100g, respectivamente. El término vitamina C incluye ácido ascórbico, ácido dehidroascórbico y sales de ascorbato. El ácido ascórbico posee una estructura de enodiol que se halla conjugada

con el grupo carbonilo en el anillo lactona. Los dos átomos de hidrógeno enólicos son los que dan a este compuesto su carácter ácido y proporcionan los electrones para su función como antioxidante. En presencia de oxígeno, el ácido ascórbico se oxida a ácido dehidroascórbico, el cual tiene su misma actividad vitamínica, que se pierde después de la hidrólisis de la lactona para formar el ácido 2,3-dicetogulónico (Figura 1).

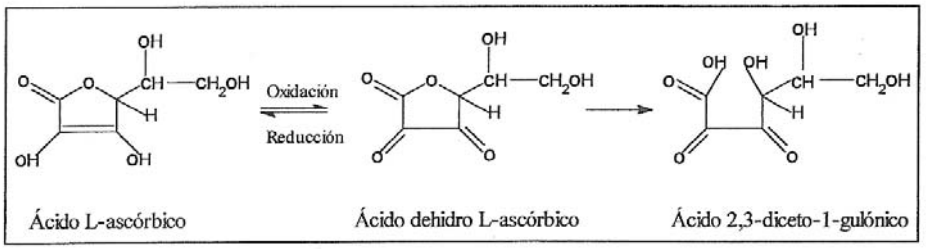


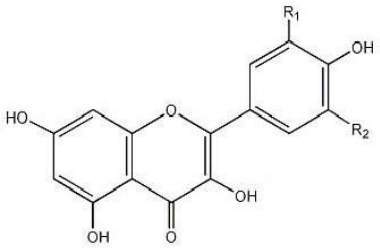
Figura 1.-Oxidación del ácido ascórbico

La vitamina C es esencial para los humanos ya que no podemos sintetizarla por la falta de la enzima gulonolactona oxidasa. La mayor fuente de vitamina C son las frutas y verduras, aunque la cantidad de vitamina C que contienen depende de la variedad, las condiciones del suelo, el clima, madurez, las condiciones de almacenamiento y de procesado (Talanen, 1995). Esta vitamina tiene propiedades reductoras y actúa como un potente antioxidante que es capaz de reaccionar efectivamente con radicales superóxido e hidroxilo y juega un papel importante en la regeneración de la vitamina E. La ***vitamina E*** es una vitamina liposoluble que protege las membranas celulares de los efectos de la peroxidación. Además, esta vitamina está implicada en la estabilización de las membranas, agregación plaquetaria, hemólisis y ciertas actividades enzimáticas. En las pepitas del tomate se encuentra vitamina E en concentraciones que varían entre 0,32-1,2 mg/100g dependiendo de la variedad, madurez del fruto, época de cultivo entre otros factores.

Los **compuestos fenólicos** son un grupo de metabolitos secundarios que son sintetizados por las plantas durante su desarrollo, como respuesta a diversas condiciones adversas como infecciones, heridas, radicaciones, etc. (Dixon y Paiva, 1995). Existen aproximadamente 8000 compuestos naturales conocidos hasta ahora, los cuales se caracterizan por tener como mínimo un anillo fenólico en su estructura molecular. Los compuestos fenólicos son sintetizados por la vía del ácido masónico o por la del ácido shikímico, o por las dos como en el caso de los flavonoides (Taiz y Zeiger, 2006). Los compuestos fenólicos se dividen en dos grandes grupos, flavonoides y no flavonoides.

Flavonoides es el término genérico con que se identifica a compuestos polifenólicos caracterizados por una estructura química basada en un esqueleto C6-C3-C6, es decir, un anillo bencénico unido a una cadena propánica y ésta a su vez a otro anillo bencénico. Los

flavonoides generalmente se encuentran unidos a moléculas de azúcar. Suelen encontrarse también parcialmente polimerizados dando lugar a dímeros, trímeros, etc., hasta formar complejos multienlazados como los taninos condensados. Estos compuestos se encuentran de manera natural en los vegetales. En general el sabor que aportan a los alimentos suele ser amargo llegando incluso a provocar sensaciones de astringencia dependiendo de lo condensados que sean los taninos. El sabor puede variar en función de las sustituciones presentadas en el esqueleto pudiéndose usar como edulcorantes cientos de veces más dulces que la glucosa. Los flavonoides comprenden varios miles de compuestos y se dividen en flavonoles, flavonas, flavanonas, antocianos, isoflavonas y flavanoles. Entre los *flavonoles* los principales son kaempferol, quercitina y miricetina (Figura 2.1). Tanto los tomates como las fresas se caracterizan por poseer altos contenidos en estos flavonoles, no obstante la concentración depende de varios factores como la variedad, las condiciones de crecimiento del fruto, origen geográfico, época de cosecha entre otros.



R_1 = H; R_2 = H: **Kaempferol**
R_1 = OH; R_2 = H: **Quercitina**
R_1 = OH; R_2 OH: **Miricetina**

Figura 2.1.-Estructura de los principales flavonoles presentes en tomate y fresa

Dentro del grupo de *antocianos*, se encuentran las antocianinas, formas glucosídicas de las antocianidinas. Éstos compuestos son pigmentos naturales que se encuentran en frutas y dan tonalidad desde rojas a azules según el pH (Figura 2.2). Pelargonidina-3-glucósido, cianidina-3-glucósido y pelargonidina-3-rutinósido son las principales antocianinas presentes en fresas, siendo responsables de su color rojo. Dentro del grupo de compuestos fenólicos flavonoides, las flavonas, flavanonas, isoflavonas y flavanoles no suelen encontrarse en tomates y fresas. Las *flavonas* más importantes son apigenina y luteolina que se encuentran en apio, olivas y plantas aromáticas. Las *flavanonas* son responsables del amargor de los cítricos, las principales son la hesperetina y naringenina. Las *isoflavonas* se encuentran principalmente en granos de soja y derivados, las más comunes son la genisteína y daidzeína. Por último los el grupo de los *flavanoles* comprenden las catequina y epicatequina que se encuentran en vinos, chocolate y té.

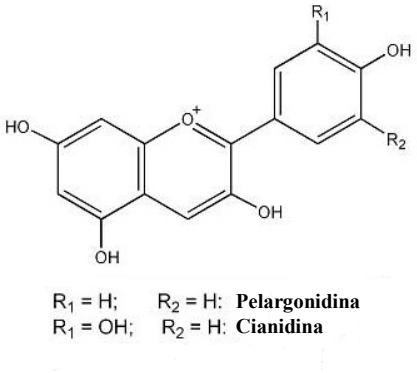
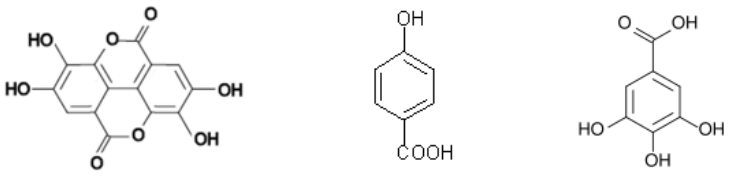


Figura 2.2.-Estructura de las principales antocianidinas presentes en fresas

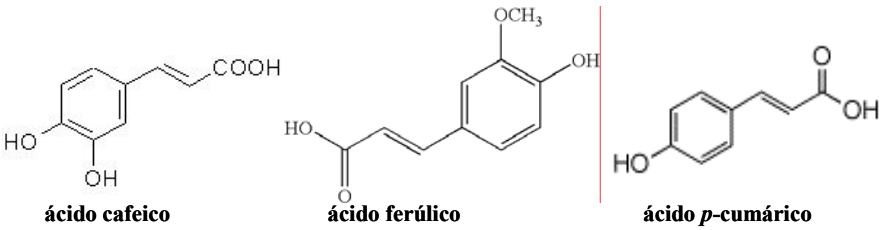
Dentro del grupo de los ***No Flavonoides*** se encuentran los ácidos fenólicos y los estilbenos. Los *ácidos fenólicos* son los compuestos no flavonoides más estudiados y se caracterizan por tener un ácido carboxílico funcional (Macheix et al., 1990). Los ácidos fenólicos forman un grupo diverso que incluyen los derivados de ácido hidroxibenzoico y del ácido hidroxicinámico. Ejemplos de los derivados del ácido hidroxibenzoico son el ácido *p*-hidroxibenzoico, gálico y elágico (Figura 3.1). Dentro de los ácidos hidroxicinámico los que se encuentran en mayor proporción en frutas son los ácidos *p*-cumárico, cafeico y ferúlico (Figura 3.2). Las fresas son una fuente importante de ácido elágico, además poseen alto contenido en ácido *p*-cumárico y *p*-hidroxibenzoico, mientras que el tomate posee además de ácido p-cumárico, altos contenidos en ácido cafeico y ferúlico. Generalmente los ácidos fenólicos están presentes en diversas formas conjugadas, siendo más frecuentes como ésteres que como glucósidos. El ácido clorogénico es el principal derivado de los ácidos hidroxicinámicos. Este ácido fenólico es el principal compuesto fenólico presente en tomates, en concentraciones que oscilan entre 0,14 y 8 mg/100g.

Del grupo de los *estilbenos* el más estudiado es el resveratrol, fitoalexina con carácter antiinflamatorio, protector cardiovascular y participa en la profilaxis del cáncer.



ácido elágico **ácido p-hidroxibenzoico** **ácido gálico**

Figura 3.1.- Estructura de los principales ácidos derivados del ácido hidroxibenzoico presentes en tomate o fresa.



ácido cafeico **ácido ferúlico** **ácido *p*-cumárico**

Figura 3.2.- Estructura de los principales ácidos derivados del ácido hidroxicinámico presentes en tomate o fresa.

Dentro del grupo de los **terpenoides o isoprenoides**, el isopreno es el precursor biológico de estos compuestos que se pueden dividir en tres grupos carotenoides, fitoesteroles y saponinas. Los compuestos ***carotenoides*** son un grupo numeroso de pigmentos liposolubles que se encuentran ampliamente difundidos en el reino vegetal. Se dividen en dos grupos, carotenos (compuestos hidrocarbonatos) y xantofilas (derivados oxigenados de los carotenos). Todos estos compuestos están formados por largas cadenas de dobles enlaces conjugados siendo su estructura de naturaleza isoprénica. Así, los compuestos carotenoides tienen la característica de poseer un esqueleto bilateralamente simétrico de cuarenta átomos de carbono donde cada mitad puede ser considerada desde un punto de vista formal, como constituida por cuatro unidades isoprénicas unidas cabeza a cola, de forma que los grupos metilo que sobresalen de la cabeza están en posiciones 1,5. Estas dos mitades de C_{20}, sin embargo, se hallan unidas cola a cola de forma que los dos metilos centrales de la molécula están en posiciones 1,6. Los principales carotenos presentes en tomates son el *licopeno*, *neurosporeno*, *γ-caroteno*, *ξ-caroteno*, *δ-caroteno*, *β-caroteno*, *luteína*, *fitoeno* y *fitoflueno* (Figura 4). El β-caroteno y γ-caroteno tienen gran importancia desde el punto de vista nutricional debido a su actividad como precursores de la vitamina A. En general, los carotenoides protegen a las células vegetales de la oxidación y, por consiguiente, de su descomposición. En el organismo humano actúan como antioxidantes, que protegen las membranas celulares de la acción de los radicales libres. Se ha demostrado que existe una asociación inversa entre la ingestión de carotenoides y el riesgo de padecer determinados tipos de cáncer (estómago y pulmón) y frente a la arteriosclerosis. Los carotenoides dan lugar a que se forme en nuestro organismo una mayor cantidad de anticuerpos que actúan de forma específica contra las sustancias o elementos extraños que pueden afectarnos.

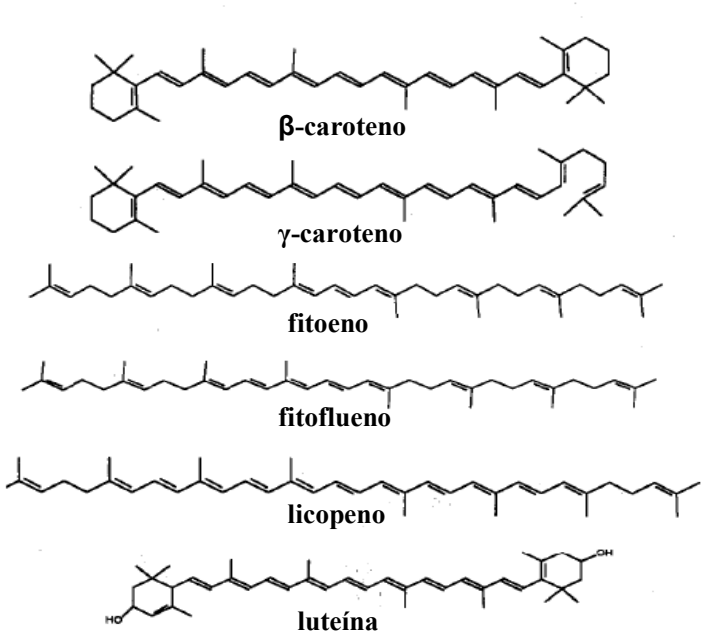


Figura 4.- Estructura de algunos de los principales compuestos carotenoides presentes en tomate.

Se denomina **fibra dietética** al material complejo de las plantas resistente a la digestión por los enzimas producidas por el tracto gastrointetinal. El término fibra se asocia botánicamente con los compuestos de la pared celular vegetal que poseen estructura fibrosa, es decir, celulosa, hemicelulosa, lignina, pectinas, gomas, mucílagos y taninos. La fibra dietética se caracteriza por su capacidad para captar moléculas de agua y formar coloides tipo gel que aumentan la motilidad intestinal y el volumen fecal. Además, gran parte de ella sufre un proceso bacteriano de fermentación en el colon con producción de hidrógeno, metano, dióxido de carbono y ácidos grasos de cadena corta, de gran interés en la fisiología colónica y en el metabolismo. Por otra parte, la fibra dietética regula el metabolismo lipídico, tiene efecto hipocolesterolémico, reduce la absorción de colesterol y grasas del intestino, regula el metabolismo de la glucosa y tiene un efecto preventivo frente al cáncer.

La mayoría de compuestos bioactivos poseen una marcada **capacidad antioxidante** que se pone de manifiesto en su capacidad de atrapar radicales de oxígeno, de nitrógeno y radicales orgánicos.

Un radical libre es una especie química definida que tiene en su estructura uno o más electrones desapareados, lo que lo convierte en un compuesto muy inestable, altamente reactivo con gran capacidad de formar otros radicales libres y dañar estructuras celulares (Kaur y Kapoor, 2001). Estos compuestos buscan aparear los electrones desapareados con el fin de estabilizarse por lo que, cuando la molécula que ha sido atacada ha perdido un electrón, se convierte en un radical libre, generándose así una reacción en cadena en la cual se forman más radicales libres o se forman otras sustancias tóxicas. Generalmente los radicales libres atacan las moléculas estables más cercanas. Los radicales libres se generan de forma natural durante el metabolismo por medio de la reducción parcial de la molécula de oxígeno, formándose así especies reactivas como el hidroperóxido de hidrógeno (H_2O_2), superóxido ($O_2^{\bullet-}$), hidroperoxilo ($HO_2^{\bullet}$) e hidoxilo ($OH^{\bullet}$) entre otros. La producción puede incrementar frente a diferentes estados de estrés fisiológico. A concentraciones elevadas pueden dañar la mayoría de los constituyentes celulares. Los radicales libres son capaces de dañar de forma reversible o irreversible todo tipo de compuestos bioquímicos. La acumulación de estas especies provoca la aparición de daños oxidativos en el ADN, así como en las proteínas y los lípidos de las membranas celulares (peroxidación de lípidos), acontecimientos íntimamente relacionados con los procesos de envejecimiento de tejidos y la aparición de enfermedades degenerativas (Ames, et al. 1993). Los radicales libres y otros compuestos de oxígeno altamente reactivos se cree que contribuyen a causar una amplia variedad de enfermedades, especialmente enfermedades crónicas relacionadas con la edad tales como cáncer, Alzheimer, parkinson, cataratas, arteriosclerosis entre otras.

TECNOLOGIAS DE PROCESADO DE FRUTAS Y HORTALIZAS TRANSFOMADAS

El cambio en los hábitos de consumo de la población ha tenido como resultado que los consumidores demanden alimentos naturales, de apariencia y valor nutricional semejantes a los productos frescos, sin aditivos sintéticos, microbiológicamente seguros y que, además, sean fáciles de preparar y de consumir. Por tanto, la industria, consciente de la importancia que tiene el hecho de satisfacer estas exigencias para mantener y aumentar el número de sus clientes, intenta mejorar de forma continua sus procesos productivos y busca alternativas tecnológicas a los tratamientos tradicionales. Una respuesta a la demanda de este tipo de productos son las frutas y verduras mínimamente procesadas, que abren un nuevo mercado como producto preparado y listo para su consumo a aquellas personas que, por falta de tiempo o por simple comodidad no tienden a consumir frutas y hortalizas. El procesado mínimo consiste en la aplicación de una serie de tecnologías que, combinadas o no, mantengan las características del alimentos lo más cercanas posibles a las del producto fresco y que aumenten su vida útil, en términos microbiológicos, sensoriales y nutricionales. Dentro de este contexto surge el empleo de las nuevas tecnologías de procesado no térmico de alimentos como los pulsos eléctricos de alta intensidad de campo (PEAIC) que permiten contrarrestar los efectos negativos producidos por el procesado térmico.

ZUMOS PASTEURIZADOS

La forma más habitual de alargar la vida útil de los zumos es el tratamiento térmico. El calor destruye microorganismos e inactiva enzimas que pueden alterar los alimentos. Aunque esta técnica ha demostrado ser muy eficaz para estos fines, presenta algunos inconvenientes

como la pérdida de componentes nutricionales y una considerable reducción de la calidad organoléptica respecto al producto fresco. La aplicación de pulsos eléctricos de alta intensidad de campo puede resultar una buena alternativa para conservar los zumos de fruta ya que permite reducir o eliminar la carga microbiológica y los enzimas responsables del deterioro del alimento, alterando mínimamente la calidad. Hasta ahora, la mayoría de estudios, se centran en determinar el efecto que producen los PEAIC sobre microorganismos y enzimas. Sin embargo, existen pocos estudios que evalúen el efecto de los PEAIC sobre compuestos bioactivos.

Producción y consumo de zumos

El consumo europeo de zumos y néctares en el 2007 fue aproximadamente de 11,2 billones de litros, con un consumo per cápita de 22,9 litros por persona y año. Según Canadean Wisdom Annual Series (2008), el consumo de zumos en nuestro país es de 28,6 litros por habitante y año, muy por encima de la media europea, similar a Austria (29,7 litros) y superior al de países tan significativos como Francia (25,3 litros), el Reino Unido (24,7 litros), Bélgica (22,4 litros) e Italia (14,6), aunque inferior al de Alemania (33,5 litros), Finlandia (32,1 litros) o Estados Unidos (30 litros).

Según las cifras de consumo en España del año 2007 dadas por el Ministerio de Agricultura, Pesca y Alimentación, el 2% del total de los alimentos consumidos son transformados de frutas y hortalizas donde se encuentran incluidos los zumos. La evolución del consumo alimentario en España entre los años 2006 y 2007 refleja que los zumos de fruta han experimentado un incremento del 5%. No obstante, el grupo que más está creciendo, en proporción, son los nuevos zumos funcionales y sin azúcar o de frutas exóticas. El consumo total de zumos y néctares en España se sitúa en aproximadamente 508 millones de kilos, contra una producción nacional de unos 1.500 millones de kilos, aproximadamente un tercio se destina a la exportación (MAPA, 2008). De acuerdo con la Asociación Española de Fabricantes de zumos, se exportaron 652.748 toneladas de zumos en 2006, más de la mitad de cítricos (y entre estos 238.730 toneladas de naranja) y poco más de 100.000 toneladas de zumos de uva. Aunque en España se importaron unas 200.668 toneladas de zumos en el mismo periodo, sobretodo de zumos de piña.

Por lo que respecta a los zumos tratados mediante PEAIC, existen algunos equipos comerciales para la elaboración de zumos mediante PEAIC que ya han recibido la aprobación de la FDA y están actualmente en el mercado para procesar zumos. Sin embargo, no se pueden encontrar datos estadísticos de la producción y consumo de zumos tratados mediante PEAIC. En Estados Unidos, Genesis Juice Corp., vende zumos y mezclas de zumos orgánicas pasteurizadas por PEAIC con una vida útil de 4 semanas. Estos zumos tratados por PEAIC se venden en envases de vidrio y refrigerados, y su precio supera el de otros productos similares.

Aspectos Legislativos

El empleo de nuevas tecnologías de procesado de alimentos en la Comunidad Económica Europea se regula según el Reglamento (CE) núm. 258/1997 sobre nuevos alimentos y nuevos ingredientes alimentarios. Dicho Reglamento se aplicará a la puesta en el mercado de alimentos e ingredientes alimentarios que, hasta el momento, no hayan sido utilizados para el consumo humano en la Comunidad Económica Europea, y que hayan sido sometidos a un proceso de

producción no utilizado habitualmente, que pueda provocar en su composición o estructura cambios significativos de su valor nutritivo, de su metabolismo o de su contenido en sustancias indeseables. Por otra parte, el etiquetado de estos nuevos alimentos e ingredientes alimentarios debe cumplir además de los requisitos generales establecidos en la legislación comunitaria, una serie de requisitos adicionales tales como indicar la presencia de materias no presentes en el alimento convencional, indicar las características o propiedades modificadas respecto al tradicional, especificar el método de obtención del producto e indicar la presencia de organismos genéticamente modificados.

La FDA, institución que regula los aspectos de seguridad alimentaria en Estados Unidos, publicó el 12 de Septiembre de 2002 una guía dirigida a las industrias productoras de zumos con los requisitos legales para implantar el sistema de APPCC en dichas industrias. Este sistema se aplica con el fin de prevenir, reducir o eliminar cualquier contaminación microbiológica, química o física que pueda sufrir el zumo. Además, la tecnología de procesado utilizada debe conseguir al menos, una reducción de cinco unidades logarítmicas en la población de un microorganismo patógeno seleccionado para cada tipo de zumo, en función de su mayor resistencia a ser inactivado, pudiéndose emplear métodos distintos a la pasteurización en los que están incluidos los PEAIC, siempre que consigan dicho nivel de reducción microbiana.

En España, recientemente se ha aprobado el Real Decreto núm. 1518/2007, de 16 noviembre, por el que se establecen parámetros mínimos de calidad en zumos de frutas y los métodos de análisis aplicables. El objetivo de esta norma es establecer los parámetros analíticos de autenticidad y calidad, que permitan evaluar la composición de los zumos de frutas, a fin de asegurar el control de su calidad comercial y evitar el fraude al consumidor y la competencia desleal. La nueva norma señala que además del cumplimiento de estos parámetros mínimos, tendrán que ajustarse también a otros que afecten a su autenticidad y calidad, y especialmente los recogidos en la Norma del Codex Alimentarius y en el Código de Prácticas para evaluación de zumos de frutas y vegetales de la Asociación de la Industria de Zumos y Néctares de Frutas y Vegetales de la Unión Europea (AIJN). Esta norma es una demanda del sector y de las comunidades autónomas, que desean mantener la calidad y el prestigio de los zumos españoles en el mercado internacional, y dotarse de instrumentos para controlar y velar por estos zumos colaborando así en mantener la situación privilegiada de producción y de exportación del sector español en el contexto internacional.

Elaboración de zumos

La estacionalidad y el carácter perecedero de las frutas y vegetales explican la necesidad de mejorar las tecnologías de procesado. El objetivo es combinar tratamientos que aumenten la vida útil del producto intentado mantener las características nutritivas y sensoriales del alimento.

Para la elaboración de zumos de frutas y hortalizas, en concreto zumo de tomate y fresa, primeramente las frutas son ***lavadas*** y ***cepilladas*** para eliminar de su superficie partículas de suciedad y productos químicos que aún queden adheridos. Después, en la mesa de inspección, personal cualificado ***selecciona*** los frutos descartando aquellos que no son adecuados.

Una vez ***triturado***, el zumo se somete al proceso de ***clarificación*** cuya finalidad es eliminar pulpa, semillas u otros residuos mediante filtración o centrifugación. La presencia de oxígeno

puede provocar oxidación, pérdida de vitamina C y desarrollo de pardeamiento. Para evitar esto se efectúa la ***desaireación*** que consiste en hacer pasar el zumo, en capa fina o ducha ligera por un depósito bajo vacío produciéndose una breve ebullición que elimina el gas disuelto. También se puede desairear por burbujeo de nitrógeno. Para reducir o eliminar la carga microbiana de estos zumos, es necesaria la aplicación ***de tratamientos de pasteurización o esterilización***. Una vez los zumos son tratados, éstos se ***envasan*** asépticamente en caliente. Posteriormente se enfrían y se almacenan para su posterior comercialización (Braddock, 1999)

Tratamientos de pasteurización o esterilización

Hasta la actualidad, el calor es la técnica más eficaz para lograr una conservación adecuada de los alimentos ya que es capaz de destruir los microorganismos y enzimas alteradores de los alimentos. Sin embargo, el tratamiento térmico destruye gran cantidad de componentes bioactivos presentes en los alimentos. Por esta razón, la industria alimentaria intenta optimizar los métodos térmicos para minimizar los inconvenientes como consecuencia de su aplicación.

Tratamiento térmico

La esterilización es un tratamiento térmico severo, cuya finalidad es eliminar la mayoría de los microorganismos presentes en el alimento, resultando un alimento comercial estéril. La ventaja de este tratamiento es que proporciona una mayor vida útil al alimento, pudiéndose incluso almacenar a temperatura ambiente; no obstante, la esterilización tiene muchas desventajas, ya que produce cambios de textura, color, aroma, sabor y pérdida de calidad nutricional del producto. Los tiempos y temperaturas de calentamiento varían, pero el tratamiento por calor debe ser suficiente para esterilizar el alimento. Generalmente, se aplican temperaturas muy elevadas (entre 135 y 150 ºC) que permiten tiempos de calentamiento cortos (4-15 segundos). Una forma de elevar rápidamente la temperatura es utilizando vapor en los sistemas de procesado aséptico también llamados UHT (ultra high temperature). Esta forma de conservación por calor está muy extendida en la industria alimentaria. Su calidad final es comparable a la de los productos refrigerados pero con una vida útil como mínimo de 6 meses sin refrigeración.

La pasteurización es un tratamiento suave por calor, cuya finalidad es eliminar selectivamente los microorganismos patógenos, reducir el recuento microbiano e inactivar las enzimas del alimento, proporcionando las mínimas pérdidas de aroma, sabor, textura y calidad nutritiva. Este tipo de tratamiento tiene inconvenientes, ya que el producto resultante tiene una corta vida útil y requiere de otro medio de conservación adicional, como la refrigeración o la congelación. La pasteurización se suele realizar en intercambiadores de placas, el zumo es sometido a temperaturas de entre 90-95 ºC durante un tiempo de 15-60 segundos.

Tratamiento mediante PEAIC

La aplicación de PEAIC es una tecnología prometedora para la conservación de alimentos. Esta tecnología consiste en la aplicación de pulsos de alta intensidad de campo (kV/cm) durante un periodo de tiempo corto (μs). Las principales ventajas de esta forma de procesado es que además de alargar la vida útil del producto, mantiene las propiedades nutricionales de los alimentos. No obstante, los PEAIC únicamente pueden aplicarse a alimentos líquidos, los cuales deben ser homogéneos y tener un tamaño de partícula pequeño para no obstruir las

conducciones, ni la cámara de tratamiento. Es importante evitar que el alimento contenga burbujas de aire ya que puede causar arcos eléctricos en el equipo.

El equipo típico de procesado de alimentos líquidos mediante PEAIC consta de una fuente de alimentación de alta tensión cuya misión es almacenar energía en un condensador y un interruptor que permite la descarga de esa energía en pulsos rápidos de alta tensión a un recipiente que contiene el alimento a tratar (Barbosa-Cánovas et al., 1998). El **generador de pulsos** es el componente encargado de proporcionar los pulsos de alto voltaje en la forma, duración, e intensidad requeridas. La acción de este equipo se puede dividir en tres secciones, la generación de corriente directa de alto voltaje a la intensidad requerida por una fuente de energía, el almacenamiento de dicha energía eléctrica en un condensador o un grupo de condensadores, y la liberación del alto voltaje en forma de pulso, con forma y amplitud características. La **cámara de tratamiento** es un elemento clave en el sistema de PEAIC cuya función principal es contener el producto a tratar mientras se aplican los pulsos. Las características de diseño de la cámara no sólo definen su capacidad de tratamiento, si no que también influyen sobre la máxima intensidad de campo alcanzada y sobre la intensidad de tratamiento, así como en la homogeneidad del tratamiento. Los **sistemas de bombeo** por PEAIC están equipados con tuberías y bombas que permiten desplazar el líquido desde el tanque de almacenamiento de materia prima hasta el tanque de producto tratado. Los sistemas de PEAIC están conectados a un ordenador central que **controla y monitoriza** la generación de los pulsos de alto voltaje, estableciendo las condiciones de adecuadas, así como la operación de las bombas y válvulas del sistema.

La efectividad del procesado mediante PEAIC depende de factores críticos entre los que se encuentran parámetros que están relacionados con el tratamiento y otros que dependen del producto a tratar.

Dentro de los factores de procesado se encuentran, la intensidad de campo, el tiempo de tratamiento, la anchura de pulso, la frecuencia de pulso, la forma del pulso, la polaridad, la energía y temperatura aplicada.

La **intensidad de campo**, se define como la diferencia de potencial que hay entre dos electrodos dividido entre la distancia que los separa. No obstante, una intensidad de campo demasiado alta puede causar la rotura dieléctrica de líquido que se procesa. El **tiempo de tratamiento** es el tiempo durante el cual el producto está sometido al tratamiento de PEAIC. Este parámetro se obtiene multiplicando la anchura de pulso por el número de pulsos. La intensidad de campo eléctrico y el tiempo de tratamiento se han identificado como el factor más relevante para definir la inactivación microbiana y enzimática al aplicar un tratamiento con PEAIC (Elez-Martínez et al., 2005; 2006a). La **anchura de pulso** se define como la duración del pulso aplicado. Cuando se usan ondas cuadradas, la anchura de éstas es igual a su duración, mientras que si son de caída exponencial su anchura corresponde al tiempo en el que el voltaje es superior al 37% del valor máximo de descarga. La **frecuencia** de pulso es el número de pulsos aplicados por unidad de tiempo. La frecuencia determina el tiempo de permanencia del alimento en la cámara de tratamiento, una vez fijados los valores de anchura de pulso y tiempo de tratamiento. La **forma del pulso** varía según el proceso de descarga del condensador. Las más habituales son las de caída exponencial y de onda cuadrada, habiendo también oscilatorias. El modo de aplicación de los pulsos se denomina **polaridad** y puede ser monopolar o bipolar. En los tratamientos monopolares la intensidad de campo siempre se descarga en el mismo sentido, y en los bipolares se descarga alternando el sentido de la

polaridad del pulso. La **energía suministrada** al alimento es un parámetro que incluye el campo eléctrico y el tiempo de tratamiento. Se ha demostrado que la aplicación de PEAIC aumenta **la temperatura** del producto. No obstante este calor generado durante la aplicación de PEAIC no se considera significativo ya que no se suelen alcanzar temperaturas por encima de 40 ºC.

Por lo que respecta a los factores inherentes a los alimentos, la presencia de componentes como grasas o proteínas se ha relacionado con un efecto protector de los microorganismos contra los PEAIC, por lo que la inactivación de microorganismos en los alimentos complejos representa un mayor reto cuando se compara con suspensiones simples de microorganismos (Martín et al., 1994). El **pH** o la **presencia de antimicrobianos** naturales no son factores relacionados directamente con la efectividad del tratamiento, pero sí contribuyen al procesado incrementando la efectividad de los PEAIC para conservar el producto (Barbosa-Cánovas y Sepúlveda, 2005).

La **conductividad eléctrica** de un medio es una variable importante y se define como la capacidad de conducir la corriente eléctrica. Así, los alimentos con elevada conductividad producen pequeños campos eléctricos y no son adecuados para el tratamiento mediante PEAIC. La presencia de partículas en suspensión puede causar un aumento o una reducción de la intensidad de tratamiento.

La aplicación de PEAIC para la pasteurización de alimentos data de principios del siglo XX, pero la mayoría de avances se están produciendo en las últimas décadas. Los primeros estudios se realizaron sobre soluciones reguladoras o modelo y posteriormente en leche y algunos zumos. En cuanto a los aspectos considerados en los estudios, la mayoría de las investigaciones se han centrado en su efecto sobre microorganismos alterantes (Qin et al., 1995; Harrisson et al., 1997; Elez-Martínez et al., 2005). Algunos estudios demuestran que el tratamiento de zumos mediante PEAIC alcanza niveles de destrucción de microorganismos parecidos a los conseguidos por pasteurización térmica en diferentes productos (Min et al., 2003a; Evrendilek et al., 2000; Yeom et al., 2000a; Elez-Martínez et al., 2006b).

El número de trabajos acerca del efecto de PEAIC sobre microorganismos patógenos en zumos es realmente muy reducido, siendo *Escherichia coli* el patógeno objeto de estudio tras su inoculación en zumo de manzana (Evrendilek et al., 1999), de naranja (McDonald et al., 2000) y de melón y sandía (Mosqueda-Melgar et al., 2007). La máxima inactivación alcanzada fue de 5.0 reducciones logarítmicas de *E. coli* O157:H7 en zumo de manzana tratada con 172 μs a 30 kV/cm.

En cuanto a los enzimas, no existen tantos estudios como en el caso de los microorganismos. Los resultados dependen del enzima estudiado y del producto en el que se encuentran. Con tratamientos en un rango de intensidades de campo eléctrico de 25 a 35 kV/cm y tiempos de tratamiento desde 59 μsec a 8 msec, se ha conseguido disminuir entre un 45% y 100% la actividad de algunos enzimas como pectin metilesterasas (PME) en extracto de tomate (Giner et al., 2000) y zumo de naranja (Yeom et al., 2000a); polifenoloxidasas (PPO) en extractos de manzana (Giner et al., 2001), pera (Giner et al., 2001), melocotón (Giner et al., 2002) y uva (Marsellés-Fontanet y Martín-Belloso, 2007); peroxidasa (POD) en zumo de naranja (Elez-Martínez et al., 2006a) y uva (Marsellés-Fontanet y Martín-Belloso, 2007); lipoxigenasa (LOX) en tomate (Min et al., 2003a) y β-glucosidasa en fresa (Aguiló-Aguayo et al., 2008).

Los primeros resultados obtenidos del efecto de los PEAIC sobre las propiedades fisicoquímicas y sensoriales en zumos de fruta se han centrado, principalmente, en el contenido y evolución de varias propiedades fisicoquímicas como el contenido en azúcares, el pH, la acidez o el pardeamiento (Yeom et al, 2000b, Evrendilek et al., 2000). En zumo de manzana no se ven modificados los valores de sólidos solubles, pH y acidez observándose un ligero pardeamiento después del tratamiento con PEAIC (Ortega-Rivas et al., 1998). Sin embargo, otros investigadores no encontraron cambios físicos y químicos aparentes tanto en el zumo fresco como tampoco en el procedente de concentrados (Qin et al., 1995). También se han realizado comparaciones mediante técnicas de análisis sensorial entre alimentos no procesados y los procesados térmicamente o por PEAIC. En los primeros ensayos, se obtuvieron diferencias significativas comparando el sabor del zumo de manzana procesado mediante PEAIC y sin tratar, siendo el primero de ellos el que obtuvo mejor calificación (Qin et al., 1995). Posteriores trabajos han confirmado estos resultados en zumo de naranja (Qiu et al., 1998; Yeom et al., 2000b; Min et al., 2003b), tomate (Min et al., 2003a) o zumo de naranja-zanahoria (Esteve et al., 2001).

Efectos de los tratamientos por PEAIC en los compuestos bioactivos y capacidad antioxidante

La vitamina C ha sido la más estudiada de las vitaminas presentes en los zumos de fruta, dado que es una de las más abundantes y, al mismo tiempo, más termosensibles. Existen varios trabajos en los que se demuestra que zumos tratados mediante PEAIC retienen mayor cantidad de vitamina C que los procesados térmicamente. En zumo de naranja, el tratamiento mediante PEAIC provocó una disminución del 8.8% en su contenido, mientras que tratamientos de pasteurización térmica la destruyeron hasta en un 17.2% (Elez-Martínez et al., 2006b). Estos resultados concuerdan con los obtenidos por Qiu et al. (1998) y Min et al. (2003b) quienes observaron que en zumo de naranja pasteurizado por calor se redujo entre dos o tres veces más el contenido en vitamina C comparado con zumo tratado por PEAIC. Además, estudios de vida útil sugieren que zumos de naranja tratados mediante PEAIC mantiene una mayor retención de vitamina C durante el almacenamiento comparado con el zumo tratado por calor (Yeom et al., 2000b; Min et al., 2003b; Sánchez-Moreno et al., 2005a; Elez-Martínez et al., 2006b; Cortés et al., 2008). Del mismo modo, Torregrosa et al. (2006) y Min y Zhang (2003) obtuvieron mayores niveles de vitamina C durante la refrigeración cuando el zumo de naranja-zanahoria y tomate se trataron mediante por PEAIC en vez de por calor. En otro tipo de alimento, como la leche, también se ha investigado la variación del contenido vitamínico después de haber sido tratada con PEAIC (22,6 kV/cm durante 400 μs); los resultados obtenidos muestran que no hay cambios ni en las vitaminas liposolubles estudiadas (colecalciferol y tocoferol) ni en riboflavina y tiamina, sólo un descenso en la vitamina C, aunque por debajo del nivel producido por los tratamientos térmicos (Bendicho et al., 2002). Por el contrario, Evrendilek et al. (2000) sugirieron que el tratamiento de PEAIC (35 kV/cm durante 94 μs) no afecta significativamente al contenido en vitamina C en zumo de manzana y sidra.

Existen algunos estudios del efecto de las distintas variables de procesado mediante PEAIC en el contenido de la vitamina C. Elez-Martínez y Martín-Belloso (2007) obtuvieron menores retenciones de vitamina C en zumo de naranja y gazpacho cuando los tratamientos se aplicaron con elevadas intensidades de campo, frecuencias, anchuras de pulso y tiempos de tratamiento en modo bipolar. Estos resultados coinciden con los obtenidos por Cortés et al. (2008) en zumo

de naranja y Torregrosa et al. (2006) en zumo de naranja-zanahoria, quienes demostraron que el contenido en vitamina C disminuye con el tiempo de tratamiento y la intensidad de campo eléctrico aplicada. Además, estos autores propusieron un modelo de primer orden para describir los cambios cinéticos del contenido en vitamina C durante los tratamientos a distintas intensidades de campo eléctrico.

Por otra parte, se ha estudiado la biodisponibilidad de la vitamina C en los zumos tratados por PEAIC. Al consumir 500 ml/día de zumo de naranja (Sánchez-Moreno et al., 2004) o gazpacho (Sánchez-Moreno et al., 2005b) existió un aumento de la vitamina C y una disminución de los isoprostanos en el plasma sanguíneo. Además, no existieron diferencias significativas entre la disponibilidad de los zumos tratados con PEAIC y los frescos.

Existen muy pocos estudios acerca de la influencia de los PEAIC en los compuestos fenólicos. Sánchez-Moreno et al. (2005a) determinaron el efecto de tratamientos por PEAIC a 35 kV/cm durante 750 μs con pulsos bipolares de 800 Hz en el contenido en flavononas en zumo de naranja. Estos autores no observaron cambios en el contenido de flavononas totales, así como en el de las agliconas hesperetina y naringenina después del tratamiento. Por otra parte, Zhang et al. (2007) estudiaron el efecto de distintos tratamientos mediante PEAIC (1,2, 2,2 y 3,0 kV/cm con 300 pulsos) en el contenido de cianidina-3-glucósido en una solución metanólica, observando que a medida que aumentó la severidad del tratamiento disminuyó el contenido en antocianina. Zhang et al. (2008) han propuesto modelos de primer orden para describir la cinética de degradación de cianidina-3-glucósido en una solución metanólica durante el almacenamiento.

Los estudios de investigación realizados sobre el efecto de los PEAIC en los compuestos derivados del isopreno son escasos. Min et al. (2003a) comprobaron que las concentraciones de licopeno en zumo de tomate no variaron al someter al zumo a un tratamiento con PEAIC. De igual manera, el tratamiento por PEAIC (35kV/cm durante 750 μs) no modifica significativamente el contenido en carotenoides individuales (β-cryptoxanthin, α-cryptoxanthin, zeaxanthin, lutein, β-carotene, α-carotene) y vitamina A respecto al zumo de naranja tratado térmicamente (Sánchez-Moreno et al., 2005a). En cambio, Cortés et al. (2006a) sugirieron que las concentraciones de carotenos individuales se veían ligeramente aumentadas después de llevar a cabo tratamientos a 35 kV/cm en zumo de naranja. Torregosa et al. (2005) observaron que distintos tratamientos de PEAIC aumentaron el contenido de algunos carotenos en zumo de naranja-zanahoria. Además, estos autores sugirieron que la vitamina A de los zumos se incrementaba con la intensidad de campo eléctrico y tiempo de tratamiento. Mayor cantidad de vitamina A durante la conservación (7 semanas) de zumo de naranja se obtuvo en zumos tratados mediante PEAIC comparados con los tratados por calor o frescos (Cortés et al., 2006b).

Estudios realizados en leche demuestran que los tratamientos mediante PEAIC aplicando altas intensidades de campo y tiempo cortos de tratamiento no afectan ni al contenido en α-lactoalbumina y β-lactoglobulina (Odriozola-Serrano et al., 2006) ni a la estructura de la inmunoglobulina G (Li et al., 2003). Además, Li et al. (200) estudiaron el efecto de los PEAIC, comparándolos con un tratamiento térmico, en la estabilidad de la Inmunoglobulina G en leche de soja enriquecida, propuesta como alimento funcional infantil para prevenir infecciones virales y microbianas, particularmente las intestinales (Facon et al., 1993). Los resultados obtenidos revelaron que un tratamiento mediante PEAIC no produce cambios en la actividad de la IgG, mientras que el tratamiento térmico produjo una pérdida del 86% en la actividad de

la inmunoglobulina G. Por otra parte, Rivas et al. (2007) estudiaron el efecto de los PEAC en los péptidos inhibidores de la enzima convertidora de Angiotensina (ACE), que actúan como reguladores de la actividad hormonal y tienen un efecto antihipertensivo. Sus resultados confirman que los PEAIC no afectan a la actividad inhibidora de la ACE de los péptidos. Por otra parte, Gardé-Cerdán et al. (2007) evaluaron el efecto de un tratamiento de pasteurización mediante PEAIC sobre los aminoácidos de zumo de uva, indicando que el contenido de algunos aminoácidos (histidina, triptófano, asparagina, fenilalanina y ornitina) aumentó respecto al fresco.

Por lo que respecta a la clorofila, existen muy pocos estudios que evalúen el efecto de los pulsos en estos compuestos. Yin et al. (2007) propusieron el uso combinado de tratamientos de PEAIC con soluciones de Zn^{2+} para prevenir la pérdida de la clorofila en puré de espinacas. Estos autores también demostraron que al aumentar la intensidad de campo eléctrico hasta 60 kV/cm se incrementaba el color verde del puré como consecuencia de la destrucción de microorganismos y enzimas que participan en la degradación de las clorofilas.

Apenas existen estudios de la influencia de los PEAIC sobre los ácidos grasos. Zulueta et al. (2007) no encontraron diferencias significativas en el contenido de ácidos grasos saturados, monosaturados y poliinsaturados de zumo de naranja-leche procesados a 35 kV/cm durante 40-180 µs con pulsos bipolares de 2,5-µs, comparado con las concentraciones del zumo fresco. Estos resultados coinciden con los obtenidos por Odriozola-Serrano et al. (2006) quienes observaron que el contenido en ácidos grasos de la leche tratada mediante PEAIC (35 kV/cm durante 300 o 1000 µs con pulsos monopolares de 7-µs a 111 Hz) no varió respecto al fresco. El tratamiento por PEIAC con altas intensidades de campo (35 kV/cm) y largos tiempos de tratamiento (1 ms) no modificó el contenido inicial en ácidos grasos de zumo de uva, a excepción del ácido laurico que disminuyó considerablemente después del procesado (Gardé-Cerdán et al., 2007).

Por otra parte, el tratamiento con PEAIC de zumo de naranja y gazpacho no modificó sustancialmente su capacidad antioxidante (Elez-Martínez y Martín-Belloso, 2007). Sin embargo, un tratamiento de pasteurización por calor (90 ºC, 1 min) redujo la capacidad antioxidante del zumo de naranja respecto al fresco o tratado mediante PEAIC (Elez-Martínez et al., 2006b). En zumo de naranja, no se observaron diferencias apreciables en la capacidad antioxidante durante el tiempo de almacenamiento entre zumos tratados por PEAIC con los tratados por calor, por altas presiones o frescos (Plaza et al., 2006).

FRUTOS FRESCOS CORTADOS

Las frutas y hortalizas frescas cortadas son un importante segmento del mercado en rápido y constante crecimiento, que atrae el interés del sector alimentario, incluyendo la restauración. Las frutas frescas cortadas se definen como aquellas obtenidas mediante la aplicación de una o varias operaciones unitarias de preparación, tales como pelado, cortado, reducción de tamaño y envasado. La atmósfera modificada es una tecnología de envasado de alimentos que permite alargar significativamente su vida útil, es decir, el período durante el cual mantienen las propiedades organolépticas y de seguridad requeridas para su consumo.

Producción y consumo de frutos frescos cortados

En España, en 2005, se constituyó la Asociación de Frutas y Hortalizas Lavadas Listas para su Empleo (AFHORLA), con el objetivo de responder a las necesidades de esta actividad productiva y económica, que se encuentra en pleno proceso de expansión. Dicha asociación, que representa el 90% de la producción nacional, está constituida por las mayores empresas españolas de este sector. Entre las empresas que elaboran y comercializan los productos hortofrutícolas frescos cortados en España, destacan Vega Mayor, S.A., Verdifresh S.L., Kernel Export S.L. y Sogesol que representan aproximadamente el 90%, seguidas de Tallo Verde S.L., Primaflor S.A.T., Lorca Maria Rosa, El Vergel S.L. y Hortibas S.L. (Lobo y González, 2006). Según Artés-Henández et al. (2005), la producción Española de hortalizas frescas cortadas está orientada principalmente a lechugas de ensalada (60%), además de mezcla de lechugas y otras hortalizas para ensalada (17%). Actualmente el volumen de fruta fresca cortada que se comercializa en el mercado Español es muy limitado y se destina principalmente al sector HORECA. Según los últimos datos disponibles por AFHORLA, la comercialización de frutas y hortalizas frescas cortadas experimentó un incremento de un 6% desde enero y hasta agosto de 2008 con respecto al mismo período de 2007, alcanzando las 42.859 toneladas. De este modo, según la Asociación, se mantiene la evolución positiva registrada en el sector durante los últimos años. Del total comercializado, unas 40.443 correspondieron a hortalizas, donde el 78% se destinó a la distribución, mientras que el 22% restante se dirigió a la restauración.

Si bien el consumo total en España de vegetales frescos cortados se ha cuadruplicado desde 1998, estimándose en la actualidad en un 1,5 a 2 kg por persona y año, las frutas ocupan una posición minoritaria. Muy lejos queda el consumo británico, líder absoluto en Europa, con más de 12 kilos por habitante, el consumo francés que llega a 6 kilos y el holandés, belga y alemán que superan los 3 kilos per cápita al año. No obstante, en EEUU se llega a consumir unos 30 kilos de frutas y hortalizas frescas cortados por persona y año.

En Europa los mercados líderes en frutos frescos cortados son el británico y el francés. La difusión de los productos frescos cortados en el mercado francés e inglés ha sido más veloz que en Italia y España quizá a causa de las diferentes culturas alimenticias de estos países, donde se considera más seguro y natural el producto fresco o a granel. La distribución británica y francesa de frutas y hortalizas frescas cortadas representa un 8% de la venta total de los productos hortofrutícolas, mientras que en Italia y España es de un 4-5%. En EEUU el mercado de productos frescos cortados está consolidado debido a la mayor variedad de productos en los supermercados, el desarrollo del espacio expositivo y el aumento de la vida media de los productos frescos cortados (Revista Mercados, 2007). Según la United Fresh Produce Association (2007), la venta de estos productos frescos cortados fue de hasta 15 billones de dólares en el año 2007, representando el 15% de las ventas.

Aspectos Legislativos

En la actualidad no existen legislaciones específicas para productos frescos cortados. La mayoría de regulaciones en Estados Unidos y la Unión Europea se refieren exclusivamente a aspectos de seguridad y calidad microbiológica. En España, este tipo de productos están regulados a través del Real Decreto 3484/2000 por el que se establecen las normas de higiene para la elaboración, distribución y comercio de comidas preparadas. La legislación española fija un nivel máximo de contaminación de 5×10^7 ufc/g a la fecha de caducidad y prohíbe la

presencia de microorganismos patógenos como Salmonella, mientras que los recuentos de *Escherichia coli* o *Listeria monocytogenes* están restringidos.

La manipulación inadecuada de los productos frescos cortados puede conllevar riesgos microbiológicos. La implantación del sistema de análisis de puntos de control crítico (APPCC) es obligatoria según el reglamento (CE) núm. 852/2004 para garantizar la seguridad microbiológica de los alimentos y está enfocado al control no sólo del producto final sino de todo el proceso de elaboración. La Comisión del Codex Alimentarius desarrolló un documento titulado "Código de prácticas de higiene para las frutas y hortalizas frescas" (FAO, 2003a) con un anexo sobre frutas y hortalizas frescas precortadas con el propósito de orientar en la implantación de buenas prácticas de fabricación para ayudar al control de riesgos microbiológicos, físicos, y químicos asociados al procesado de frutas y hortalizas frescas cortadas. El anexo se centra específicamente en los principios generales de higiene de alimentos (FAO, 2003b) pero con especial énfasis en algunos aspectos de control referentes a las instalaciones, programas de capacitación de personal así como el mantenimiento de documentación y registros adecuados durante el periodo de fabricación y almacenamiento del producto. La U.S. Food and Drug Administration ha desarrollado un documento similar, "Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-cut Produce", en el que se establecen los riesgos potenciales y se proponen métodos de intervención para reducir los riesgos microbiológicos en productos frescos cortados (FDA, 2001). Una nueva regulación de la Unión Europea en materia alimentaria (Reglamento núm 2072/2005) establece un límite de 1×10^2 ufc/g para *E.coli* como indicativo de la aceptabilidad del proceso de elaboración. Este criterio se aplica independientemente a otras normativas específicas referentes a microorganismos, y sirve a los fabricantes como herramienta para decidir si un producto está listo para ser comercializado.

Elaboración de frutos frescos cortados

Los productos frescos cortados son un importante segmento del mercado en rápido y constante crecimiento, que atrae el interés del sector alimentario, incluyendo la industria, distribución y restauración (Martín-Belloso et al., 2006). Las frutas y hortalizas mínimamente procesadas son aquellas obtenidas mediante la aplicación de una o varias operaciones unitarias de preparación, tales como pelado, cortado, reducción de tamaño, lavado y que son sometidas a una combinación de tratamientos parciales de conservación tales como el uso de calentamiento mínimo, el empleo de agentes conservadores, la inmersión en agua clorada, la aplicación de radiación, control de pH, el envasado en atmósferas modificadas, y el uso de temperaturas de refrigeración (Wiley, 1997). Los consumidores demandan productos libres de defectos, que tengan un grado de madurez óptimo y que posean una elevada calidad organoléptica y nutricional, además de que sean inocuos y estén libres de compuestos tóxicos (Watada y Qu, 1999). Para asegurar la calidad final de estos productos, se debe conocer el efecto que el procesado tiene en su elaboración, así como las técnicas necesarias para evitar el deterioro, ya sea durante el procesado como en su conservación, transporte y distribución.

Previo al procesado es necesario realizar una adecuada selección del material vegetal, de manera que éste posea las mejores aptitudes al procesamiento al que posteriormente va a ser sometido. La correcta selección de la materia prima es de vital importancia, ya que no todas las variedades se comportan frente al procesado de la misma manera. Así la optimización de dicho

aspecto puede simplificar los procesos de conservación que han de ser aplicados a continuación (Weller et al., 1997). Por otro lado la selección del estado de madurez de los frutos es esencial sobre todo en aquellos que son climatéricos. Dichos frutos se recolectan cuando alcanzan la madurez fisiológica, punto que no coincide con su madurez comercial y por lo tanto con el punto de consumo (Gorny et al., 2000). Para el procesado hay que seleccionar la fruta suficientemente madura para que los aromas y sabores propios se hayan desarrollado, pero no sobremadura, ya que ésta se deterioraría rápidamente pudiendo presentar incluso aromas y sabores indeseados.

Debido a las operaciones de preparación, los productos vegetales frescos cortados manifiestan, generalmente, respuestas fisiológicas más aceleradas que los productos enteros. La ruptura del tejido por el corte, supone un incremento de la respiración y transpiración que conducen a un rápido deterioro del producto, con pérdidas de sus características sensoriales y nutricionales. Además, el corte aumenta la superficie del tejido y lo hace más susceptible a alteraciones microbiológicas (Martín-Belloso y Rojas-Graü, 2005). La aplicación de técnicas que permitan controlar los factores alterantes en frutas y hortalizas frescas cortadas es actualmente el objetivo principal de muchas investigaciones en el campo de la ciencia y tecnología de alimentos. En este sentido, deben aplicarse técnicas de conservación que combinadas o no, puedan mantener o mejorar las características originales del producto, alargando su vida útil sin que se pierdan las características sensoriales y nutricionales, asegurando además su estabilidad microbiológica. La aplicación de tratamientos de conservación como el envasado en atmósfera modificada y la refrigeración ayuda a alargar considerablemente la vida comercial de estos productos (Gorris y Peppelenbos, 1999).

El lavado después del pelado/cortado con agua y agentes químicos como cloro, ácido cítrico u ozono elimina los microorganismos y los fluidos celulares, y por este motivo provoca una reducción del crecimiento microbiano y de las oxidaciones enzimáticas posteriores durante el almacenamiento de los productos procesados (González y Lobo, 2005). Unas concentraciones del 100-200 ppm de cloro o ácido cítrico son efectivas para lograr este objetivo (Dong et al. 2000). No obstante el cloro puede representar un riesgo para el consumidor ya que se descompone con la consiguiente formación de sustancias tóxicas; por tanto el producto tratado con agua clorada debe ser enjuagado. Las alternativas al empleo de cloro son el uso de ozono, fosfato trisódico o peróxido de hidrogeno. En particular, el uso de peróxido de hidrogeno y de ozono como alternativas al cloro para desinfectar los productos vegetales frescos cortados son muy prometedoras (Cano y de Ancos, 2004).

Aunque las condiciones higiénicas de la materia prima original tienen un impacto importante en la calidad final del producto, también hay que tener en cuenta el grado de limpieza de las instalaciones de procesado, así como la temperatura a la cual se está trabajando. Durante las operaciones de transformación de los productos vegetales se liberan hacia el exterior sustancias ricas en nutrientes, propiciando condiciones idóneas para el crecimiento microbiano; por lo que la maquinaria empleada para el pelado y troceado del producto debe de ser de acero inoxidable y dotada de un sistema que permita la limpieza en profundidad de aquellas zonas donde el acceso sea difícil, ya que la dificultad de acceso permite la acumulación de restos del alimento procesado.

En general, la inmersión de la fruta fresca cortada en disoluciones de agentes antioxidantes y sales de calcio es el método más efectivo para el control del oscurecimiento y ablandamiento de los tejidos vegetales. La alteración del color es debida al pardeamiento enzimático, en el que

los compuestos fenólicos de la fruta son oxidados hasta quinonas mediante reacciones catalizadas por la enzima polifenoloxidasa (Rocha, 1998). Tradicionalmente, se ha recurrido a la adición de sulfitos para controlar este problema de calidad (Brennan et al., 1999). Sin embargo, su empleo presenta problemas relacionados con el desencadenamiento de crisis asmáticas en personas alérgicas. Por tanto, en algunos países se ha restringido su empleo, lo que ha potenciado la investigación con muchos otros productos para su sustitución. Uno de los componentes ampliamente estudiados como antioxidante, es el ácido ascórbico y alguna de sus sales, ya que previene las reacciones de pardeamiento considerablemente y en diferente medida en función del producto (Martín-Belloso et al., 2006). Otros compuestos químicos también utilizados frente al pardeamiento enzimático son ácido eritórbico, 4-hexilresorcinol, ácido cítrico, málico y los aminoácidos cisteina y N-acetilcisteína entre otros (González y Lobo, 2005).

Existen tratamientos químicos que previenen la contaminación microbiana o eliminación de microorganismos ya presentes en los productos entre los que destacan los higienizantes y los aditivos conservantes. Otro posible tratamiento de conservación es la utilización de antimicrobianos naturales como los aceites o aromas esenciales, fracciones de éstos o compuestos individuales aislados y purificados (Raybaudi-Massilia, 2007), así como bacterias lácteas (Gómez et al., 2002).

El envasado en atmósferas modificadas se considera, después de la refrigeración del producto, la etapa más determinante para prolongar la vida comercial de las frutas y hortalizas frescas cortadas (Schlimme y Rooney, 1994). El principio en el que se basa el envasado en atmósferas modificadas puede ser conseguido de forma pasiva, mediante la utilización de materiales de envasado que presenten permeabilidad adecuada, o de forma activa utilizando una mezcla de gases junto con un envase también permeable. El objetivo de ambos procesos es crear una concentración óptima de gases dentro del envase, en la cual la intensidad respiratoria del producto vegetal sea lo más baja posible y por otro lado, los niveles de oxígeno y dióxido de carbono no sean perjudiciales para el mismo (Cano y de Ancos, 2004). El uso incorrecto de dichas atmósferas puede desencadenar el metabolismo anaeróbico, favoreciendo la aparición de olores no deseables debido a la producción de etanol y acetaldehído, el hinchamiento de los envases debido a la excesiva producción de dióxido de carbono, así como el rápido deterioro microbiano. Las propuestas más innovadoras para el envasado en atmósfera modificada van dirigidas al empleo de concentraciones altas de oxígeno, las cuales han demostrado tener un efecto beneficioso en la inhibición del pardeamiento enzimático y en el control de microorganismos aerobios y anaerobios en frutas y hortalizas frescas cortadas (Day, 2000). Sin embargo, cada uno de los productos frescos cortados requiere un estudio detallado de los efectos de las atmósferas modificadas en los cambios fisiológicos, bioquímicos y nutricionales del producto para determinar aquélla que es óptima, ya que esta puede ser totalmente diferente de la del producto entero del que procede, como consecuencia de los cambios fisiológicos que se inducen durante el procesado.

Cambios en los compuestos bioactivos y capacidad antioxidante de frutos frescos cortados

Existe poca información acerca del efecto del procesado mínimo en la retención de aquellos compuestos presentes en los frutos con efecto beneficioso para la salud. Gil et al. (2006) no detectaron diferencias significativas en el contenido de vitamina C, compuestos fenólicos, y carotenoides tras el procesado mínimo de piñas, mangos, melón, sandía, fresas y kiwis. En

cambio, se han detectado cambios significativos de estos compuestos bioactivos durante el almacenamiento de productos frescos cortados dependiendo del tipo de producto. En limones frescos cortados, se observó una buena retención de vitamina C durante 10 días a 5 ºC (Artés-Hernández et al., 2007), mientras que la vitamina C se mantuvo durante 14 días de almacenamiento a 5 y 10 ºC en pimiento fresco cortado (González-Aguilar et al., 2004). En cambio, se apreció una pérdida del 13% de la vitamina C inicial en mandarina después de 12 días de conservación a 4 ºC bajo atmósfera modificada (Piga et al., 2002). Los tejidos vegetales con pH ácido ejercen un efecto protector frente a la degradación de la vitamina C. Así, en rodajas de fresa (pH = 3.5) no se observaron diferencias significativas en el contenido en vitamina C durante el almacenamiento. Sin embargo, en las mismas condiciones, las rodajas de caqui (pH = 5.4), sufrieron una pérdida significativa de vitamina C (20%) (Wright y Kader, 1997a). La concentración de vitamina C también depende de la variedad seleccionada. En patatas frescas cortadas se observó un mayor contenido en vitamina C en muestras de la variedad Cara en relación con otras variedades tales como Agria, Liseta, Monalisa y Spunta durante los 6 días de almacenamiento a 4 ºC en aire (Tudela et al., 2002). Parece que los niveles de ácido ascórbico se mantienen constantes a niveles de oxígeno bajos mientras que disminuyen en otras condiciones. Según algunos trabajos realizados por Soliva-Fortuny y Martín-Belloso (2003) y Soliva-Fortuny et al. (2004), la conservación de manzana y pera fresca cortada en ausencia de aire previno eficazmente las pérdidas de ácido ascórbico. Agar et al. (1999) estudiaron la influencia de varias condiciones de envasado sobre el contenido de ácido ascórbico en rodajas de kiwi. El contenido de ácido ascórbico en las rodajas envasadas en 0,5, 2 y 4 kPa O_2 disminuyó alrededor de un 7, 12, 18%, respectivamente, después de 12 días de conservación. En cambio, los niveles de ácido ascórbico tras 12 días de almacenamiento en aire en combinación con 5, 10, 20 kPa CO_2 disminuyeron alrededor de 14, 22 y 34%, respectivamente, en relación a los niveles iniciales de ácido ascórbico. Altas concentraciones de CO_2 (> 5 kPa) en la atmósfera de envasado podrían ser perjudiciales en la conservación del contenido en ácido ascórbico (Gil et al., 1998; Wright y Kader, 1997a). En cambio, existe muy poca información sobre los efectos de elevadas concentraciones de O_2 sobre el contenido nutritivo de la fruta fresca cortada. No obstante, estudios en pera y melón sugieren que las concentraciones iniciales de O_2 altas podrían reducir el contenido de ácido ascórbico en comparación con el uso de niveles de O_2 bajos o aire (Oms-Oliu et al., 2008a; 2008b).

Dentro del grupo de vitaminas antioxidantes, Robles-Sánchez et al. (2008) estudiaron el efecto del procesado mínimo sobre el contenido de vitamina E de mango fresco cortado. Estos autores concluyeron que el contenido en vitamina E se mantiene mejor durante el almacenamiento (15 días) en muestras tratadas con una solución antioxidante (ácido ascórbico y ácido cítrico) respecto a las no tratadas.

El nivel de compuestos fenólicos en la fruta fresca cortada tiene importancia por la participación de éstos como substratos del pardeamiento enzimático, además de por su contribución al valor nutritivo de los productos frescos cortados. La disponibilidad de O_2 en las atmósferas de envasado afectó significativamente al contenido de compuestos fenólicos en manzanas frescas cortadas, debido a la rápida oxidación de estos compuestos presentes en la superficie del tejido (Gil et al., 1998). Estos resultados coinciden con los obtenidos por Oms-Oliu et al. (2008b) quienes observaron una clara reducción del contenido en ácido clorogénico en pera fresca cortada envasada en atmósferas ricas en O_2. Por el contrario, en productos como zanahoria y naranja cortadas, Alasalvar et al. (2005) observaron un incremento progresivo en la concentración de compuestos fenólicos durante la conservación frigorífica en atmósfera modificada. No obstante, las variaciones en el contenido de compuestos bioactivos durante el

almacenamiento de fruta fresca cortada dependen no solo del producto y de la atmósfera de envasado sino también de la variedad seleccionada. Así, el contenido de flavonoides aumentó en naranjas frescas cortadas 'Shamouti' mientras en naranjas 'Salustiana' se detectó una disminución significativa durante los 12-15 días de almacenamiento refrigerado (Del Caro et al. 2004). Por otra parte, la aplicación de tratamientos antioxidantes mejoró la retención de compuestos fenólicos durante el almacenamiento (14 días) en piña (González-Aguilar et al., 2005), manzana (Cocci et al., 2006) y mango frescos cortados (Robles-Sánchez et al., 2008) envasados en aire.

En rodajas de caqui y melocotón, se detectaron cambios mínimos en el contenido de carotenoides (con y sin actividad de provitamina A) durante un almacenamiento de 8 días a 5 ºC en 2 kPa O_2 + 12 kPa CO_2 (Wright y Kader, 1997b). Un comportamiento similar se observó en tomate fresco cortado conservado a 5 ºC en aire, ya que después de 15 días el contenido en licopeno prácticamente fue similar al inicial (Ayala-Zavala et al., 2008). Sin embargo, al almacenar sandía fresca cortada en condiciones atmosféricas, el contenido en licopeno disminuyó entre un 6 y un 11% a los 7 días de almacenamiento, en función de la variedad (Perkins-Veazie y Collins, 2004). La concentración de carotenos en naranja y zanahoria frescas cortadas se vio influenciada negativamente por la presencia de oxígeno el envase (Alasalvar et al. 2005). En las condiciones adecuadas, la vida útil comercial en fruta cortada finaliza antes de que se produzca una pérdida significativa de compuestos carotenoides, y por tanto de vitamina A (Cano et al., 2005).

En lo referente a derivados azufrados, su contenido no se vio afectado de forma significativa durante la conservación frigorífica de cebolletas verdes cortadas. La combinación de un tratamiento térmico por inmersión en agua a 55 ºC durante 2 minutos previo al almacenamiento en una atmósfera controlada (0,1-0,2% O_2 ó 0,1-0,2% O_2 + 7,5-9% CO_2) mantuvieron el contenido en tiosulfinatos comparado con las muestras cortadas almacenadas en aire que sufrieron una pérdida del 10% de estos compuestos (Hong et al., 2000). La conservación a temperaturas de refrigeración inferiores a 4 ºC combinada con el envasado en atmósfera modificada (16-18% O_2 + 3,4-4% CO_2) mantuvo la concentración inicial de glucosinolatos prácticamente inalterada durante 23 días en floretes de brócoli (de Ancos et al., 2007).

El contenido en aminoácidos en fruta fresca cortada varía en función de la temperatura de conservación. Así, la degradación de aminoácidos en melón fresco cortado diminuyó significativamente al bajar la temperatura de conservación, siendo la temperatura de 4 ºC la que mantuvo mejor estos compuestos (Lamikanra et al., 2000).

Actualmente se requiere de la evaluación de la capacidad antioxidante para determinar la eficacia de los antioxidantes naturales, en relación a la protección de productos vegetales contra el daño oxidativo y pérdida de su valor comercial y nutricional (Sánchez-Moreno, 2002). Lana y Tijskens (2006), en un estudio realizado en tomate fresco cortado, demostraron que la capacidad antioxidante del fruto se reduce después del procesado mínimo. No obstante, no se han detectado cambios en la capacidad antioxidante en mango (Robles-Sánchez, et al., 2008), naranjas y zanahoria (Alasalvar et al., 2005) frescas cortadas durante el almacenamiento frigorífico. La disponibilidad de O_2 en la atmósfera de envasado afectó significativamente a la capacidad antioxidante de mandarinas y pera frescas cortadas (Piga et al., 2002; Oms-Oliu et al., 2008b). Varios estudios sobre la evaluación de la capacidad antioxidante en frutos frescos cortados han revelado aspectos interesantes en relación al comportamiento de los

constituyentes antioxidantes. Estudios realizados en melón (Oms-Oliu et al., 2008a) y pera (Oms-Oliu et al., 2008b) frescos cortados han demostrado que existe una correlación significativa entre la capacidad antioxidante y los compuestos fenólicos totales. Por otra parte, Del Caro et al. (2004) y Cocci et al. (2006) realizaron estudios en naranja y manzanas fresca cortadas, respectivamente, demostrando que la mayor parte de la actividad antioxidante proviene principalmente del contenido en vitamina C, en lugar del contenido en flavonoides y otros compuestos fenólicos.

BIBLIOGRAFIA

Agar, I.T., Massantini, R., Hess-Pierce, B., Kader, A.A. (1999). Postharvest CO_2 and ethylene production and quality maintenance of fresh-cut kiwifruit slices. *Journal of Food Science*, 64, 433-440.

Aguiló-Aguayo, I., Sobrino-López, A., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric fields processing on lipoxygenase and β-glucosidase activities in strawberry juice. *Innovative Food Science and Emerging Technologies*, 9, 455-462.

Alasalvar, C., Al-Farsi, M., Quantick, P.C., Shalhidi, F., Wiktorowicz, R. (2005). Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. *Food Chemistry*, 89, 69-76.

Ames, B.M., Shigena, M.K., Hagen, T.M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. *Proceeding of the National Academy of Sciences of the United States of America*, 90, 7915-7922.

Artés-Hernández, F., Aguayo, E., Artés, E. (2005). Evolución y tendencias de la industria española de procesado mínimo en fresco de frutas y hortalizas. Mercados, 62.

Artés-Hernández, F., Rivera-Cabrera, F., Kader, A.A. (2007). Quality retention and potential shelf-life of fresh-cut lemons as affected by cut type and temperature. *Postharvest Biology and Technology*, 43, 245-254.

Ayala-Zavala, J.F., Oms-Oliu, G., Odriozola-Serrano, I., González-Aguilar, G.A., Álvarez-Parrilla, E., Martín-Belloso, O. (2008). Bio-preservation of fresh-cut tomatoes using natural antimicrobials. *European Food Research and Technology,*226, 1047-1055.

Barbosa-Cánovas, G., Pothakamury, U., Palou, E., Swanson, B. (1998). Biological effects an application of pulsed electric fields fort the preservation of foods. En: Barbosa-Canovas, G., Pothakamury, U., Palou, E., Swanson, B. Non thermal preservation of foods. Marcel Dekker, New York. pp. 73-112.

Barbosa-Cánovas, G.V., Sepúlveda, D. (2005). Present status and the future of PEF technology. En: Barbosa-Cánovas, G.V., Tapia, M.S., Cano, M.C. Novel Food Processing Technologies. CRC Press, Boca Raton, Florida. pp. 1-44.

Bendicho, S., Espachs, E., Arántegui, J., Martín, O. (2002). Effect of high intensity pulsed electric fields and heat treatments on vitamins of milk. *Journal of Dairy Research*, 69, 116-123.

Braddock, R.J. (1999). Handbook of citrus by-products and processing technology. John Wiley & Sons, New York.

Brennan, M., Le Port, G., Pulvirenti, A., Gromley, R. (1999). The effect of sodium metabisulphite on the whiteness and keeping quality of slices mushrooms. *LWT-Food Science and Technology*, 32, 460-463.

Canadean Wisdom Annual Series. (2008). Available at http:// www.aijn.org. Accessed October 21, 2008.

Cano, M.P., de Ancos, B. (2004). Estado actual de la comercialización e innovaciones en la producción de productos vegetales precortados frescos en España. En: González-Aguilar G., Fabiola Cuamea Navarro M.C. Estado actual del mercado en frutas y vegetales cortados en Iberoamérica. CIAD, Hermosillo, Sonora, México. pp. 17-23.

Cano, M.P., Sánchez-Moreno, C., Pascual-Teresa, S., de Ancos, B. (2005). Procesado mínimo y valor nutricional. En: González-Aguilar, G.A., Gardea, A.A., Cuamea-Navarro, F. Nuevas tecnologías de conservación. Productos vegetales frescos cortados. Centro de Investigación en Alimentación y Desarrollo, Mexico. pp.119-152.

Cocci, E., Rocculi, P., Romani, S., Rosa, M.D. (2006). Changes in nutritional properties of minimally processed apples during storage. *Postharvest Biology and Technology*, 39, 265-271.

Cortés, C., Esteve, M.J., Rodrigo, D., Torregrosa, F., Frígola, A. (2006a) Changes of colour and carotenoids contents during high-intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology,* 44, 1932-1939.

Cortés, C., Torrgrosa, F., Esteve, M.J., Frígola, A. (2006b). Carotenoid profile modification during refrigerated stoarge in untreated and pasteurized orange juice and orange juice treated with high intesity pulsed electric fields. *Journal of Agricultural and Food Chemistry*, 54, 6247-6254.

Cortés, C., Esteve, M.J., Frigola, A. (2008). Effect of refrigerated storage on ascorbic acid content of orange juice treated by pulsed electric fields and thermal pasteurization. *European Food Research and Technology*, 227, 629-635.

Day, B. (2000). Modified atmosphere packaging (MAP) of fresh fruit and vegetables-an overview. En: 4th International Conference on Postharvest Science. Jerusalen, Isarael.

De Ancos, B., Sánchez-moreno, C., De Pascual-Teresa, S., Martínez, J.A. Crespo, I., Cano, M.P. (2007). Efecto del procesado de elaboración de frutas y hortalizas frescas cortadas sobre el potencial saludable de floretes de brócoli y gajos de naranja. Simposio Avances Tecnológicos en el procesado mínimo hortofrutícola. Aspectos nutricionales y sensoriales. Proyecto CYTED XI.22 Desarrollo de tecnologías para la conservación de vegetales frescos cortadas. Cartagena, Murcia, España.

Del Caro, A., Piga, A., Vacca, V., Agabbio, M. (2004). Changes of flavonoids and antioxidant capacity in minimally processed citrus segments ad juices during storage. *Food Chemistry*, 84, 99-105.

Dixon, R.A., Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell*, 7, 1085-1097.

Dong, X., Wrolstad, R., Sugar, D. (2000). Extending shelf life of fresh cut pears. *Journal of Food Science*, 65, 181-186.

Elez-Martínez, P., Escolà-Hernández, J., Espachs-Barroso, A., Barbosa-Cánovas, G. V., Martín-Belloso, O. (2005). Inactivation of *Lactobacillus brevis* in orange juice by high intensity pulsed electric fields. *Food Microbiology*, 22 (4) 311-319.

Elez-Martínez, P., Aguiló-Aguayo, I., Martín-Belloso, O. (2006a). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of Agricultural and Food Chemistry***,** 86, 71-81.

Elez-Martínez, P., Soliva-Fortuny, R.C., Martín-Belloso, O. (2006b). Comparative study on shelf-life of orange juice processed by high intensity pulsed electric fields or heat treatments. *European Food Research and Technology,* 222(3-4), 321-329.

Elez-Martínez, P., Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102, 201-209.

Esteve, M. J., Frígola, A., Rodrigo, D.,Torregrosa, F. (2001). Pulsed electric fields inactivation kinetics of carotenes in mixed orange-carrot juice. European Conference on Advanced Technology for Safe and High Quality Foods, Berlín, Alemania.

Evrendilek, G., Zhang, Q. H., Richter, E. (1999). Inactivation of *Escherichia coli* O157:H7 and *Escherichia coli* 8739 in apple juice by pulsed electric fields. *Journal of Food Protection*, 62, 793-796.

Evrendilek, G.A., Jin, Z.T., Ruhlman, K.T., Qiu, X., Zhang, Q.H., Richter, E.R. (2000). Microbial safety and shelf-life of an apple juice and cider processed by bench and pilot scale PEF systems. *Innovative Food Science and Emerging Technologies*, 1, 77-86.

Facon, M., Skura, B.J., Nakai, S. (1993). Potential for immunoglogical supplementation of foods. *Food and Agriculture Immunology*, 5, 85-91.

FAOSTAT. (2008). FAO Statiscal Database. Available at http:// www.faostat.fao.org. Accessed October 20, 2008.

Food Agricultural Organization of the United Nations. (2003a). Code of hygienic practice for fresh fruits and vegetables CAC/RCP 53. Available at http.www.codexalimentarius.net/web/standard_list.do. Accessed September, 7, 2006.

Food Agricultural Organization of the United Nations. (2003b). Recommended international code of practice. General principles of food hygiene. CAC/RCP 1-1969, Rev. 4-2003.Available at http.www.codexalimentarius.net/web/standard_list.do. Accessed September, 7, 2008.

Food Drug Administration. (2001). Analysis and evaluation of preventive control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. Accessed September, 7, 2008. from http://vm.cfsan.fda.gov/~commift3-toc.html.

Garde-Cerdán, T., Arias-Gil, M., Marsellés-Fontanet, A.R., Ancín-Azpilicueta, C., Martín-Belloso, O. (2007). Effects of thermal and non thermal processing treatments on fatty acids and free amino acids of grape juice. *Food Control*, 18, 473-479.

Gil, M.I., Gorny, J.R., Kader, A.A. (1998). Responses of 'Fuji' apple slices to ascorbic treatments and low-oxygen atmospheres. *HortScience*, 33, 305-309.

Gil, M.I., Aguayo, E., Kader, A.A. (2006). Quality and nutrient retention in fresh-cut versus whole fruit during storage. *Journal Agricultural and Food Chemistry*, 54, 4284-4296.

Giner, J., Gimeno, V., Espachs, A., Elez, P., Barbosa-Cánovas, G.V., Martín, O. (2000). Inhibition of tomato (*Licopersicon esculentum* Mill.) pectin methylesterase by pulsed electric fields. *Innovative Food Science and Emerging Technologies*, 1, 57-67.

Giner, J., Gimeno, V., Barbosa-Cánovas, G.V.; Martín, O. (2001). Effects of pulsed electric field processing on apple and pear polyphenoloxidases. *Food Science and Technology International*, 7, 339-345.

Giner, J., Gimeno, V., Ortega, M., Mesegué, M., Barbosa-Cánovas, G.V., Martín, O. (2002). Inactivation of peach polyphenoloxidase by exposure to pulsed electric fields. *Journal of Food Science*, 67, 1467-1472.

Gómez, R., Muñoz, M., de Ancos, B., Cano, M.P. (2002). New procedure for the detection of lactic cid bacteria in vegetables producing antibacterial substances. *LWT-Food Science and Technology*, 35, 284-288.

González, M., Lobo, M.G. (2005). Técnicas de procesamiento. En: Nuevas tecnologías de conservación. Productos vegetales frescos cortados. González-Aguilar, G.A., Gardea, A.A., Cuamea-Navarro, F. Centro de Investigación en Alimentación y Desarrollo, Mexico. pp. 97-115.

González-Aguilar, G.A., Ayala-Zavala, J.F., Ruiz-Cruz, E., Acedo-Félix, M.E., Díaz-Cinco. (2004). Effect of temperature and modified atmosphere packaging on overall quality of fresh-cut bell peppers. *LWT-Food Science and Technology*, 37, 817-826.

González-Aguilar, G.A., Ruiz-Cruz, S., Soto-Valdez, H., Vasquez-ortiz, F., Pacheco-Aguilar, R., Wang, C.Y. (2005). Biochemical changes of fresh-cut pineapple slices treated with antibrowning agents. *International Journal of Food Science and Technology*, 40, 377-383.

Gorny, J.R., Cifuentes, R.A., Hess-Pierce, B., Kader, AA. (2000). Quality changes in peach slices as affected by cultivar, ripeness stage, fruit size, and storage regime. *Journal of Food Science*, 65, 541-544.

Gorris, L.G.M., Peppelenbos, H.W. (1999). Modified atmosphere packaging of produce. En: Handbook of Food Preservation. Rahmaan M.S. Marcel Deckker Inc, New York, USA. pp-316-333.

Harrison, S. L., Barbosa-Cánovas, G. V., Swanson, B. G. (1997). *Saccharomyces cerevisiae* structural changes induced by pulsed electric field treatment. *LWT-Food Science and Technology*, 30, 236-240.

Heinonen, I.M., Meyer, A.S., Frankel, E.N. (1998). Antioxant activity of berry oxidation. *Journal of Agricultural and Food Chemistry,* 46, 4107-4112.

Hong, G., Peiser, G., Cantwell, M.I. (2000). Use of controlled atmospheres and heat treatments to maintain quality of intact and minimally processed green onions. *Postharvest Biology and Technology*, 20, 53-61.

Jeffery, E.H., Brown, A.F., Kurilich, A.C., Keck, A.S., Matusheski, N., Klein, B.P., Juvik, J.A. (2003). Variation in content of bioactive components in broccoli. *Journal of Food Composition and Analysis*, 16, 323-330

Kaur, C., Kapoor, H.C. (2001). Antioxidants in fruits and vegetables – the millennium's health. *International Journal of Food Science and technology*, 36, 703-7025

Kris-Etherton, P.M., Hecker, K.D., Bonamone, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine*, 113, 71-88, S.9B.

Lamikanra, O., Chen, J.C., banks, D., Hunter, P.A. (2000). Biochemical and microbial changes during the storage of minimally processed cantaloupe. *Journal of Agricultural and Food Chemistry*, 48, 5955-5961.

Lana, M.M., Tijskens, L.M.M. (2006). Effect of cutting and maturity on antioxidant activity of fresh-cut tomatoes. *Food Chemistry*, 97, 203-211.

Li, S.Q., Zhang, Q.H., Lee, Y.Z., Pham, T.V. (2003). Effects of pulsed electric fields and thermal processing on the stability of bovine immunoglobulin G (IgG) in enriched soymilk. *Journal of Food Science*, 68, 1201-1207.

Li, S.Q., Bomser, J.A., Zhang, H. (2005). Effects of pulsed electric fields and heat treatments on stability and secondary structure of bovine Immunoglobulin G. *Journal of Agricultural and Food Chemistry*, 53, 663-670.

Lobo, M.G., González, M. (2006). Estado actual de los productos mínimamente procesados en España. En: Actas del IV Congreso Nacional sobre Processamento Mínimo de Frutas e Hortalizas. I Simposio Iberoamericano de Vegetales Frescos Cortados. Universidade de Sao Paulo, Escola Superior de Agricultura Luis de Queiroz. Sao Pedro, Brasil. pp. 77-80.

Macheix, J.J., Fleuriet, A., Billot, J. (1990). Fruit phenolics. CRC Press, Boca Raton, Forida.

MAPA. (2008). Análisis de Consumo Alimentario. Ministerio de Agricultura, Pesca y Alimentación. Available at http://www.mapa.es. Accessed October 20, 2008.

Martín, O., Zhang, Q., Castro, A.J., Barbosa-Cánovas, G.V., Swanson, B.G. (1994). Revisión: Empleo de pulsos eléctricos de alto voltaje para la conservación de alimentos. Microbiología e ingeniería de proceso. *Revista de Ciencia y Tecnología de los Alimentos*, 1, 34, 1-34.

Martín-Belloso, O., Rojas-Graü, M.A. (2005). Factores que afectan la calidad. En Nuevas tecnologías de conservación. Productos vegetales frescos cortados. Ed: González-Aguilar, G.A., Gardea, A.A., Cuamea-Navarro, F. Centro de Investigación en Alimentación y Desarrollo, Mexico. pp.77-93.

Martín-Belloso, O., Soliva-Fortuny, R., Oms-Oliu, G. (2006). Fresh-cut fruits. En: Hui, Y.H. Handbook of fruits and fruit processing. Blackwell Publishing, Ames, Iowa, USA, pp. 129-144.

Marsellés-Fontanet, A.R., Martín-Belloso, O. (2007). Optimization and validation of PEF processing conditions to inactivate enzymes of grape juices. *Journal of Food Engineering*, 83, 452-462.

Mc Donald, C.J., Lloyd, S.W., Vitale, M.A., Petersson, K., Innings, F. (2000). Effects of pulsed electric fields on microorganisms in orange juice using electric fields stregths of 30 and 50 kV/cm. *Journal of Food Science,* 65, 984-989.

Min, S., Jin, Z.T., Zhang, Q.H. (2003a). Commercial scale pulsed electric field processing of tomato juice. *Journal of Agricultural and Food Chemistry*, 51(11), 3338-3344.

Min, S., Jin, Z.T., Min, S.K., Yeom, H., Zhang, Q.H. (2003b). Commercial-scale pulsed electric field processing on orange juice. *Journal of Food Science,* 68, 1265-1271

Min, S., Zhang, Q.H. (2003). Effects of commercial-scale pulsed electric fields processing on flavor and color of tomato juice. *Journal of Food Science,*68, 1600-1606.

Mosqueda-Melgar, J., Raybaudi-Massilia, R.M., Martín-Belloso, O. (2007). Influence of treatment time and frequency on *Salmonella* Enteritidis, *Escherichia coli* and *Listeria monocytogenes* populations inoculated in melon and watermelon juices treated by pulsed electric fields. *International journal of Food Microbiology*, 117, 192-200.

Odriozola-Serrano, I., Bendicho-Porta, S., Martín-Belloso, O. (2006). Comparative study on shelf life of whole milk processed by high intensity pulsed electric field or heat treatments. *Journal of Dairy Science*, 89, 905-911.

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008a). The role of peroxidase on the antioxidant potential of fresh-cut Piel de Sapo" melon packaged under different modified atmospheres. *Food Chemistry*, 106, 1085-1092.

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008b). Antioxidant content of fresh-cut pears stored in high-O_2 active packages compared with conventional low-O_2 active and passive modified atmosphere packaging. *Journal of Agricultural and Food Chemistry*, 56, 932-940.

Ortega-Rivas, E., Zárate-Rodríguez, E., Barbosa-Cánovas, G. V. (1998). Apple juice pasteurization using ultrafiltration and pulsed electric fields. *Transactions of the Institute of Chemical Engineering*, 76 (C): 193-198.

Perkins-Veazie, P., Collins, J.K. (2004). Flesh quality and lycopene stability o fresh-cut watermelon. *Postharvest Biology and Technology*, 31, 159-166.

Periago, M.J., Ros, G., López, G., Martínez, M.C., Rincón, F. (1993). Componentes de la fibra dietética y sus efectos fisiológicos. *Revista Española de Ciencia y Tecnología de los Alimentos*, 33, 229-245.

Piga, A., Agabbio, M., Gambella, F., Nicoli, M.C. (2002). Retention of antioxidant activity in minimally processed mandarin and satuma fruits. *LWT-Food Science and Technology*, 35, 344-347.

Plaza, L., Sánchez-Moreno, C., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., Cano, M.P. (2006) Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. *European Food Research and Technology,* 223**,** 487-493.

Qin, B. L., Chang, F. J., Barbosa-Cánovas, G. V., Swanson, B. G. (1995). Non-thermal inactivation of *Saccharomyces cerevisiae* in apple juice using pulsed electric fields. *LWT-Food Science and Technology*, 28, 564-568.

Qiu, X., Sharma, S., Tuhela, L., Jia, M., Zhang, Q. (1998). An integrated PEAIC pilot plant for continuous nonthermal pasteurization of fresh orange juice. *Agricultural Engineering*, 41, 1069-1074.

Raybaudi-Massilia, R.M. (2007). Uso de sustancias antimicrobianas naturales en combinación con compuestos estabilizadores de la calidad para controlar microorganismos patógenos y extender la vida útil de frutas frescas cortadas. Tesis Doctoral, Octubre, 2007.

Revista Mercados. Agosto-Setiembre 2007 nº 72. Movimientos en la cuarta gama de la lechuga a la macedonia con zumo. Available at http://www.revistamercados.com. Accessed October 15, 2008

Rivas, A., Rodrigo, D., Company, B., Sampedro, F., Rodrigo, M. (2007). Effect of pulsed electric fields on water-soluble vitamins and ACE inhibitory peptides added to a mixed orange juice and milk beverage. *Food Chemistry*, 104, 1550-1559.

Robles, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G.A., Martín-Belloso, O. (2008). Effect of minimal processing on bioactive compounds and antioxidant capacity of fresh-cut 'Kent' Mango (Mangifera indica L.). *Postharvest Biology and Tecnology*. DOI: 10.1016/j.postharvestbio.2008.09.003.

Rocha, A.M.C.N., Brochado, C.M., Morais, A.M.M.B. (1998). Influence of chemical treatments on quality of cut apple (cv. Jonagored). *Journal of Food Quality*. 21, 13-18.

Sánchez-Moreno, P., Cano, M.P., De Ancos, B., Plaza, L., Olmedilla, B., Granado, F., Elez-Martínez, P., Martín-Belloso, O., Martín, A. (2004). Pulsed electric fields-processed orange juice consumption increases plasma vitamin C and decreases F2-isoprostanes in healthy humans. *Journal of Nutritional Biochemistry*, 15, 601-607.

Sánchez-Moreno, C. (2002). Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International*, 8, 121-137.

Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., Cano, P. (2005a). Impact o high pressure and pulsed electric fields on bioactive compounds and antioxidant capacity of orange juice in comparison with traditional thermal processing. *Journal of Agricultural and Food Chemistry*, 53(11), 4403-4409.

Sánchez-Moreno, P., Cano, M.P., De Ancos, B., Plaza, L., Olmedilla, B., Granado, F., Elez-Martínez, P., Martín-Belloso, O., Martín, A. (2005b). Intake of Mediterranean soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *International Journal of Food Sciences and Nutrition*, 56, 115-124.

Schlimme, D.V., Rooney, M.L. (1994). Packaging of minimally processed fruits and vegetables. En: Wiley, R.C. Minimally processed refrigerated fruits and vegetables. Chapman and Hall, New York, USA. pp. 135-182.

Soliva-Fortuny, R.C., Martín-Belloso, O. (2003). Microbiological and biochemical changes in minimally processed fresh-cut Conference pears. *European Food Research and Technology*, 217, 4-9.

Soliva-Fortuny, R.C., Elez-Martínez, P., Martín-Belloso. (2004). Microbial and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. *Innovative Food Science and Emerging Technologies*, 5, 215-224.

Taiz, L., Zeiger, E. (2006). Secondary Metabolites and Plant Defense. En: Taiz, L., Zeiger, E. Plant Physiology. Sinauer Associates, Inc.

Talanen, M. (1995). Vitaminas y minerales en la salud y en la nutrición. Ed. Acribia, Zaragoza.

Torregrosa, F., Cortés, C., Esteve, M.J., Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry*, 53, 9519-9525.

Torregrosa, F., Esteve, M.J., Frígola, A., Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*, 73, 339-345.

Tudela, J.A., Espín, J.C., Gil, M.I. (2002). Vitamin C retention in fresh-cut potatoes. *Postharvest Biology and Technology*, 26, 75-84.

United Fresh Produce Association. (2007). Available at http://www.unitedfresh.org. Accessed March 31, 2007.

Watada, A.E., Qu, L. (1999). Quality of fresh-cut produce. *Postharvest Biology and Technology*, 15, 201-205.

Weller, A., Sims, C.A., Matthews, R.F., Bates, R.P., Brecht, J.K. (1997). Browning susceptibility and changes in composition during storage of carambola slices. *Journal of Food Science*, 62, 256-260.

Wiley, R. (1997). Frutas y hortalizas mínimamente procesadas y refrigeradas. Editorial Acribia, Zaragoza, España.

Willcox, L.K., Catignani, G.L., Lazarus S. (2003). Tomatoes and cardiovascular health. *Critical Reviews in Food Science and Nutrition,* 43, 1-18.

Wright, K.P., Kader, A.A. (1997a). Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. *Postharvest Biology and Technology*, 10, 39-48.

Wright, K.P., Kader, A.A. (1997b). Effect of controlled-atmosphere storage on the quality and carotenoid content of sliced persimmons and peaches. *Postharvest Biology and Technology*, 10, 89-97.

Yeom, H. W., Streaker, C. B., Zhang, Q. H., Min, D. B. (2000a). Effects of pulsed electric fields on the activities of microorganisms and pectin methyl esterase in orange juice. *Journal of Food Science*, 65, 1359-1363.

Yeom, H.W., Streaker, C.B., Zhang, H., Min, D.B. (2000b). Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *Journal of Agricultural and Food Chemistry*, 48, 4597-4605.

Ying, Y., Han, Y., Liu, J. (2007). A novel protecting method for visual green color in spinach puree by high intensity pulsed electric fields. *Journal of Food Engineering*, 79, 1256-1260.

Zhang, Y., Liao, X. J., Ni, Y.Y., Wu, J.H., Hu, X.S., Wang, Z.F., Chen, F. (2007). Kinetic analysis of the degradation and its color change of cyanidin-3-glucoside exposed to pulsed electric field. *European Food Research and Technology*, 224, 597-603.

Zhang, Y., Hu, X.S., Chen, F., Wu, J.H., Liao, X. J., Wang, Z.F. (2008). Stability and colour characteristics of PEF-treated cyanidin-3-glucoside during storage. *Food Chemistry*, 106, 669-676.

Zulueta, A., Esteve, M.J., frasquet, I., Frígola, A. (2007). Fatty acids changes during orange juice-milk beverage processing by high-pulsed electric field. *European Journal of Lipid Science and Technology*, 109, 25-31.

Objetivos

OBJETIVOS

Con la realización de esta tesis se pretende establecer las condiciones de procesado no térmico (pulsos eléctricos de alta intensidad de campo o cortado/envasado en atmósfera modificada) para obtener zumos y frutos cortados con alto potencial antioxidante. Los compuestos bioactivos estudiados en estos productos fueron, vitamina C, ácidos fenólicos y flavonoles; también se determinó la capacidad antioxidante de los mismos. Además, en tomate se evaluó el contenido en carotenoides individuales, mientras que en fresa se determinó la concentración de antocianinas totales e individuales. Con esta finalidad se plantearon los siguientes objetivos específicos:

- Establecer las condiciones óptimas de procesado mediante PEAIC para obtener zumos de tomate y fresa con alto potencial antioxidante.
- Modelizar el efecto de los parámetros de procesado por PEAIC sobre los componentes beneficiosos para la salud y la capacidad antioxidante de los zumos de tomate y fresa.
- Comparar el efecto de los tratamientos de pasteurización mediante PEAIC con el tratamiento térmico en las propiedades antioxidantes de zumos de tomate y fresa.
- Estimar la vida útil de los zumos de tomate y fresa pasteurizados mediante PEAIC o calor en función de su potencial antioxidante.
- Evaluar la influencia de la variedad en el potencial antioxidante de tomate fresco cortado durante el almacenamiento.
- Determinar la evolución a lo largo del almacenamiento del potencial antioxidante de tomates y fresas frescos cortados envasados en distintas atmósferas modificadas y temperaturas de conservación.

Material y Métodos

MATERIAL Y METODOS

Recepción de la muestra y preparación

Las variedades de tomate (*Lycopersicum esculentum* Mill.) escogidas para la realización de este trabajo fueron las más habituales en el mercado: Rambo, Durinta, Bodar, Pitenza, Cencara y Bola. Por lo que respecta a la fresa (*Fragaria ananassa* Duch), la variedad utilizada fue Camarosa. Todos los frutos se adquirieron en el Mercado Central de Frutas y Hortalizas de Lleida. Antes de ser transportados, se seleccionaron y se descartaron aquellos frutos defectuosos.

El transporte se realizó en condiciones de refrigeración. Una vez recibidos los frutos en el laboratorio, se seleccionaron y se almacenaron a una temperatura de 5 ºC ± 1 ºC.

Zumos de tomate/fresa

Los zumos de tomate y fresa se obtuvieron a partir de frutos enteros, mediante trituración, centrifugación (sólo en el zumo de fresa) y filtrado. Antes del tratamiento, el zumo se caracterizó mediante la determinación de diferentes propiedades físico-químicas. El procesado por PEAIC del zumo se llevó a cabo en un equipo de flujo continuo OSU-4F (Ohio State University, Columbus, Ohio, EEUU). El equipo consta de un sistema de varias cámaras (sección de 2,3 mm de diámetro y 3,0 mm de largo) con electrodos colineales dispuestos en serie. Los tratamientos de PEAIC aplicados a los zumos variaron en intensidad de campo, frecuencia, anchura de pulso, tiempo de tratamiento y polaridad según los estudios realizados. Se estableció un caudal volumétrico del zumo de 60 cm^3/min para que la temperatura no superara los 40 ºC. El control de temperaturas del zumo se realizó mediante termopares situados a la entrada y a la salida de las cámaras de tratamiento.

Por otra parte, los tratamientos térmicos de referencia (90 ºC-1 min y 90 ºC-30 s) se llevaron a cabo en un intercambiador de calor tubular de flujo continuo. Se utilizó una bomba peristáltica para mantener la velocidad adecuada de flujo a través del serpentín de acero inoxidable, el cual fue sumergido en un baño de agua caliente con agitación (Universitat de Lleida, Lleida, España). Inmediatamente después del tratamiento térmico, el zumo fue enfriado con hielo.

Los zumos tratados tanto por PEAIC como por calor fueron envasados inmediatamente después del procesado en recipientes estériles y posteriormente almacenados a 4 ± 0.5 ºC hasta la realización de las correspondientes determinaciones analíticas. Los análisis se realizaron en muestras por duplicado. Para los estudios de vida útil, la determinación de los compuestos bioactivos se realizó una vez por semana durante 9-13 semanas.

Tomate/Fresa frescos-cortados

Tanto tomates como fresas se caracterizaron antes de ser procesados. Para la preparación del producto fresco cortado, las frutas enteras se lavaron con agua y hipoclorito sódico a concentración de 200 ppm, se secaron y trocearon en rodajas de aproximadamente 7 mm de espesor (para tomate fresco cortado) y en cuartos (para fresa fresca cortada). Después, se

colocaron 100 g en cada bandeja de polipropileno, modificando la atmósfera interior mediante una termoselladora de vacío compensado ILPRA Food Pack Basic V/6 (Ilpra S. CP. Vigevono, Italia). Las bandejas se almacenaron en ausencia de luz. La evolución de la atmósfera en los envases y el contenido en compuestos bioactivos se realizó justo después del envasado y posteriormente cada 2-3 días durante un periodo total de 14-21 días. Los análisis se realizaron por duplicado.

Caracterización

Los zumos frescos recién extraídos, así como los tomates y fresas enteros fueron caracterizados mediante la determinación de distintas propiedades físico-químicas tales como color, pH, acidez y sólidos solubles. Además, en los zumos se midió la conductividad eléctrica de los mismos, mientras que en frutos enteros se determinó la textura y el coeficiente de respiración. Los métodos de análisis utilizados fueron los siguientes:

-Color. El color se determinó mediante un colorímetro Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) para los frutos frescos cortados, mientras que se utilizó un colorímetro Macbeth Colour-Eye 3000 (Macbeth-kollmorgen Inst Corp., newburg, NY, USA) en el caso de zumos. Estos equipos, a partir de valores de refractancia, proporcionaron las coordenadas del sistema cromático uniforme CIELab (L*, a*, b*). El iluminante fue el D_{75} y el ángulo de observación de 10 ºC.

-**pH**. El pH se determinó por medida potenciométrica según BOE (1988) con un pH-metro Crisol 2001 (Crison Instruments SA, Alella, Barcelona, España).

-**Acidez**: La acidez se determinó a través de un análisis potenciométrico, según método de BOE (1988).

-**Sólidos solubles**. Los sólidos solubles (ºBrix) de las muestras se determinaron mediante un refractómetro digital Atajo RX-100 (Atago Company Ldt., Japón).

-**Conductividad.** La conductividad eléctrica de los zumos de determinó con un conductímetro Testo-240 (TestoGmBh&Co, Lenzkirch, Alemania).

-**Textura.** La textura de los tomates y fresas enteros se determinó mediante un texturómetro TA-XT2 (Stable Micro Systems Ltd., Surrey, Inglaterra).

La resistencia a la penetración se midió como la fuerza necesaria para que una sonda de 4 mm de diámetro y base plana penetrase en el fruto 5 mm. Se fijaron los siguientes parámetros en el texturómetro: velocidad pre-ensayo, 2 mm/s; velocidad ensayo, 5 mm/s; velocidad post-ensayo, 5 mm/s; distancia de penetración, 5 mm.

Mediante el test de Kramer se evaluó el esfuerzo necesario para deformar un 50 % el volumen inicial de los trozos de frutos (cubos de 2,5 cm de largo, 2,5 cm de ancho y 1 cm de altura). Se fijaron los siguientes parámetros en el texturómetro: velocidad pre-ensayo, 5 mm/s; velocidad ensayo, 3 mm/s; velocidad post-ensayo, 10 mm/s; porcentaje de deformación, 5 mm.

-**Coeficiente de respiración**. El coeficiente de respiración de los frutos enteros se determinó siguiendo el método propuesto por Soliva-Fortuny et al. (2005). Para ello se colocaron 50 g de muestra en un recipiente cerrado de vidrio, éste se mantuvo a 4 ºC en la

oscuridad durante 1 hora. Después de este tiempo, y con la ayuda de un septum situado en la parte superior del recipiente, se determinó el contenido en gases en el interior del envase con un cromatógrafo de gases MICRO-GC CP2002 (Chrompack Intl., Middelburg, Holanda). El coeficiente de respiración se calculó teniendo en cuenta la concentración inicial de gases en el interior del envase antes y después de 1 hora.

Determinaciones Analíticas

Gases del espacio de cabeza

Para la determinación de la atmósfera en el espacio de cabeza de las bandejas se usó un analizador de gases MICRO-GC CP 2002 (Chrompack Internacional, Middelburg, Holanda) equipado con un detector de conductividad térmica. Se efectuó la medida de la concentración de O_2, CO_2 y etileno en dos bandejas de cada lote, realizando dos repeticiones medidas en cada una de ellas. Se inyectaron 0,25 y 0,33 µL de muestra para la determinación del oxígeno y dióxido de carbono/etileno, respectivamente. Para la cuantificación del oxígeno se utilizó una columna CP-Molsieve 5 Å (4m×0,32 mm,10µm) a 55 ºC y 90 kPa, mientras que la columna utilizada para la separación de dióxido de carbono y etileno fue una Poraplot Q (10m×0,32 mm,10µm) a 75 ºC y 140 kPa. El método de la recta patrón permitió obtener rectas que relacionaron el área obtenida en el cromatograma de gases con la concentración de cada gas con R^2 =0.9977-9998.

Microorganismos

Para realizar el recuento de microorganismos aerobios mesófilos se llevó a cabo el protocolo establecido según la norma ISO 4833 (1991) y para el recuento de mohos y levaduras se siguió la norma ISO 7954 (1988). En condiciones estériles, 10 g de tomate cortado se homogenizaron durante 2 minutos con 90 ml de peptona salina al 0,1% en agua con un Stomacher Lab Blender (Steward medical, London, England). En ambos casos, se preparó un banco de diluciones mezclando 0,1 ml de esta solución con 0,9 mL de peptona salina (PS) previamente esterilizada. Una vez realizado el banco de diluciones, se procedió a realizar la siembra de placas de Petri con PCA (Palte Count Agar), en el caso de microorganismos aerobios mesófilos, mientras que para el recuento de mohos y levaduras, la siembra se realizó en placas Petri con CGA (Chloramphenicol Glucosa Agar). En cada placa se colocó 0,1 ml de la dilución correspondiente y se extendió con la ayuda de un asa Drigalski. Finalmente las placas fueron almacenadas a 30 ºC ± 1 ºC durante 3 días en el caso de recuento aerobios mesófilos y 25 ºC ± 1 ºC durante 5 días para el recuento de mohos y levaduras.

Vitamina C

El método utilizado para la extracción y determinación de la vitamina C de las muestras fue el validado por Odriozola-Serrano et al. (2007) (Anexo). Se introdujeron 25 g de muestra en un vaso de precipitados y se añadió 25 g de una solución que contenía 45 g/l de ácido metafosfórico y 7,2 g/l de DL-ditiotreitol (DTT) como agente reductor. La mezcla se trituró y se centrifugó en una centrifuga Avanti TM J-14 (Beckman Instrument, Inc. Fullertow, California, USA) a 12500 rpm durante 15 minutos a 4 ºC. Pasado este tiempo, el sobrenadante

se filtró al vacío con papel Whatman nº 1 y se pasó por un filtro de nylon de 0,45 µm. Una alícuota de 20 µl se inyectó en el sistema cromatográfico (Waters, Milford, MA), el cual consta de un controlador, un desgasificador, un detector de absorbancia y un integrador. La separación se realizó en una columna de fase reversa C18 Hypersil ODS de 250 mm de largo, 4,6 mm de diámetro y 5 µm de tamaño de partícula interna. Se empleó una fase móvil constituida por una solución de ácido sulfúrico al 0.01% ajustada a pH 2,6. La columna de separación se mantuvo a temperatura ambiente y el flujo de la fase móvil fue de 1 ml/min. La detección se realizó a longitud de onda de 245 nm. Para la cuantificación se construyó la curva de calibrado de cinco niveles de concentración del ácido ascórbico (5, 10, 15, 30 y 50 mg/kg) con R^2 = 0,9871-0,9992.

Licopeno

El contenido en licopeno se determinó espectrofotométricamente siguiendo el método propuesto por Davis et al. (2003). Aproximadamente 0,6 g de muestra se mezclaron con 5 ml de butilato hidroxitolueno (BHT) en acetona al 0,05% (w/v), 5 ml de etanol al 95% y 10 ml de hexano. La mezcla se centrifugó a 200 rpm durante 15 minutos a 4 ºC. Posteriormente, se le añadieron 3 ml de agua destilada, y se procedió a la lectura de la absorbancia de la parte orgánica a 503 nm. El contenido en licopeno se estimó a partir de la ecuación 1 (Fish et al., 2002).

$$Licopeno = \frac{A_{503} \times PM \times DF \times 1000}{\varepsilon \times L} \qquad (1)$$

Donde:

PM: peso molecular del licopeno (536,9 g/mol)

DF: factor de dilución

ε: coeficiente de extinción (172.000 L/mol×cm)

L: longitud de la cubeta en cm (1 cm)

Carotenos individuales

Extracción

La extracción de carotenos de los tomates y sus zumos se realizó mediante el método propuesto por Tonucci et al. (1995). Primero, se homogenizaron 25 g de muestra, 25 ml de tetrahidrofurano (THF), 2,5 g de carbonato magnésico, 2,5 g de arena sílica de Celite y 0,5 mg de patrón interno (β-apo-8'-carotenal) en un Omni Mister, con baño exterior de hielo, durante 20 min. Después, la mezcla se filtró con papel Whatman nº 1. El residuo que quedó en el filtro se lavó dos o tres veces más con THF para eliminar el color rojo/anaranjado de la misma. Posteriormente, se reunieron los extractos de THF y se concentraron hasta 10 ml en un rotavapor a 35 ºC. A continuación, el extracto se vertió en un embudo de decantación junto con

250 ml de diclorometano y 150 ml de agua saturada con cloruro sódico. La fase acuosa se lavó con diclorometano para extraer los carotenos y la fase orgánica se lavó con una solución de cloruro sódico para eliminar sustancias solubles en agua. Las fases orgánicas se combinaron, se secaron sobre sulfato de sodio anhidro y se filtraron a través de filtro Whatman nº 42. El volumen de filtrado se redujo a 25 ml en un rotavapor a 35 ºC; y posteriormente el volumen se enrasó a 50 ml en un matraz aforado con diclorometano. Antes de inyectar 20 µl de la muestra en el sistema cromatográfico, ésta se filtró con nylon de 0,45 µm.

Condiciones cromatográficas

La separación de los carotenos se llevó a cabo mediante el método descrito por Khachik et al. (1992). Para ello se utilizó un cromatógrafo de líquidos de alta eficacia (HPLC) equipado con un controlador, un desgasificador y un detector de UV/visible (Waters, Milford, MA) a una longitud de onda de 470 nm. La separación se realizó en una columna de fase reversa C18 Hypersil ODS de 250 mm de largo, 4,6 mm de diámetro y 5 µm de tamaño de partícula interna. La columna de separación se mantuvo a temperatura ambiente y el flujo de la fase móvil fue de 0,7 ml/min. La separación de los distintos carotenos comenzó con un flujo isocrático de acetonitrilo (85%), metanol (10%), acetonitrilo (3%) y hexano (2%) que se mantuvo durante 10 minutos. Transcurrido este tiempo, continuó un gradiente lineal durante 30 minutos hasta niveles de (45%) acetonitrilo, (10%) metanol, (23%) diclorometano y (22%) hexano. Finalmente, la columna se reequilibra durante 20 minutos hasta alcanzar las condiciones iniciales. La cuantificación se llevó a cabo mediante patrones externos. Para ello se construyó la curva de calibrado de cinco niveles (0, 4, 8, 12, 16 y 20 mg/kg) de concentración de licopeno, neurosporeno, γ-caroteno, ξ-caroteno, δ-caroteno, β-caroteno, luteína, fitoeno y fitoflueno con R^2 = 0,9768-0,9870.

Vitamina A

La vitamina A se expresó como Equivalentes de Retinol (RE). Para el cálculo de los RE en tomates se empleó ecuación 2 (Trumbo et al., 2003),

$$RE = \left[\frac{\beta - caroteno(\mu g)}{12}\right] + \left[\frac{\gamma - caroteno(\mu g)}{24}\right] \quad (2)$$

Compuestos fenólicos totales

La extracción de fenoles solubles totales del tomate y del zumo de tomate se llevó a cabo siguiendo el método descrito por Singleton et al. (1999). Los fenoles solubles se extrajeron con una disolución de metanol/agua (50:50). Los fenoles totales se cuantificaron usando el reactivo de Folin-Ciocalteu. Se mezclaron 0,5 ml del extracto polifenólico y 0,5 ml del reactivo de Folin-Ciocalteu durante 3 minutos a temperatura ambiente; se añadieron 10 ml de una solución de carbonato sódico saturado a la mezcla y agua destilada hasta un volumen de 25 ml. Después de una hora a temperatura ambiente en la oscuridad, se midió la absorbancia a 725 nm. Las concentraciones de fenoles totales de las muestras se obtuvieron mediante comparación con las rectas de calibrado realizada con ácido gálico. Para ello, se construyó la curva de calibrado de

seis niveles de concentración de ácido gálico (0, 100, 200, 300, 400 y 500 mg/kg) con R^2 = 0,9897-0,9939

Compuestos fenólicos individuales

Extracción de ácidos fenólicos y flavonoles

El método utilizado para extraer ácidos fenólicos y flavonoles fue el propuesto por Hertog et al. (1992). En un matraz de 50 ml se mezclaron 40 ml de metanol acuoso al 62,5% que contenía 2 g/l de butil hidroquinona (TBHQ), 10 ml de ácido clorhídrico 6 M y 0,5 g de muestra liofilizada. Después se realizó su hidrólisis en un baño de agua con reflujo a 90 ºC durante 2 horas. A continuación, el hidrolizado se enfrió a temperatura ambiente, se enrasó a 100 ml con metanol y se sonicó. De la solución anterior se filtró una alícuota a través de un filtro de celulosa de 0,45 µm y se inyectaron 20 µm en el sistema cromatográfico.

Extracción de antocianinas

Para la extracción de antocianinas de las fresas y sus zumos, se siguió el método propuesto por Gómez-Plaza et al. (2008) con algunas modificaciones. Primero, la mezcla de 2 ml de metanol con 100 mg de muestra liofilizada se sonicó durante 20 minutos y después se centrifugó a 200 rpm durante 10 minutos. El sobrenadante se filtró con nylon de 0,45 µm antes de inyectar 20 µl de muestra en el sistema cromatográfico.

Condiciones cromatográficas

Para la separación de fenoles individuales, flavonoles y antocianinas se utilizó un cromatógrafo de líquidos de alta eficacia (HPLC) equipado con un controlador y un detector de UV/visible (Waters, Milford, MA) a una longitud de onda de entre 200 y 600 nm. La separación se realizó en una columna de fase reversa C18 Hypersil ODS de 250 mm de largo, 4.6 mm de diámetro y 5 µm de tamaño de partícula interna. La columna de separación se mantuvo a temperatura ambiente y el flujo de la fase móvil fue de 1 ml/min. Para la separación de los compuestos fenólicos se utilizó un gradiente formado por una mezcla de 2,5% de ácido fórmico en agua (Fase A) y una mezcla de 2,5% de ácido fórmico en acetonitrilo (Fase B). La separación de los compuestos fenólicos comenzó con un gradiente hasta el minuto 15 desde 5% a 13% de fase B, seguido de otro gradiente del minuto 15 al 20 del 13% al 15% de fase B. Después, se continuó con un gradiente del 15% al 30% de fase B hasta el minuto 25, un flujo isocrático de 30 a 45% de fase B del minuto 28 al 32, otro gradiente lineal de 45 a 90% de fase B entre los 35 y 40 minutos y finalmente un flujo isocrático de 90% de fase B hasta el minuto 45. Transcurrido este tiempo, la columna se reequilibró a las condiciones iniciales durante 15 minutos. La cuantificación se llevó a cabo mediante el método de patrón externo. Para ello se realizó la recta de calibrado de cinco niveles (0, 4, 8, 12, 16 y 20 mg/kg) de concentración de ácido clorogénico, ácido ferúlico, ácido cafeico, ácido elágico, ácido gálico, ácido *p*-cumárico, ácido *p*-hidroxibenzoico, kaempferol, quercitina, miricetina, pelargonidina-3-glucósido, cianidina-3-glucósido y pelargonidina-3-rutinósido con R^2 = 0,9581-0,9980.

Antocianinas totales

El contenido en antocianinas totales de fresas cortadas o en zumo se determinó mediante el método espectrofotométrico propuesto por Meyers et al. (2003). Primero, se homogenizaron 25 mg de muestra con 25 ml de metanol y se centrifugaron a 12500 rpm durante 4 ºC. A continuación, se colocaron 10 ml de sobrenadante en dos matraces aforados de 50 ml, uno de ellos se enrasó con cloruro de potasio 0,025 M a pH 1 y el otro con acetato de sodio 0,4 M a pH 4,5. La absorbancia de cada una de las mezclas se determinó con un espectrofotómetro (CECIL CE 2021: Cecil Instruments Ltd., Cambridge, UK) a 510 y 700 nm. El contenido en antocianinas se calculó a partir de la ecuación 3,

$$TA = \frac{\left[\left(A_{510} - A_{700}\right)_{pH1.0} - \left(A_{510} - A_{700}\right)_{pH4.5}\right] \times PM \times DF \times 1000}{\varepsilon \times L} \qquad (3)$$

Donde:

PM: peso molecular de la pelargonidina-3-glucósido (433.0 g/mol)

DF: factor de dilución

ε: coeficiente de extinción (22.400 L/mol×cm)

L: longitud de la cubeta en cm (1 cm)

Capacidad Antioxidante

La actividad antioxidante se determinó por el método del secuestro del radical estable 2,2-difenil-1-picrilhidrazilo (DPPH) según el método descrito por Brand-Williams et al. (1995) y a través del radical estable 2,2'-azino-di-(3-etil-benzotiazolina-6-sulfonato) (ABTS) según el método propuesto por Re et al. (1999) con algunas modificaciones. Aproximadamente 5 g de muestra se centrifugaron a 10000 rpm durante 15 minutos a 4 ºC (Centrifuga AVANTI^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA) con 25 ml de metanol o hexano para obtener el extracto hidrofílico o lipofílico de la muestra, respectivamente (Lennuci et al., 2006). Una alícuota de 0,010 ml del sobrenadante lipofílico o hidrofílico obtenido se mezcló con 3,9 ml de una solución metanólica de DPPH o ABTS (20 mg/ml) y 0,09 ml de agua. Después de 30 minutos (tomate) y 60 minutos (fresa) en la oscuridad, se midió la absorbancia a 515 nm en el caso del DPPH y 734 nm del ABTS. El porcentaje de inhibición con respecto a la absorbancia inicial obtenida por la solución metanólica de DPPH o ABTS se expresó como micromoles de equivalente de Trolox por kg de muestra. Para ello, se llevó a cabo una ecuación de regresión relacionando la concentración de Trolox y el % de inhibición para cada de las soluciones metanólicas.

Modelos Matemáticos

Para establecer las condiciones óptimas de procesado por PEAIC para obtener zumos con alto potencial antioxidante se llevó a cabo un análisis de superficie de respuesta. Las variables numéricas independientes fueron frecuencia, anchura de pulso y polaridad. La función de segundo orden se predijo mediante la ecuación 4, donde Y es la variable independiente, β_0 es el punto central del sistema β_i, β_{i1} y β_{ij} los coeficientes de la regresión correspondientes a los efectos lineales, cuadráticos y de las interacciones, respectivamente; X_i, X_i^2 and X_iX_j los efectos lineales, cuadráticos y de las interacciones de las variables independientes.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_1 + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{i=i+1}^{3} \beta_{ij} X_i X_j \qquad (4)$$

Por otra parte, para describir la cinética de los cambios de las propiedades antioxidantes de los zumos de tomate y fresa el modelo de primer orden (ecuación 5) y la función de distribución de Weibull (ecuación 6) se ajustaron a los resultados,

$$RC = RC_0 \cdot exp(-k_1 \cdot t) \qquad (5)$$

$$RC = RC_0 \, exp\left[-\left(\frac{t}{\alpha}\right)^{\gamma}\right] \qquad (6)$$

donde *RC* (%) es el contenido relativo del compuesto bioactivo o la capacidad antioxidante relativa, RC_0 (%) valor relativo inicial de la propiedad antioxidante (100%), t es el tiempo de tratamiento (μs), k_1 es la constante de primer orden, α es el factor de escala (μs), γ es el parámetro de forma (adimensional).

A partir de la ecuación de Weibull se determino el tiempo de tratamiento (t_m) a partir del cual existe una destrucción completa de un determinado compuesto bioactivo (ecuación 7),

$$t_m = \alpha \cdot \Gamma\left(1 + \frac{1}{\gamma}\right) \qquad (7)$$

donde α es el factor de escala (μs), γ es el parámetro de forma (adimensional) y Γ es la función de gamma.

Además, se empleó un modelo de extracción sólido líquido propuesto por Peleg (ecuación 8) para describir la cinética de los cambios de la concentración de licopeno del zumo de tomate para cada una de la intensidad de campo aplicada.

$$LR = LR_0 + \frac{t}{K_1 + K_2 \cdot t} \qquad (8)$$

donde *LR* (%) es el contenido relativo de licopeno, LR_0 (%) valor relativo inicial de licopeno (100%), *t* es el tiempo de tratamiento (μs), K_1 (μs/%) es la constante cinética de Peleg y K_2 es la constante de capacidad de Peleg (μs^{-1}).

A partir de la constante de capacidad del modelo de Peleg (K_2) se pudo calcular la retención del licopeno en el zumo de tomate después de tiempo prolongados (RC_∞) a partir de la ecuación 9,

$$RC_\infty = 100 + \frac{1}{K_2} \qquad (9)$$

Una vez obtenidas las constantes para cada una de los modelos cinéticos, éstas se ajustaron a modelos de primer orden (ecuación 5) dependientes de la intensidad de campo eléctrico. El efecto combinado de la intensidad de campo eléctrico y del tiempo de tratamiento en las propiedades antioxidantes de los zumos se obtuvo sustituyendo cada una de las variables de los modelos cinéticos por su respectiva ecuación de primer orden.

Para el estudio del efecto del tiempo y la temperatura de almacenamiento sobre las propiedades antioxidantes de las fresas, el modelo de primer orden (ecuación 5) y la función de distribución de Weibull (ecuación 6) se ajustaron a los resultados; aunque cabe destacar que en estos estudios la variable *t* es el tiempo de almacenamiento (en días) en vez del tiempo de tratamiento (en μs). Además, se utilizó el modelo de primer orden (ecuación 10) para predecir los cambios del potencial antioxidante durante el tiempo de almacenamiento.

$$RC = RC_0 - k_0 \cdot t \qquad (10)$$

donde *RC* (%) es el contenido relativo del compuesto bioactivo o la capacidad antioxidante relativa, RC_0 (%) valor relativo inicial de la propiedad antioxidante (100%), *t* es el tiempo de almacenamiento (días) y k_0 es la constante de orden cero.

El efecto de las temperaturas de almacenamiento en las constantes cinéticas se predijo mediante la ecuación de Arrhenius (ecuación 11), donde A_o (dias^{-1}) es la constante de Arrhenius (factor de frecuencia), E_a es la energía de activación (J/mol) de la reacción química y R es la constante universal de los gases (8,32 J/mol·K),

$$k = A_o \, exp\left(\frac{-Ea}{R \cdot T}\right) \qquad (11)$$

Análisis Estadístico

Los análisis de varianza permitieron evaluar si existían diferencias significativas ($p \leq 0,05$) entre muestras y tratamientos aplicados. Mediante el test de LSD de separación de medias se estableció la dirección de las diferencias. En algunos casos se determinaron correlaciones entre las variables estudiadas a través de test de Person. En otros casos, se realizó un análisis de componentes principales (PCA) para establecer posibles relaciones entre variables.

A través del programa informático Design Expert 6.0.1 (Stat Ease Inc., Minneapolis, USA), se realizaron las superficies de respuesta y se obtuvieron las ecuaciones polinómicas de segundo orden.

Para determinar la adecuación de los modelos para describir la cinética de los cambios de las propiedades antioxidantes se evaluaron los coeficientes de regresión ajustados (R^2_{adj}), el factor de exactitud (A_f) (Ross, 1996), el error cuadrático medio (RMSE), X^2 (chi-cuadrado reducida) y MBE (error de sesgo) (Hayaloglu et al., 2007).

Bibliografía

B.O.E. (1988). Orden de 29 de enero de 1988 por la que se aprueban los Métodos oficiales de análisis de zumos de frutas y otros vegetales y sus derivados.

Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel–Wissenschaft und Technologie,* 28, 25-30.

Davis, A.R., Fish, W.W., Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biology and Technology* 28, 425-430.

Fish, W.W., Perkins-Veazie, P., Collins, J.K. (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of Food Composition and Analysis*, 15, 309-317.

Gómez-Plaza, E., Gil-Muñoz, R., Hernández-Jiménez, A., López-Roca, J.M., Ortega-Regules, A., Martínez-Cutillas, A. (2008). Studies on anthocyanins profile of Vitis Vinifera intraspecific hybrids (Monastrell × Cabernet Sauvignon). *European Food Research and Technology*, 227, 479-484.

Hayaloglu, A.A., Karabulut, I., Alpaslan, M., Kelbaliyev, G. (2007). Mathematical modeling of drying characteristics of strained yoghurt in a convective type tray-dryer. *Journal of Food Engineering*, 78, 109-117.

Hertog, M., Hollman, P., Venema, D. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, 40, 1591-1598

ISO, 4833. (1991). Methods for microbiological examination of food and animal feeding stuffs. Geneva.

ISO, 7954. (1988). General guidance for enumeration of yeast and moulds. Geneva.

Khachik, F., Goli, M.B., Beecher, G., Holden, J., Lusby, W.R., Tenorio, M.D., Barrera, M.R., 1992. Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *Journal of Agricultural and Food Chemistry*, 40, 390-398.

Lenucci, M.S., Codinu, D., Taurina, M., Piro, G., Dalessandro, G. (2006). Antioxidant composition in cherry and high-pigment tomato cultivar. *Journal of Agricultural and Food Chemistry*, 54, 2606-2613.

Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry*, 51, 6887-6892.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits. *Food Chemistry,* 105, 1151-1158.

Re, R., Pellegrin, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free Radical Biology and Medicine*, 26, 1231-1237.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, 81, 501-508.

Singleton, V.L., Orthofer, R.M., Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.

Soliva-Fortuny, R.C., Ricart-Coll, M., Martín-Belloso, O. (2005). Sensory quality and internal atmosphere of fresh-cut Golden Delicious apples. *International Journal of Food Science and Technology*, 40: 369-375.

Tonucci, L.H., Holden, J.M., Beecher, G.R., Khachik, F., Davis, C.S., Mulokozi, G. (1995). Carotenoid content of thermally processed tomato-based food products. *Journal of Agricultural and Food Chemistry*, 43, 579-586.

Trumbo, P.R., Yates, A.A., Schlicker-Renfro, S., Suitor C. (2003). Dietary Reference Intakes: revised nutritional equivalents for folate, vitamin E and provitamin A carotenoids. *Journal of Food Composition and Analysis*, 16, 379-382.

Publicaciones

Capítulo I

Lycopene, vitamin C and antioxidant capacity of tomato juice as affected by high-intensity pulsed electric fields critical parameters

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ABSTRACT

The effect of HIPEF treatment variables (frequency, pulse width and pulse polarity) on lycopene, vitamin C and antioxidant capacity of tomato juice were evaluated using a response surface methodology. An optimization of the HIPEF treatment conditions was carried out to obtain tomato juice with the highest content of bioactive compounds possible. Samples were subjected to electric field intensity set at 35kV/cm during 1000μs using squared wave pulses, frequencies from 50 to 250 Hz and pulse width from 1 to 7μs, in monopolar or bipolar mode. Data fitted significantly ($P < 0.001$) the proposed second-order response functions. Pulse frequency, width and polarity significantly affected lycopene, vitamin C and antioxidant capacity of HIPEF-treated tomato juice. Maximal relative lycopene content (131.8%), vitamin C (90.2%) and antioxidant capacity retention (89.4%) were attained with HIPEF treatments of 1-μs pulse duration applied at 250 Hz in bipolar mode. Therefore the application of HIPEF may be appropriate to achieve nutritious tomato juice.

Key words: high intensity pulsed electric field, tomato juice, bioactive compounds, vitamin C, carotenoids and antioxidant capacity

INTRODUCTION

Reactive oxygen species (ROS) could cause a number of human diseases, including coronary heart diseases and cancers. It has been suggested that a high intake of fruits and vegetables, the main source of antioxidants in the diet, could decrease the potential stress caused by ROS (Pellegrini et al., 2000). Tomato is a widely consumed vegetable, either fresh or industrially processed as juices, pastes, purees, sauces and soups (Shi and Le Maguer, 2000). In addition, tomato is rich in health-related compounds as it is a good source of carotenoids (in particular, lycopene) and ascorbic acid (Sahlin et al., 2004). However, processed fruits and vegetables have been considered to have lower nutritional value than their respective fresh commodities due to the loss of antioxidant compounds during processing (Murcia et al., 2000). Thermal processing is the most common method used to extend the shelf-life of tomato juices by inactivating microorganisms and enzymes that curb the product quality during storage (Vega-Mercado et al., 1997), but this treatment can induce undesirable changes in color, flavor and nutritional value (Qin, et al., 1996). The increased demand for fresh-like and highly nutritious products has raised the concern of the food industry for the development of milder preservation technologies that can replace traditional pasteurization methods. High intensity pulsed electric fields (HIPEF) is a non-thermal technology in which food industry is increasingly interested because it can lead to high inactivation levels of both spoilage and pathogenic microorganisms while maintaining the quality and freshness of juices (Mertens and Knorr, 1992). Up to now, most of the studies evaluating the effects of HIPEF processing conditions on juices have been focused on microbial and enzymes inactivation. Process parameters such as electric field strength, pulse width, frequency, pulse polarity and treatment time are important to optimize the inactivation of microorganisms (Wouters and Smelt, 1997; Ortega-Rivas et al., 1998; Elez-Martínez et al., 2004, 2005) and enzymes (Giner et al., 2000; Espachs-Barroso, et al., 2006; Elez-Martínez et al., 2006). In comparison to the extensive research devoted to the destruction of microorganisms and enzymes by HIPEF, there are very few works studying about the effect of HIPEF treatment parameters on the bioactive compounds of juices. Cortés et al. (2006) reported that electric field strength and treatment time had a significant effect on orange juice carotenoids. In this way, Torregrosa et al. (2005) observed that HIPEF processing caused a significant rise in the carotenoids content as treatment time increased in orange-carrot juice. Elez-Martínez and Martín-Belloso (2007) concluded that vitamin C retention in orange juice and gazpacho depended on processing factors such as pulse polarity, electric field strength, treatment time, pulse frequency and pulse width. However, little research has been carried out evaluating the effect of HIPEF critical parameters on bioactive compounds of tomato juice. In addition, at the stage of development of HIPEF technology, evaluating the influence of process variables on the antioxidant properties of tomato juice is a key factor in defining treatment conditions necessary to prevent undesirable changes in these properties.

On the other hand, the use of an adequate experimental design is particularly important in assessing the effect of HIPEF treatment on health-related compounds. Response surface methodology (RSM) consists of mathematical and statistical procedures to study the relationship between one or more responses (Diniz and Martin, 1996). The multivariate approach reduces the number of experiments, improves statistical interpretations, and indicates whether parameters interact (Myers and Montgomery, 2002).

The aim of this research was to study the effect of pulse width, frequency and polarity on the main bioactive compounds (lycopene and vitamin C) as well as on the antioxidant capacity of HIPEF-treated tomato. Moreover an optimization of HIPEF-processing critical parameters was carried out in order to obtain tomato juice with high retention of bioactive compounds and antioxidant capacity.

MATERIALS AND METHODS

Reagents

Metaphosphoric acid, DL-1,4 -dithiotreitol (DTT) were purchased from Acros Organics (NJ, USA); butylated hydroxytoluene (BHT), USP grade ethanol, hexane, acetone, ascorbic acid, sulphuric acid, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Scharlau Chemie, SA (Barcelona, Spain).

Tomato juices

Tomato fruits (*Licopersicon esculentum* Mill, cultivar Bodar) at commercial maturity were purchased from a local supermarket (Lleida, Spain). The fruits were chopped and then filtered through 2-mm of diameter steel sieves. Electric conductivity (Testo 240 conductivimeter ; Testo GmBh & Co, Lenzkirch, germany), pH (crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan) and color (Macbeth-Kollmorgen inst. Corp., Newburg, NY) of tomato juice were determined (Table 1).

Table 1.-Analytical characteristics of tomato juice

Parameters[a]		**Tomato juice**
pH		4.38 ± 0.01
Soluble solids (ºBrix)		4.52 ± 0.1
Color	**L***	22.41 ± 0.07
	a*	6.98 ± 0.12
	b*	5.26 ± 0.06
Electrical conductivity (S/m)		0.67 ± 0.02

[a]Results are the mean ± SD of three measurement

Pulsed electric fields Equipment

HIPEF treatments were carried out using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that holds positive monopolar and bipolar squared wave pulses. The treatment chamber device consists of eight co-linear chambers disposed in series, each one containing two stainless steel electrodes separated by a gap of 0.29cm with a treatment volume of 0.012cm^3.

The treatment flow rate was 60mL/min and was controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). Treated tomato juice was passed through a cooling coil connected between each pair of chambers and submerged in an ice-water shaking bath, so that samples temperatures never exceeded 40ºC. Tomato juice was treated using pulses of 35 kV/cm for 1000 μs at frequencies ranging from 50 to 250, and pulse width between 1 and 7 μs in monopolar or bipolar mode.

Bioactive Compounds

Lycopene

Total lycopene content was measured spectrophotometrically following the method proposed by Davis et al. (2003). This method determines the content of lycopene and other derivates such as hydroxy lycopene and lycopene epoxides. Approximately 0.6g tomato juice was weighed and added to 5ml of 0.05% (w/v) BHT in acetone, 5 ml of 95% USP (Unitated Stated Pharmacopeia) grade ethanol and 10 ml of hexane. The homogenate was centrifuged at 320xg for 15min at 4ºC. After shaking, 3 ml of distilled water were added. The vials were then agitated for 5 min and left at room temperature to allow phase separation. The absorbance of the upper, hexane layer, was measured in a 1-cm pathlength quartz cuvette at 503nm blanked with hexane. The lycopene content of each sample was estimated using the absorbance at 503nm and the sample weight (Fish et al., 2002). Lycopene was expressed as percentage of lycopene content compared to the untreated samples.

Vitamin C

The extraction procedure was based on a method validated by Odriozola-Serrano et al. (2007). A portion of 25g of tomato juice was added to 25ml of a solution containing 45g of metaphosphoric acid and 7.2g of DTT per liter. The mixture was centrifuged at 22100xg for 15min at 4 ºC. The supernatant was vacuum-filtered through Whatman No. 1 paper. The sample was then passed through a Millipore 0.45μm membrane and injected into the HPLC system.

A Waters 600E multisolvent delivery system was used for the analysis. Samples were introduced onto the column via a manual injector equipped with a sample loop (20μl). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb® ODS2 (5μm) stainless steel column (4.6mm x 250cm). The mobile phase was a 0.01% solution of sulphuric acid adjusted to pH=2.6. The flow rate was fixed at 1.0ml/min. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245nm. Identification of the ascorbic acid was carried out comparing the retention time and UV-visible absorption

spectrum with those of the standards. Results were expressed as vitamin C concentration compared to the untreated sample.

Antioxidant capacity

The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. This determination was based on the method proposed by de Ancos et al. (2002). An aliquot of 0.01ml of tomato juice was mixed with 3.9ml of methanolic DPPH solution (0.025g/l) and 0.090ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515nm against a blank of methanol without DPPH. Results were expressed as antioxidant capacity related to the untreated sample.

Experimental Design

A response surface analysis was used to evaluate the effect of the different variables of the HIPEF treatment on the bioactive compounds and antioxidant capacity of tomato juice. A face-centered, central composite design with 3 factors was the proposed experimental design (Myers and Montgomery, 2002). The independent variables were frequency, pulse width and polarity. The pulse repetition rate was set up from 50 to 250Hz, each pulse had a duration between 1 to 7-μs, in monopolar or bipolar mode. Samples were treated at a field strength of 35kV/cm for 1000 μs irrespective of the frequency, pulse width and pulse mode applied according to previous studies where pasteurization levels were achieved in orange juice processed by HIPEF (Elez-Martínez et al., 2004, 2005). The levels for each independent parameter were chosen considering sample and equipment limitations. The selected responses were lycopene, vitamin C and antioxidant capacity. The experimental design along with each experimental condition is shown in Table 2. A duplicate was performed, resulting in two blocks of experiments. The order of assays within each block was randomized and performed in triplicate. The effect of each HIPEF variables was modeled using a polynomial response surface. The second-order response function was predicted by Equation 1, where Y is the dependent variable, β_0 is the constant, β_i, β_{i1} and β_{ij} represent the coefficients of the linear, quadratic and interactive effect, respectively; X_i, X_i^2 and X_iX_j represent the linear, quadratic and interactive effect of the independent variables, respectively.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_1 + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{i=1}^{3} \beta_{ij} X_i X_j \qquad (1)$$

Three-dimensional surface plots and contour plots were drawn to illustrate the interactive effects of two factors on the dependent variable, while keeping constant the other variables. After carrying out an analysis of variance (ANOVA), the non significant terms were deleted from the second-order polynomial model. Then, a new polynomial was recalculated to obtain the coefficients for the initial equation. Design Expert 6.0.1 software (Stat Ease Inc.,

Minneapolis, Minn.) was used to generate models that fit the experimental data and to draw the response surface plots. The optimization was done according to the method proposed by Derringer and Suich (1980). All the individual desirability functions obtained for each response were combined into an overall expression, which is defined as the geometrical mean of the individual functions. The higher the desirability value, the more adequate is the system. In the present study, desirability functions were developed in order to obtain tomato juice with the highest levels of lycopene, vitamin C and antioxidant capacity.

Table 2.-Central composite response surface design for bioactive compounds and antioxidant capacity on tomato juice treated under different HIPEF treatment

		Variables					
f (Hz)	τ (μs)	Monopolar	Bipolar	Monopolar	Bipolar	Monopolar	Bipolar
		lycopene relative content (%)[2]		vitamin C retention (%)[2]		AC retention (%)[2]	
50	1	101.0 ± 0.2	103.2 ± 2.7	99.0 ± 0.1	98.3 ± 0.5	50.7 ± 1.1	66.5 ± 0.8
50	7	112.3 ± 2.6	114.2 ± 0.3	84.4 ± 1.1	82.1 ± 0.9	81.8 ± 2.5	86.1 ± 2.7
250	1	113.4 ± 1.1	129.1 ± 2.1	93.8 ± 0.9	86.1 ± 0.7	77.5 ± 0.2	90.4 ± 0.2
250	7	135.1 ± 3.0	146.2 ± 0.5	66.0 ± 0.9	60.6 ± 1.6	67.8 ± 0.2	75.9 ± 2.2
50	4	110.2 ± 0.1	109.1 ± 0.4	92.2 ± 0.9	88.4 ± 0.7	62.3 ± 1.1	74.6 ± 0.5
150	1	107.0 ± 1.5	118.7 ± 0.1	98.6 ± 0.3	89.2 ± 0.7	60.4 ± 0.9	80.1 ± 0.7
150	7	118.9 ± 1.1	128.1 ± 0.3	80.2 ± 0.7	58.2 ± 0.7	78.3 ± 0.7	81.3 ± 0.1
250	4	127.0 ± 0.4	142.7 ± 1.8	82.6 ± 0.4	78.8 ± 1.2	72.6 ± 0.3	81.0 ± 0.1
150	4	109.6[3] ± 0.5	119.4[3] ± 2.4	76.6[3] ± 1.2	74.8[3] ± 1.6	81.3[3] ± 1.1	92.3[3] ± 1.8

f = frequency; τ = pulse width; AC = antioxidant capacity
[1] Order the assays was randomized, and HIPEF treatment was set up at 35kV/cm for 1000 μs
[2]Data shown are the mean ± SD of 2 treatment repetitions, each assay was performed in triplicate;
[3]Data shown are the mean of 5 repetitions

RESULTS AND DISCUSSION

Results of the analysis of variance (F-test) for each dependent variable and their corresponding coefficients of determination (R^2) obtained by fitting the second-order response model to the experimental data are shown in Table 3.

Table 3.-Analysis of variance of the second-order models for bioactive compounds and antioxidant capacity

	F-value		
Source[1]	**Lycopene**	**Vitamin C**	**Antioxidant Capacity**
Quadratic model	36.32**	11.26**	13.15**
f	160.50**	16.41*	7.05*
τ	52.94**	50.04**	6.36*
p	47.55**	6.92*	29.44**
f^2	10.85*	8.10*	13.34*
τ^2	0.12	1.32	6.27*
$f \cdot \tau$	5.16*	2.11	26.93**
$f \cdot p$	12.17*	0.29	$7.61 \cdot 10^{-4}$
$\tau \cdot p$	0.45	0.41	4.32
Lack of fit	0.95	1.07	1.95
Std dev.	3.27	5.45	4.95
Mean	117.73	80.27	78.95
Coefficient of variation	2.78	6.79	6.27
R-squared	0.9447	0.8413	0.8609
Adj R-squared	0.9187	0.7666	0.7954

[1]f= frequency, τ = pulse width, p= pulse polarity
*Significant at $p < 0.05$
**Significant at $p < 0.001$

Effect of HIPEF-processing critical parameters on lycopene

Lycopene is an isoprenoid compound which provides red color to tomato and is used as an index of quality for tomato products (Abushita, et al., 2000). However, this compound is susceptible to oxidation in the presence of light, oxygen and low pH (Shi and Le Maguer, 2000). The effect of HIPEF processing on the concentration of lycopene in the tomato juice is shown in Table 2. Fresh tomato juice had a lycopene content of 7.5mg/100g. Higher lycopene concentrations were achieved in HIPEF-processed tomato juice than in the untreated. The increase ranged from 1.0% to 46.2% after applying different HIPEF treatments. The maximum lycopene relative content (146.2%) was observed when HIPEF treatment was carried out at 35kV/cm during 1000μs with bipolar pulses of 7-μs at 250Hz. Sánchez-Moreno et al. (2005) observed that the content of total carotenoids in "gazpacho", a cold vegetable soup where tomato is the major component, increased approximately a 62% after applying bipolar 4-μs pulses of 35 kV/cm for 750 μs at 800 Hz. Torregrosa et al. (2005) reported that HIPEF treatment set up at 25 or 30kV/cm caused a significant enhancement in the content of carotenoids in orange-carrot juice. In addition, Cortés et al. (2006) observed that carotenoids concentration rose slightly after applying an intensive treatment (35kV/cm) to orange juice. The lycopene in tomato juice treated by HIPEF might increase from other carotenoids in tomato juice such as phytoene, phytofluene, ζ-carotene and neurosporene throughout desaturation, isomerization and cyclization (Bramley et al., 1992). The formation of lycopene is dependent on temperature and takes place between 12 and 32ºC (Dumas et al., 2003). As a

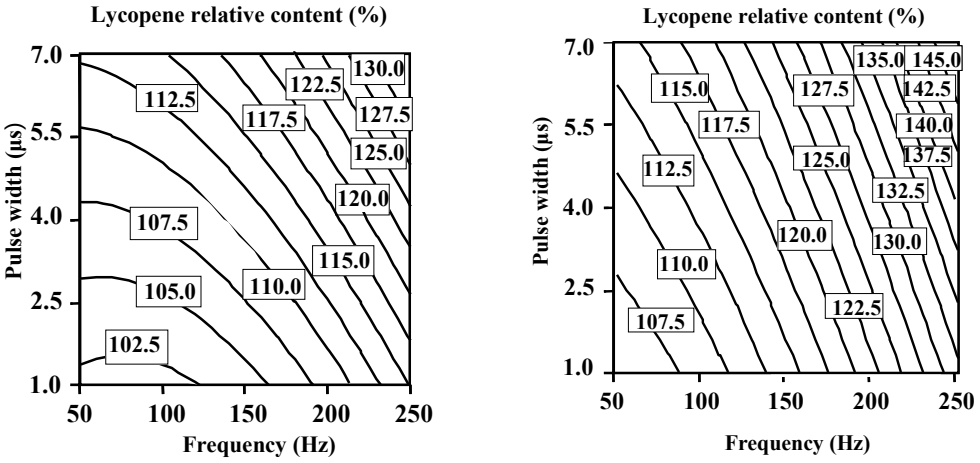
result, HIPEF treatment conducted at temperature lower than 40ºC might stimulate the conversion of some carotenoids into lycopene.

The analysis of variance revealed that a second-order model adequately fitted the experimental data ($P < 0.001$). The determination coefficient (R^2) was 0.94 and the lack-of-fit was not significant (Table 3). Frequency, pulse width and pulse polarity affected the lycopene content linearly, whereas only the quadratic term of frequency was significant (Table 3). The combined effects of frequency and pulse width, as well as frequency and pulse polarity, were included in the model as interaction terms. Pulse polarity was considered a categorical factor. As a result, lycopene relative content in tomato juice using monopolar pulses can be fitted through Equation 2 and in the case of bipolar pulses the relative content can be modeled by Equation 3, where *Lyc* is lycopene relative content, f is the frequency (Hz) and τ is pulse width (μs).

$$Lyc(\%) = +98.007 - 0.051 \cdot f + 1.472 \cdot \tau + 4.766 \cdot 10^{-4} f^2 + 6.845.10^{-3} f \cdot \tau \qquad (2)$$

$$Lyc(\%) = +108.533 - 0.051 \cdot f + 1.051 \cdot \tau + 4.766 \cdot 10^{-4} f^2 + 6.845.10^{-3} f \cdot \tau \qquad (3)$$

Pulse polarity resulted in a marked effect on lycopene content ($P < 0.001$) (Table 3). Bipolar treatments resulted in higher lycopene concentrations in tomato juices than monopolar treatments. A difference of 7.6% in lycopene was observed when using bipolar over monopolar pulses after applying a HIPEF treatment set up at 35kV/cm for 1000μs at 150Hz and 7μs pulse width (Figure 1 and 2). On the other hand, frequency had also a significant effect ($P < 0.001$) on the lycopene content of tomato juice. The linear coefficient of the frequency variable was negative, meaning that the lycopene content depleted with the increase of frequency (Equations 2 and 3). However, the linear negative effect of frequency may be masked by the positive effect of either the quadratic term of frequency or the interaction of frequency with pulse width (Equations 2 and 3). Pulse width also had an important effect on the lycopene content of tomato juice ($P < 0.001$) (Table 3). Thus, the higher the pulse width, the greater the lycopene retention achieved in tomato juice. The lycopene content was affected by the combined effect of frequency and pulse width (Equations 2 and 3). The positive value of the interaction coefficient suggests that increasing treatment frequency and pulse width resulted in higher lycopene relative content. The simultaneous increase in the two variables from 50Hz, 1-μs to 250Hz, 7-μs resulted in an increment of lycopene of 43%, whereas rising one variable and keeping the other constant lycopene content rose between 8.4% (50Hz, 7-μs) and 26.4% (250Hz, 1-μs) (Figure 1b). In addition, interaction allowed combinations of frequency and pulse width for monopolar (Figure 1a) and bipolar (Figure 1b) mode to achieve tomato juice with similar lycopene levels. As observed in Figure 1, tomato juice treated at 110 Hz using 7-μs monopolar pulses had the same lycopene relative content (115%) than that treated at 250Hz with 1-μs pulses, also applied in monopolar mode (Figure 1b).



(a) (b)

Figure 1.-Counter plots for the combined effect of frequency and pulse width on lycopene relative content of tomato juice treated at 35 kV/cm during 1000 µs in (a) monopolar or (b) bipolar mode.

Effect of HIPEF-processing critical parameters on vitamin C

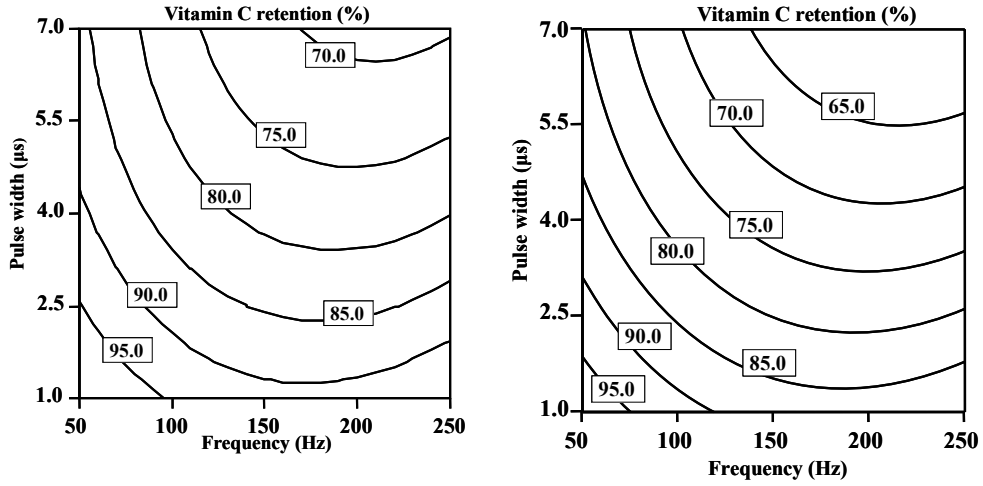
Vitamin C content of untreated tomato juice was 15.2mg/100g. The concentration of vitamin C expressed as vitamin C retention ranged between 58.2% and 99.0% in HIPEF-treated tomato juice (Table 2). Consistently, Torregrosa et al. (2006) reported vitamin C retentions between 87.5 and 97% in orange-carrot juice treated at different electric field strengths (25, 30, 35 and 40kV/cm) for different treatment times (from 30 to 340µs) using 2.5-µs bipolar pulses. Vitamin oxidation and loss during processing and cooking is of great concern for nutritionists, processors and consumers. Vitamin C is used as an index of the health-related quality of fruits, since, as compared to other beneficial compounds, it is more sensitive to degradation by processing (Davey et al., 2000). The analysis of variance showed that the second-order regression model adequately fitted vitamin C retention ($P < 0.001$) explaining 84.13% of the total variation without significant lack of fit (Table 3). Vitamin C content was affected by the linear terms of frequency and pulse width, as well as by pulse mode and the quadratic term of frequency. HIPEF treatments applied at the same frequency and pulse width maintained more vitamin C when performed in monopolar than in bipolar mode. Vitamin C retention in bipolar mode was a 7.3% lower than in monopolar mode for HIPEF treatment set up at 35 kV/cm for 1000 µs at 250 Hz and 4-µs pulse width (Figure 3 and 4). Vitamin C retention is represented by the following polynomial quadratic equations for monopolar (Equation 4) and bipolar (Equation 5) mode.

$$VitC(\%) = +121.099 - 0.292\,f - 3.710 \cdot \tau + 7.616 \cdot 10^{-4} f^2 \quad (4)$$

$$VitC(\%) = +115.478 - 0.292\,f - 3.710 \cdot \tau + 7.616 \cdot 10^{-4} f^2 \quad (5)$$

where *vitC* is vitamin C retention, *f* is the frequency (Hz) and τ is the pulse width (μs).

The lineal terms of frequency and pulse width are negative; thus the higher the frequency or pulse width, the lower the content of vitamin C is. Consistently, Elez-Martínez and Martín-Belloso (2007) reported that vitamin C retention in orange juice and gazpacho increased with a decrease in pulse frequency or pulse width. The high pulse width coefficient in the fitted model indicates that this parameter is more relevant for vitamin C retention than frequency. A difference of about 15% was observed between HIPEF treatments applying 1 and 7-μs monopolar pulses at 35kV/cm for 1000μs at 50Hz (Figure 2a). In contrast, higher variation (≈ 30%) was obtained between 1 and 7-μs bipolar pulse treatments applied at 250Hz (Figure 2b). Consequently, the differences in vitamin C retention of tomato juice treated using different pulse widths depended on both frequency and polarity mode.



(a) (b)

Figure 2.-Counter plots for the combined effect of frequency and pulse width on vitamin C retention of tomato juice treated at 35 kV/cm during 1000 μs in (a) monopolar or (b) bipolar mode.

On the other hand, the positive value of the quadratic term ($P < 0.05$) indicated that vitamin C retention reached a minimum as frequency rose. In fact, increasing frequency beyond 150Hz (monopolar mode) and 200 Hz (bipolar mode) provided decreasing vitamin C retentions than using lower frequencies (Figures 2a and 2b).

Effect of HIPEF-processing critical parameters on antioxidant capacity

Table 2 shows the antioxidant capacity of HIPEF-processed tomato juices treated under the studied experimental conditions. The maximum antioxidant capacity retention, 92.3%, was achieved with a treatment of 35kV/cm for 1000μs at 150Hz applying 4-μs bipolar pulses. In contrast, the combination of 50 Hz and 1-μs monopolar pulses width resulted in the least antioxidant capacity retention (50.7%).

The analysis of variance test revealed that a second-order model fits well the antioxidant capacity results (Table 3). The determination coefficient, R^2, was 0.86, and the lack of fit was not significant, indicating that the model is sufficiently accurate for predicting the response. Table 3 indicates that the linear terms of frequency, pulse width and pulse polarity affected antioxidant capacity. The quadratic terms of frequency and pulse width, as well as the interaction of frequency with pulse width, also exerted a significant influence on the antioxidant capacity of HIPEF-treated tomato juice. The antioxidant capacity was represented by polynomial quadratic equations in terms of the studied HIPEF parameters. Equation 6 fits the relative antioxidant capacity using monopolar pulses. The antioxidant capacity can be calculated using Equation 7 in the case of bipolar pulses.

$$AC(\%) = +20.398 + 0.390 \cdot f + 11.414 \cdot \tau - 7.688 \cdot 10^{-4} f^2 - 0.586 \tau^2 - 0.030 f \cdot \tau \quad (6)$$

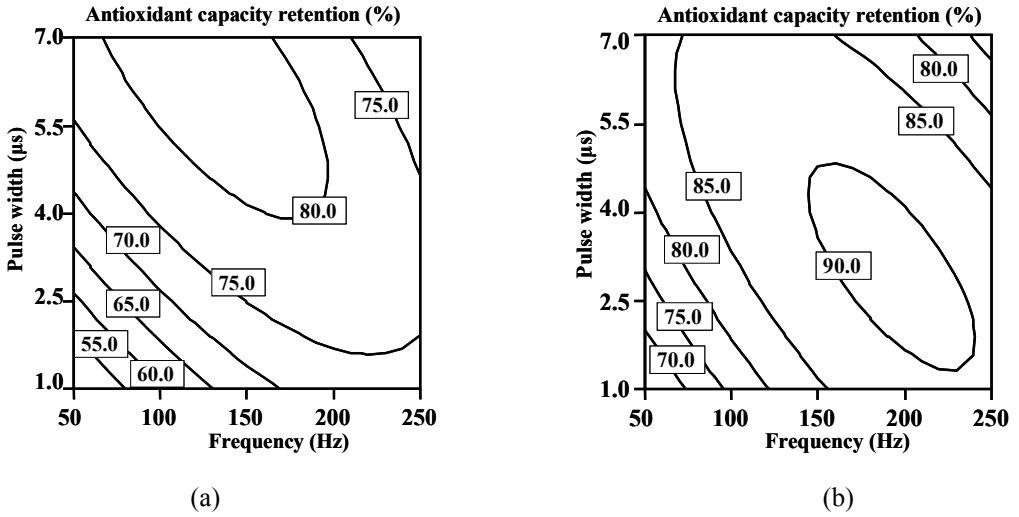
$$AC(\%) = +38.841 + 0.389 \cdot f + 9.436 \cdot \tau - 7.688 \cdot 10^{-4} f^2 - 0.586 \tau^2 - 0.030 f \cdot \tau \quad (7)$$

where AC (%) is the relative antioxidant capacity, f is frequency (Hz) and τ is pulse width (μs).

Pulse polarity was the most important variable ($P < 0.001$) affecting antioxidant capacity retention in HIPEF-treated tomato juice. As can be seen in Figures 3a and 3b, tomato juices treated with bipolar pulses had greater antioxidant capacity than those processed using monopolar treatment. On the other hand, frequency and pulse width coefficients are positive, meaning that an increase in frequency or pulse width results in a lower antioxidant capacity (Equations 6 and 7). However, the expected behavior of frequency and pulse width was modified by their negative quadratic terms. The negative coefficients of the quadratic terms of frequency and pulse width indicate a rise in antioxidant capacity when frequency and pulse width are slightly increased above 50 Hz and 1-μs, respectively, although the antioxidant capacity might decrease if frequency or pulse width increased further. In addition, an increment in either frequency or pulse width resulted in lower increments in antioxidant capacity (Figure 3a and 3b). Antioxidant capacity retention rose from 68.1 to 75.8% when frequency was

increased from 50 to 250Hz, applying 4-μs monopolar pulses at 35kV/cm during 1000μs. Nevertheless the maximal antioxidant capacity retention (80.2%) under these conditions was reached at approximately 175 Hz. When bipolar pulses were applied, the maximum was observed also at 175 Hz (Figure 3b).

The coefficient sign of the interaction frequency with pulse width is negative, thus indicating that these factors act in opposite directions. An increase of both parameters may lead to a decrease in antioxidant capacity. Moreover it is possible to exchange different combinations of the variables frequency and pulse width to achieve the same antioxidant capacity of tomato juice (Figure 3a and 3b). The maximum values of antioxidant capacity in monopolar mode were obtained by combining frequencies between 100 and 200Hz with pulse widths higher than 4μs, whereas frequencies higher than 150Hz and pulse widths lower than 5μs achieved the maximum values of antioxidant capacity in bipolar mode.



(a) (b)

Figure 3.- Counter plots for the combined effect of frequency and pulse width on antioxidant capacity retention of tomato juice treated at 35 kV/cm during 1000 μs in (a) monopolar or (b) bipolar mode.

Optimization of HIPEF-processing critical parameters

Optimal conditions for tomato juice HIPEF-processing were determined to obtain juices with high content of bioactive compounds as well as high antioxidant capacity. To this purpose, the same priority was assigned to each variable, seeking maximum levels of lycopene, vitamin C and antioxidant capacity. The desirability of each HIPEF treatment was shown in Figure 4. The maximal desirability was achieved at high frequencies and low pulse widths in bipolar mode. An overall score of 0.748 was obtained when the treatment was carried out at

35kV/cm during 1000μs using 1-μs squared wave pulses at 250Hz in bipolar mode. At these optimal conditions, predicted lycopene relative content, vitamin C and antioxidant capacity retention were 131.8, 90.2 and 89.4%, respectively. On the other hand, the second highest desirability (0.528) was obtained when processing at 35kV/cm for 1000 μs, using 2.75-μs monopolar pulses at 250 Hz, reaching a 120.3% predicted lycopene relative content, 85.7% vitamin C retention and 75.9% antioxidant capacity retention. The results of the optimization of HIPEF critical parameters were validated by repeating the experiment at the conditions predicted. Treating tomato juices at 250 Hz with 1-μs pulse width in bipolar mode, lycopene relative content, vitamin C and antioxidant capacity retention were 129.3%, 90.2% and 90.1%, respectively. Hence, no significant differences between the predicted and experimental results were observed.

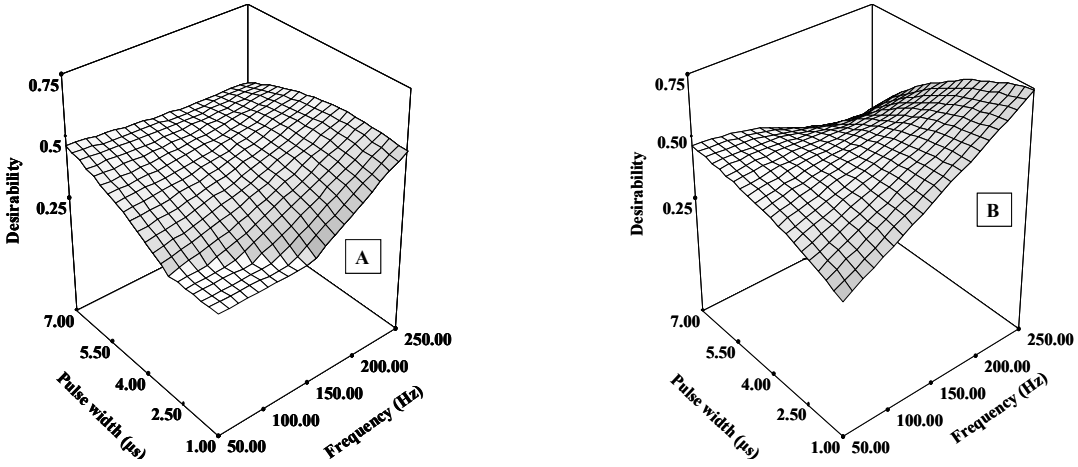


Figure 4.-Response surface of desirability of HIPEF-treated tomato juice as a function o maximal lycopene, vitamin C and antioxidant capacity retention for monopolar (A) and bipolar mode (B).

Conclusions

Lycopene retention, vitamin C and antioxidant capacity depend on the pulse frequency, width and polarity mode applied. Higher frequency and pulse width result in greater lycopene relative content, but lower vitamin C retention, although the effect of these variables is nonlinear. The use of bipolar pulses raises lycopene and decreases vitamin C in tomato juice more than monopolar treatments. The maximum values of antioxidant capacity are obtained by combining frequencies around 150Hz with pulse widths higher than 6μs in monopolar mode or HIPEF treatments with frequencies higher than 150Hz and pulses widths lower than 5μs applying bipolar pulses. In addition, different combination of frequencies and pulse widths lead

to equivalent lycopene relative content and antioxidant capacity retention. The evaluation of the effects of HIPEF critical parameters on bioactive compounds as well as on the antioxidant capacity of tomato juice is useful to achieve the optimal processing conditions to obtain tomato juice with high nutritional quality. However, further research is required to determine the effect of other critical HIPEF parameters such as electric field strengths and treatment time on the bioactive compounds of tomato juice.

ABBREVIATIONS USED

HIPEF, high intensity pulsed electric fields; *f,* frequency; τ, pulse width; *p*, pulse polarity.

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REFERENCES

Abushita, A.A., Daood, H.G., Biacs, P. (2000). Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *Journal of Agricultural and Food Chemistry*, 48, 2075-2081.

Bramley, P., Teuliers, C., Blain, I., Bird, C., Schuch, W. (1992). Biochemical characterization of transgenic tomato plants in which carotenoids synthesis has been inhibited through the expression of antisense RNA to p TOM5. *Plant Journal*, 2, 343-349.

Cortés, C., Esteve, M.J., Rodrigo, D., Torregrosa, F., Frígola, A. (2006). Changes of colour and carotenoids contents during high-intensity pulsed electric field treatment in orange juices. *Food Chemistry and Toxicology,* 44, 1932-1939.

Davey, M.W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science and Food Agriculture,* 80, 825-860.

Davis, A.R., Fish, W.W., Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biology and Technology*, 28, 425-430.

De Ancos, B., Sgroppo, S., Plaza, L.; Cano, M.P. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science and Food Agriculture*, 82, 790-796.

Derringer, G., Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technol*ogy, 12, 214-219.

Diniz, F.M., Martin, A.M. (1996). Use of response surface methodology to describe the combined effects of pH, temperature and E/S ratio on the hydrolysis of dogfish (*Squalus acanthias*) muscle. *International Journal of Food Science and Technology*, 31, 419-426.

Dumas, Y., Dadomo, M., Di Lucca, G., Grolier, P. (2003) Review: Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *Journal of the Science and Food Agriculture*, 83, 369-382

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R.C., Martín-Belloso, O. (2004). Inactivation of *Saccharomyces cerevisiae* suspended in orange juice using high-intensity pulsed electric fields. *Journal of Food Protection*, 67, 2596-2602.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R.C., Martín-Belloso, O. (2005). Inactivation of *Lactobacillus brevis* in orange juice by high-intensity pulsed electric fields. *Food Microbiology*, 22, 311-319.

Elez-Martinez, P., Aguiló-Aguayo, I., Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of the Science and Food Agriculture*, 86, 71-81.

Elez-Martínez, P., Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102, 201-209.

Espachs-Barroso, A., Van-Loey, A., Hendrickx, M., Martín-Belloso, O. (2006). Inactivation of plant pectin methylesterase by thermal or high intensity pulsed electric field treatments. *Innovative Food Science and Emerging Technology*, 7, 40-48.

Fish, W.W., Perkins-Veazie, P., Collins, J.K. (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of Food Composition and Analysis*, 15, 309-317.

Giner, J., Gimeno, V., Espachs, A., Elez, P., Barbosa-Cánovas, G.V., Martín, O. (2000). Inhibition of tomato (Licopersicon esculentum Mill.) pectin methylesterase by pulsed electric fields. *Innovative Food Science and Emerging Technology*, 1, 57-67.

Mertens, B., Knorr, D. (1992). Developments of nonthermal processes for food preservation. *Food Technology*, 46, 124-133.

Myers RH, Montgomery C. (2002). Response surface methodology: process and product optimization using design experiments. John Wiley & Sons, New York.

Murcia, M.A., López-Ayerra, B., Martinez-Tomé, M., Vera, A.M., García-Carmona, F. (2000). Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. *Journal of the Science and Food Agriculture*, 80, 1882-1886.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits. *Food Chemistry*, 105, 1151-1158.

Ortega-Rivas, E., Zarate-Rodríguez, E., Barbosa-Cánovas, G.V. (1998). Apples juice pasteurization using ultrafiltration and pulsed electric fields. *Food and Bioproducts and Processing,* 76, 193-198.

Pellegrini, N., Riso, P., Porrini, M. (2000). Tomato consumption does not affect the total antioxidant capacity of plasma. *Nutrition*, 16, 268-271.

Qin, B.L., Pothakamury, U.R., Barbosa-Cánovas, G.V., Swanson, B.G. (1996). Nonthermal pasteurization of liquid foods using high-intensity pulsed electric fields. *Critical Reviews in Food Science and Nutrition*, 36, 603-627.

Sahlin, E., Savage, G.P., Lister, C.E. (2004). Investigation of the antioxidant properties of tomatoes after processing. *Journal of Food Composition and Analysis*, 17, 635-647.

Sánchez-Moreno, P., Cano, M.P., De Ancos, B., Plaza, L., Olmedilla, B., Granado, F., Elez-Martínez, P., Martín-Belloso, O., Martín, A. (2005). Intake of Mediterranean soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *International Journal of Food Science and Nutrition*, 56,115-124.

Shi, J., Le Maguer, M. (2000). Lycopene in tomatoes: Chemical and physicochemical properties affected by food processing. *Critical Reviews in Food Science and Nutrition*, 40, 1-42.

Torregrosa, F., Cortés, C., Esteve, M.J., Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry*, 53, 9519-9525.

Torregrosa, F., Esteve, M.J., Frígola, A., Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*, 73, 339-345.

Vega-Mercado, H., Martín-Belloso, O., Qin, B.L., Chang, F.J., Góngora-Nieto, M.M., Barbosa-Cánovas, G.V., Swanson, B.G. (1997). Non-thermal food preservation: pulsed electric fields. *Trends in Food Science Technology*, 8, 151-157.

Wouters, P.C., Smelt, J.P.P.M. (1997). Inactivation of microorganisms with pulsed electric fields: potential for food preservation. *Food Biotechnology*, 11, 193-229.

Capítulo II

Modeling changes in health-related compounds of tomato juice treated by high-intensity pulsed electric fields

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ABSTRACT

Changes in some health-related compounds (lycopene and vitamin C) and antioxidant capacity of tomato juice treated by high intensity pulsed electric fields (HIPEF) were modeled as a function of the electric field strength and treatment time. Samples were subjected to electric field strengths from 20 to 35 kV/cm for up to 2000 μs using bipolar 1-μs pulses at 250 Hz. Weibull kinetic models predicted vitamin C and antioxidant capacity retention of HIPEF-treated tomato juice with good accuracy ($R^2_{adj} \geq 0.836$; A_f = 1.001-1.010). A model used to describe moisture sorption processes was the most accurate for describing lycopene changes through the HIPEF treatment time. The combined effect of treatment time and electric field strength on health-related compounds of tomato juice were successfully predicted ($R^2_{adj} >$ 0.948; A_f = 1.016-1.017) through secondary expressions. Information from this study would be useful in determining optimal HIPEF-conditions to produce tomato juices with a high retention of bioactive compounds.

Key words: modeling, high intensity pulsed electric fields, tomato juice, health-related compounds, antioxidant capacity

INTRODUCTION

Regular intake of tomatoes and tomato based products has been associated with lower incidence of various forms of cancer, in particular prostate cancer, and heart diseases (Arab and Steck, 2000). This beneficial effect is believed to be due, at least partially, to the action of antioxidant compounds, which reduce oxidative damage in the body. However, the nutritional value of tomato products depends on several factors such as processing and storage conditions (Willcox, et al., 2003). Consumers demand high quality nutritious foods with fresh flavor, texture and color as well as with minimal or no addition of chemical preservatives (Bull, et al., 2004). Consequently, emerging technologies for food processing and preservation, such as high-intensity pulsed electric fields (HIPEF), are being investigated (Deliza et al., 2003). HIPEF processing, as a non-thermal technology, has been shown to effectively inactivate microorganisms in tomato juice, thus leading to microbial inactivation levels similar to those achieved with heat pasteurization (Min et al., 2003). In addition, HIPEF treatments can achieve high rates of tomato juice peroxidase (POD), pectin methylesterase (PME) and polygalacturonase (PG) inactivation (Aguiló-Aguayo, et al., 2007, 2008). Several authors have studied the evolution of quality parameters in tomato juice after HIPEF treatments and promising results have been obtained regarding the maintenance of health-related compounds and color attributes compared to heat treatments (Min, et al., 2003; Odriozola-Serrano, et al., 2008). Process parameters such as electric field strength and treatment time are important variables to be controlled in order to optimize the inactivation of microorganisms (Elez-Martínez, et al., 2004, 2005) and enzymes (Giner, et al., 2000; Elez-Martínez, et al., 2006) by HIPEF. In addition, there are several works studying the effect of HIPEF treatment parameters on health-related compounds in juices. For instance, Cortés et al. (2006) reported that electric field strength and treatment time had a significant effect on HIPEF-treated orange juice carotenoids. In this way, Elez-Martínez and Martín-Belloso (2007) concluded that vitamin C retention and the antioxidant capacity in orange juice and "gazpacho" cold vegetable soup mostly depended on electric field strength and treatment time. On the other hand, several models have been used to describe the microbial destruction (Rodrigo et al., 2001; Elez-Martínez et al., 2004) and enzymatic inactivation (Giner, et al., 2005; Elez-Martínez, et al., 2006) as a function of the HIPEF critical parameters. Although retention of health-related compounds can be a limiting factor when defining process conditions, little information is available on modeling the content of antioxidant compounds as affected by HIPEF treatment parameters (Bendicho, et al., 2002; Torregrosa, et al., 2006). Therefore, the aim of this work was to propose mathematical models that properly relate changes in health-related compounds, namely lycopene and vitamin C, and antioxidant capacity of tomato juice to electric field strength and HIPEF treatment time.

MATERIALS AND METHODS

Tomato juices

Tomatoes *(Lycopersium esculentum* Mill. cultivar Bodar) at commercial maturity were bought from a local supermarket and kept at 4 ºC before being processed. The fruits were chopped, crushed and then filtered through a 2-mm diameter steel sieve. Electric conductivity (Testo 240 conductivimeter ; Testo GmBh & Co, Lenzkirch, germany), pH (crison 2001 pH-

meter; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan) and color (Macbeth-Kollmorgen inst. Corp., Newburg, NY) of tomato juice were determined. The physico-chemical characteristics of just filtered tomato juice were: soluble solids = 4.1 ± 0.2 ºBrix, pH = 4.32 ± 0.33, electric conductivity = 0.63 ± 0.21 S/m, and color L* = 22.21 ± 0.53, a* = 6.88 ± 0.33 and b* = 5.14 ± 0.23.

Pulsed electric fields equipment

HIPEF treatments were carried out in a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA). The treatment system consists of eight collinear chambers in series, each one with two stainless steel electrodes separated by a gap of 0.29 cm, thus defining a treatment volume of 0.012 cm^3. The flow rate of the process was adjusted to 60 mL/min and controlled with a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). Treated tomato juice was passed through a cooling coil was connected between each pair of chambers and submerged in an ice-water shaking bath. Thermocouples were attached to the surface of the stainless-steel coils, 2.5 cm away from the HIPEF zones along the flow direction. The thermocouples were connected to temperature readers and isolated from the atmosphere with an insulation tape. The temperatures of the inlet and outlet of each pair of chambers were recorded every 0.1 s during HIPEF treatment and the samples never exceeded 40 ºC. Samples of tomato juice were subjected to field strengths of 20, 25, 30 and 35 kV/cm during 100, 300, 600, 1000, 1500 and 2000 μs, using 1-μs square-wave bipolar pulses at 250 Hz. Treatment conditions were selected according to a previous study (Odriozola-Serrano et al., 2007a).

Bioactive Compounds

Lycopene

Lycopene concentration in tomato juice was measured spectrophotometrically (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) following the method proposed by Davis et al. (2003). About 0.6 g of tomato juice was weighted and added to 5 mL of 0.05% (w/v) butylated hydroxytoluene in acetone, 5 mL of 95% USP grade methanol, and 10ml of hexane. The homogenate was centrifuged at 320xg for 15 min at 4 ºC. Then, 3 mL of distilled water were added to each vial and the samples were shaken for 5 min at 4 ºC. Afterwards, the vials were left at room temperature for 5 min to allow separation. The absorbance of the upper, hexane layer was measured in a 1-cm pathlength quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample was estimated according to Equation 1:

$$lycopene(mg/kg) = \frac{A_{503} \times MW \times DF \times 1000}{\varepsilon \times L} \qquad (1)$$

where MW is the molecular weight of lycopene (536.9 g/mol), DF is the dilution factor, L is the pathlength in cm and ε is the molar extinction coefficient for lycopene (172000 L/mol·cm). Results were expressed as lycopene retention compared to the untreated sample.

Vitamin C

Vitamin C content of tomato juice was analyzed by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (2007b). A sample of 25 ml of tomato juice was mixed with 25 ml of a solution containing 45g/L metaphosphoric acid and 7.2g/L DL-1,4-dithiotreitol. The homogenate was centrifuged at 22100xg for 15 min at 4 ºC (Centrifuge Avanti^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vacuum-filtered through Whatman No. 1 paper. Then, the samples were filtered with a Millipore 0.45 μm membrane. An aliquot of 20 μL was injected into the HPLC system consisting of a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm x 250 cm) and a 486 Absorbance Detector (Waters, Milford, MA). A 0.01% solution of sulphuric acid adjusted to pH = 2.6 was used as eluent. The flow was isocratic at a rate of 1 mL/min at room temperature. Detection was performed at 245 nm. Identification of the ascorbic acid was carried out comparing the retention time and UV-visible absorption spectrum of the juice samples with those of the standards. Results were expressed as vitamin C retention related to the untreated sample.

Antioxidant capacity

The antioxidant capacity of tomato juice was studied through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the method described by Odriozola-Serrano et al. (2007a). Samples of tomato juice were centrifuged at 6000xg for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and aliquots of 0.01 mL of the supernatant were mixed with 3.9 mL of methanolic DPPH (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured with a spectrophotometer at 515 nm against a blank of methanol without DPPH. Results were expressed as antioxidant capacity retention related to the untreated sample.

Data Analysis

Each processing condition was assayed in duplicate and two replicate analyses were carried out in order to obtain the mean value. Several models such as first-order, first-order fractional conversion, Weibull distribution, Fermi and Hülsheger model have been used to describe the microbial destruction and enzymatic inactivation as a function of the HIPEF critical parameters. These different models were fitted to the experimental data and it was found that first-order model (Equation 2), Weibull distribution function (Equation 3) and a model proposed by Peleg (Equation 5), best relate the changes in antioxidant properties of tomato juices to HIPEF processing parameters.

First-order kinetics (Equation 2) are commonly used to fit the variation of health-related compounds in juices and nectars as a function of treatment time for heat processing (Vieira, et

al., 2000; Vikram, et al., 2005; Wang and Xu, 2007). Bendicho et al. (2002) proposed a first-order model to describe the vitamin C changes in milk as affected by HIPEF treatment time.

$$RC = RC_0 \cdot exp(-k_1 \cdot t) \qquad (2)$$

where RC (%) is the relative content of health-related compounds or relative antioxidant capacity, RC_0 (%) is the intercept of the curve, k_1 is the first-order kinetic constant (μs^{-1}) and t is the treatment time (μs).

Weibull distribution (Equation 3) has been used to describe destruction of microorganisms (Rodrigo et al., 2001) and enzyme inactivation (Rodrigo, et al., 2003; Giner et al., 2005; Soliva-Fortuny, et al., 2006) under HIPEF. The use of Weibull distribution function to describe the retention of health-related compounds and antioxidant capacity has not been reported yet.

$$RC = RC_0 \, exp\left[-\left(\frac{t}{\alpha}\right)^{\gamma}\right] \qquad (3)$$

where RC (%) is the relative content of health-related compounds or relative antioxidant capacity, RC_0 (%) is the intercept of the curve, t is the treatment time (μs), α is the scale factor (μs), and γ is the shape parameter that indicates concavity (tail-forming) or convexity (shoulder-forming) of the curve when it takes values below and above 1, respectively. Derived from the Weibull distribution function parameters (α, γ), t_m was defined as the mean processing time to achieve complete destruction/inactivation of the health-related compound or antioxidant capacity and can be used as a measurement of the resistance of these compounds to HIPEF treatments (Equation 4):

$$t_m = \alpha \cdot \Gamma\left(1 + \frac{1}{\gamma}\right) \qquad (4)$$

where α and β are the parameters of the Weibull distribution and Γ is the gamma function.

Because of the shape similarity between solid-liquid extraction curves in sorption processes and the changes in lycopene retention, the fit of a model proposed by Peleg (1988) (Equation 5) to lycopene content as affected by HIPEF treatment time was evaluated. This model has been used to describe sorption processes in various foods (Turhan, et al., 2002; Palou, et al., 1994) and has been shown to properly fit the solid-liquid extraction kinetics of total polyphenols from grape seeds (Bucić-Kojić, et al., 2007).

$$RC = RC_0 + \frac{t}{K_1 + K_2 \cdot t} \qquad (5)$$

where RC (%) is the relative lycopene content, RC_0 (%) is the intercept of the curve, t is the treatment time (μs), K_1 (μs/%) is the Peleg rate constant indicating the lycopene formation rate at initial treatment time ($t=t_0$), and K_2 is the Peleg capacity constant, which is related to the steady value reached for prolonged treatment times (Equation 6).

$$RC_\infty = 100 + \frac{1}{K_2} \qquad (6)$$

Additionally, kinetic rate constants obtained for each model were related, when possible, to the applied electric field strength through mathematical expressions. The combined effect of the electric field strength and treatment time on tomato juice antioxidant properties was described by rearranging these mathematical expressions into the best kinetic model for each compound.

The fit of the models to experimental data was evaluated by nonlinear regression procedures, using the Statgraphics Plus v.5.1 Windows package. Fitting accuracy of the models was evaluated through the analysis of the adjusted regression coefficients (R^2_{adj}), the accuracy factor (A_f) (Ross, 1996), the root mean square error (RMSE), the reduced Chi-square (χ^2) and mean bias error (MBE) (Hayaloglu, et al., 2007). The higher the R^2_{adj} value as well as the lower the RMSE, χ^2 and MBE, the better the model fits experimental data.

RESULTS AND DISCUSSION

Lycopene

The effect of HIPEF treatments on the lycopene content of tomato juice is shown in Figure 1. Fresh tomato juice had a lycopene content of 77 mg/L. Lycopene concentration was enhanced significantly after HIPEF processing from 3.8% to 37.7% compared to the untreated juice Our results agree with those of other studies reporting an enhancement in the carotenoids content of health-related compounds in HIPEF-processed juices (Torregrosa, et al., 2005; Cortés et al., 2006). In addition, Odriozola-Serrano et al. (2008) reported a rise of 4.67 % in tomato juice treated by heat at 90ºC for 60 s compared to the fresh juice. This rise in lycopene content might be attributed to the conversion of other carotenoids such as phytoene, phytofluene, ζ-carotene and neurosporene through desaturation, isomerization and cyclization into lycopene (Bramley, Teulieres, Blain, Bird, & Schuch, 1992). Further investigations are still needed to explain the mechanisms that mediate this HIPEF-induced conversion of carotenoids to lycopene.

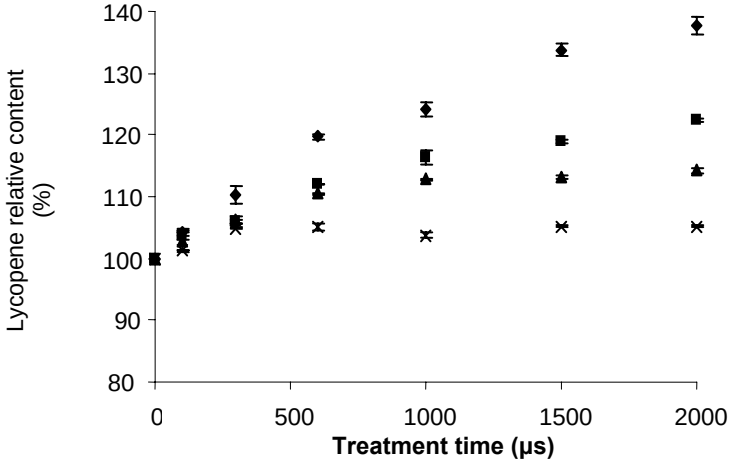

Figure 1.- Effect of treatment time and electric field strength on the lycopene retention of tomato juice (mean ± SD). Treatments were performed at 250 Hz and square bipolar pulses of 1-µs. Electric field strengths: (♦) 35 kV·cm^{-1}, (■) 30 kV·cm^{-1}, (▲) 25 kV·cm^{-1}, and (×) 20 kV·cm^{-1}.

Electric field strength and treatment time had a significant effect on tomato juice lycopene content (Figure 1). The kinetic constants estimated by the models and determination coefficients for treatments of different electric field strength are shown in Table 1. The fitting performance of both the Weibull function and Peleg model was good irrespective of the electric field strength (R^2_{adj} = 0.759-0.992). Although A_f-values for the Weibull equations and Peleg approach were quite similar (1.007-1.032), the RMSE, MBE and χ^2 were lower using the latter model. Therefore, from a quantitative point of view, data were best described by the Peleg model (Equation 5), followed by Weibull function (Equation 3). Weibull distribution shape parameter (γ) and scale factor (α) were influenced by the electric field strength (Table 1). The higher the electric field strength, the greater the shape factor and the nearer the scale factor to 0 was. The γ took values from 2.3×10^{-1} to 5.8×10^{-1}.Values of the γ below 1 could be regarded as evidence that lycopene formation became increasingly lower over time.

Table 1.- Kinetic constants of Weibull distribution function (Equation 3) and Peleg model (Equation 5) of lycopene content as a function of the pulsed electric field strengths.

E	Weibull distribution function[1]						
	$\alpha \times 10^{-4}$ (μs)	$\gamma \times 10$	R^2_{adj}	A_f	RMSE	MBE	χ^2
35	-1.3 ± 0.2	5.8 ± 0.3	0.986	1.012	0.810	0.363	1.347
30	-3.5 ± 0.7	5.5 ± 0.3	0.985	1.007	0.589	0.078	0.424
25	-24.4 ± 15.6	4.1 ± 0.5	0.947	1.009	0.651	-0.046	0.674
20	-6203 ± 29500	2.3 ± 0.8	0.759	1.032	1.197	1.434	6.298
E	Peleg model[1]						
	$K_1 \times 10^{-1}$ (μs × %$^{-1}$)	$K_2 \times 10^2$ (%$^{-1}$)	R^2_{adj}	A_f	RMSE	MBE	χ^2
35	2.38 ± 0.16	1.45 ± 0.12	0.992	1.001	0.588	0.013	0.510
30	3.46 ± 0.25	2.79 ± 0.19	0.990	1.005	0.515	-0.022	0.246
25	2.73 ± 0.27	5.55 ± 0.27	0.984	1.004	0.452	0.023	0.128
20	2.69 ± 1.02	18.1 ± 1.66	0.835	1.006	0.528	0.013	0.319

[1]Mean values ± standard error
E: Electric field strength (kV/cm)
R^2_{adj}: adjusted regression coefficients A_f: accuracy factor RMSE: root mean square error
MBE: mean bias error χ^2: reduced chi-square

As can be seen in Table 1, the Peleg rate constant (K_1) was not linearly correlated by the applied electric field strength, thus indicating similar rates of lycopene formation at initial treatment time ($t = t_0$) irrespective of the intensity of the HIPEF treatments. In contrast, Peleg capacity constant (K_2), which is related to the steadily value reached for prolonged treatment time, increased as electric field strength rose, following an exponential trend that could be described with good accuracy by a first-order model ($R^2_{adj} = 0.988$, $A_f = 1.296$, RMSE = 0.147, MBE = 0.002, $\chi^2 = 6.8 \times 10^{-5}$) (Equation **7**).

$$K_2 = \left(1.2 \cdot 10^{1}\right) \cdot exp\left[-\left(2.1 \cdot 10^{-1}\right)\right] \qquad (7)$$

Lycopene content for prolonged treatment times (RC_∞), which was calculated from K_2 (Equation 6), took values from 105 to 169%. The greater the electric field strength, the higher the relative lycopene content after prolonged HIPEF treatment time. Consistently, Cortés et al. (2006) observed that the carotenoids content in HIPEF-treated orange juice increased significantly when the most intensive treatments (35 or 40 kV/cm) were conducted. Carotenoids concentration rose as treatment time increased when HIPEF treatments at 25 or 30kV/cm were applied to orange-carrot juice (Torregrosa et al., 2005).

The combined effect of treatment time and electric field strength on tomato juice lycopene content is described by Equation 8, where the K_2 in the Peleg model (Equation 5) is replaced by Equation 7., and K_1 is substituted by the mean value, calculated from those obtained for each

electric field. The new model fitted well the experimental data and exhibited good accuracy (R^2_{adj} = 0.976, A_f = 1.017, RMSE = 1.004, MBE = 0.561 and χ^2 = 4.493).

$$RC = 100 + \frac{t}{\left(2.82\times10^{1}\right)+\left(\left[1.2\cdot10^{1}\right]\cdot exp\left(-\left[2.1\cdot10^{-1}\right]\cdot E\right)\cdot t\right)} \qquad (8)$$

Lycopene relative content estimated by the model (Equation 8) is compared with the experimental data in Fig. 2. The small deviation from the bisector in this plot is related to the good fitting accuracy of the model.

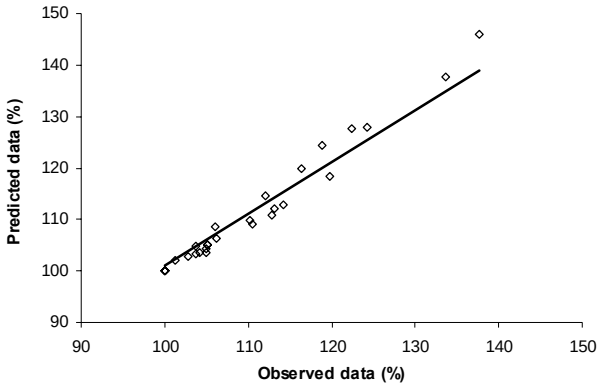

Figure 2.-Plot of the observed values of lycopene relative content after HIPEF treatments versus the predicted values by a secondary model (Equation 8).

Vitamin C

Vitamin C content of untreated tomato juice was 130 mg/L. The effect of HIPEF processing parameters on the concentration of vitamin C in tomato juice is shown in Figure 3. The results are in agreement with those obtained by Torregrosa et al. (2006), who reported vitamin C retentions between 87.5 and 97% in orange-carrot juice treated at different electric field strengths (25, 30, 35 and 40 kV/cm) for different treatment times (from 30 to 340 μs) using 2.5-μs bipolar pulses. On the other hand, lower vitamin C retention just after treatment were obtained in heat-treated tomato juice at 90 ºC for 30 or 60 s (79.2%-80.4%) compared to the HIPEF-treated juices (Odriozola-Serrano et al., 2008). Vitamin C content significantly depended on the HIPEF treatment time and electric field strength applied during HIPEF-processing of the juice, so that the lower the treatment time and electric field strength, the greater the vitamin C retention (Figure 3). Elez-Martínez and Martín-Belloso (2007) studied the variation of vitamin C content after applying different HIPEF treatments to "gazpacho", a

cold vegetable soup where tomato is the major component, and obtained similar results to those found in the present study. Vitamin C is an unstable compound and under undesirable conditions it decomposes easily (Lee and Coates, 1999). Degradation of ascorbic acid depends upon many factors such as oxygen, light, processing temperature and time. In general, the milder the treatment, the better the vitamin C retention (Davey, et al., 2000). Therefore, the high vitamin C destruction for prolonged intense HIPEF treatments may be explained through the instability of vitamin C to temperature and time.

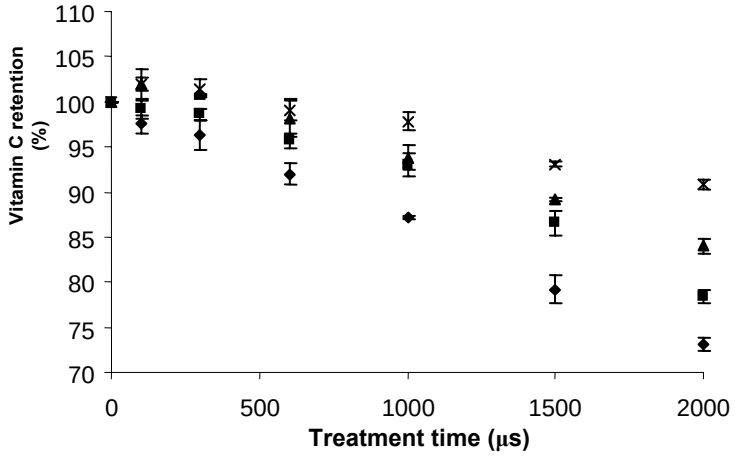

Figure 3.- Effect of treatment time and electric field strength on the vitamin C retention of tomato juice (mean ± SD). Treatments were performed at 250 Hz and square bipolar pulses of 1-μs. Electric field strengths: (♦) 35 kV·cm^{-1}, (■) 30 kV·cm^{-1}, (▲) 25 kV·cm^{-1}, and (×) 20 kV·cm^{-1}.

The estimated parameters and the R^2_{adj}, A_f, RMSE, MBE and χ^2 of the models used to describe vitamin C variation at different electric field strengths are displayed in Table 2. Based on these coefficients, it can be concluded that both models fit well the data. However, it appears that the Weibull model is most suitable because have the highest R^2_{adj}, the lowest RMSE, MBE and χ^2 and the A_f values are the closest to 1 (Table 2). First-order rate constants were statistically influenced by the electric field strength and took values in the range of 4.1×10^{-5} to 1.52×10^{-4} μs^{-1}. The degradation of ascorbic acid studied by other authors was also fitted to first-order kinetics (Ariahu, et al., 1997; Esteve, et al., 1998). Bendicho et al. (2002) used a first-order model to describe the vitamin C depletion in HIPEF-treated milk. They obtained rate constants (k_1) ranging from 1.8×10^{-4} to 1.27×10^{-3} μs^{-1} using HIPEF treatments of up to 400 μs and electric field strengths from 18.3 to 27.1 kV/cm. Differences in the loss of vitamin C due to HIPEF treatments can be attributed not only to factors intrinsic to the food product, which can greatly condition the sensitivity of vitamin C to the applied treatments, but

also to other factors such as HIPEF system, treatment chambers, pulse characteristics and electrical conditions.

Table 2.- Kinetic constants of first-order (Equation 1) and Weibull distribution function (Equation 3) of vitamin C retention as a function of the pulsed electric field strengths.

E	First-order model[1]					
	$k_1.10^5$ (μs^{-1})	R^2_{adj}	A_f	RMSE	MBE	χ^2
35	15.2 ± 0.4	0.987	1.021	0.608	-0.239	0.462
30	10.2 ± 0.6	0.932	1.018	0.845	-0.266	1.760
25	7.5 ± 0.6	0.881	1.020	0.905	-0.501	2.165
20	4.1 ± 0.6	0.747	1.018	1.225	-0.435	8.007
E	**Weibull distribution function[1]**							
	$\alpha \times 10^{-4}$ (μs)	**$\gamma \times 10$**	**t_m**	**R^2_{adj}**	**A_f**	**RMSE**	**MBE**	**χ^2**
35	0.57 ± 0.04	11.0 ± 0.6	11252	0.987	1.007	0.882	-0.659	1.854
30	0.48 ± 0.03	16.0 ± 0.3	7940	0.985	1.006	0.482	-0.079	0.167
25	0.55 ± 0.08	16.9 ± 0.4	8793	0.952	1.009	0.630	-0.227	0.624
20	0.66 ± 0.23	19.1 ± 1.5	10028	0.836	1.010	0.808	-0.256	2.340

[1]Mean values ± standard error

E: Electric field strength (kV/cm)

R^2_{adj}: adjusted regression coefficients A_f: accuracy factor RMSE: root mean square error MBE: mean bias error χ^2: reduced chi-square

The scale parameter (α) and the shape parameter (γ) of the Weibull model were obtained by fitting Equation 3 to the experimental data. The γ parameter, which ranged from 1.1 to 1.9, was inversely dependent on the electric field strength, suggesting that the higher the electric field strength the greater the effect of HIPEF treatment time. Values of γ parameter above 1 indicate that vitamin C became increasingly destroyed overtime. In contrast, α was independent from the electric field strength and took values from 5741 to 6587 μs. The values of mean time (t_m), which can be defined as the mean processing time to achieve complete destruction the vitamin C of tomato juice, were calculated from Equation 4, and varied from 7939 to 11252 μs. Because no references have been found reporting the use of Weibull equation to describe health-related compounds retention in HIPEF-treated products, the Weibull parameters were compared with those reported by Sampedro et al. (2006) for microorganisms and by Elez-Martinez et al. (2006) for enzymes. Considering the t_m parameter defined by the Weibull model, tomato juice vitamin C was more resistant to HIPEF treatments than *Lactobacillus plantarum* in orange juice-milk (15-40 kV/cm, up to 180 μs) but less than orange juice peroxidase treated at similar conditions to those used in the present work.

Although both tried models displayed good fit (R^2_{adj} = 0.747-0.987 and A_f = 1.006-1.021), the Weibull function was slightly more accurate in describing vitamin C changes as affected by HIPEF treatment time. First-order kinetics was adjusted to the γ estimated by the Weibull model for different electric field strength conditions. The model adequately fitted the shape

constants, explaining 80.24% of the variability with good accuracy (A_f = 1.016, RMSE = 0.152, MBE = 0.001 and χ^2 = 0.006) (Equation 9).

$$\gamma = (3.6) \cdot exp\left[-(3.1 \cdot 10^{-2}) \cdot E\right] \quad (9)$$

Moreover, substitution of γ by Equation 10 into the Weibull distribution (Equation 3) and replacing α by a mean constant value, calculated from those obtained for each electric field transforms the equation into a function dependent on both the electric field strength (E) and treatment time (t) (Equation 10). The model performed well under the whole range of applied conditions, with high determination coefficients (R^2_{adj} = 0.941) and good accuracy (A_f = 1.016, RMSE = 0.781, MBE = 0.042 and χ^2 = 1.202).

$$RC = RC_0 \, exp\left[-\left(\frac{t}{5.65 \cdot 10^3}\right)^{(3.6)exp\left[-(3.1 \cdot 10^{-2}) \cdot E\right]}\right] \quad (10)$$

The plot of the observed retention values versus the predicted data by Equation 10 is shown in Figure 4. Differences between the points of the graph and the line of equivalence are small and no local overestimation or underestimation of vitamin C retention is illustrated.

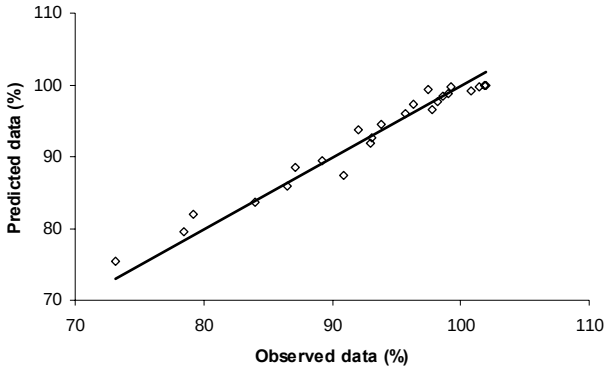

Figure 4.- Plot of the observed values of vitamin C retention after HIPEF treatments versus the predicted values by a secondary model (Equation 10)

Antioxidant Capacity

Figure 5 shows the antioxidant capacity of tomato juices treated under the studied experimental conditions. Antioxidant capacity significantly depended on electric field strength and treatment time (Figure 5). HIPEF-treated tomato juice processed at 20 kV/cm had the highest antioxidant capacity, followed by that treated at 35 kV/cm. There was no statistical difference in the antioxidant capacity between tomato juices processed at 25 and 30 kV/cm. Changes in the antioxidant capacity of HIPEF-treated tomato juices are consistent with the variation in health-related compounds throughout processing. Vitamin C acts as a scavenging agent of reactive oxygen species before they participate in oxidative reactions (Carr and Frei, 1999). In this way, vitamin C reduction as a result of HIPEF treatments was reflected in a depletion of antioxidant capacity in tomato juice (Figure 3 and 5).

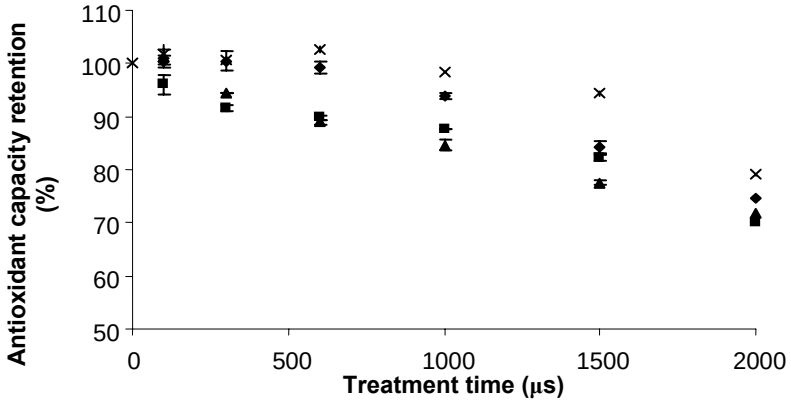

Figure 5.- Effect of treatment time and electric field strength on the antioxidant capacity of tomato juice (mean (SD). Treatments were performed at 250 Hz and square bipolar pulses of 1-μs. Electric field strengths: (♦) 35 kV·cm^{-1}, (■) 30 kV·cm^{-1}, (▲) 25 kV·cm^{-1}, and (×) 20 kV·cm^{-1}.

On the other hand, it has been reported that also lycopene accounts for the changes in antioxidant capacity of tomato products (Gahler et al., 2003). In this study, the extent of the changes in antioxidant capacity could be also associated with the variation in lycopene relative content. As can be seen in Figure 1 and Figure 5., tomato juice having the greatest lycopene concentration, had the second highest antioxidant capacity. Therefore, these results suggest that changes in antioxidant capacity of the juices might be due to vitamin C concentrations, rather than lycopene content.

In order to adequately relate changes in antioxidant capacity to treatment time, first-order (Equation 2) and Weibull functions (Equation 3) were fitted to the experimental data. Estimated first-order rates, Weibull parameters and regression coefficients for both models, obtained for different electric field strength, are shown in Table 3. The fitting performance of the first-order kinetic model was high for treatments with an electric field strength between 25 and 35 kV/cm but dramatically decreased for 20 kV/cm treatments (R^2_{adj} = 0.592-0.984). In contrast, adequacy of Weibull distribution seemed to be consistently good regardless of the applied electric field strength (R^2_{adj} = 0.912-0.984) (Table 3). As can be seen in Table 3, α and γ varied upon electric field strength. However, changes in Weibull parameters could not be well fitted by any model, provided that no clear influence of the electric field strength on α and γ were observed.

Table 3.- Kinetic constants of first-order (Equation 1) and Weibull distribution function (Equation 2) of antioxidant capacity retention as a function of the pulsed electric field strengths.

E	First-order model[1]					
	$k_1.10^5$ (μs^{-1})	R^2_{adj}	A_f	RMSE	MBE	χ^2
35	11.3 ± 1.2	0.836	1.039	1.227	-0.718	7.500
30	15.8 ± 0.8	0.916	1.031	0.446	0.326	3.560
25	16.9 ± 0.4	0.984	1.010	0.185	-0.088	0.792
20	6.7 ± 1.6	0.592	1.053	0.775	-0.850	12.661
E	Weibull distribution function[1]							
	α ×10^{-4} (μs)	γ × 10	t_m	R^2_{adj}	A_f	RMSE	MBE	χ^2
35	0.34 ± 0.02	22.1 ± 1.6	5004	0.984	1.008	0.561	-0.157	0.355
30	0.7 ± 0.2	8.9 ± 1.3	15971	0.912	1.003	0.501	0.185	3.541
25	0.62 ± 0.06	9.7 ± 0.6	12512	0.983	1.009	0.135	-0.121	0.842
20	0.27 ± 0.01	47.9 ± 6.2	3271	0.967	1.001	0.034	-0.311	0.913

[1]Mean values ± standard error

E: Electric field strength (kV/cm)

R^2_{adj}: adjusted regression coefficients A_f: accuracy factor RMSE: root mean square error MBE: mean bias error χ^2: reduced chi-square

Values of mean time to achieve complete loss of antioxidant capacity (t_m) were calculated according to Equation 4. The results ranged from 3271 to 15970 μs. Elez-Martínez et al. (2006) used the Weibull model to describe the inactivation of peroxidase by HIPEF treatments of up to to 35 kV/cm for 1500 μs and they reported t_m ranging from 100 to 43000 μs. Rodrigo et al. (2001) reported t_m values of 0.71-72 μs for *Lactobacillus plantarum* and *Byssochlamys fulva* in orange-carrot juice treated at electric field strength within 15-40 kV/cm. Therefore, in a first approach, according to Weibull model, tomato juice antioxidant capacity was shown to be less sensitive to HIPEF treatments than microorganisms, but more than enzymes.

On the other hand, Aguiló-Aguayo et al. (2007) reported that treatments carried out at 35 kV/cm for 1500 μs, supplying an energy density of 8269 kJ/L, achieved 5 log reductions for *Lactobacillus brevis*, whereas an inhibition of 97% peroxidase, 82% pectin methylesterase and 50% polygalacturonase activity in tomato juice compared to the untreated juice was observed. In addition, Mosqueda-Melgar (2007) observed that the naturally-occurring microbial population in tomato juice was maintained below 1 $\log_{10}$ for at least 28 days of storage at 5 ºC when applying a HIPEF treatment of 35 kV/cm for 1000 μs. Microbial counts of 10^7 CFU/mL were reached after 50 days of storage. Therefore, the most intensive conditions applied in this study led to safe and stable tomato juices, with minimal changes in their health-promoting properties. HIPEF-treated (35 kV/cm for 1500 μs with 1-μs bipolar pulses at 250 Hz, energy input of 8269 kJ/L) tomato juice exhibited higher lycopene content (133%) and slightly lower vitamin C concentration (87%) compared to the fresh juice.

CONCLUSIONS

The evaluation of the effects of HIPEF critical parameters on the antioxidant potential of tomato juice contributes to determine the optimal processing conditions in order to obtain tomato juices with high nutritional quality. Retention of health-related compounds and antioxidant capacity depend on the HIPEF processing parameters. Generally, the greater the electric field strength and treatment time, the lower the vitamin C and the higher lycopene contents. The model based on Weibull distribution function is likely to be a useful tool for describing vitamin C and antioxidant capacity changes in tomato juice treated by HIPEF treatments. The Peleg model most accurately described kinetics of lycopene in HIPEF treated tomato juice. The proposed mathematical models may help to predict the variation of the antioxidant potential of tomato juice as affected by key parameters involved in HIPEF treatments.

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REFERENCES

Aguiló-Aguayo, I., Soliva-Fortuny, R., Martín-Belloso, O. (2007). Effects of pulsed electric field or heat treatments on pectin methylesterase and polygalacturonase activities and viscosity during storage of tomato juice. IFT Annual Meeting, Chicago, IL. Institute of Food Technologists.

Aguiló-Aguayo, I., Odriozola-Serrano, I, Quintão-Teixeira, L.J., Martín-Belloso, O. (2008). Inactivation of tomato juice peroxidase by high-intensity pulsed electric field as affected by process conditions. *Food Chemistry*, 107, 949-955.

Arab, L., Steck, S. (2000). Lycopene and cardiovascular disease. *American Journal of Clinical Nutrition,* 71(6), 1691S-1695S.

Ariahu, C.C., Adekunle, D.E., Nkpa, N. (1997). Kinetics of heat/enzymatic degradation of ascorbic acid in fluted pumpkin (*Telfairia occidentalis*) leaves. *Journal of Food Processing and Preservation*, 21, 21-32.

Bendicho, S., Espachs, E., Arántegui, J., Martín, O. (2002). Effect of high intensity pulsed electric fields and heat treatments on vitamins of milk. *Journal of Dairy Research*, 69, 116-123.

Bramley, P., Teulieres, C., Blain, I., Bird, C., Schuch, W. (1992). Biochemical characterization of transgenic tomato plants in which carotenoids synthesis has been inhibited through the expression of antisense RNA to p TOM5. *Plant Journal*, 2, 343-349.

Bucić-Kojić, A., Planinić, M., Tomas, S., Bilić, M., Velić, D. (2007). Study of solid-liquid extraction kinetics of total polyphenols from grape seeds. *Journal of Food Engineering*, 81, 236-242.

Bull, M.K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E.A., Johnson, R.L., & Steward, C.M. (2004). The effect of high-pressure processing on the microbial, physical, and chemical properties of Valencia and Navel orange juice. *Innovative Food Science and Emerging Technologies*, 5, 135-149.

Carr A., Frei B. (1999). Does vitamin C act as a pro-oxidant under physiological conditions? . *Faseb Journal*, 13, 1007-1024.

Cortés, C., Esteve, M.J., Rodrigo, F., Torregrosa, F., Frígola, A. (2006). Changes of colour and carotenoids contents during high-intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology*, 44(11), 1932-1939.

Davey, M.W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science of Food and Agriculture,* 80, 825-860.

Davis, A.R., Fish, W.W., Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biology and Technology,* 28(3), 425-430.

Deliza, R., Rosenthal, A., Silva, A.L.S. (2003). Consumer attitude towards information on non conventional technology. *Trends in Food Science and Technology*, 14(1-2), 43-49.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R.C., Martín-Belloso, O. (2004). Inactivation of *Saccharomyces cerevisiae* suspended in orange juice using high-intensity pulsed electric fields. *Journal of Food Protection*, 67, 2596-2602.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R.C., Martín-Belloso, O. (2005). Inactivation of *Lactobacillus brevis* in orange juice by high-intensity pulsed electric fields. *Food Microbiology*, 22, 311-319.

Elez-Martínez, P., Aguiló-Aguayo, I., Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of Agricultural and Food Chemistry***,** 86, 71-81.

Elez-Martínez, P., Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102(1), 201-209.

Esteve, M.J., Frígola, A., Martorell, L., Rodrigo, C. (1998) Kinetics of ascorbic acid degradation in green asparagus during heat processing. *Journal of Food Protection*, 61, 1518-1521.

Gahler S., Otto K., Böhm V. (2003). Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products *Journal of Agricultural and Food Chemistry*, 51, 7962-7968.

Giner, J., Gimeno, V., Espachs, A., Elez, P., Barbosa-Cánovas, G.V., Martín, O. (2000). Inhibition of tomato (Licopersicon esculentum Mill.) pectin methylesterase by pulsed electric fields. *Innovative Food Science and Emerging Technologies***,** 1, 57-67.

Giner, J., Grouberman, P., Gimeno, V., Martín, O. (2005). Reduction of pectinesterase activity in a commercial enzyme preparation by pulsed electric fields: Comparison of inactivation kinetic models. *Journal of the Science of Food Agriculture,* 85, 1613-1621.

Hayaloglu, A.A., Karabulut, I., Alpaslan, M., Kelbaliyev, G. (2007). Mathematical modeling of drying characteristics of strained yoghurt in a convective type tray-dryer. *Journal of Food Engineering*, 78, 109-117.

Lee H.S., Coates G.A. (1999). Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: a storage study. *Food Chemistry*, 65, 165-168.

Min, S., Jin, Z.T., & Zhang, Q.H. (2003). Commercial scale pulsed electric field processing of tomato juice. *Journal of Agricultural and Food Chemistry*, 51(11), 3338-3344.

Mosqueda-Melgar, J. (2007). Aplicación de pulsos eléctricos de alta intensidad de campo en combinación con sustancias antimicrobinas para garantizar la calidad e inocuidad microbiológica de zumos de frutas. Doctoral thesis. University of Lleida, Spain.

Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., Gimeno-Añó, V., Martín-Belloso, O. (2007a). Lycopene, vitamin C, and antioxidant capacity of tomato juice as affected by high-intensity pulsed electric fields critical parameters. *Journal of Agricultural and Food Chemistry*, 55, 9036-9042.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007b). Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits. *Food Chemistry*, 105, 1151-1158.

Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. *Innovative Food Science and Emerging Technologies,* 9, 272-279.

Palou, E., Lopezmalo, A., Argaiz, A., Welti, J. (1994). The use of Pelegs equation to model osmotic concentration of papaya. *Drying Technology*, 12, 965-978.

Peleg, M. (1988). An empirical model for the description of moisture sorption curves. *Journal of Food Science*, 53, 1216-1219.

Rodrigo, D., Martínez, A., Harte, F., Barbosa-Cánovas, G.V., Rodrigo, M. (2001). Study of inactivation *Lactobacillus plantarum* in orange-carrot juice by means of pulsed electric fields. *Journal of Food Protection*, 64, 259-263.

Rodrigo, D., Barbosa-Cánovas, G.V., Martínez, A., Rodrigo, M. (2003). Pectin methyl esterase and natural microflora of fresh mixed orange and carrot juice treated with pulsed electric fields. *Journal of Food Protection*, 66, 2336-2342.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, 81, 501-508.

Sampedro, F., Rivas, A., Rodrigo, D., Martínez, A., Rodrigo, M. (2006). Effect of temperature and substrate on Pef inactivation ol Lactobacillus plantarum in an orange juice-milk beverage. *European Food Research and Technology,* 223, 30-34.

Soliva-Fortuny, R., Bendicho-Porta, S., Martín-Belloso, O. (2006). Modeling high-intensity pulsed electric fields inactivation of a lipase from *Pseudomonas fluoresecens*. *Journal of Dairy Science*, 89, 4096-4104.

Torregrosa, F., Cortés, C., Esteve, M.J., Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry,* 53, 9519-9525.

Torregrosa, F., Esteve, M.J., & Frígola, A., Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*, 73, 339-345.

Turhan, M., Sayar, S., Gunasekaran, S. (2002). Application of Peleg model to study water absorption in chickpea during soaking. *Journal of Food Engineering*, 53, 153-159.

Vieira, M.C., Teixeira, A.A., Silva, C.L.M. (2000). Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuaçu (theobroma grandiflorum) nectar. *Journal of Food Engineering*, 43, 1-7.

Vikram, V.B., Ramesh, M.N., Prapulla, S.G. (2005). Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. *Journal of Food Engineering*, 69, 31-40.

Wang, W.D., Xu, S.Y. (2007). Degradation kinetics of anthocyanins in blackberry juice and concentrate. *Journal of Food Engineering*, 82, 271-275.

Willcox, J.K., Catignani G.L., Lazarus, S. (2003). Tomatoes and cardiovascular health . *Critical Reviews in Food Science and Nutrition*, 43, 1-18.

Capítulo III

Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments

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ABSTRACT
The effect of high intensity pulsed electric fields (HIPEF) processing (35 kV/cm for 1500 μs in bipolar 4-μs pulses at 100 Hz, with an energy density of 8269 kJ/L) on the main bioactive compounds and antioxidant capacity of tomato juice was investigated and compared to heat pasteurization (90 ºC for 1 min or 30 s) having the fresh juice as a reference. HIPEF and heat treated tomato juices showed higher lycopene and lower vitamin C levels than the untreated juice. However, no significant changes in the total phenolic content and antioxidant capacity were observed between treated and fresh juices just after processing. Lycopene, vitamin C and antioxidant capacity of both treated and untreated juices decreased exponentially during storage following a first order kinetics (R^2 = 0.763-0.987), whereas tomato juices maintained their initial phenolic content. HIPEF-treated tomato juice maintained higher lycopene and vitamin C content than the thermally treated juices during the storage time. Hence, the application of HIPEF may be appropriate to achieve nutritious and fresh like tomato juice.

Key words: thermal treatment, high intensity pulsed electric fields, tomato juice, bioactive compounds, antioxidant capacity

INTRODUCTION

Regular consumption of tomatoes and tomato based products has been associated with lower incidence of various forms of cancer, in particular prostate cancer, and heart diseases (Arab and Steck, 2000). These beneficial effects have been attributed to the antioxidant components tomato contains such as carotenoids, vitamin C and phenolic compounds (Dumas, et al., 2003). The main carotenoid present in tomato, is lycopene which provides its color (Stahl and Sies, 1996). The ability of lycopene to act as a potent antioxidant is thought to protect cells against oxidative damage (Rao and Agarwal, 1999). On the other hand, vitamin C may prevent free radical-induced damage to DNA quenching oxidants (Fraga, et al., 1991), that overcome cell dysfunction and decrease low-density lipoprotein induced leukocyte adhesion (Lehr, et al, 1995). Several studies demonstrated that a diet rich in phenolic compounds correlates with reduced risk of coronary heart diseases (Amiot, et al., 1997). This association was partially explained on the basis of the fact that phenols interrupt lipid peroxidation induced by reactive oxygen species (ROS). Although tomatoes are commonly consumed in fresh, over 80% of the tomato consumption comes from processed products such as tomato juices (Gould, 1992).

Thermal processing is the most common method for extending the shelf-life of tomato juices, by inactivating microorganisms and enzymes. However, heat treatments can reduce the sensory and nutritional qualities of juices (Braddock, 1999). Therefore, consumer demands for healthy and nutritious food products with a fresh-like appearance has raised the aware of the food industry for the development of milder preservation technologies to replace the existing pasteurization methods (Linneman et al., 1999). High-intensity pulsed electric fields (HIPEF) processing of liquid foods is being investigated to avoid the negative effects of heat pasteurization (Deliza et al., 2003). HIPEF treatment is efficient enough to destroy microorganisms in fruit juices at levels equivalent to heat pasteurization (Yeom et al., 2000a). In addition, the enzymes commonly present in fruit juices are inactivated (Espachs-Barroso et al., 2003). Giner et al. (2000) reported a reduction of 93.8% of PME from tomato juice after applying a HIPEF treatment set up at 24 kV/cm with 400 0.02-ms pulses. Yeom et al., (2000b) and Ayhan et al. (2002) reported that quality parameters of juices are kept after applying HIPEF treatments. However, knowledge about the effects of this emerging technology on the antioxidant potential of fruit juices is scarce and generally focused on orange juices (Min et al., 2003a; Elez-Martínez et al., 2006). Min and Zhang (2003) and Min et al. (2003b) studied the evolution of some quality parameters and bioactive compounds of hot break tomato juice, which was treated for 2 min at 88 ºC before HIPEF treatment. Nevertheless, no information is currently available about the effect of HIPEF pasteurization on bioactive compounds as well as antioxidant capacity of fresh tomato juice. In addition, little is known about the evolution of the antioxidant properties during the commercial shelf-life of HIPEF-processed juices. Therefore the aim of the present work was to evaluate and compare the effects of HIPEF processing and heat pasteurization on lycopene, vitamin C and phenolic content as well as the antioxidant capacity of tomato juice. In addition, the effect of storage (4 ºC) on the concentration of bioactive compounds in the tomato juices was investigated.

MATERIALS AND METHODS

Tomato juices

Tomato fruits (*Licopersicon esculentum* Mill, cultivar Bodar) were purchased at commercial maturity from a local supermarket. The fruits were ground and then filtered through 2-mm steel sieves. Electrical conductivity (Testo 240 conductivimeter ; Testo GmBh & Co, Lenzkirch, germany), pH (crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan) and color measurement (Macbeth-Kollmorgen inst. Corp., Newburg, NY) of tomato juice were determined (Table 1).

Table 1.-Analytical characteristics of tomato juice

Parameters[1]		**Tomato juice**
pH		4.28 ± 0.02
Soluble solids (ºBrix)		4.48 ± 0.1
Color	**L***	21.98 ± 0.08
	a*	7.02 ± 0.22
	b*	5.21 ± 0.05
Electrical conductivity (S/m)		0.65 ± 0.02

[1]Results are the mean ± DS of three measurement

Pulsed electric fields equipment

A continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that held bipolar squared wave pulses was used to treat tomato juice samples. The juice was pumped at a flow rate of 60 mL/min through a system of eight collinear chambers connected in series. Each chamber had a treatment volume of 0.012 cm^3 that was delimited by two stainless steel electrodes separated by a gap of 0.29 cm. The flow was controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). Treated tomato juice was passed through a cooling coil connected between each pair of chambers and submerged in an ice-water shaking bath. Thermocouples were attached to the surface of the stainless-steel coils, 2.5 cm away from the HIPEF zones along the flow direction. The thermocouples were connected to temperature readers and isolated from the atmosphere with an insulation tape. The temperatures of the inlet and outlet of each pair of chambers were recorded every 0.1 s during HIPEF treatment and the samples never exceeded 40 ºC. Tomato juice was subjected to HIPEF treatment consisted of bipolar square-wave pulses of 4μs, with a frequency of 100 Hz, and 35 kV/cm field strength during 1500 μs, supplying an energy density of 8269 kJ/L. The main undesirable changes in juices are due to compounds produced by the

growth of lactic acid bacteria (Lactobacillus and Leuconostoc) (Hendrix and Redd, 1995). Therefore treatment conditions were selected to reach a logarithmic reduction for *Lactobacillus brevis* of 5 log (data not shown).

Thermal treatment

Mild (90 ºC, 30 s) and high (90 ºC, 60 s) heat pasteurizations were applied as reference treatments that allow to compare the effectiveness of HIPEF treatments on bioactive compounds and antioxidant capacity. These conditions were selected based on literature, where typical heat treatments of juices vary from 95 ºC to 90 ºC for 15-60 s (Nagy et al., 1993). Tomato juice was thermally processed in a tubular heat exchanger. A gear pump was used to maintain the juice flow rate through a stainless steel heat exchange coil, which was immersed in a hot water shaking bath (Universitat de Lleida, Lleida, Spain). After thermal processing, the juice was immediately cooled in a heat exchange coil immersed in an ice water-bath.

Sample packaging and storage

HIPEF and thermal fluid handling system was disinfected first with 4 % NaOH and then with 10 % chlorine and 20 % ethanol solutions prior to processing. The first 200 ml treated liquid was discarded to ensure stationary treatment conditions. Polypropylene bottles of 100 ml previously sterilized at 121 ºC for 30 min were used to store tomato juice. The juice was bottled directly from the treatment system, leaving the minimum amount of headspace volume. Once filled, the receptacle was tightly closed and stored, up to analysis, under refrigeration at 4 ºC in darkness until spoilage took place in samples.

Lycopene

Total lycopene content was measured spectrophotometrically following the method proposed by Davis et al. (2003). Approximately 0.6 g of sample were weighed with precision from each cultivar and added to a mixture consisting of 5 ml of 0.05 % (w/v) butylated hydroxytoluene in acetone, 5 ml of 95 % USP grade ethanol and 10 ml of hexane. The homogenate was centrifuged at 320 x g for 15 min on ice. Afterwards, 3 ml of deionized water were added. The vials were then agitated for 5 min and left at room temperature to allow phase separation. The absorbance of the upper hexane layer, was measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample was estimated using the absorbance at 503 nm and the sample weight (Fish et al., 2002). Results were expressed as mg of lycopene per 100 g of tomato juice.

Vitamin C

Vitamin C content in tomato juice was analysed by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (2007). A sample of 25 g of juice was mixed with 25 ml of a solution containing 45 g of metaphosforic acid and 7.2 g of DL-1,4-dithiotreitol per litre. The mixture was centrifuged at 22100 x g for 15 min at 4 ºC. The supernatant was vacuum-filtered through Whatman No. 1 paper. The sample was then passed

through a Millipore 0.45 μm membrane. An aliquot of 20 μs was injected into the HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm x 250 cm). The mobile phase was a 0.01 % solution of sulphuric acid adjusted to pH = 2.6. The flow rate was fixed at 1.0 ml/min at room temperature. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. The quantification of vitamin C was carried out comparing the samples with a calibration line built with 0, 5, 10, 15, 30 and 50 mg ascorbic acid/100 g (Scharlau Chemie, SA., Barcelona, Spain). Results were expressed as mg of vitamin C per 100 g of tomato juice.

Total Phenolic content

Total phenols were determined by the colorimetric method of Singleton et al. (1999) using the Folin-Ciocalteu reagent. Samples of tomato juice were centrifuged at 6000 x g for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and filtered through Whatman No 1 filter paper. Afterwards, 0.5 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 10 ml of saturated Na_2CO_3 solution. Samples were allowed to stand for 1 h at room temperature before the absorbance at 725 nm was measured. Concentrations were determined by comparing the absorbance of the samples with a calibration line built with 0, 10, 20, 30, 40 and 50 mg gallic acid/100 g (Scharlau Chemie, SA.,Barcelona, Spain). Results were expressed as milligrams of gallic acid per 100 g of tomato juice.

Antioxidant capacity

The antioxidant capacity was studied through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by de Ancos et al. (2002). Samples of tomato juice were centrifuged at 6000 x g for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH (0.025 g/l) and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm against a blank of methanol without DPPH. Results were expressed as percentage decrease with respect to the absorption value of a reference DPPH solution.

Statistical analysis

Treatments were conducted in duplicate and two replicate analyses were carried out for each sample in order to obtain the mean value (n = 4). Significance of the results and statistical differences were analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md). Analysis of variance (ANOVA) was performed to compare treatment mean values. The least significant difference test was employed to determine differences between means at a 5 % significance level. Correlations between antioxidant capacity and the studied bioactive compounds were evaluated with Pearson's test.

Experimental data were fitted to a first-order kinetic model (Equation 1) to describe the evolution of the health-related compounds as well as the antioxidant capacity during cold storage.

$$C = C_0 e^{(-k \cdot t)} \qquad (1)$$

where C is the residual content of bioactive compound (mg/100g) or antioxidant capacity (%), C_0 is the initial content of the bioactive compound (mg/100g) or antioxidant capacity (%), k is a first-order rate constant (days^{-1}) and t the storage time (days).

RESULTS AND DISCUSSION

Lycopene

The effects of processing and storage time on the concentration of lycopene in tomato juices are shown in Figure 1. Lycopene concentration was enhanced significantly after thermal or HIPEF processing compared to the untreated juice. The increase in lycopene content ranged from 4.67 % in high thermally processed to 7.6 % in HIPEF-treated tomato juice. It has been reported that food processing such as cooking or grinding might improve lycopene bioavailability by breaking down cell walls (Gartner et al. 1997). Nguyen and Schwartz (1999) suggested that homogenization and heat treatment disrupt cell membranes and protein-carotenoids complex, making carotenoids more accessible for extraction. Nevertheless further investigations are still needed to explain this enhancement in lycopene content of tomato juice treated by HIPEF. Some authors reported higher levels of carotenoids in juices processed with HIPEF compared to the untreated tomato juice. For instance, Sánchez-Moreno et al. (2005a) observed that in "gazpacho" a soup where tomato is the major component, the content of total carotenoids increased after applying 35 kV/cm in bipolar mode, 800 Hz pulse frequency, 4 μs pulse width during 750 μs in comparison to the untreated juice. Consistently, Torregrosa et al. (2005) and Cortés et al. (2006) reported a significant rise in some carotenoids in HIPEF-treated juices.

The concentration of lycopene in tomato juices depleted with storage time irrespective of the treatment applied (Figure 1). Lycopene content decreased in high thermally and HIPEF processed tomato juice from 6.94 to 2.05 mg/100g and from 7.15 to 2.02 mg/100g, respectively, after 91 days at 4 ºC. This trend is in accordance with that observed by other authors. Min et al. (2003b) reported significant losses of lycopene in hot break tomato juice treated by HIPEF (40 kV/cm for 57 μs) and thermally processed (92 ºC for 90s) over 112 days of study. As can be seen in Figure 1, the lycopene content of the tomato juice processed with HIPEF was 2.21 mg/100g at 56 days. At the same day, thermally processed juice showed a lycopene concentration of 2.03 and 2.20 mg/100g for high and mild pasteurization, respectively. These changes in lycopene content throughout the storage might be due to the oxygen availability in the headspace of the bottles during the early storage period. Rodriguez-Amaya (1993) found that the stability of lycopene in foods greatly depends on oxygen availability and packaging conditions. Shi and Le Maguer (2000) observed that carotenoids are susceptible to oxidation in the presence of light, oxygen and low pH. On the other hand,

HIPEF-treated tomato juice had higher lycopene content than the thermally treated during the storage time. Cortés et al. (2006) reported that the decrease in the concentration of total carotenoids was greater in untreated and pasteurized juice (90 ºC, 20 s) than in HIPEF-treated (30 kV/cm for 100 μs) orange juice during cold storage. However, Min et al. (2003b) did not observe differences in the lycopene content between thermally and HIPEF processed hot break tomato juices, which was heat processed (88 ºC for 2 min) before HIPEF treatment, during the storage period.

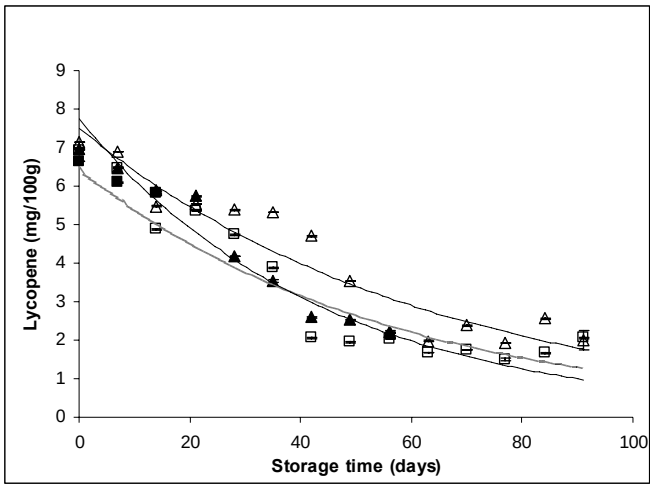

Figure 1.- Effects of HIPEF treatment and heat pasteurization on lycopene content of tomato juice throughout storage at 4ºC. Tomato juices: (■) untreated, (Δ,——) HIPEF, (▲,.....) heat pasteurized at 90ºC for 30s and (□,– – –) heat pasteurized at 90ºC for 60s. Data shown are mean ± standard deviation.

The experimental data values of lycopene content as a function of storage time were described by a first-order kinetic model. The regression parameters of the fitted model ($P < 0.05$) are given in Table 2, which shows that the first-order kinetic model displayed high determination coefficients (R^2) and fits well the experimental data. The first-order model rate constant ranged from 1.59×10^{-2} (HIPEF processing) to 2.27×10^{-2} (mild pasteurization), indicating higher destruction of lycopene in thermally treated than in HIPEF-treated tomato juice with storage time. Comparing the intercepted point of the first-order kinetic models, the lycopene content just after applying the HIPEF treatment was 0.5-1.6 mg/100ml higher than that treated at 90 ºC, 60 s (Table 2). In addition, an increment over 14 % of lycopene was obtained for juices treated by HIPEF compared to high heat treated juice, during the storage period (Table 2). No significant differences in lycopene concentration were observed between HIPEF and mild heat treated juices immediately after the treatments as well as during the first week of storage. Nevertheless, the difference in lycopene content between HIPEF- and mild

heat treated tomato juice rose when storage increase. In this way, tomato juices treated by HIPEF had 0.8-1.7 % more lycopene presence the day 7 of storage than that treated by mild heat, whereas this difference increased to 26.7-29.5 % at 56 storage days (Table 2).

Parameters	Treatments	C_0	k ($days^{-1}$)	R^2
Lycopene (mg/100g)	HIPEF	7.50 ± 0.27	$1.59\times10^{-2} \pm 1.11\times10^{-3}$	0.866
	HP	6.45 ± 0.28	$1.77\times10^{-2} \pm 1.42\times10^{-3}$	0.834
	MP	7.74 ± 0.21	$2.27\times10^{-2} \pm 1.30\times10^{-3}$	0.959
Vitamin C (mg/100g)	HIPEF	10.37 ± 0.18	$2.41\times10^{-2} \pm 7.43\times10^{-4}$	0.968
	HP	9.07 ± 0.40	$3.04\times10^{-2} \pm 2.70\times10^{-3}$	0.987
	MP	11.68 ± 0.27	$3.75\times10^{-2} \pm 1.54\times10^{-3}$	0.968
Antioxidant Capacity (%)	HIPEF	7.17 ± 0.37	$1.32\times10^{-2} \pm 1.46\times10^{-3}$	0.901
	HP	7.49 ± 0.39	$1.37\times10^{-2} \pm 1.50\times10^{-3}$	0.906
	MP	8.14 ± 0.65	$1.22\times10^{-2} \pm 2.96\times10^{-3}$	0.763

Table 2.- First-order kinetic rate constants (k) and determination coefficients (R^2) for the degradation of the health-related compounds and antioxidant capacity of treated tomato juice during storage at 4ºC.

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1000 μs; bipolar 4-μs pulses at 100 Hz
High pasteurization (HP) = treatment at 90ºC for 1min
Mild pasteurization (MP) = treatment at 90ºC for 30s

Vitamin C

Vitamin C content of treated and untreated tomato juice measured directly after treatment ranged from 10.17 to12.8 mg/100g (Figure 2). The highest content of this vitamin was observed in fresh tomato juice. The results obtained in the present work for vitamin C content are in the range of those published in literature (Davey et al., 2000; Sánchez-Moreno et al., 2006). Vitamin C content of both HIPEF and thermally processed tomato juices decreased drastically after being treated compared to untreated juices. However, HIPEF treated tomato juice showed higher concentration of vitamin C than the thermally processed juice (Figure 2). Vitamin C retention just after treatment in heat-treated tomato juice was 79.2 % (high pasteurization) and 80.4 % (mild pasteurization), whereas in HIPEF-treated tomato juice a 86.5 % retention was attained. This is in agreement with results observed by Elez-Martínez and

Martín-Belloso (2007), who reported a vitamin C retention of 84.3 % in gazpacho treated under HIPEF conditions similar to those used in the present work. Most differences between HIPEF and heat treatments can be explained through the temperatures reached through processing. Ascorbic acid is a heat-sensitive bioactive compound in the presence of oxygen. Thus, high temperatures during processing can greatly affect the rates of its degradation through an aerobic pathway. The maximum temperature achieved during HIPEF processing was 40 ºC. Therefore the higher retention of Vitamin C of HIPEF treated tomato juice compared to the thermally processed samples might be due to the lower processing temperatures of the HIPEF treatment. Different studies have proven the effectiveness of HIPEF in achieving higher vitamin C retention in comparison with heat treatments in juices. Min et al. (2003b) did not find difference between fresh and HIPEF processed tomato juice treated at 40 kV/cm for 57 μs with bipolar pulses of 2-μs and a maximum temperature of 45 ºC. These authors observed similar vitamin C retention in thermally and HIPEF treated hot break tomato juices, which might be due to the heat pasteurization (88 ºC for 2 min) applied before HIPEF treatment.

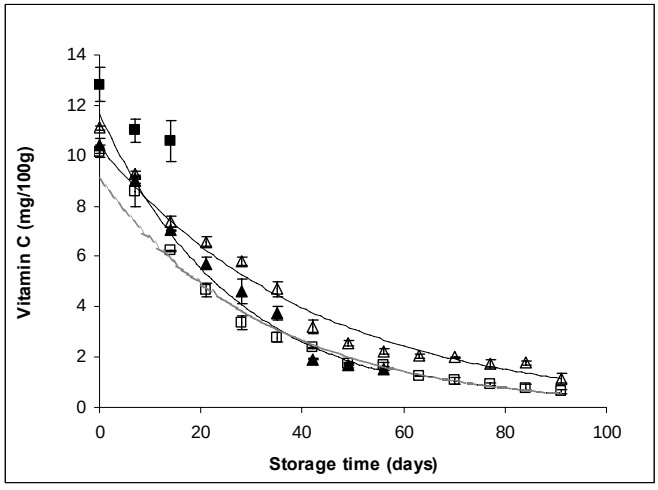

Figure 2.- Effects of HIPEF treatment and heat pasteurization on vitamin C retention of tomato juice throughout storage at 4ºC. Tomato juices: (■) untreated, (Δ,——) HIPEF, (▲,·····) heat pasteurized at 90ºC for 30s and (□,– – –) heat pasteurized at 90ºC for 60s. Data shown are mean ± standard deviation.

The concentration of vitamin C in thermally processed, HIPEF-processed and untreated juices decreased as storage time increased (Figure 2). However, tomato juice treated by HIPEF retained more vitamin C than thermally processed tomato juice for 91 days at 4 ºC. Vitamin C retentions in HIPEF and high thermally processed tomato juices were 8.9 % and 5.0 %, respectively, at 91 days of storage at 4ºC. Consistently, Min et al. (2003b) observed significantly higher contents of vitamin C in HIPEF-treated than in thermally pasteurized hot

break tomato juice. Vitamin C retention has been used as indicator of shelf-life for chilled orange juices. It has been considered that juices with 50 % of the initial vitamin C are at the end of its shelf-life (Shaw, 1992). As can be seen in Figure 2, the concentration of vitamin C in the tomato juice treated by HIPEF or mild pasteurization was reduced about 50 % after 28 days of storage at 4 ºC. However, the losses of vitamin C were grater than 50 % in juices subjected to high pasteurization after 21 days of storage. Min et al. (2003b) reported a reduction of 50 % of vitamin C in HIPEF and heat treated hot break tomato juices during the first 30 days of the storage period. The great reduction of vitamin C within the first month of storage might be due to the presence of oxygen in the head space of the packages. Atmospheric oxygen is responsible for most losses during long-term storage. Vitamin C is usually degraded by oxidative processes which are stimulated in the presence of light, oxygen, heat peroxides and enzymes (especially ascorbate oxidase and peroxidase) (Davey et al., 2000). The rate of vitamin C depletion during storage depends on type of processing, storage conditions and packaging (Ayhan et al., 2001). The contents of vitamin C as a function of the storage time were described by a first-order kinetic model. The model fitted well the experimental data ($R^2 \geq 0.968$) (Table 2) and the first-order model rate constant values varied from 2.41×10^{-2} (HIPEF processing) to 3.75×10^{-2} (mild pasteurization). Significantly higher vitamin C content (15.1-59.8 %) was obtained in HIPEF-treated tomato juice compared to that high heat treated during the storage period. Although during the first week of storage the concentration of vitamin C was greater in tomato juice treated by mild pasteurization than that treated by HIPEF, no significant differences were found between 7 and 21 days of storage and lower content of vitamin C (15.9-49.4 %) were observed in the former juice compared to HIPEF-treated juice after 21 days of storage at 4 ºC (Figure 2).

Total Phenolic Compounds

The concentration of total phenolic compounds in tomato juices varied from 31.1 mg/100g (high pasteurization) to 32.8 mg/100g (untreated) (Figure 3). These values are within the range observed in other studies. Podsędek et al. (2003) reported a concentration of phenolic content between 26.77 to 52.26 mg/100g in different tomato juices. On the other hand, no significant differences were observed in the phenolic content of processed and unprocessed tomato juices. In accordance with our results, Dewanto et al. (2002) did not find significant changes in total phenolic content between thermally treated and fresh tomato puree.

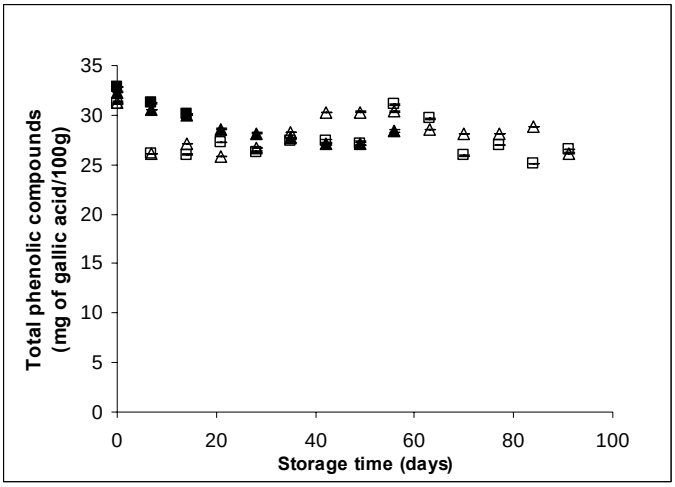

Figure 3.- Effects of HIPEF treatment and heat pasteurization on total phenolic content of tomato juice throughout storage at 4ºC. Tomato juices: (■) untreated, (Δ) HIPEF (▲) heat pasteurized at 90ºC for 30s and (□) heat pasteurized at 90ºC for 60s. Data shown are mean ± standard deviation.

As can been seen in Figure 3, tomato juice treated at 90 ºC for 30 s retained the initial total phenolic content for a period of 56 days at 4 ºC. In the same way, the tomato juices subjected to both high thermal treatment and to HIPEF-processing retained their initial phenolic content during 91 days. Tomato juices showed phenolic compounds values of 26.2 mg/100g (HIPEF) and 26.5 mg/100g (high pasteurization) at 91 days of storage. As far as we know, there are no published works quantifying total phenolic content throughout storage of tomato juices. However, Pérez-Vicente et al. (2004) reported insignificant changes in total phenolic compounds of pomegranate juices stored during 160 days at 18 ºC. The maintenance of total phenolic compounds during storage might be due to the inactivation of the enzymes responsible for its degradation. Peroxidase is the main enzyme implicated in reactions that are associated with loss of quality in tomato juice. In addition, this enzyme is involved in the oxidative degradation of phenolic compounds (Amiot et al., 1997). It has been demonstrated that both thermal and HIPEF treatments could inhibit peroxidase in juices. Anthon et al. (2002) observed a residual peroxidase activity of 10 % after applying a thermal treatment (72 ºC-30 s) in tomato juices. A reduction of 96.87 % of the initial POD activity of tomato juice were obtained after applying a HIPEF treatment similar to that used in the present study (Aguayo-Aguiló et al. 2007). In this way, Aguiló-Aguayo et al. (2006) reported a 65 % inactivation of peroxidase activity in "gazpacho" soup, where tomato is the main component, treated under HIPEF conditions similar to the used in the present work.

Antioxidant Capacity

The antioxidant capacity, measured on the basis of the DPPH stable radical, of fresh and processed tomato juices ranged from 9.8 to 10.4 % of DPPH inhibition (Figure 4), with no significant differences between treated and untreated products. Therefore the treatment did not affect the DPPH inhibition of tomato juices versus the fresh juice. Elez-Martínez and Martín-Belloso (2007) reported that HIPEF treatments of 15 or 35 kV/cm for 1000 μs at 200 Hz applying bipolar or monopolar 4-μs pulses led to similar levels of antioxidant capacity in orange juice and gazpacho with respect to the thermal treatment. On the other hand, Sánchez-Moreno et al. (2005b) observed that the antioxidant capacities of untreated and HIPEF-treated orange juices were not significantly different. It has been demonstrated that heat processing causes no change in the antioxidant capacity of fruit and vegetables due to the formation of novel compounds such as Maillard reaction products having antioxidant activity (Manzocco et al., 2001). The antioxidant capacity of tomato juice depleted with storage time at 4 ºC irrespective of the treatment applied (Figure 4). Antioxidant capacity of tomato juice subjected to mild heat treatment was 46.52 % of the initial value at 56 days, whereas antioxidant capacity decreased to 29.6 % and 35.7 % of the initial DPPH inhibition for HIPEF-treated and high pasteurized juice respectively, at the same storage day. No significant differences in antioxidant capacity were observed between tomato juice treated by HIPEF and high heat pasteurization.

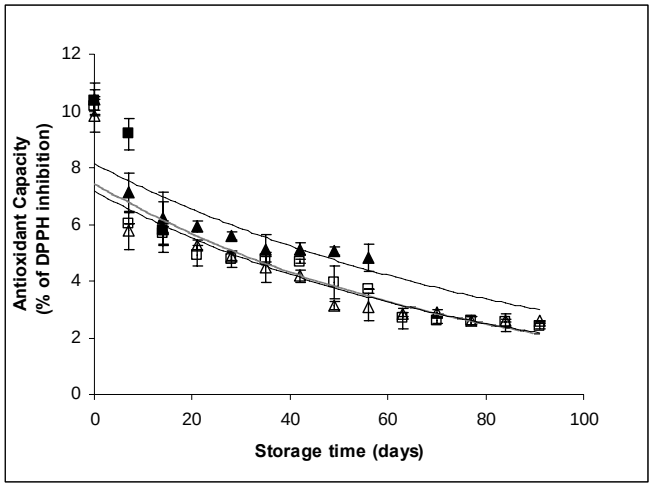

Figure 4.- Effects of HIPEF treatment and heat pasteurization on antioxidant capacity of tomato juice throughout storage at 4ºC. Tomato juices: (■) untreated, (Δ,——) HIPEF (▲,·····) heat pasteurized at 90ºC for 30s and (□,– – –) heat pasteurized at 90ºC for 60s. Data shown are mean ± standard deviation.

The antioxidant capacity is related to the amount and composition of bioactive compounds present in food (Sánchez-Moreno et al., 2005b). Eberhardt et al. (2000) indicated that most antioxidant capacity comes from the natural combination of different phytochemicals. Vitamin C and phenols are reported to be the major antioxidant components in tomato (Takeoka, et al., 2001). However, the magnitude of the changes in the antioxidant capacity could not be associated with the maintenance of the total phenolic compounds during storage (R^2 = 0.524) (Table 3). There was a significant correlation between antioxidant capacity and vitamin C (R^2 = 0.859) as well as between lycopene content (R^2 = 0.808) and antioxidant capacity (Table 3). These correlations seem to indicate that changes in antioxidant capacity might be modulated by vitamin C and lycopene. The decrease of antioxidant capacity during refrigeration could be attributed to vitamin C and lycopene losses. Sánchez-Moreno et al. (2006) observed that vitamin C and lycopene were the main compounds responsible for the changes of antioxidant capacity of tomato juices during the storage time. As can be seen in Table 3, there was a strong correlation (R^2 = 0.913) between lycopene and vitamin C content. Thus, the higher the lycopene content of tomato juice, the greater the vitamin C concentration was.

The first-order kinetic model predicted well the antioxidant capacity as indicated by the obtained good determination coefficients (R^2 = 0.763-0.906). Nevertheless, the model did not describe adequately the fast decrease in antioxidant capacity that occurred during the first 2 days of storage at 4 ºC. First-order kinetic rates took values from 1.22×10^{-2} to 1.37×10^{-2} (Table 2). No references about modeling the effect of the storage time on the antioxidant capacity of heat or HIPEF treated tomato juice through the first-order kinetic model have been published up now.

Table 3.- Correlation coefficients among different bioactive compounds and antioxidant capacity of tomato juice subjected to different treatments and storage at 4ºC

	Lycopene	**Vitamin C**	**Phenolic compounds**	**Antioxidant capacity**
Lycopene	----	0.913[1]	0.344	0.808[1]
Vitamin C	0.913[1]	----	0.467[1]	0.859[1]
Phenolic compounds	0.344	0.467[1]	-----	0.524[1]
Antioxidant capacity	0.808[1]	0.859[1]	0.524[1]	----

[1]significant differences at $P < 0.001$, using Pearson's correlation coefficients

CONCLUSIONS

HIPEF processing can produce tomato juice with higher nutritional value than conventional thermal processing. HIPEF-treated (35 kV/cm for 1500 μs with 4-μs bipolar pulses at 100 Hz, energy input of 8269 kJ/L) tomato juice shows higher lycopene and vitamin C content just after the treatment and during the storage time than thermally treated (90 ºC-30 s and 90 ºC-60 s) tomato juice. A rise in lycopene content and a depletion of vitamin C is observed after HIPEF and thermal treatments compared to the fresh tomato juice. Nevertheless no significant differences are observed in phenolic content and antioxidant capacity between treated and untreated samples just after processing. In addition, storage time shows a significant effect on the studied compounds. Lycopene, vitamin C and antioxidant capacity deplete with time irrespective of the treatment applied, whereas the initial content of total phenolic compounds is kept during storage. Therefore HIPEF technology could be an alternative to thermal treatment to obtain tomato juice with high nutritional quality. Further research is needed in order to know more about the effects of HIPEF on the mechanisms involving bioactive changes.

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REFERENCES

Aguiló-Aguayo, I., Elez-Martínez, P., Soliva-Fortuny, R., Martín-Belloso, O. (2006). Effects of high-intensity pulsed electric fields on the inactivation of peroxidase in Mediterranean vegetable soup. In: Workshop on Applications of Novel Technologies in Food and Biotechnology, Cork, 11-13 September, 2006.

Aguiló-Aguayo, I., Soliva-Fortuny, R., Martín-Belloso, O. (2007). Comparative study on peroxidase inactivation and color parameters of tomato juice processed by pulsed electric fields or heat treatment during storage. In: IFT Annual Meeting, Chicago, 28 July to 1 August, 2007.

Amiot, M.J., Fleuriet, A., Cheynier, V., Nicolas, J. (1997). Phenolic compounds and oxidative mechanisms in fruits and vegetables. In: Tomás-Barberán, F.A., Robins, R.J. Phytochemistry of fruit and vegetables. Science Publications, Oxford. pp. 51-85.

Anthon, G.E., Sekine, Y., Watanabe, N., Barret, D.M. (2002). Thermal inactivation of pectin methylesterase, polygalacturonase and peroxidase in tomato juice. *Journal of Agricultural and Food Chemistry*, 50(21), 6153-6159.

Arab, L., Steck, S. (2000). Lycopene and cardiovascular disease. *American Journal of Clinical Nutrition,* 71(6), 1691S-1695S.

Ayhan, Z., Yeom, H.W., Zhang, Q.H., Min, D.B. (2001). Flavor, color and vitamin C retention of pulsed electric field processed orange juice in different packaging materials. *Journal of Agricultural and Food Chemistry*, 49(2), 669-674.

Ayhan, Z., Zhang, Q.H., & Min, D.B. (2002). Effects of pulsed electric field processing and storage on the quality of single-strength orange juice. *Journal of Food Protection*, 65(10), 1623-1627.

Braddock, R.J. (1999). Handbook of citrus by-products and processing technology. John Wiley & Sons, New York. pp. 53-83.

Cortés, C., Esteve, M.J., Rodrigo, F., Torregrosa, F., Frígola, A. (2006). Changes of colour and carotenoids contents during high-intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology*, 44(11), 1932-1939.

Davey, M.W., Van Montagu, M., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science of Food and Agriculture,* 80(7), 825-860.

Davis, A.R., Fish, W.W., Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biology and Technology,* 28(3), 425-430.

De Ancos, B., Sgroppo, S., Plaza, L., Cano, M.P. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science of Food and Agriculture*, 82(8), 790-796.

Deliza, R., Rosenthal, A., & Silva, A.L.S. (2003). Consumer attitude towards information on non conventional technology. *Trends in Food Science and Technology*, 14(1-2), 43-49.

Dewanto, V., Wu, X., Adom, K., Liu, R. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing the total antioxidant activity. *Journal of Agricultural and Food Chemistry*, 50(10), 3010-3014.

Dumas, Y., Dadomo, M., Di Lucca, G., Grolier, P. (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *Journal of the Science of Food and Agriculture*, 83(5), 369-382.

Eberhardt, M.V., Lee, C.Y., Liu, R.H. (2000). Antioxidant activity of fresh apples. *Nature*, 405(6789), 903-904.

Elez-Martínez, P., Soliva-Fortuny, R.C., Martín-Belloso, O. (2006). Comparative study on shelf-life of orange juice processed by high intensity pulsed electric fields or heat treatments. *European Food Research and Technology,* 222(3-4), 321-329.

Elez-Martínez, P., Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102(1), 201-209.

Espachs-Barroso, A., Barbosa-Cánovas, G.V., Martín-Belloso, O. (2003). Microbial and enzymatic changes in fruit juice induced by High-intensity Pulsed Electric Fields. *Food Reviews International*, 19(3), 253-273.

Fish, W.W., Perkins-Veazie, P., Collins, J.K. (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of Food Composition and Analysis*, 15(3), 309-317.

Fraga, C.G., Motchnik, P.A., Shigenaga, M.K., Helbock, H.J., Jacob, R.A., Ames, B.N. (1991). Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proceeding of the National Academy of the Sciences of the United Stated of America*, 88, 11003-11006.

Gartner, C., Stahl, W., Sies, H. (1997). Lycopene is more bioavailability from tomato paste than from fresh tomatoes. *American Journal of Clinical Nutrition*, 66(1), 116-122.

Giner, J., Gimeno, V., Espachs, A., Elez, P., Barbosa-Cánovas, G.V., Martín, O. (2000). Inhibition of tomato (*Licopersicon esculentum* Mill.) pectin methylesterase by pulsed electric fields. *Innovative Food Science and Emerging Technologies*, 1, 57-67.

Gould, W.V. (1992). Tomato production, processing and technology. CTI Publications, Baltimore.

Hendrix, C.M., Redd, J.B. (1995). Chemistry and technology of citrus juices and by-products. In: Ashurst, P.R. Production and packaging of non-carbonated fruit juices and fruit beverages. Blackie Academic & Professional, Glasgow. pp. 53-87.

Lehr, H.A., Frei, B., Olofsson, A.M., Carew, T.E., Arfors, K.E. (1995). Protection from oxidized LDG-induced leukocyte adhesion to microvascular and macrovascular endothelium in vivo by vitamin C but not vitamin E. *Circulation*, 91(5), 1525-1532.

Linneman, A.R., Meerdink, G., Meulenberg, M.T.C., Jongen, W.M.F. (1999). Consumer-oriented technology development. *Trends in Food Science and Technology*, 9(11-12), 409-414.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M.C., Lerici, C.R. (2001). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science and Technology*, 11(9-10), 340-346.

Min, S., Zhang, Q.H. (2003). Effects of Commercial-scale pulsed electric field processing on flavour and color of tomato juice. *Food Chemistry and Toxicology*, 68(5), 1600-1606.

Min, S., Jin, Z.T., Min, S.K., Yeom, H., Zhang, Q.H. (2003a). Commercial-scale pulsed electric field processing of orange juice. *Food Chemistry and Toxicology*, 68(4), 1265-1271.

Min, S., Jin, Z.T., Zhang, Q.H. (2003b). Commercial scale pulsed electric field processing of tomato juice. *Journal of Agricultural and Food Chemistry*, 51(11), 3338-3344.

Nagy, S., Chen, C.S., Shaw, P.E. (1993). *Fruit juice processing technology*. Florida: Auburndale, Agscience.

Nguyen, M.L., Schwartz, S.J. (1999). Lycopene: chemical and biological properties. *Food Technology*, 53(2), 38-45.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105, 1151-1158.

Pérez-Vicente, A., Serrano, P., Abellán, P., García-Viguera, C. (2004). Influence of packaging material on pomegranate juice colour and bioactive compounds, during storage. *Journal of the Science of Food and Agriculture*, 84(7), 639-644.

Podsędek, A., Sosnowska, D., Anders, B. (2003). Antioxidative capacity of tomato products. *European Food Research and Technology*, 217(4), 296-300.

Rao, A.V., Agarwal, S. (1999). Role of lycopene as antioxidant carotenoid in the preservation of chronic diseases: A review. *Nutritional Research*, 19(2), 305-323.

Rodriguez-Amaya, D.B. (1993). Stability of carotenoids during the storage of foods. In G. Charalambous, *Shelf-life studies of foods and beverages* (pp. 591-628). New-York: Elsevier.

Sánchez-Moreno, P., Cano, M.P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F., Élez-Martínez, P., Martín-Belloso, O., Martín, A. (2005a). Intake of Mediterranean soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *International Journal of Food Sciences and Nutrition*, 56(2), 115-124.

Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., Cano, P. (2005b). Impact o high pressure and pulsed electric fields on bioactive compounds and antioxidant capacity of orange juice in comparison with traditional thermal processing. *Journal of Agricultural and Food Chemistry*, 53(11), 4403-4409.

Sánchez-Moreno, C., Plaza, L., de Ancos, B., Cano, P.M. (2006). Nutritional characterization of commercial traditional pasteurized tomato juices: carotenoids, vitamin C and radical-scavenging capacity. *Food Chemistry*, 98(4), 749-756.

Shaw, P.E. (1992). Shelf-life and aging of citrus juices, juice drinks and related soft drinks. In: Redd, J.B., Shaw, P.E., Hendrix, Jr., C.M, Hendrix, D.L. Quality control manual for citrus processing plants. Auburndale, Agscience, Florida. 173-199.

Shi, J., Le Maguer, M. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition*, 40(1), 1-42.

Singleton, V.L., Orthofer, R.M., Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.

Stahl, W., Sies, H. (1996). Lycopene: a biologically important carotenoid for human? *Archives of Biochemistry and Biophysics*, 336(1), 1-9.

Takeoka, G.R., Dao, L., Flessa, S., Gillespie, D.M., Jewell, W.T., Huebner, B., Bertow, D., Ebeler, S.E. (2001). Processing effects on lycopene content and antioxidant activity of tomatoes. *Journal of Agricultural and Food Chemistry*, 49(8), 3713-3717.

Torregrosa, F., Cortés, C., Esteve, M.J., Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry*, 53 (24), 9519-9525.

Yeom, H.W., Streaker, C.B., Zhang, Q.H., Min, D.B. (2000a). Effect of pulsed electric fields on the activities of microorganism and pectin methyl esterase in orange juice. *Journal of Food Science*, 65(8), 1359-1363.

Yeom, H.W., Streaker C.B., Zhang, Q.H., Min, D.B. (2000b). Effects of Pulsed Electric Fields on the Quality of orange juice and comparison with hat pasteurization. *Journal of Agricultural and Food Chemistry*, 48(10), 4597-4605.

Capítulo IV

Carotenoid and phenolic profile of tomato juices processed by high intensity pulsed electric fields compared with conventional thermal treatments

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ABSTRACT
The effect of high intensity pulsed electric fields (HIPEF) processing (35 kV/cm for 1500 μs of overall treatment time with bipolar pulses of 4-μs at 100 Hz) and heat pasteurization (90 ºC for 30 s or 60 s) on carotenoids and phenolic compounds as well as on some quality attributes (pH, soluble solids and colour parameters) of tomato juice was evaluated and compared, having the untreated juice as a reference. Processing enhanced some carotenoids (lycopene, β-carotene and phytofluene) and the red colour of juices, whereas no significant changes in phenolic compounds, pH and soluble solids were observed between treated and untreated juices. A slight decrease in overall health-related compounds was observed over time, with the exception of some carotenoids (β-carotene and phytoene) and caffeic acid. However, HIPEF-processed tomato juices maintained higher content of carotenoids (lycopene, neurosporene and γ-carotene) and quercetin through the storage time than thermally and untreated juices. Hence, the application of HIPEF may be appropriate to achieve not only safe but also nutritious and fresh like tomato juice.

Key words: high intensity pulsed electric fields, thermal treatment, tomato juice, carotenoids and phenolic compounds

INTRODUCTION

Regular consumption of tomatoes and tomato based products has been associated with reduced incidence of some types of cancer and heart diseases (Clinton, 1998). These beneficial properties have been attributed in part to their content in various bioactive compounds such as carotenoids. Tomato and tomato products are the predominant source of lycopene, which exhibits a high oxygen-radical scavenging and quenching capacities, and β-carotene, which is the main carotenoid with provitamin A activity (Beecher, 1998). However, carotenoids are highly unsaturated compounds with an extensive conjugated double-bonds system and they are susceptible to oxidation, isomerisation and other chemical changes during processing and storage (Shi and Le Maguer, 2000). On the other hand, carotenoids are mainly responsible for the red color of tomato which is the first quality factor that the consumer appreciates and has a remarkable influence on its acceptance (Abushita et al., 2000). In addition to carotenoids, other antioxidant compounds such as phenolics also contribute to the beneficial effects of tomato products. Phenolics possess reducing character, capacity of sequestering reactive oxygen species (ROS) and several electrophiles, tendency to self-oxidation and capacity to modulate the activity of some cell enzymes (Robards et al., 1999). Thus, consumption of tomato and tomato based product is being considered as a nutritional indicator of good dietary habits and healthy lifestyle.

Thermal processing is the most common method to extend the shelf-life of juices, by inactivating microorganisms and enzymes. However, heat treatments reduce the sensory and nutritional qualities of these products (Braddock, 1999). Therefore, high intensity pulsed electric fields (HIPEF) is being developed as a non-thermal emerging technology for the preservation of foods. Up to now, studies have suggested that HIPEF treatment is efficient enough to destroy microorganisms in fruit juices at levels equivalent to those achieved by heat pasteurization without greatly affecting their nutritional and sensory properties (Yeom et al., 2000; Min and Zhang, 2003). In addition, the enzymes commonly present in fruit juices are partially or totally inactivated (Marsellés-Fontanet and Martín-Belloso, 2007). In this regard, Aguiló-Aguayo et al. (2008a) reported complete POD inactivation in tomato juice after applying 5.5-μs bipolar pulses of 35 kV/cm for 2000 μs at 200 Hz. On the other hand, some studies have suggested that HIPEF processing may enhance the antioxidant properties of juices comparing to those untreated (Torregrosa et al. 2005; Odriozola-Serrano et al. 2007). However, little is known about how HIPEF treatments can alter or modify the individual profile of health-related compounds in processed vegetable foods. Therefore, evaluating the influence of HIPEF processing and storage on individual carotenoids and phenolic compounds is a key factor to obtain tomato juices with antioxidant potential similar or higher than that of fresh juices.

The aim of the present work was to evaluate and compare the effects of HIPEF processing and heat pasteurization on individual carotenoids and phenolic compounds of tomato juice. In addition, the effect of storage (4 ºC) on the concentration of these bioactive compounds was investigated. Colour, pH and soluble solids content were also considered as quality parameter and related to health-related compounds.

MATERIALS AND METHODS

Tomato juice preparation

Tomatoes *(Lycopersium esculentum* Mill. cultivar Bodar) at commercial maturity were bought from a local supermarket, and kept at 4 ºC before being processed. The fruits were washed, sorted and chopped and then filtered through a 2-mm diameter steel sieve to remove peel and seeds. pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), titratable acidity, soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Tokyo, Japan), color measurement (Macbeth-Kollmorgen inst. Corp., Newburg) and hardness of the mesocarp of whole tomato (TA-XT2 Texture Analyzer; Stable Micro Systems Ltd, Surrey, UK) were determined. The physico-chemical characteristics of tomato were: pH = 4.32 ± 0.33, titratable acidity = 0.34 ± 0.01, soluble solids = 4.5 ± 0.1 ºBrix, color L* = 42.2 ± 1.8, a* = 21.8 ± 2.5 and b* = 25.1 ± 1.9, hardness of mesocarp = 28.6 ± 1.3 N.

Pulsed electric fields equipment

Pulse treatments were carried out using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that provides squared-wave pulses within eight co-field flow chambers in series. Each chamber had a treatment volume of 0.012 cm^3, delimited by two stainless steel electrodes and separated by a gap of 0.29 cm. The flow rate of the process was adjusted to 60 mL/min and controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). The treatment temperature was kept below 40 ºC using a cooling coil, which was connected before and after each pair of chambers and submerged in an ice-water shaking bath. Thermocouples were attached to the surface of the stainless-steel coils, 2.5 cm away from the HIPEF zones along the flow direction. The thermocouples were connected to temperature readers and isolated from the atmosphere with an insulation tape. The temperatures of the inlet and outlet of each pair of chambers were recorded every 0.1 s during HIPEF treatment. HIPEF treatment was set up at 35 kV/cm for 1500 μs using bipolar squared-wave pulses of 4-μs and a frequency of 100 Hz.

Thermal treatment

In order to compare the effect of HIPEF treatment to that of the conventional thermal treatment, tomato juice was subjected to heat processes at 90 ºC for 30 s (mild heat pasteurization) or 90 ºC for 60 s (high heat pasteurization). These conditions were selected based on literature (Nagy et al., 1993). Tomato juice was thermally processed in a tubular stainless steel heat exchange coil of 2.2 mm of internal diameter and 11 m of length immersed in a hot water shaking bath (Universitat de Lleida, Lleida, Spain). A gear pump was used to maintain the desirable juice flow rate. After thermal processing, the juice was immediately cooled in a heat exchange coil immersed in an ice water-bath.

Packaging and storage conditions

HIPEF and thermal fluid handling system were sanitized prior to processing. Polypropylene sterile 100-mL bottles were filled directly from the outlet of the treatment systems leaving as less headspace as possible. Afterwards, the container was tightly closed and stored at 4 ºC ± 1 ºC for 56 days. Treatments were conducted in duplicate and two replicate analyses were carried out for each sample in order to obtain the mean value.

Carotenoids

Extraction

The extraction method was based on a procedure for extraction of carotenoids from thermally processed tomato products (Tonucci et al., 1995). First, a 2.5 g of magnesium carbonate and 2.5 g of Celite, used as filter aid, were added to 25 mL of tomato juice and 0.5 mg of internal standard (β-apo-8'-carotenal). The mixture was blended for 20 min in an Omni Mixer with 25 mL of tetrahydrofuran (THF) and then filtered through Whatman No. 1 filter paper using a Büchner funnel. The solid material was extracted two or three more times until it was devoid of red/orange colour. The THF extracts were combined, and the volume was reduced by about two-thirds under vacuum at 35ºC with a rotary evaporator. Components of the combined extract were portioned into 250 mL of methylene chloride and 150 mL of saturated sodium chloride water in a separatory funnel. The water layer was washed with methylene chloride until carotenoids were completely removed. The methylene chloride layer containing carotenoids was dried over anhydrous sodium sulfate and filtered through Whatman No. 42 filter paper. The volume of the filtrate was reduced under vacuum to approximately 25 mL and brought up to 50 mL with methylene chloride. Then the extracts were passed through a Millipore 0.45 µm membrane and injected into the HPLC system.

Chromatography conditions

Conditions for the HPLC separations were those reported by Khachik, Goli, Beecher, Holden, Lusby, Tenorio and Barrera (1992). The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 470nm. Samples were introduced into a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6mm x 250mm) through a manual injector equipped with a sample loop (20 µl). The flow rate was fixed at 0.7 mL/min at room temperature. An isocratic elution of acetonitrile (85%), methanol (10%), methylene chloride (3%) and hexane (2%) was maintained from 0 to 10 min, followed by a linear gradient to acetonitrile (45%), methanol (10%), methylene chloride (23%) and hexane (22%) from 10 to 40 min. At the end of the gradient, the column was equilibrated under the initial conditions for 20 min. Carotenoids were quantified by comparison with external standards of lycopene, neurosporene, γ-carotene, ζ-carotene, β-carotene, phytofluene and phytoene. Results were expressed as milligrams of phenolic compounds in 100 mL of fw tomato juice.

Vitamin A quantification

Vitamin A was expressed as retinol equivalents (RE), according to Equation 1 (Trumbo et al., 2003):

$$RE = \left[\frac{\beta - carotene(\mu g)}{12}\right] + \left[\frac{\gamma - carotene(\mu g)}{24}\right] \quad (1)$$

Phenolic compounds

Extraction and Hydrolysis

The extraction was carried out following the method validated by Hertog et al. (1992). 20 mL of 62.5% aqueous methanol with 2g/L of tert-bytlhydroquinone and 5 mL of 6 M HCl were carefully mixed with 2.5 g of freeze-dried strawberry juice. After refluxing at 90 ºC for 2 h with regular swirling, the extract was cooled and subsequently made up to 25 mL with methanol and sonicated for 5 min. The extract was then passed through a 0.45 µm filter prior to injection.

Chromatography conditions

HPLC system was equipped with a 600 Controller and a diode array detector (Waters, Milford, MA) which was set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6mm x 250mm) at room temperature with a flow rate of 1 mL/min. A gradient elution was employed with a solvent mixture of 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B) as follows: linear gradient from 5 to 13% B, 0-15 min; linear gradient from 13 to 15% B, 15-20 min; linear gradient from 15 to 30% B, 20-25 min; isocratic elution 30% B, 25-28 min; linear gradient from 30 to 45% B, 28-32 min; isocratic elution 45% B, 32-35 min; linear gradient 45 to 90% B, 35-40 min; isocratic elution 90% B, 40-45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection. Individual phenolics were quantified by comparison with external standards of chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, kaempferol and quercetin. The results were expressed as milligrams of phenolic compounds in 100 mL of fw tomato juice.

Quality attributes

The juice colour was measured using a Macbeth Color-Eye 3,000 colorimeter (Macbeth-Kollmorgen Inst Corp., Newburg, NY, USA) at room temperature. The CIE L* (lightness), CIE a* (red-green) and CIE b* (yellow-blue) were read using a D_{75} light source and the observer angle at 10º. Hue angle (hº) was calculated using Equation 2:

$$h^{\circ} = tan^{-1}\frac{b^*}{a^*} \qquad (2)$$

A temperature-compensated refractometer Atago RX-1000 (Atago Company Ltd., Tokyo, Japan) was used to determinate the soluble solids content of tomato juice. In addition, tomato juice pH was measured using a pH-meter (Crison Instruments SA, Alella, Barcelona, Spain).

Statistical analysis

Significance of the results and statistical differences were analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md). Data were analyzed by multifactor analysis of variance. Duncan multiple-range test was employed to determine differences among means, with a level of significance of 0.05. Principal component analysis (PCA) was carried out to obtain correlations among variables. PCA is a multivariate statistical technique based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be transformed to new coordinate system called principal components (PCs) that allow to explain the greatest capacity. A correlation matrix is used to standardize the variables which are not measured on the same scale. The loadings plot summarises the main relationship between variables and principal components and also highlights relationships between different variables themselves. Variables that appear close together in this plot correlated positively. On the other hand, the score plot represents the projection of each sample into PC, defining different groups.

RESULTS AND DISCUSSION

Effect of processing and storage on individual carotenoids

The effects of processing and storage time on total and individual carotenoid concentration of tomato juice are shown in Table 1. Considering total carotenoids, HIPEF-treated tomato juices showed the highest amount (14.7 mg/100 mL fw), whereas the lowest values were found in the fresh juice (14.1 mg/100 mL fw). Our results are in the range of those reported in other studies which vary from 5 to 17 to mg/100 g fw (Khachik et al., 2002; Podsędek et al., 2003). On the other hand, HIPEF-treated tomato juice maintained total carotenoids better than heat treatments during the storage period (Table 1). Total carotenoid content decreased over the time in mild and high thermally processed tomato juice from 14.4 to 7.3 mg/100 mL fw, after 56 days at 4 ºC, without significant differences between treatments. Regarding the individual carotenoids, lycopene was found in higher concentration, reaching values from 7.13 to 7.84 mg/100 mL fw. Lycopene content was enhanced significantly (7.7%-10%) after thermal or HIPEF processing compared to the untreated juice. In this way, other minor individual carotenoids which are lycopene precursors such as phytoene (1.83-2.07 mg/100 mL fw), phytofluene (1.29-1.37 mg/100 mL fw) and neurosporene (1.19-1.30 mg/100 mL fw) were affected by processing. However, ζ-carotene content of tomato juices (0.19-0.20 mg/100 mL fw) was similar between fresh and treated juices (Table 1). It has been reported that thermal treatment may imply an increase in some individual carotenoids, owing to greater stability, enzymatic degradation, and unaccounted losses of moisture, which concentrate the sample (Rodriguez-Amaya, 1997). Nguyen and Schwartz (1999) suggested that homogenization and heat treatment disrupt cell membranes and protein-carotenoids complex, making carotenoids

more accessible for extraction. However, the increase in lycopene just after processing coincided with a depletion of phytoene and neurosporene contents compared to the untreated juice (Table 1). Thus, HIPEF and heat treatments might stimulate the transformation of some carotenoids into lycopene. Phytoene undergoes a series of desaturation reactions, each of which creates a new double bond and extends the chromophore by two conjugated double bonds; the end product is lycopene, produced via the intermediates phytofluene, ζ-carotene and neurosporene (Britton and Hornero-Méndez, 1997). In accordance with our results, other authors have reported an enhancement of carotenoids after HIPEF processing of juices other than tomato (Cortés et al., 2006; Sánchez-Moreno et al., 2005). In addition, Torregrosa et al. (2005) observed that the concentration of some carotenoids rose, whereas the content of other decrease when thermal pasteurization and HIPEF treatments were applied to orange-carrot juice, reporting that conversion among carotenoids took place during processing. On the other hand, β-carotene and γ-carotene were found in the tomato juices in amounts ranging from 0.29 to 0.40 mg/100 mL fw and from 1.67 to 1.77 mg/100 mL fw, respectively (Table 1). Both carotenoids were affected by treatments so that significant differences in β-carotene and γ-carotene between treated and fresh tomato juices just after processing were observed. As can be seen in Table 1, β-carotene in treated tomato juice underwent a significant increase (31%-38%), whereas γ-carotene content was depleted (3%-6%) when HIPEF and thermal treatments were conducted. A plausible explanation for this fact is that γ-carotene may undergo cyclization to form six membered rings at one end of the molecule, giving β-carotene as a product (Britton and Hornero-Méndez, 1997). A decrease in the amounts of all individual carotenoids was observed over time, with the exception of β-carotene and phytoene content which were maintained for 56 days, regardless of the treatment applied (Table 1). Lycopene content decreased much more considerably than other carotenoids through the storage period, leading to levels of 2.43-2.71 mg/100 mL fw at 56 days of storage. Vitamin A content is related to the amount of β-carotene and γ-carotene in the samples (Rodriguez-Amaya, 1997). Vitamin A in tomato juices also underwent a slight depletion, reaching contents in HIPEF and thermally processed juices between 0.77-0.91 RE/mL, at 56 days of storage at 4 ºC (Table 1). The major cause of carotenoid losses in vegetable products is the oxidation of the highly unsaturated carotenoid structure (Kidmose et al., 2002). Oxidation may occur by autooxidation, which is a spontaneous free-radical chain reaction in the presence of oxygen, or by photooxidation produced by oxygen in the presence of light. These oxidative reactions may result in carotene bleaching, which is the cause of formation of colourless end-products (Gross, 1991). During autooxidation of carotenoids, alkylperoxyl radicals are formed and these radicals attack the double bonds resulting in formation of epoxides. The severity of oxidation depends on the structure of carotenoids and the environmental conditions, and the compounds being formed depend on the oxidation process and the carotenoids structure (Ramakrishnan and Francis, 1980). On the other hand, HIPEF-processed tomato juices kept higher amounts of carotenoids (lycopene, neurosporene, and γ-carotene) and vitamin A than juices treated by mild and high heat pasteurization for 56 days at 4 ºC. However, non significant differences in ζ-carotene and β-carotene content were observed between high thermally and HIPEF-treated tomato juices (Table 1). In this way, some authors (Cortés et al., 2006; Odriozola-Serrano et al., 2008) studied the evolution of some carotenoids in HIPEF-treated juices through the storage time, reporting higher maintenance of these health-related compounds in comparison to thermally pasteurized juices.

Table 1.- Effects of high-intensity pulsed electric fields and heat pasteurization on carotenoids of tomato juice throughout storage at 4ºC

ST	Process	Individual carotenoids (mg/100 mL fw)							vit A (RE/ml)	TC[1]
		Lyc	Neu.	γ-carot.	ζ-carot.	β-carot.	Phytof	Phytoe		
0	Fresh	7.13^{dA}	1.30^{aA}	1.77^{aA}	0.20^{aA}	0.29^{bA}	1.29^{bA}	2.07^{aA}	0.99^{bA}	14.1^{cA}
	HIPEF	7.84^{aA}	1.19^{cA}	1.70^{bA}	0.20^{aA}	0.40^{aA}	1.37^{aA}	1.98^{bB}	1.04^{aA}	14.7^{aA}
	MP	7.46^{cA}	1.27^{bA}	1.67^{bA}	0.19^{aA}	0.38^{aA}	1.37^{aB}	2.02^{bBC}	1.01^{abA}	14.4^{bA}
	HP	7.64^{bA}	1.23^{bcA}	1.71^{bA}	0.19^{aA}	0.39^{aA}	1.36^{aB}	1.83^{cC}	1.04^{aA}	14.4^{bA}
7	Fresh	6.59^{cB}	1.26^{aA}	1.34^{cB}	0.16^{aB}	0.28^{bA}	1.26^{bA}	1.97^{bB}	0.79^{cB}	12.9^{cB}
	HIPEF	7.39^{aB}	1.13^{bA}	1.68^{aA}	0.16^{aB}	0.37^{aB}	1.38^{aA}	1.95^{bB}	1.01^{aA}	14.1^{aB}
	MP	6.78^{bB}	1.17^{bB}	1.62^{bB}	0.17^{aA}	0.36^{aA}	1.43^{aA}	2.16^{aA}	0.98^{bAB}	13.7^{bB}
	HP	6.97^{bB}	1.24^{aA}	1.71^{aA}	0.16^{aB}	0.39^{aA}	1.40^{aA}	1.86^{cB}	1.04^{aA}	13.7^{bB}
14	Fresh	6.31^{aC}	1.24^{aA}	1.34^{bB}	0.08^{bC}	0.24^{cB}	0.92^{cB}	1.98^{abB}	0.75^{cB}	12.1^{cC}
	HIPEF	6.32^{aC}	1.11^{bA}	1.63^{aB}	0.15^{aB}	0.37^{aB}	1.37^{bA}	1.90^{bC}	0.97^{abB}	12.9^{abC}
	MP	6.37^{aC}	1.13^{bB}	1.63^{aB}	0.14^{aB}	0.33^{bB}	1.44^{aA}	2.05^{aB}	0.95^{bB}	13.1^{aC}
	HP	5.99^{bC}	1.24^{aA}	1.65^{aB}	0.14^{aC}	0.38^{aA}	1.40^{abA}	1.88^{cA}	1.00^{aA}	12.7^{bC}
21	HIPEF	6.23^{aC}	0.90^{bB}	1.69^{aA}	0.12^{aC}	0.37^{aB}	1.37^{bA}	1.98^{bB}	1.01^{aAB}	12.7^{aC}
	MP	6.23^{aC}	0.65^{cC}	1.46^{bC}	0.12^{aC}	0.33^{bB}	1.45^{aA}	2.11^{aA}	0.87^{bC}	12.3^{bD}
	HP	5.85^{bC}	1.15^{aB}	1.69^{aA}	0.13^{aC}	0.37^{aB}	1.37^{bB}	1.85^{cC}	1.02^{aA}	12.4^{bC}
28	HIPEF	5.87^{aD}	0.75^{aC}	1.64^{aB}	0.11^{aCD}	0.38^{aB}	1.38^{bA}	2.04^{bA}	1.00^{aAB}	12.2^{aD}
	MP	4.66^{bD}	0.47^{bD}	1.46^{bC}	0.11^{aC}	0.32^{bB}	1.43^{aA}	2.16^{aA}	0.87^{bC}	11.6^{bE}
	HP	5.24^{cD}	0.70^{aC}	1.70^{aA}	0.12^{aCD}	0.37^{aB}	1.33^{cC}	1.89^{cA}	1.02^{aA}	11.4^{bD}
35	HIPEF	5.81^{aD}	0.62^{aD}	1.60^{aC}	0.11^{aCD}	0.37^{aB}	1.36^{aA}	2.07^{aA}	0.97^{aB}	12.0^{aD}
	MP	4.46^{bD}	0.38^{bD}	1.47^{bC}	0.09^{bD}	0.31^{bB}	1.38^{aB}	2.01^{aBC}	0.88^{bC}	10.1^{bF}
	HP	4.38^{bD}	0.64^{aCD}	1.63^{aB}	0.11^{aD}	0.37^{aB}	1.26^{bD}	1.85^{bC}	0.99^{aB}	10.3^{bE}
42	HIPEF	5.20^{aE}	0.60^{aD}	1.65^{aB}	0.11^{aCD}	0.38^{aB}	1.33^{aB}	2.01^{aAB}	0.99^{aB}	11.3^{aE}
	MP	3.09^{bE}	0.15^{bE}	1.47^{cC}	0.09^{bD}	0.28^{bC}	1.38^{bB}	2.04^{aB}	0.84^{cC}	8.5^{bG}
	HP	2.56^{cE}	0.56^{aD}	1.57^{bC}	0.11^{aD}	0.37^{aB}	1.30^{aC}	1.82^{bD}	0.96^{bC}	8.3^{bF}
49	HIPEF	2.71^{aF}	0.60^{aD}	1.57^{aC}	0.10^{aD}	0.36^{aC}	1.21^{aC}	1.90^{bC}	0.95^{aC}	9.8^{aF}
	MP	2.70^{aF}	nd	1.40^{cD}	0.08^{bD}	0.26^{bC}	1.33^{bC}	2.07^{aB}	0.80^{bD}	8.2^{bG}
	HP	2.43^{bF}	0.54^{bD}	1.50^{bD}	0.10^{aD}	0.36^{aB}	1.24^{aD}	1.83^{cC}	0.93^{aD}	8.1^{bG}
56	HIPEF	2.71^{aF}	0.60^{aD}	1.44^{aD}	0.09^{aD}	0.36^{aC}	1.03^{aD}	1.96^{aB}	0.91^{aD}	8.2^{aG}
	MP	2.70^{aF}	nd	1.34^{bE}	0.07^{bD}	0.25^{bC}	0.99^{aD}	1.99^{aC}	0.77^{cD}	7.3^{bH}
	HP	2.43^{bF}	0.52^{bD}	1.37^{bE}	0.09^{aD}	0.36^{aB}	0.85^{bE}	1.81^{bD}	0.87^{bE}	7.4^{bH}

HIPEF = high intensity pulsed electric fields at 35 kV/cm for 1000 µs; bipolar 4-µs pulses at 100 Hz;
ST: storage time (days); **HP**:90 ºC for 60 s; **MP**:90 ºC for 30 s; **Lyc**: lycopene; **Neu**: neurosporene; **γ-carot**: γ-carotene **ζ-carot**: ζ-carotene; **β-carot:** β-carotene; **Phytof**: phytofluene; **Phytoe:** phytoene; **nd**: not detected
[1]**TC**: Total carotenoids quantified by HPLC. The values are the result of the sum of each component (mg/100 ml fw)
[a]Different lower case letter in the same column for each day indicate significant differences among treatments($p<0.05$). Different capital letters in the same column for each treatment correspond to significant differences with time ($p<0.05$).

Effect of processing and storage on individual phenolic compounds

The initial total phenolic content of tomato juices ranged from 8.9 to 9.1 mg/100 mL fw (Table 2). In this way, Odriozola-Serrano et al. (2008) and Podsędek et al. (2003) reported a concentration of total phenolic between 26.8 to 52.3 mg/100 g in different processed tomato juices. The higher phenolic concentrations found in the above-mentioned studies compared to those obtained in the present work, could be attributed to the analytical method used to determine these compounds. The Folin-Ciocalteu reagent usually overestimates the content of phenolic compounds compared with the sum of the individual phenolics, since other reducing agents present in food, such as ascorbic acid can interfere (Martínez-Valverde et al., 2002). On the other hand, no significant differences in total phenolic content were observed among tomato juices just after processing. However, HIPEF-processed and mild pasteurized tomato juices showed higher total phenolic compounds throughout the storage period than those treated at 90 ºC for 60 s (Table 2). In accordance with our results, Dewanto et al. (2002) did not find significant changes in total phenolic content in either thermally treated or fresh tomato purees. As expected, chlorogenic acid was the main hydroxycinnamic acid derivative in tomato juices, obtained in concentrations of 4.4 mg/100 mL fw (Table 2). Chlorogenic acid was also found to be in greater concentrations over the time in tomato juices treated by mild and HIPEF processes than by high pasteurization. In addition, tomato juices underwent a substantial loss of chlorogenic acid from 4.4 to 3.5-3.8 mg/100 mL fw at 56 days of storage at 4ºC. Peroxidase is involved in the oxidative degradation of phenolic compounds (Amiot et al., 1997). Thus, the degradation of phenolic compounds during storage might be associated to the residual activity of peroxidase. It has been demonstrated that both thermal and HIPEF treatments could partially inhibit peroxidase in tomato juices. Aguiló-Aguayo et al. (2008b) reported residual peroxidase activities of 10% and 21% in tomato juices treated at 90 ºC for 60 s and 30 s, respectively. In this product, a reduction of 96.87% of the initial POD activity of tomato juice was also obtained after applying a HIPEF treatment similar to that used in the present study. Tomato juices have been found to be a rich source of flavonoids, obtaining as the main flavonols quercetin and kaempferol (Stewart et al., 2000). The initial concentrations of quercetin in the studied tomato juices were 1.98-2.05 mg /100 mL fw, whereas kaempferol was found at concentrations of 0.56-0.57 mg /100 mL fw just after processing. Stewart et al. (2000) observed that tomato juice had high levels of quercetin, which ranging from 2.8 to 3.7 mg/100 mL fw. In this way, Martínez-Valverde et al. (2002) reported concentrations of kaempferol between 0.12 and 0.21 mg/100 g for different commercial cultivars. Quercetin content of tomatoes varies according to fruit cultivar, country of origin, harvesting seasons and growing condition (Crozier et al., 1997). Quercetin and kaempferol content depleted significantly throughout the storage of tomato juices irrespective of the treatment conducted, thus reaching values of 1.32-1.64 mg/100 mL fw and 0.40-0.47 mg/100 mL fw, respectively (Table 2). HIPEF-treated tomato juices showed significantly greater quercetin content than thermally treated juices during storage for 56 days at 4 ºC.

Table 2.- Effects of high-intensity pulsed electric fields and heat pasteurization on phenolic compounds of tomato juice throughout storage at 4ºC

ST	process	Individual phenolic (mg/100 mL fw)						TP[1]
		chlorogenic acid	ferulic acid	p-coumaric acid	caffeic acid	quercetin	kaempferol	
0	Fresh	4.41^{aA}	0.88^{aA}	0.64^{aA}	0.43^{aA}	2.05^{aA}	0.57^{aA}	9.0^{aA}
	HIPEF	4.42^{aA}	0.86^{aA}	0.62^{a}	0.44^{aC}	2.01^{aA}	0.57^{aA}	8.9^{aA}
	MP	4.43^{aB}	0.89^{aAB}	0.64^{a}	0.43^{aD}	1.97^{aA}	0.56^{aA}	9.1^{aA}
	HP	4.44^{aA}	0.87^{aA}	0.62^{a}	0.45^{aB}	1.98^{aA}	0.57^{aA}	9.0^{aA}
7	Fresh	4.34^{bB}	0.82^{bB}	0.65^{aA}	0.43^{aA}	2.04^{aA}	0.46^{cB}	9.0^{aA}
	HIPEF	4.32^{bB}	0.87^{bA}	0.63^{a}	0.44^{aC}	1.85^{abB}	0.57^{aA}	8.7^{aB}
	MP	4.53^{aA}	0.92^{aA}	0.63^{a}	0.43^{aD}	1.67^{bC}	0.54^{bAB}	8.7^{aB}
	HP	4.16^{cB}	0.86^{bA}	0.58^{b}	0.45^{aB}	1.73^{bB}	0.54^{bB}	8.4^{bB}
14	Fresh	4.04^{cC}	0.77^{cC}	0.58^{bB}	0.44^{cA}	1.89^{aB}	0.46^{cB}	8.1^{bB}
	HIPEF	4.43^{abA}	0.85^{bA}	0.61^{a}	0.50^{aB}	1.72^{bC}	0.55^{aA}	8.5^{aB}
	MP	4.57^{aA}	0.90^{aA}	0.62^{a}	0.46^{bC}	1.62^{cC}	0.52^{bB}	8.7^{aB}
	HP	4.18^{bcB}	0.86^{bA}	0.57^{b}	0.47^{bAB}	1.63^{cC}	0.55^{aB}	8.3^{bB}
21	HIPEF	4.42^{aA}	0.80^{aB}	0.61^{a}	0.43^{aC}	1.77^{aC}	0.51^{aB}	8.5^{aC}
	MP	4.53^{aA}	0.85^{aB}	0.61^{a}	0.47^{aBC}	1.62^{bC}	0.52^{aB}	8.5^{aB}
	HP	4.09^{bBC}	0.88^{aA}	0.54^{b}	0.44^{aB}	1.60^{bC}	0.54^{aB}	8.0^{bC}
28	HIPEF	4.26^{aC}	0.83^{aB}	0.53^{b}	0.42^{bC}	1.67^{aD}	0.51^{aB}	8.2^{aD}
	MP	4.09^{bD}	0.86^{aB}	0.61^{a}	0.48^{aB}	1.67^{aC}	0.54^{aAB}	8.1^{aC}
	HP	4.13^{bB}	0.83^{aB}	0.51^{b}	0.43^{bB}	1.68^{aBC}	0.55^{aB}	8.2^{aBC}
35	HIPEF	4.21^{aC}	0.76^{aC}	0.52^{a}	0.52^{aB}	1.74^{aC}	0.50^{bB}	8.3^{aD}
	MP	4.26^{aC}	0.79^{aC}	0.52^{a}	0.50^{aB}	1.75^{aB}	0.50^{bB}	8.2^{aC}
	HP	4.12^{bB}	0.81^{aB}	0.50^{a}	0.50^{aA}	1.73^{aB}	0.57^{aA}	8.2^{aBC}
42	HIPEF	4.29^{aB}	0.79^{aBC}	0.49^{a}	0.57^{aA}	1.82^{aB}	0.52^{aB}	8.5^{aC}
	MP	4.10^{bD}	0.80^{aC}	0.49^{a}	0.55^{aA}	1.75^{bB}	0.51^{aB}	8.1^{bC}
	HP	4.08^{bBC}	0.77^{aC}	0.49^{a}	0.50^{bA}	1.62^{cC}	0.47^{bC}	8.1^{bBC}
49	HIPEF	4.10^{aD}	0.63^{bD}	0.51^{a}	0.55^{aA}	1.70^{aD}	0.48^{aC}	8.0^{aE}
	MP	3.84^{aE}	0.70^{aD}	0.52^{a}	0.54^{aA}	1.74^{aB}	0.42^{bC}	7.8^{bD}
	HP	3.92^{aC}	0.67^{aD}	0.51^{a}	0.51^{aA}	1.57^{bD}	0.44^{bC}	7.7^{bC}
56	HIPEF	3.78^{aE}	0.59^{aD}	0.34^{a}	0.57^{aA}	1.64^{aE}	0.47^{aC}	7.4^{aF}
	MP	3.74^{aE}	0.64^{aE}	0.32^{a}	0.55^{abA}	1.58^{bD}	0.40^{cC}	7.2^{aE}
	HP	3.49^{bD}	0.61^{aE}	0.34^{a}	0.51^{bA}	1.32^{cE}	0.43^{bC}	6.8^{bD}

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1000 µs; bipolar 4-µs pulses at 100 Hz
ST: storage time (days)**, HP**: 90 ºC for 60 s; **MP**: 90 ºC for 30 s;
[1]Total phenolic quantified by HPLC. The values are the result of the sum of each component (mg/100 mL fw
[a]Different lower case letter in the same column for each day indicate significant differences among treatments ($p<0.05$)
Different capital letters in the same column for each treatment correspond to significant differences with time ($p<0.05$)

Changes in minor phenolic acids such as ferulic acid (0.86-0.89 mg/100 mL fw), *p*-coumaric acid (0.62-0.64 mg /100 mL fw) and caffeic acid (0.43-0.45 mg /100 mL fw) are shown in Table 2. Tomato juices underwent a substantial depletion of *p*-coumaric acid during storage, since values of 0.32-0.34 mg/100mL fw at 56 days of cold storage, which may be a consequence of its conversion to caffeic acid. As can be seen in Table 2, the caffeic acid content was slightly enhanced during the storage time, regardless of the processing treatment, reaching maximal values (0.51-0.57 mg/100 mL fw) at 56 days. Hydroxylation of *p*-coumaric acid into caffeic acid takes place in food as a consequence of the introduction of a second hydroxyl group into *p*-coumaric acid, probably catalyzed by monophenol monooxygenases (Macheix et al., 1990). Therefore, the increase of caffeic acid in tomato juices after 28 days of storage may be directly associated with residual hydroxylase activities which convert *p*-coumaric acid in caffeic acid. Nevertheless, further research would be needed to know more about the effect of HIPEF treatments on this kind of enzymes.

Effect of processing and storage on quality attributes

The effects of HIPEF and heat treatments on lightness (L*) and hue angle (h*) of tomato juice as well as changes in these colour parameters during storage at 4 ºC are shown in Table 3. Fresh tomato juice was statistically brighter than processed juices, but exhibited higher hº than the treated. Changes in the CIELab parameters between HIPEF and heat treatments appeared to be significant. Generally, HIPEF treatments preserved better the colour of tomato juice than heat treatments (Min and Zhang, 2003). HIPEF-treated tomato juice showed significantly higher L* values compared to high pasteurized (90 ºC, 60 s) tomato juices through the storage period. However, no changes in L* values were observed between mild heat treated (90 ºC, 30 s) and HIPEF-processed juices (Table 3).

Table 3.- Effects of high-intensity pulsed electric fields and heat pasteurization on whiteness (L*) and hue angle (hº) of tomato juice throughout storage at 4ºC

ST	L*				hº			
	Fresh	HIPEF	MP	HP	Fresh	HIPEF	MP	HP
0	22.8^{aB}	22.6^{bD}	22.5^{bcE}	22.6^{bE}	38.1^{aA}	36.5^{bF}	37.1^{bE}	36.7^{bG}
7	22.7^{bB}	23.0^{aC}	22.5^{cE}	22.6^{cE}	37.2^{bcA}	36.6^{cF}	37.6^{bDE}	36.8^{cG}
14	23.1^{aA}	23.0^{bC}	22.9^{bD}	22.6^{cE}	37.7^{cA}	39.0^{aE}	38.6^{abCD}	38.2^{bcF}
21		23.3^{bB}	23.6^{aC}	22.9^{cD}		42.3^{aD}	38.6^{cCD}	39.9^{bE}
28		23.4^{abB}	23.7^{aBC}	23.1^{bC}		44.0^{aC}	38.9^{bC}	43.8^{aD}
35		23.7^{aA}	23.8^{aAB}	23.4^{bB}		47.5^{aB}	42.3^{bB}	46.3^{aC}
42		23.8^{aA}	23.9^{aA}	23.5^{bAB}		48.7^{aAB}	43.1^{bB}	48.2^{aB}
49		23.7^{aA}	23.9^{aA}	23.6^{aA}		49.8^{aAB}	43.3^{bB}	50.1^{aA}
56		24.0^{aA}	24.0^{aA}	23.6^{bA}		49.0^{aA}	45.0^{bA}	50.3^{aA}

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1000 μs; bipolar 4-μs pulses at 100 Hz
HP: 90 ºC for 60 s; **MP**: 90 ºC for 30 s; **ST**: storage time (days)
[a]Different lower case letter in the same row indicate significant differences among treatments ($p<0.05$).
Different capital letters in the same column correspond to significant differences with time ($p<0.05$).

A decrease in L* values in thermal treated juices is associated with the formation of dark colour compounds due to nonenzymatic browning reactions, thus reducing the acceptance of the juices (Klim and Nagy, 1988). Enzymatic browning of non-treated and HIPEF processed tomato juices may take place during storage, diminishing the brightness of juices. However, HIPEF treatment at 35 kV/cm for 1500µs using 4-µs bipolar pulses at 100 Hz has been shown to deplete a 97% of POD activity compared to fresh tomato juice (Aguiló-Aguayo, et al., 2008b). In this study, an increase in L* values was observed during the storage at 4 ºC irrespective of the treatment conducted. L* value rose in mild thermally and HIPEF processed tomato juice from 22.5-22.6 to 24 after 56 days at 4 ºC. At the same day, high thermally processed juice exhibited an L* value of 23.6 (Table 3). According to these results, Aguiló-Aguayo et al. (2008b) reported an enhancement of brightness in processed tomato juices after prolonged storage. In their study, statistically significant differences in colour parameters were also found between HIPEF and thermally treated tomato juice during storage at 4 ºC. The kind of treatment applied led to significant differences on hº during storage. Lower hº values were obtained in tomato juices treated by mild heat pasteurization compared to the HIPEF-treated and high thermally processed (Table 3). The hº values rose dramatically from 36-37º to 45-50º throughout the storage at 4 ºC in processed tomato juices. Thus, treated juices depleted their redness as storage time increased irrespective of the treatment applied.

Table 4.- Effects of high-intensity pulsed electric fields and heat pasteurization on pH and soluble solids content of tomato juice throughout storage at 4ºC.

ST	pH				soluble solids content (ºBrix)			
	Fresh	HIPEF	MP	HP	Fresh	HIPEF	MP	HP
0	4.38^{aA}	4.38^{aA}	4.38^{aA}	4.39^{aA}	5.8^{aA}	5.9^{aA}	5.9^{aAB}	5.9^{aA}
7	4.35^{bB}	4.38^{aA}	4.38^{aA}	4.38^{aA}	5.6^{bB}	5.9^{aA}	5.8^{aB}	5.9^{aA}
14	4.31^{bC}	4.38^{aA}	4.38^{aA}	4.38^{aA}	5.5^{cB}	5.9^{bA}	5.9^{aAB}	6.0^{bA}
21		4.37^{aB}	4.34^{aB}	4.38^{aA}		6.0^{aA}	6.0^{aA}	5.9^{aA}
28		4.36^{abB}	4.34^{bB}	4.38^{aA}		6.0^{aA}	6.0^{aA}	6.0^{aA}
35		4.36^{aB}	4.33^{aB}	4.37^{aAB}		6.1^{aA}	6.0^{aA}	5.9^{bA}
42		4.36^{aB}	4.33^{bB}	4.37^{aAB}		6.1^{aA}	5.8^{bB}	5.9^{bA}
49		4.36^{aB}	4.33^{aB}	4.35^{aB}		6.1^{aA}	5.8^{bB}	5.9^{bA}
56		4.34^{aC}	4.32^{aB}	4.35^{aB}		6.1^{aA}	5.8^{bB}	6.0^{aA}

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1000 µs; bipolar 4-µs pulses at 100 Hz

HP: 90 ºC for 60 s; **MP**: 90 ºC for 30 s; **ST**: storage time (days)

[a]Different lower case letter in the same row indicate significant differences among treatments ($p<0.05$). Different capital letters in the same column correspond to significant differences with time ($p<0.05$).

On the other hand, values of pH and soluble solids content for the different processed tomato juices are exhibited in Table 4. Processing had no significant effect on these physical properties of tomato juice. Little effect of HIPEF and thermal treatments on physical properties (pH and soluble solids) of orange juice was reported by Elez-Martínez et al. (2006). Yeom et al. (2000) observed that pH and soluble solids were neither affected by HIPEF (35 kV/cm for 59 μs) nor by heat treatment (94.6 ºC for 30 s). No significant differences in pH values were observed through the storage period among the treated juices (Table 4). Although statistical differences between treatments on soluble solids content were obtained, pH values for HIPEF and thermally treated tomato juices were similar throughout the storage (Table 4). pH values of thermally and HIPEF-pasteurized tomato juice were slightly decreased during the storage from 4.38 to 4.32-4.34 (at 56 days). This depletion in pH may be related to microbial spoilage growth. Microorganisms cause fruit juice spoilage by reduction of acidity and organic acid fermentation (Sodeko et al., 1987).

Correlation between health-related compounds and quality attributes

A principal components analysis (PCA) was performed on all samples and variables (total and individual carotenoids, total and individual phenolic compounds, pH, soluble solids and colour parameters) to obtain relationships among the studied parameters. Two principal components (PC1 and PC2) were obtained. They accounted for 74.17% of the variability in the original data (Figure 1). The statistical analysis for the data showed a correlation between carotenoids and hº and L* variables. It is noted a positive correlation between hº value and total carotenoids content of tomato juices, whereas a negative correlation was observed for lycopene or total carotenoids concentrations and L*. On the contrary, vitamin A did not appear to correlate adequately with colour parameters, whereas there was an outstanding correlation between this vitamin and pH. On the other hand, PC2 correlated well with soluble solids content, meaning that this quality attribute was positively related to β-carotene, but negatively associated to phytoene. Nevertheless, soluble solids were weakly related to total bioactive compounds, demonstrating that soluble solids value is a bad indicator of the nutritional quality of juices. The score plot of PC1 versus PC2 from the full-data PCA model plotted in Figure 2 describes differences between treatments and storage days. It can be observed that the majority of the samples stored for up to 28 days are situated in the left part of the score plot, whereas those samples preserved for over 28 days appear on the right-hand side. Therefore, total heath-related compounds content in tomato juice samples depleted as storage time increased. In addition, the plot allows to discriminate among differently processed tomato juices (Figure 2). HIPEF-processed and high heat pasteurized samples located in the upper part of the plot are well related with soluble solids content, pH, hº, vitamin A and β-carotene, whereas fresh and mild heat pasteurized samples scored the lowest values of these parameters as they were located at the bottom of the plot (Figure 2).

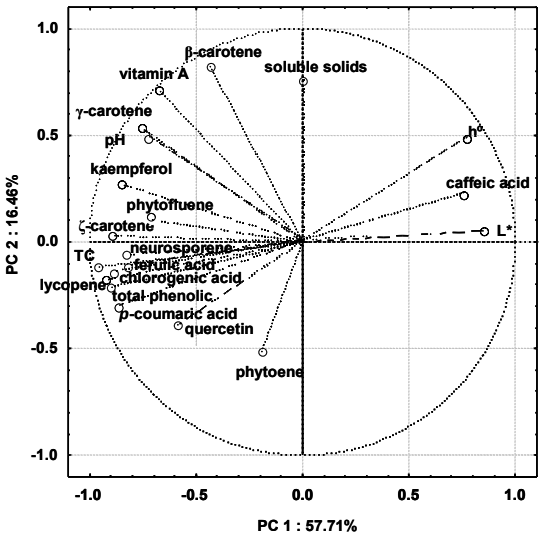

Figure 1.- Principal components plot of fresh, HIPEF-treated and heat pasteurized tomato juices stored for 56 days at 4 ºC. TC: total carotenoids

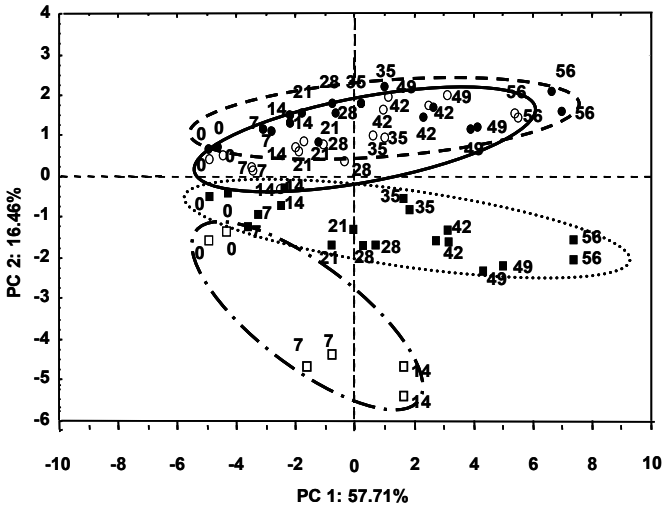

Figure 2.- Score plot of PC1 vs. PC2 of the samples for fresh and treated tomato juices stored during 56 days at 4 ºC. Tomato juices: □ untreated, ○ HIPEF treatment (35kV/cm for 1500 μs

in bipolar mode using 4-μs pulses at 100 Hz), ■ mild heat pasteurized (90 ºC for 30 s) and ● high heat pasteurized (90 ºC for 60 s). Numbers next to each marker stand for the storage day.

CONCLUSIONS

HIPEF-treated tomato juices maintained better nutritional value than those thermally pasteurized just after processing and during the storage period. HIPEF (35 kV/cm for 1500 μs with 4-μs bipolar pulses at 100 Hz) and thermal treatments (90 ºC-30 s and 90 ºC-60 s) led to tomato juices with higher total and individual carotenoids (lycopene, β-carotene and phytofluene) and redder colour than fresh juices, demonstrating that processing may improve not only quality attributes but also the antioxidant properties of juices. However, this enhancement in health-related compounds was greater in HIPEF-processed tomato juices than in those heat-treated. The amounts of individual health-related compounds in fresh and treated tomato juices underwent a substantial loss during storage, with the exception of β-carotene, phytoene and caffeic acid content. However, HIPEF-processed tomato juices better maintained over time the individual carotenoids (lycopene, neurosporene and γ-carotene) and quercetin than thermally-treated and untreated juices. Therefore HIPEF technology could be an alternative to thermal treatments in order to obtain tomato juices with high antioxidant properties.

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REFERENCES

Aguiló-Aguayo, I., Odriozola-Serrano, I, Quintão-Teixeira, L.J., Martín-Belloso, O. (2008a). Inactivation of tomato juice peroxidase by high-intensity pulsed electric field as affected by process conditions. *Food Chemistry*, 107, 949-955.

Aguiló-Aguayo, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008b). Comparative study on color, viscosity and related enzymes of tomato juice processed by pulsed electric fields or heat treatment. *European Food Research and Technology*, doi: 10.1007/s00217-007-0761-2.

Abushita, A.A., Daood, H.G., Biacs, P. (2000). Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *Journal of Agricultural and Food Chemistry*, 48, 2075-2081.

Amiot, M.J., Fleuriet, A., Cheynier, V., Nicolas, J. (1997). Phenolic compounds and oxidative mechanisms in fruits and vegetables. In Tomás-Barberán, F.A. Robins, R.J. Phytochemistry of fruit and vegetables. Science Publications, Oxford. (pp. 51-85).

Beecher, G.R. (1998). Nutrient content of tomatoes and tomato products. *Proceedings of the Society for Experimental Biology and Medicine*, 218, 98-100.

Braddock, R.J. (1999). Handbook of citrus by-products and processing technology. John Wiley & Sons, New York. pp. 53-83.

Britton, G., Hornero-Mendez, D. (1997). Carotenoids and colour in fruit and vegetables. In:. Tomas-Barberan, F.A., Robins, R. J. Phytochemistry of fruit and vegetables, Science Publications, Oxford. pp. 11–27.

Clinton, S.K. (1998). Lycopene: chemistry, biology, and implications for human health and disease. *Nutrition Reviews*, 56, 35-51.

Cortés, C., Esteve, M.J., Rodrigo, F., Torregrosa, F., Frígola, A. (2006). Changes of colour and carotenoids contents during high-intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology*, 44, 1932-1939.

Crozier, A., Lean, M.E.J., McDonald, M.S., Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery . *Journal of Agricultural and Food Chemistry*, 45, 590-595.

Dewanto, V., Wu, X., Adom, K., Liu, R. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing the total antioxidant activity. *Journal of Agricultural and Food Chemistry*, 50(10), 3010-3014.

Elez-Martínez, P., Soliva-Fortuny, R.C., Martín-Belloso, O. (2006). Comparative study on shelf-life of orange juice processed by high intensity pulsed electric fields or heat treatments. *European Food Research and Technology,* 222(3-4), 321-329.

Gross, J. (1991). Pigments in Vegetables. Chlorophylls and carotenoids. Van Nostrand Reinold, New York.

Hertog, M., Hollman, P., Venema, D. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, 40, 1591-1598

Khachik, F., Goli, M.B., Beecher, G., Holden, J., Lusby, W.R., Tenorio, M.D., Barrera, M.R. (1992). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *Journal of Agricultural and Food Chemistry*, 40, 390-398.

Khachik, F., Carvalho, L., Bernstein, P.S., Muir, G.J., Zhao, D.Y., Katz, N.B. (2002). Chemistry, distribution, and metabolism of total carotenoids and their impact on human health. *Experimental Biology and Medicine,* 227, 845-851.

Kidmose, U., Edelenbos, M., Nøbæk, R., Christensen, L. P. (2002).Colour stability in vegetables. In: MacDougall, D.B. Colour in Food: Improving quality. CRC Press, Boca Raton, Florida. pp. 179- 232.

Klim, M., Nagy, S. (1988). An improved method to determine nonenzymic browning in citrus juices. *Journal of Agricultural and Food Chemistry*, 36, 1271–1274.

Macheix, J.J., Fleuriet, A., Billot, J. (1990). Fruit phenolics. CRC Press, Boca Raton, Florida.

Marsellés-Fontanet, A. R., Martín-Belloso, O. (2007). Optimization and validation of PEF processing conditions to inactivate oxidative enzymes of grape juice. *Journal of Food Engineering*, 83, 452-462.

Martínez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant capacity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture*, 82, 323-330.

Min, S., Zhang, Q.H. (2003). Effects of Commercial-scale pulsed electric field processing on flavour and color of tomato juice. *Food Chemistry and Toxicology*, 68, 1600-1606.

Nagy, S., Chen, C.S., Shaw, P.E. (1993). *Fruit juice processing technology*. Florida: Auburndale, Agscience.

Nguyen, M.L., Schwartz, S.J. (1999). Lycopene: chemical and biological properties. *Food Technology*, 53(2), 38-45.

Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., Gimeno-Añó, V., Martín-Belloso, O. (2007). Lycopene, vitamin C, and antioxidant capacity of tomato juice as affected by high-intensity pulsed electric fields critical parameters. *Journal of Agricultural and Food Chemistry*, 55, 9036-9042.

Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. *Innovative Food Science and Emerging Technologies,* 9, 272-279.

Podsędek, A., Sosnowska, D., Anders, B. (2003). Antioxidative capacity of tomato products. *European Food Research and Technology*, 217(4), 296-300.

Ramakrishnan, T.V., Francis, F.J. (1980). Autooxidation of carotenoids and their relative polarity. *Journal of Food Quality*, 3, 25-34.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P. Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, 66, 401-436.

Rodriguez-Amaya, D.B. (1997). Carotenoids and food preservation: the retention of provitamin A carotenoids in prepared, processed and stored food. USAID, OMNI Project.

Sánchez-Moreno, C., Cano, M.P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F., Elez-Martínez, P., Martín-Belloso, O., Martín, A. (2005). Intake of mediterranian soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *International Journal of Food Sciences and Nutrition*, 56, 115-124.

Shi, J., Le Maguer, M. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition*, 40(1), 1-42.

Sodeko, O.O., Izuagde, Y.S., Ukhun, M.E. (1987). Effect of different preservative treatments on the microbial population of Nigerian orange juice. *Microbios*, 51, 133-143.

Stewart, A.J., Bozonnet, S., Mullen, W., Jenkins, G.I., Lean, M.E.J., Crozier, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *Journal of Agricultural and Food Chemistry*, 48, 2663-2669.

Tonucci, L.H., Holden, J.M., Beecher, G.R., Khachik, F., Davis, C.S., Mulokozi, G. (1995). Carotenoid content of thermally processed tomato-based food products. *Journal of Agricultural and Food Chemistry*, 43, 579-586.

Trumbo, P.R., Yates, A.A., Schlicker-Renfro, S., Suitor C. (2003). Dietary Reference Intakes: revised nutritional equivalents for folate, vitamin E and provitamin A carotenoids. *Journal of Food Composition and Analysis*, 16, 379-382.

Torregrosa, F., Cortés, C., Esteve, M.J., Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry*, 53, 9519-9525.

Yeom, H.W., Streaker C.B., Zhang, Q.H., Min, D.B. (2000). Effects of Pulsed Electric Fields on the Quality of orange juice and comparison with hat pasteurization. *Journal of Agricultural and Food Chemistry*, 48, 4597-4605.

Capítulo V

Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice

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ABSTRACT

A response surface methodology was used to determine the combined effect of HIPEF critical processing conditions on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. Keeping constant the electric field strength at 35 kV/cm and the treatment time at 1000 μs, the treatments were set at frequencies from 50 to 250 Hz, pulse width from 1 to 7 μs using monopolar or bipolar mode. A second order response function covering the whole range of experimental conditions was obtained for each health-related compound. Strawberry juice antioxidant potential was affected linearly by frequency, pulse width and pulse polarity. The quadratic term of frequency and the combined effect of frequency and pulse width were also significant. HIPEF treatments conducted at 232 Hz with bipolar pulses of 1-μs led to strawberry juices with the greatest presence of health-related compounds. The evaluation of the HIPEF critical parameters influence on health-related compounds can contribute to achieve optimal processing conditions to obtain strawberry juices with high antioxidant potential.

Key words: high intensity pulsed electric field, strawberry juice, vitamin C, anthocyanins, antioxidant capacity.

INTRODUCTION

Citrus and apple juices have dominated the fruit juice market for many years, but within the past decade, new types of fruit juice products, including strawberry juices, have come onto the market. Strawberry juice is a good source of health related compounds such as anthocyanins and vitamin C, thus having high antioxidant activities (Cordenunsi, et al., 2005). Anthocyanins are a widespread group of plant phenolic compounds which have been regarded as a natural alternative to replace synthetic food colorants. Recently, increased attention has been given to their potential health benefits in preventing heart diseases and cancers due to their powerful antioxidant properties (Hannum, 2004). On the other hand, vitamin C is commonly recognized as a major, naturally occurring antioxidant in diet with protective effects against various oxidative stress-related diseases (Omaye and Zhang, 1998).

Heat is the most widely used technology for preserving and extending the shelf-life of juices. However, it has been demonstrated that heat degrades anthocyanins and vitamin C, diminishing the nutritional quality and the antioxidant properties of juices (Klopotek et al., 2005). Processed fruits and vegetables have been considered to have lower nutritional value than their respective fresh commodities due to the loss of antioxidant compounds during processing (Múrcia et al., 2000). The increasing demand for fresh-like products has promoted the effort for developing innovative non thermal food preservation methods. High intensity pulsed electric fields (HIPEF) is a non-thermal technology extensively studied as an alternative to thermal treatments (Elez-Martínez, et al., 2006; Cortés, et al., 2008). High microbial destruction levels and little losses of flavor, color, taste or nutrients have evinced the suitability of HIPEF technology to obtain high quality fresh-like foods (Mertens and Knorr, 1992). Up to now, the studies evaluating the effects of HIPEF processing conditions on juices have demonstrated that electric field strength, pulse width, pulse frequency, pulse polarity, treatment time or pulse shape are among the most important HIPEF processing parameters affecting microbial and enzymatic inactivation (Elez-Martínez, et al., 2004, 2006; Marsellés-Fontanet and Martín-Belloso, 2007). Nevertheless, research studying the effect of HIPEF treatment parameters on health-related compounds of juices is scarce and generally focused on vitamin C. In addition, most of these studies have only evaluated the influence of electric field strength and treatment time in the antioxidant potential of juices (Torregrosa et al., 2005; Torregrosa, et al., 2006).

Therefore, the aim of this research was to study whether vitamin C, anthocyanins, and antioxidant capacity of strawberry juice are affected by HIPEF parameters in terms of frequency, pulse width and polarity mode and to asses the HIPEF processing conditions to achieve the highest health-related compounds content in strawberry juice.

MATERIALS AND METHODS

Sample preparation

Strawberry fruits (*Fragaria ananassa* Duch, cultivar Camarosa) were purchased from a local supermarket (Lleida, Spain). The fruits were washed, drained and chopped. Then, the squeezed strawberry juice was centrifuged at 24.000×g for 15 min and the supernatant was filtered using a steel sieve with an approximate mesh of 2 mm.

Pulsed electric fields processing device

HIPEF treatments were performed using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that holds monopolar and bipolar squared wave pulses. The treatment flow rate was 60 mL/min and it was controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). The treatment chamber device consisted of eight co-linear chambers disposed in series, each one containing two stainless steel electrodes separated by a gap of 0.29 cm with a treatment volume of 0.012 cm^3. The treatment temperature was kept below 40 ºC, using a cooling coil, which was connected between each pair of chambers and submerged in an iced water bath. Thermocouples were attached to the surface of the stainless-steel coils, 2.5 cm away from the HIPEF zones along the flow direction. The thermocouples were connected to temperature readers and isolated from the atmosphere with an insulation tape. The temperatures of the inlet and outlet of each pair of chambers were recorded every 0.1 s. The characteristics of the electric pulses delivered such as shape, polarity, width, difference of potential as well as the electric current generated across the electrodes and the pulse frequency were monitored using a digital oscilloscope (THS720, Tektronix Inc., Beaverton, OR, USA).

Strawberry juice was treated using pulses of 35 kV/cm for 1000 μs applied in monopolar or bipolar mode at frequencies ranging from 50 to 250 Hz and pulse width between 1 and 7 μs.

Health-related compounds

Vitamin C

Vitamin C in strawberry juice was determined following the validated method proposed by Odriozola-Serrano et al. (2007a). A sample of 25 mL of strawberry juice was mixed with 25 mL of a solution containing 45 g/L of metaphosphoric acid and 7.2 g/L of DTT. The mixture was centrifuged at 22100xg for 15 min at 4 ºC and the supernatant was vacuum-filtered through Whatman No. 1 paper. The sample was then passed through a Millipore 0.45 μm membrane into an opaque vial and kept at -42 ºC until analysis. An aliquot of 20 μL was injected into the HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm x 250 cm). The mobile phase was a 0.01% sulphuric acid solution adjusted to pH=2.6. The flow rate was fixed at 1 mL/min at room temperature. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Vitamin C was quantified throughout a calibration curve built with ascorbic acid pure standards and results were expressed as relative vitamin C concentration compared to the untreated sample.

Anthocyanins

The anthocyanins content of strawberry juice was determined with a modified pH differential method described by Meyers et al. (2003), using two buffer systems: potassium chloride 0.025 M at pH 1, and sodium acetate 0.4 M at pH 4.5. Briefly, 5 ml of strawberry juice was transferred to a 50 mL volumetric flask and made up with each buffer. The absorbance of the mixtures at pH 1 and 4.5 was then measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 510 and 700 nm against distilled water blank. The anthocyanins content was calculated according to Equation 1 and expressed as pelargonidin-3-glucoside per litre:

$$TA = \frac{\left[\left(A_{510} - A_{700}\right)_{pH1.0} - \left(A_{510} - A_{700}\right)_{pH\,4.5}\right] \times MW \times DF \times 1000}{\varepsilon \times L} \quad (1)$$

where MW is the molecular weight of pelargonidin-3-glucoside (433.0 g/mol), DF is the dilution factor, L is the pathlength in cm and ε is the molar extinction coefficient for pelargonidin-3-glucoside (22400 L/mol·cm). Results were expressed as pelargonidin-3-glucoside content compared to the untreated sample.

Antioxidant capacity

Several methods have been used to evaluate the antioxidant profile of food products and results may greatly vary depending on the experimental conditions and the specificity of the free radical used (Cao et al., 1993). In this work, the determination of the free-radical scavenging capacity was evaluated with the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by (Odriozola-Serrano et al., 2007b). The DPPH method has been proposed as an easy and accurate method for measuring the antioxidant activity of juice samples (Gil et al., 2000). In addition, DPPH assay is not specific to any particular antioxidant component, thus applying to the overall antioxidant capacity of the sample. Samples of strawberry juice were centrifuged at 6000xg for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and 0.01 mL of the supernatant was added to 3.9 mL of methanolic DPPH solution (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 60 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm against a blank of methanol without DPPH. Results were expressed as antioxidant capacity related to the untreated sample.

Experimental Design

A face-centred central composite response surface analysis was used to determine the effect of frequency (*f*), pulse width (τ) and polarity mode (*p*) on the antioxidant potential (anthocyanins, vitamin C and antioxidant capacity) of strawberry juice. The independent variables were pulse frequency (from 50 to 250 Hz), pulse width (from 1 to 7 μs) and polarity mode (monopolar or bipolar). Samples were treated at 35 kV/cm for 1000 μs irrespective of the frequency, pulse width and pulse mode applied. The levels for each independent parameter were chosen considering sample and equipment limitations. Three (maximum, minimum and central) values of each factor were considered, leading 26 experiments (Table 1).

Table 1.- Experimental central composite design for anthocyanins, vitamin C and antioxidant capacity of strawberry juice treated by different combinations of high-intensity pulsed electric field variables treatment

PARAMETERS				VARIABLES		
Assay nº.[1]	*f* (Hz)	τ (μs)	p	VCR (%)[2]	AR (%)[2]	AC (%)[2]
1	50	1	Monopolar	99.3 ± 0.2	83.2 ± 0.6	75.0 ± 0.7
2	50	7	Monopolar	98.4 ± 0.1	94.9 ± 0.8	82.2 ± 0.8
3	250	1	Monopolar	98.8 ± 0.6	99.3 ± 1.1	95.7 ± 0.3
4	250	7	Monopolar	95.6 ± 0.2	90.1 ± 0.8	75.1 ± 1.1
5	50	4	Monopolar	99.3 ± 0.3	88.0 ± 0.2	79.3 ± 0.9
6	150	1	Monopolar	99.1 ± 0.2	86.7 ± 1.2	95.6 ± 1.1
7	150	7	Monopolar	95.5 ± 0.5	96.4 ± 0.6	99.0 ± 0.3
8	250	4	Monopolar	97.3 ± 0.4	96.8 ± 0.7	88.5 ± 0.7
9	150	4	Monopolar	98.0 ± 0.5[3]	95.4 ± 3.1[3]	94.1 ± 2.0[3]
10	50	1	Bipolar	100.3 ± 0.2	85.7 ± 0.3	77.1 ± 1.4
11	50	7	Bipolar	97.6 ± 0.3	98.3 ± 0.4	85.6 ± 0.1
12	250	1	Bipolar	98.2 ± 0.3	101.9 ± 1.0	99.8 ± 0.6
13	250	7	Bipolar	91.5 ± 0.2	94.8 ± 0.3	77.6 ± 1.0
14	50	4	Bipolar	99.3 ± 0.3	90.1 ± 0.8	81.6 ± 0.5
15	150	1	Bipolar	98.5 ± 0.6	99.0 ± 0.2	97.9 ± 0.3
16	150	7	Bipolar	95.1 ± 0.5	102.1 ± 1.5	99.8 ± 0.9
17	250	4	Bipolar	97.9 ± 0.3	97.8 ± 0.9	90.7 ± 0.4
18	150	4	Bipolar	97.0 ± 0.6[3]	99.2 ± 2.6[3]	99.3 ± 1.4[3]

[1] Order the assays was randomized, and HIPEF treatment was set up at 35kV/cm for 1000 μs.
[2]Data shown are the mean ± SD of 2 treatment repetitions, each assay was performed in triplicate;
[3]Data shown are the mean of 5 repetitions
f: frequency; **τ**: pulse width; **p**: polarity mode
VCR: vitamin C retention, **AR**: anthocyanins retention, **AC**: antioxidant capacity retention

The experimental design was performed twice, resulting in two blocks of experiments. The order of assays within each block was randomized and performed in triplicate. Experimental data were fitted to a polynomial response surface. The second-order response function was predicted by Equation 2:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_1 + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{i=i+1}^{3} \beta_{ij} X_i X_j \qquad (2)$$

where Y is the dependent variable, β_0 is the centre point of the system, β_i, β_{i1} and β_{ij} represent the coefficients of the linear, quadratic and interactive effect, respectively; X_i, X_i^2 and X_iX_j represent the linear, quadratic and interactive effect of the independent variables, respectively. The non significant terms were deleted from the second-order polynomial model after an ANOVA test, and a new ANOVA was performed to obtain the coefficients of the final equation for better accuracy. Design Expert 6.0.1 software (Stat Ease Inc., Minneapolis, Minn.) was used to generate quadratic models that fit the experimental data and to draw the response

surface plots. The optimization was done following the method proposed by Derringer and Suich (1980). All the individual desirability functions obtained for each response were combined into an overall expression, which is defined as the geometrical mean of the individual functions. The nearer the desirability value to the unit, the more adequate the system is (Ross, 1996). In the present study, desirability functions were developed in order to obtain strawberry juice with the highest levels of health-related compounds.

RESULTS AND DISCUSSION

Effect of pulse frequency, width and polarity on vitamin C

The vitamin C content of the untreated strawberry juice was 49.5 mg/100g. Table 1 shows the results for vitamin C retention obtained under each experimental condition. The results are in the range of those reported by Torregrosa et al. (2006) for orange carrot juice treated at different HIPEF electric field strengths (25, 30, 35 and 40 kV/cm) for different treatment times (from 30 to 340 μs) using 2.5-μs bipolar pulses. In contrast, Odriozola-Serrano et al. (2007b) obtained vitamin C retention of tomato juice between 58.2 and 99.0% after applying the same HIPEF conditions to those reported in the present study. These differences in vitamin C retention between HIPEF-treated juices may be due to the lower pH of strawberry juice in comparison to tomato juice, as more acidic conditions are known to stabilize vitamin C (Tannenbaum et al., 1985). The statistical analysis indicates that the quadratic model proposed for vitamin C was adequate ($P < 0.0001$) with satisfactory determination coefficients ($R^2 = 0.83$) (Table 2). No significant lack of fit of the model was found, showing that it fits properly for prediction within the range of assayed conditions.

Table 2.- Analysis of variance of the second-order models for vitamin C, anthocyanins and antioxidant capacity

	F-value		
Source[a]	**Vitamin C**	**Anthocyanins**	**Antioxidant Capacity**
Quadratic model	16.1**	10.0**	45.2**
f	33.4**	19.5*	33.6**
τ	66.4**	5.3*	7.2*
p	7.4*	14.4*	7.2*
f^2	4.4*	8.6*	192**
τ^2	4.3	0.21	0.54
$f \cdot \tau$	9.5*	29.2**	79.0**
$f \cdot p$	2.3	0.01	0.02
$\tau \cdot p$	3.8	0.11	0.05
Lack of fit	2.7	0.87	2.4
Std dev.	0.73	2.7	2.3
Mean	97.5	95.3	91.6
Coefficient of variation	0.75	2.8	2.5
R-squared	0.8837	0.8249	0.9551
Adj R-squared	0.8289	0.7424	0.9340

[a] f= frequency, τ= pulse width, p= polarity

*Significant at $p < 0.05$; **Significant at $p < 0.0001$

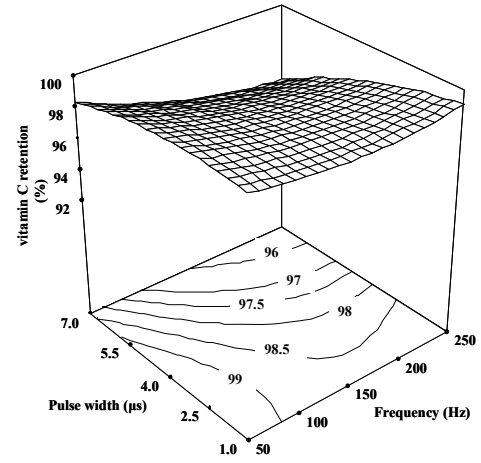
Table 3.- Significant regression coefficients of the quadratic model for anthocyanins, vitamin C and antioxidant capacity retention of HIPEF-treated strawberry juice

Source[a]	Coefficient estimate[b]					
	Vitamin C		Anthocyanins		Antioxidant Capacity	
	Monopolar	Bipolar	Monopolar	Bipolar	Monopolar	Bipolar
Intercept value	101.2 ± 0.4	100.4 ± 0.5	68.8 ± 1.0	72.9 ± 1.6	47.2 ± 0.9	49.6 ± 1.3
f	$-1.4\times10^{-2} \pm 5.8\times10^{-3}$		$2.1\times10^{-1} \pm 1.7\times10^{-2}$		$5.6\times10^{-1} \pm 1.5\times10^{-2}$	
τ	$-1.7\times10^{-1} \pm 3.4\times10^{-2}$		3.3 ± 0.1		3.1 ± 0.1	
f^2	$4.1\times10^{-5} \pm 1.6\times10^{-5}$		$-3.3\times10^{-4} \pm 4.9\times10^{-5}$		$-1.4\times10^{-3} \pm 4.3\times10^{-5}$	
$f\cdot\tau$	$-2.6\times10^{-3} \pm 5.0\times10^{-4}$		$-1.7\times10^{-2} \pm 1.5\times10^{-3}$		$-2.4\times10^{-2} \pm 1.3\times10^{-4}$	

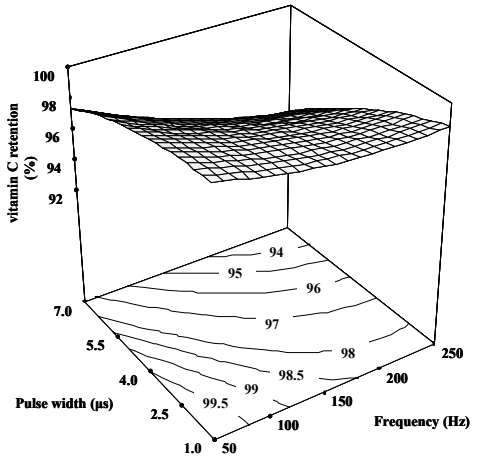
[a]f= frequency, τ = pulse width,
[b]Values ± confidence intervals (P = 0.05).

Since pulse polarity was a categorical factor, vitamin C retention was modeled separately regarding monopolar or bipolar mode (Table 3). The polarity in the application of pulses had a significant effect on vitamin C content of strawberry juices ($P < 0.05$). Bipolar treatments resulted in lower vitamin C contents than monopolar pulses, except for HIPEF treatments carried out at 1-μs where non significant differences in vitamin C were observed between polarity modes irrespective of the frequency applied. Namely, strawberry juices had 2.3% less vitamin C retention in bipolar than in monopolar mode for HIPEF treatment set up at 35 kV/cm for 1000 μs at 250 Hz and 7-μs pulse width (Figure 1A and B). Consistently, Odriozola-Serrano et al. (2007b) reported higher effect on vitamin C retention in bipolar than in monopolar mode, when tomato juice was processed at similar HIPEF conditions. Among HIPEF processing variables, frequency and pulse width have significantly influenced vitamin C retention ($P < 0.0001$). In this way, Elez-Martínez and Martín-Belloso (2007) reported that vitamin C retention significantly depended on pulse frequency and pulse width during HIPEF-processing of orange juice and "gazpacho", a cold vegetable soup. The lineal term of frequency is negative; thus the higher the frequency the lower the vitamin C content. Nevertheless, the positive value of the quadratic term ($P < 0.05$) of frequency indicated that vitamin C retention reached a minimum as the frequency rose. In fact, increasing the frequency beyond 200 Hz (monopolar) and 175 Hz (bipolar) led to strawberry juices with decreasing vitamin C retentions than using lower frequencies (Figure 1A and B). The high pulse width coefficient in the fitted model indicates that this parameter is more relevant for vitamin C retention than frequency. However, the expected behavior of pulse width was modified by the negative coefficient of the pulse width interaction with frequency. Strawberry juice showed low vitamin C retention when HIPEF treatments combining high frequencies and pulse widths were applied irrespective of the polarity mode. Nevertheless, the effect of mutual influence of frequency and pulse width on vitamin C followed a nonlinear curve (Figure 1). Therefore, different combinations of frequency and pulse width may lead to the same vitamin C levels. In this way, a 98% vitamin C retention was observed with either 7-μs at 75 Hz or 3-μs at 250 Hz, when the HIPEF treatments were carried out at 35 kV/cm for 1000 μs in monopolar mode. Considering the application of pulses in bipolar mode, the same vitamin C retention (98%) was obtained in strawberry juices

processed by 6-μs at 50 Hz or 2-μs at 250 Hz (Figure 1). Vitamin C is a heat-sensitive health-related compound in the presence of oxygen (Davey et al., 2000). Thus, high temperatures during processing can greatly affect the rates of its degradation. Although the maximum temperature reached during HIPEF treatment of samples was 40 ºC, when the HIPEF-processing parameters increased in severity, vitamin C was lower. In general, the greater the frequency and pulse width, the higher the HIPEF treatment temperature was. High temperature led to a loss of vitamin C because heat is known to speed the oxidation process of ascorbic acid (Gahler et al., 2003).



(a)



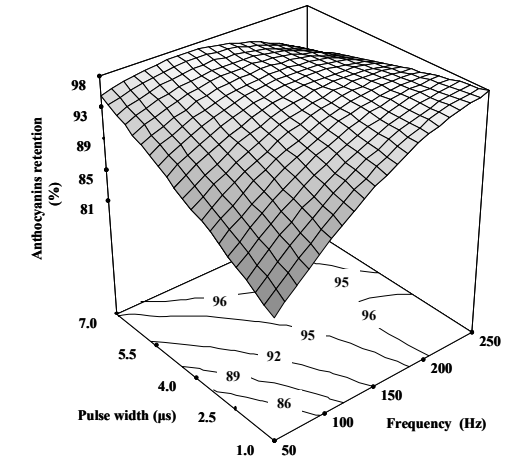
(b)

Figure 1.- Effect of pulse frequency and pulse width on vitamin C content of strawberry juice when high-intensity pulse electric field (HIEPF) treatment was set up at 35 kV/cm for 1000 μs in monopolar (a) or bipolar (b) mode.

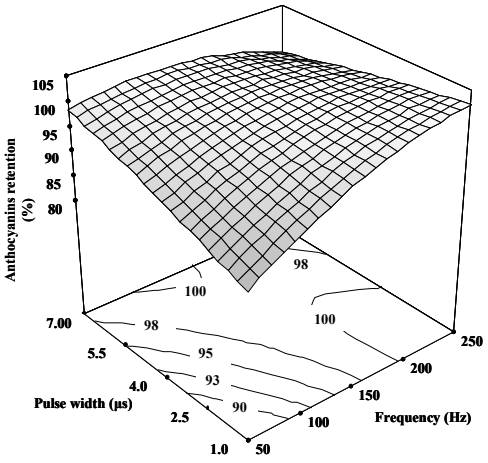
Effect of pulse frequency, width and polarity on anthocyanins

Strawberry color is due to the presence of anthocyanins (Zabetakis et al., 2000). The concentration of anthocyanins in fresh strawberry juice was 28.2 mg/100g. The anthocyanins content, expressed as relative retention, ranged between 83% and 102% in HIPEF-processed juice treated under the studied experimental conditions (Table 1). Klopotek et al. (2005) reported that pasteurization (85 ºC, 5 min) led to a 27% decrease in strawberry juice anthocyanins. The stability of anthocyanins is influenced by pH, storage temperature, presence of enzymes, light and processing conditions (Rein and Heinonen, 2004). In a fresh juice, an equilibrium exists between four anthocyanin species, including quinonoidal base, the flavylium cation, the pseudobase or carbitol, and the chalcone. Unless, structural features or others factors are successful in reversing equilibrium towards the formation of the flavylium or quinonoidal base, formation of chalcones (colorless) is favored by increasing temperature at the expense of quinoidal, flavylium and carbitol species (Brouillard, 1982). In addition, chalcones can be degraded to brown-colored polymeric structures when keeping high temperature for excessive time (Jackman and Smith, 1997). Zhang et al. (2007) observed that after processing a cyanidin-3-glucoside methanolic solution by PEF (at 1.2, 2.2 and 3.0 kV/cm, 300 number of pulses, $T^a \leq 47$ ºC), the anthocyanin was degraded and the formation of the colorless anthocyanin species, particularly chalcones took place.

The analysis of variance revealed that a second-order model ($P < 0.0001$) fits well the anthocyanins results. The determination coefficient (R^2) was 0.74 and the lack-of-fit was not significant (Table 2), indicating that the model was sufficiently accurate for predicting the response across the design space. Pulse polarity was considered a significant ($P < 0.05$) categorical factor. As a result, anthocyanins retention of HIPEF-processed strawberry juice can be predicted through two different polynomial equations in terms of the significant HIPEF parameters, concerning monopolar or bipolar mode (Table 3). As can be seen in Figure 2, bipolar treatments resulted in higher anthocyanin concentrations than monopolar treatments. For instance, a difference of over 4% in anthocyanins content was observed between monopolar and bipolar modes when HIPEF treatment set up at 150 Hz and 4-μs (Figure 2A and B). Frequency had also a significant effect ($P < 0.05$) on the anthocyanins content of strawberry juice. The negative value of its quadratic term in both modes indicates that anthocyanins retention reached a maximum value within the range of applied frequencies. Figure 2B shows a region between 100 and 250 Hz, where HIPEF-processed strawberry juice kept all its initial anthocyanins content. Nevertheless, the influence of frequency in anthocyanin retention depended on the pulse width used ($P < 0.0001$). Considering the application of pulses in monopolar mode, strawberry juice anthocyanins at 50 Hz increased quickly from 80 to 95% when pulse width rose from 1 to 7-μs. In contrast, when frequency was set up at 250 Hz, anthocyanins retention decreased from 98 to 91% after increasing pulse width from 1 to 7-μs (Figure 2A). On the other hand, the interaction between frequency and pulse width allowed combinations of these HIPEF variables that maintain the initial content of anthocyanins in strawberry juice. As can be seen in Figure 2B (bipolar mode) no changes in anthocyanins concentration of strawberry juice were induced when pulse widths lower than 4-μs with frequencies between 175 and 250 Hz were applied. The same results were observed using bipolar pulses above 6-μs and frequencies between 100 and 175 Hz.



(a)



(b)

Figure 2.- Effect of pulse frequency and pulse width on anthocyanins content of strawberry juice when high-intensity pulse electric field (HIEPF) treatment was set up at 35 kV/cm for 1000 µs in monopolar (a) or bipolar (b) mode.

To explain changes of anthocyanins in a single factor is difficult due to the complexity of chemical reactions taking place in natural systems. Although high treatment temperatures can decolorize and degrade anthocyanins, other factors such as ascorbic acid, metal ions, enzymes, light, time and oxygen during processing can accelerate anthocyanins pigment destruction (Wrolstad, 2000). As can be seen in Figure 2, strawberry juices treated at low frequencies and

narrow pulse widths showed the minimum concentration of anthocyanins. This depletion in anthocyanins may be due, not only to the HIPEF process, but also to the high levels of vitamin C they contained (Figure 1). When ascorbic acid is oxidized in the presence of oxygen to dehydroascorbic acid a molecule of hydrogen peroxide is formed (Markakis, 1982). It has been demonstrated that this hydrogen peroxide can react with anthocyanins producing colorless products (Jackman & Smith, 1997). On the other hand, β-glucosidase and peroxidase are the main enzymes implicated in anthocyanins degradation (Wrolstad, 2000). The anthocyanin destruction after low electric field strength HIPEF treatments might be also due to the low enzymatic inactivation levels reached. A residual β-glucosidase activity of 113% of strawberry juice was obtained after applying a HIPEF treatment at 50 Hz with monopolar 1-μs pulses at 35 kV/cm for 1000 μs (Aguiló-Aguayo et al., 2008).

Effect of pulse frequency, width and polarity on antioxidant capacity

Antioxidant capacities of strawberry juice were measured on the basis of the DPPH stable radical. Antioxidant capacity of fresh strawberry juice was 38.5% of DPPH inhibition. HIPEF-treated strawberry juice showed an antioxidant capacity retention between 75% and 100% (Table 1). The maximum retention was achieved with HIPEF at 35 kV/cm for 1000 μs with 1-μs bipolar pulses at 250 Hz. In contrast, HIPEF treatments of 1-μs monopolar pulses at 50 Hz led to the least retention of antioxidant capacity.

The regression analysis test revealed that a second-order model fits well the antioxidant capacity data (Table 2). The determination coefficient, R^2, was 0.93, and the lack-of-fit was not significant, indicating that the model is accurate enough for predicting the response. Provided that polarity is a categorical variable, the antioxidant capacity of HIPEF-treated strawberry juice was modeled throughout two different polynomial equations in terms of the significant HIPEF parameters (Table 3). Strawberry juices treated with bipolar pulses showed greater antioxidant capacity than those processed using monopolar pulses. The antioxidant capacity of strawberry juice treated at 150 Hz with 4-μs bipolar pulses was 97%, whereas applying the HIPEF treatment in monopolar mode strawberry juice had an antioxidant capacity of 95%. Previous results by Odriozola-Serrano et al. (2007b) in tomato juice are consistent with the fact that antioxidant capacity is better maintained in bipolar than in monopolar mode. In contrast, Elez-Martínez and Martín-Belloso (2007) reported non significant differences in orange juice and "gazpacho" antioxidant capacities, processed under similar conditions to those reported in the present study. Frequency was the most important variable ($P < 0.0001$) affecting antioxidant capacity retention in HIPEF-treated strawberry juice, whereas pulse width also had a significant effect on antioxidant capacity. The coefficients of both HIPEF parameters are positive (Table 3), meaning that an increase in frequency or pulse width results in a lower antioxidant capacity. A rise in antioxidant capacity retention was observed when frequency was increased between 50 and 150 Hz regardless of the polarity mode, although the antioxidant capacity might decrease if frequency increases further (Figure 3). Therefore, the expected behavior of frequency and pulse width was modified by either the negative quadratic terms of frequency or the interaction of frequency with pulse width. Different combinations of applied frequency and pulse width may lead to the same antioxidant capacity of strawberry juice. Strawberry juice was totally retained combining frequencies between 180-205 Hz with monopolar pulses below 1.1-μs, and frequencies from 145-240 Hz with bipolar pulses below 3.7-μs (Figure 3).

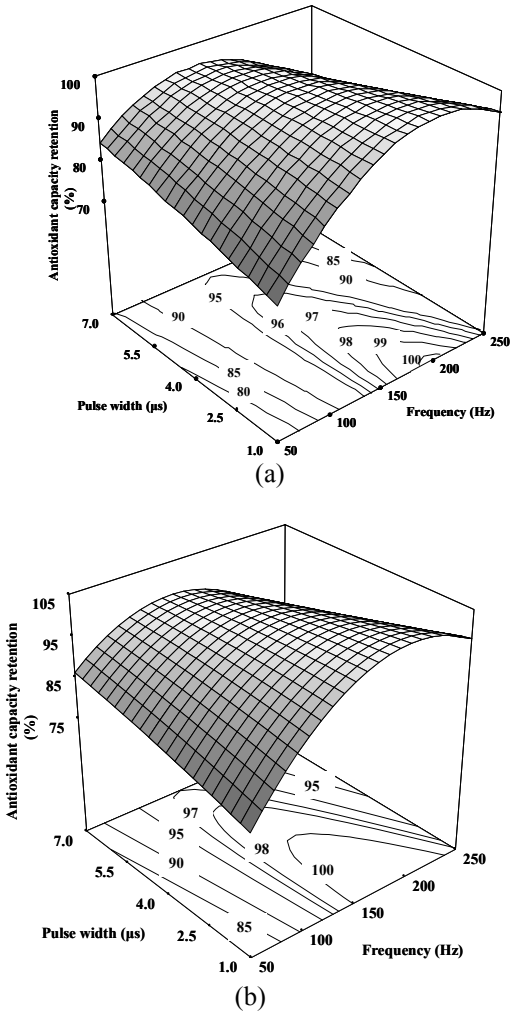


Figure 3.- Effect of pulse frequency and pulse width on antioxidant capacity of strawberry juice when high-intensity pulse electric field (HIEPF) treatment was set up at 35 kV/cm for 1000 µs in monopolar (A) or bipolar (B) mode.

The antioxidant capacity is related to the amount and composition of bioactive compounds present in food (Sánchez-Moreno et al., 2005). Vitamin C and anthocyanins are reported to be the major antioxidant components in strawberry juice (Cordenunsi et al., 2005). The magnitude of the changes in the antioxidant capacity may be associated with the health-related compounds variation due to the HIPEF processing. Strawberry juices, with the highest antioxidant capacity

(Figure 3) had the highest anthocyanins contents (Figure 2) and good levels of vitamin C (Figure 1). In contrast, HIPEF treatments conducted at low both frequency and pulse width led to strawberry juices with the lowest antioxidant capacity, although this HIPEF-treated juice had the greatest vitamin C contents. Therefore, theses results seem to indicate that changes in antioxidant capacity might be attributed mainly to anthocyanins content rather than to vitamin C concentrations

T**able 4**.-Optimal high-intensity pulsed electric field conditions to achieve maximal anthocyanins, vitamin C and antioxidant capacity in HIPEF-treated strawberry juice

HIPEF PARAMETERS				VARIABLES			
Assay nº.	***f* (Hz)**	**τ (µs)**	**p**	**VCR (%)**	**AR (%)**	**ACR (%)**	**D**
1	232	1	Bipolar	98	102	100	0.922
2	222	1	Monopolar	99	97	99	0.826
3	229	1	Monopolar	99	97	99	0.826
4	231	1	Monopolar	99	97	98	0.825

f: frequency; **τ**: pulse width; **p**: polarity mode; **VCR**: vitamin C retention; **AR**: anthocyanins retention; **ACR**: antioxidant capacity retention; **D**: desirability

Optimization and validation of the HIPEF processing conditions

The combination of HIPEF critical parameters that lead to strawberry juices with the highest nutritional quality was determined. The same priority was assigned to each dependent variable in order to obtain strawberry juices with maximal retention of vitamin C, anthocyanins and antioxidant capacity. The greatest antioxidant properties were achieved at high frequencies and low pulse widths in bipolar mode (Table 4). An overall score of 0.922 was obtained when the HIPEF treatment was conducted at 35kV/cm during 1000 µs using 1-µs squared wave bipolar pulses at 232 Hz. As can be seen in Table 4, the predicted content of vitamin C, anthocyanins and antioxidant capacity at the best HIPEF treatment conditions were 98, 102, and 100%, respectively. Nevertheless, high desirability was also obtained combining 1-µs of pulse width with frequencies above 222 Hz in monopolar mode. To complete the study, a set of experiments were carried out in order to validate the predictive models. The data comparison (Figure 4) showed that the proposed predicted expressions were accurate enough to fit experimental results. The correlation coefficients between observed and predicted retention data were 0.707, 0.857, and 0.913 for vitamin C, anthocyanins, and antioxidant capacity models, respectively.

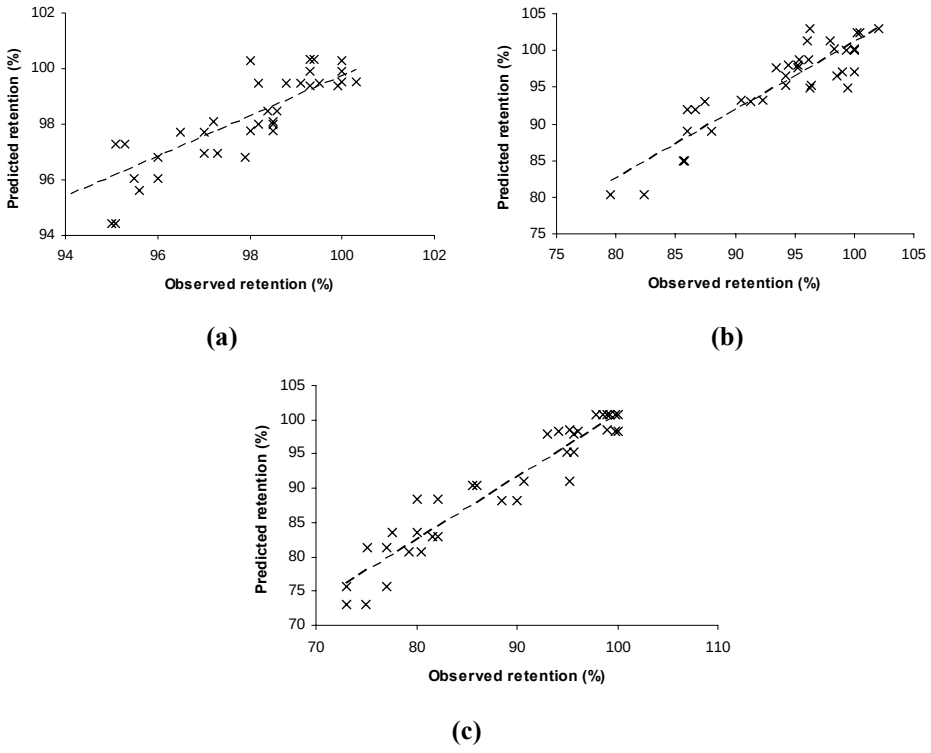


Figure 4.- Scatter plots of the observed and predicted data of strawberry juice vitamin C (a), anthocyanins (b) and antioxidant capacity (c) retention of the validated trials. The straight line indicates the correlation between both groups of data.

CONCLUSIONS

Pulse frequency, pulse width and polarity mode had a significant influence on the vitamin C, anthocyanins, and antioxidant capacity of strawberry juice. Strawberry juices submitted to bipolar pulses showed higher anthocyanins content and antioxidant capacity than that processed by applying HIPEF treatments in monopolar mode. The maximum values of health-related compounds and antioxidant capacity were obtained by combining high frequencies and low pulse widths irrespective of the pulse polarity. Different combinations of frequency and pulse width resulted in strawberry juices with equivalent antioxidant potential. The evaluation of the influence of HIPEF critical parameters on bioactive compounds as well as on the antioxidant capacity of strawberry juice is useful in achieving optimal processing conditions for obtaining juices with high nutritional quality.

ACKNOWLEDGEMENT

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REFERENCES

Aguiló-Aguayo, I., Sobrino-López, A., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric fields processing on lipoxygenase and β-glucosidase activities in strawberry juice. *Innovative Food Science and Emerging Technologies*, doi: 10.1016/j.ifset.2007.12.007.

Brouillard, R. (1982). Chemical structure of anthocyanins. In: Markakis, P. Anthocyanins as food colors. Food Science and Technology. Academic Press, London. (pp. 1-40).

Cao, G., Alessio, H.M., Cutler, R.G. (1993).Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine*, 14, 303-311.

Cordenunsi, B.R., Genovese, M.I., Nascimiento, J.R.O., Hassimotto, N.M.A., Santos, R.J., & Lajolo, F.M. (2005). Effects of temperature on the chemical composition and antioxidant capacity of three strawberry cultivars. *Food Chemistry*, 91, 113-121.

Cortés, C., Esteve, M.J., Frígola, A. (2008). Color of orange juice treated by high intensity pulsed electric fields during refrigerated storage and comparison with pasteurized juice. *Food Control*, 19, 151-158.

Davey, M.W., Van Montagu, M., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science of Food and Agriculture,* 80, 825-860.

Derringer, G., Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology,* 12, 214-219.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R.C., Martín-Belloso, O. (2004). Inactivation of *Saccharomyces cerevisiae* suspended in orange juice using high-intensity pulsed electric fields. *Journal of Food Protection,* 67, 2596-2602.

Elez-Martínez, P., Soliva-Fortuny, R.C., Martín-Belloso, O. (2006). Comparative study on shelf-life of orange juice processed by high intensity pulsed electric fields or heat treatments. *European Food Research Technology*, 222, 321-329.

Elez-Martinez, P., Aguiló-Aguayo, I., Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of the Science of Food and Agriculture*, 86, 71-81.

Elez-Martínez, P., Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102, 201-209.

Gahler S., Otto K., Böhm V. (2003). Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products *Journal of Agricultural and Food Chemistry*, 51, 7962-7968.

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, M., Kader, A. (2000). Antioxidant activity of pomegrate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry,* 48, 4581-4589.

Hannum, S.M. (2004). Potential impact of strawberries on human health: A review of the science. *Critical Review in Food Science and Nutrition*, 44, 1-17.

Jackman, R.L., Smith, J.L. (1997). Anthocyanins and betalains. Natural Food colorants. In: Hendry, G.A.F., Houghton, J.D. Natural Food Colorants. Blackie Academic & Professional, Chapman & Hall, London. (pp.244-309).

Klopotek, Y., Otto, K., Böhm, V. (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 53, 5640-5646.

Markakis, P. (1982). Anthocyanins as food colors. Food Science and Technology. Academic Press, London.

Marsellés-Fontanet, A.R., Martín-Belloso, O. (2007). Optimization and validation of PEF processing conditions to inactivate enzymes of grape juices. *Journal of Food Engineering*, 83, 452-462.

Mertens, B., Knorr, D. (1992). Developments of nonthermal processes for food preservation. *Food Technology*, 46, 124-133.

Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry*, 51, 6887-6892.

Múrcia, M.A., López-Ayerra, B., Martínez-Tomé, M., Vera, A.M., García-Carmona, F. (2000). Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. *Journal of the Science of Food and Agriculture*, 80, 1882-1886.

Odriozola-Serrano, I., Hernández-Jover, T., & Martín-Belloso, O. (2007a). Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits. *Food Chemistry,* 105, 1151-1158.

Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., Gimeno-Añó, V., Martín-Belloso, O. (2007b). Lycopene, vitamin C, and antioxidant capacity of tomato juice as affected by high-intensity pulsed electric fields critical parameters. *Journal of Agricultural and Food Chemistry*, 55, 9036-9042.

Omaye, S.T., Zhang, P. (1998). Phytochemical interactions: β-carotene, tocopherol and ascorbic acid. In: Bidlack, W.R., Omaye, S.T., Meskin, M.S., Jahner, D. Phytochemicals, a new paradigm. Technomic, Lancaster. pp. 53-75.

Rein M.J., Heinonen M. (2004). Stability and enhancement of berry juice color *Journal of Agricultural and Food Chemistry*, 52, 3106-3114.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, 81, 501-508.

Sánchez-Moreno, P., Cano, M.P., De Ancos, B., Plaza, L., Olmedilla, B., Granado, F., Elez-Martínez, P., Martín-Belloso, O., Martín, A. (2005). Intake of Mediterranean soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *International Journal of Food Sciences and Nutrition*, 56, 115-124.

Tannenbaum, S.R., Archer, M.C., Young, V.R. (1985). Vitamins and minerals. In: Fennema O.R. Food Chemistry. Marcel Dekker Inc., New York. pp. 488-493.

Torregrosa, F., Cortés, C., Esteve, M.J., Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry*, 53, 9519-9525.

Torregrosa, F., Esteve, M.J., Frígola, A., Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*, 73, 339-345.

Wrolstad, R.E. (2000). Anthocyanins. In Lauro, G.J., Francis, F.J. Natural Food Colorants. Science and Technology Marcel Dekker, New York. pp. 237-252

Zabetakis, I., Leclerc, N., Kajda, P. (2000). The effect of high hydrostatic pressure on the strawberry anthocyanins . *Journal of Agricultural and Food Chemistry*, 48, 2749-2754.

Zhang, Y., Liao, X. J., Ni, Y.Y., Wu, J.H., Hu, X.S., Wang, Z.F., Chen, F. (2007). Kinetic analysis of the degradation and its color change of cyanidin-3-glucoside exposed to pulsed electric field. *European Food Research and Technology*, 224, 597-603.

Capítulo VI

Kinetic study of anthocyanins, vitamin C, and antioxidant capacity in strawberry juices treated by high-intensity pulsed electric fields

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ABSTRACT

A kinetic study of anthocyanins, vitamin C and antioxidant capacity was carried out in strawberry juice treated with high-intensity pulsed electric fields. Samples were subjected to electric field strengths from 20 to 35 kV/cm for up to 2000 μs applying 1 μs bipolar pulses at 232 Hz. The suitability of simple first-order kinetics and an empirical model based on Weibull distribution function to describe changes in experimental data is discussed. In addition, different secondary models relating the antioxidant properties retention to the electric field strength and treatment time are given. Weibull kinetic model was the most accurate ($R^2_{adj} \geq 0.727$) to predict anthocyanins, vitamin C and antioxidant capacity changes in strawberry juice through the HIPEF treatment time. The combined effect of treatment time and electric field strength on health-related compounds of strawberry juice was successfully predicted ($R^2_{adj} \geq 0.874$) through secondary expressions. The proposed models are useful to predict the variation of the antioxidant potential of strawberry juice with the key parameters involved in HIPEF treatments.

Key words: kinetics, high intensity pulsed electric fields, strawberry juice, anthocyanins, vitamin C, antioxidant capacity

INTRODUCTION

Strawberries and strawberry based-products are a good source of vitamin C and anthocyanins, thus having high antioxidant capacities (Cordenunsi, et al., 2005). It is well known that anthocyanins are unstable pigments and can be decolorized and degraded by many factors such as temperature, pH, oxygen, enzymes, light, the presence of copigments and metallic ions, ascorbic acid, sulphur dioxide and sugars (Jackman and Smith, 1997; Mazza and Miniati, 1993). Vitamin C is a thermolabile vitamin, which is oxidized to nonantioxidant effective substances in the presence of oxygen (Davey et al., 2000). Heat processing is the most common method used to extend the shelf-life of juices, but it is one of the most important factors influencing stability of anthocyanins and vitamin C. According to Nicoli et al. (1999) the antioxidant capacity of foods may be affected by processing in several ways, including losses of water-soluble antioxidants such as phenolics compounds, alterations in the compounds that improve or reduce the antioxidant capacity of plant constituents, as well as formation of novel compounds by Maillard or other reactions that affect antioxidant activity. Therefore, the market demand for fresh-like juices with high nutritional content has increased the awareness of the food industry for the development of milder preservation technologies to replace the existing pasteurization methods (Linneman et al., 1999). HIPEF processing (35 kV/cm for 1700 μs in bipolar 4-μs pulses at 100 Hz) has been shown to effectively inactivate microorganisms in strawberry juice, thus leading to microbial inactivation levels similar to those achieved with heat pasteurization (Mosqueda-Melgar, 2007). In addition, enzymes such as lipoxygenase, which is involved in formation of undesirable flavor compounds in strawberry juice, can be partially or totally reduced (Aguiló-Aguayo et al., 2007). Up to now, the studies evaluating the effects of HIPEF processing conditions on juices have demonstrated that electric field strength and treatment time are important variables to be controlled in order to optimize the inactivation of microorganisms (Elez-Martínez et al., 2004, 2005), enzymes (Giner et al., 2000; Elez-Martínez et al., 200) and health-related compounds (Elez-Martínez et al., 2007; Torregrosa et al., 2005) by HIPEF. Therefore, to evaluate the influence of HIPEF processing on vitamin C, anthocyanins and antioxidant capacity of juices is important for the consumer, particularly with regard to recommended daily servings. This information is also of interest to processors who wish to retain or enhance health-related compounds levels in their products.

On the other hand, several models have been used to describe the microbial destruction (Elez-Martínez et al., 2004; Rodrigo et al., 2001) and enzymatic inactivation (Elez-Martínez et al., 2006; Giner et al., 2005) as a function of the HIPEF critical parameters. Although retention of health-related compounds can be a limiting factor when defining process conditions, there are few works modeling the changes in concentration of antioxidant compounds as affected by HIPEF treatment parameters (Bendicho et al., 2002; Torregrosa et al., 2006; Zhang et al., 2007). Therefore, the aim of this work was to propose mathematical models that properly relate changes in the content of health-related compounds, namely anthocyanins and vitamin C, as well as antioxidant capacity of strawberry juice to electric field strength and HIPEF treatment time.

MATERIALS AND METHODS

Reagents

Metaphosphoric acid, DL-1,4 -dithiotreitol (DTT) were purchased from Acros Organics (NJ, USA); potassium chloride, sodium acetate, ascorbic acid, sulphuric acid, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Scharlau Chemie, SA (Barcelona, Spain).

Sample preparation

Strawberry fruits (*Fragaria ananassa* Duch, cultivar Camarosa) were purchased from a local supermarket (Lleida, Spain). The fruits were washed, drained and chopped. Then, the squeezed strawberry juice was centrifuged at 24.000×g for 15 min and the supernatant was filtered using a steel sieve with an approximate mesh of 2 mm. Electric conductivity (Testo 240 conductivimeter ; Testo GmBh & Co, Lenzkirch, germany), pH (crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan) and color (Macbeth-Kollmorgen inst. Corp., Newburg, NY) of strawberry juice were determined. Analytical characteristics of fresh strawberry juice were, soluble solids 7.8 ± 0.1 ºBrix, electric conductivity 0.41 ± 0.21 S/m, pH 3.45 ± 0.22 and color L* = 18.76 ± 0.25, a* = 5.32 ± 0.12 and b* = 2.25 ± 0.1 (results are expressed as the mean ± standard deviation).

Pulsed electric fields equipment

HIPEF treatments were carried out in a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA). The treatment system consists of eight collinear chambers in series, each one with two stainless steel electrodes separated by a gap of 0.29 cm, thus defining a treatment volume of 0.012 cm^3. The flow rate of the process was adjusted to 60 mL/min and controlled with a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). A cooling coil was connected between each pair of chambers and submerged in an ice-water shaking bath. Product outlet temperatures never exceeded 40 ºC. Samples of strawberry juice were subjected to field strengths of 20, 25, 30 and 35 kV/cm during 100, 300, 600, 1000, 1500 and 2000 μs, using 1 μs square-wave bipolar pulses at 232 Hz. Treatment conditions were selected according to a previous study (Odriozola-Serrano et al., 2009). Each processing condition was assayed in duplicate and two replicate analyses were carried out in order to obtain the mean value.

Anthocyanins

The anthocyanins content of strawberry juice was determined with a modified pH differential method described by Meyers et al. (2003), using two buffer systems: potassium chloride 0.025 M at pH 1, and sodium acetate 0.4 M at pH 4.5. Briefly, 5 mL of strawberry juice were transferred to a 50 mL volumetric flask and made up with each buffer. The absorbance of the mixtures at pH 1 and 4.5 was then measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 510 and 700 nm against a blank

of distilled water. The anthocyanins content was calculated according to Equation 1 and expressed as pelargonidin-3-glucoside per liter:

$$TA = \frac{\left[\left(A_{510} - A_{700}\right)_{pH1.0} - \left(A_{510} - A_{700}\right)_{pH4.5}\right] \times MW \times DF \times 1000}{\varepsilon \times L} \quad (1)$$

where MW is the molecular weight of pelargonidin-3-glucoside (433.0 g/mol), DF is the dilution factor, L is the pathlength in cm and ε is the molar extinction coefficient for pelargonidin-3-glucoside (22400 L/mol·cm). Results were expressed as pelargonidin-3-glucoside content (plg-3-glu) compared to the untreated sample.

Vitamin C

Vitamin C content of strawberry juice was analyzed by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (2007). A sample of 25 mL of juice was mixed with 25 mL of a solution containing 45 g/L metaphosphoric acid and 7.2 g/L of DTT. The homogenate was centrifuged at 22100x*g* for 15 min at 4 ºC (Centrifuge Avanti^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vacuum-filtered through Whatman No. 1 paper. Then, the samples were filtered with a Millipore 0.45 μm membrane. An aliquot of 20 μL was injected into the HPLC system consisting of a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm x 250 cm) and a 486 Absorbance Detector (Waters, Milford, MA). A 0.01% solution of sulphuric acid adjusted to pH = 2.6 was used as eluent. The flow was isocratic at a rate of 1 mL/min at room temperature. Detection was performed at 245 nm. Identification of the ascorbic acid was carried out comparing the retention time and UV-visible absorption spectrum of the juice samples with those of the standards. Results were expressed as vitamin C retention related to the untreated sample.

Antioxidant capacity

The antioxidant capacity of strawberry juice was studied through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical, according to the method described by Odriozola-Serrano et al. (2009). Samples of strawberry juice were centrifuged at 6000xg for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and aliquots of 0.01 mL of the supernatant were mixed with 3.9 mL of methanolic DPPH (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 60 min. Absorption of the samples was measured with a spectrophotometer at 515 nm against a blank of methanol without DPPH. Results were expressed as antioxidant capacity retention related to the untreated sample.

Kinetic models

The fitting of several kinetic models to experimental data, namely simple first-order model (Equation 2) and Weibull distribution function (Equation 3) was evaluated in order to properly relate health-related compounds and antioxidant capacity retention in strawberry juices to HIPEF processing parameters.

Simple first-order model

Simple first-order kinetics have been commonly used to fit thermal degradation of anthocyanins (Kırca et al., 2003; Ahmed et al., 2004; Wang and Xu, 2007) and vitamin C (Vieira, et al., 2000; Vikram et al., 2005) in juices and nectars as a function of treatment time. In addition, Zhang et al. (2007) observed that the degradation of cyanidin-3-glu in methanolic solution exposed to PEF treatments at 1.2-3 kV/cm ($T^a \leq 47$ ºC) for 20-140 μs was well fitted by simple first-order reactions. In this way, Bendicho et al. (2002) proposed a first-order model to describe the vitamin C changes in milk as affected by HIPEF treatment time.

$$RA = RA_0 \cdot exp(-k_1 \cdot t) \qquad (2)$$

where RA (%) is the relative antioxidant property, RA_0 (%) is the intercept of the curve, k_1 is the first-order kinetic constant (μs^{-1}) and t is the treatment time (μs).

Weibull model

Weibull distribution (Equation 3) has been used to describe destruction of microorganisms (Rodrigo et al., 2001) and enzyme inactivation (Giner et al., 2005; Rodrigo et al., 2003; Soliva-Fortuny et al., 2006) under HIPEF. The use of Weibull distribution function to describe the retention of tomato health-related compounds and antioxidant capacity has been reported by Odriozola-Serrano et al. (2008)

$$RA = RA_0 \, exp\left[-\left(\frac{t}{\alpha}\right)^{\gamma}\right] \qquad (3)$$

where RA (%) is the relative antioxidant property, RA_0 (%) is the intercept of the curve, t is the treatment time (μs), α is the scale factor (μs), and γ is the shape parameter that indicates concavity (tail-forming) or convexity (shoulder-forming) of the curve when it takes values below and above 1, respectively.

Derived from the Weibull distribution function parameters (α, γ), t_m is defined as the mean processing time to achieve complete destruction/inactivation of the health-related compound or antioxidant capacity and can be used as a measurement of the resistance of these compounds to HIPEF treatments (Equation 4):

$$t_m = \alpha \cdot \Gamma\left(1+\frac{1}{\gamma}\right) \qquad (4)$$

where α and β are the parameters of the Weibull distribution and Γ is the gamma function.

Secondary models

Additionally, kinetic rate constants obtained for each model were related, when possible, to the applied electric field strength through mathematical expressions. The combined effect of electric field strength and treatment time on strawberry juice antioxidant properties was described by rearranging these mathematical expressions into the best kinetic model for each compound.

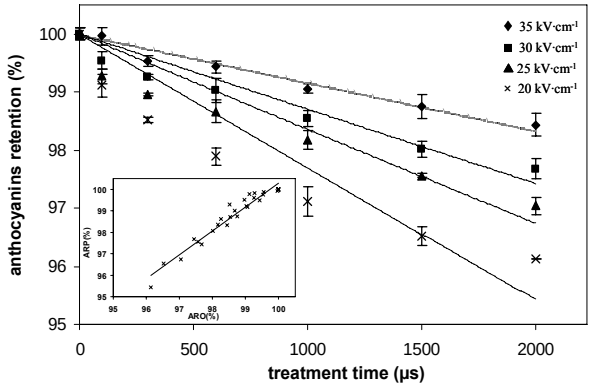
Statistical analysis

Each processing condition was assayed in duplicate and two replicate analyses were carried out in order to obtain the mean value. The analysis of variance was carried out with Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, MD). The least significant difference test was used to determine differences between treatments at a 5% significance level. The models were fitted to experimental data by nonlinear regression procedures, using the Statgraphics Plus v.5.1 Windows package. Estimated parameters are given of the estimates by the Student's-*t* adjusted at the corresponding degree of freedom. The adjusted regression coefficients (R^2_{adj}) and the statistical parameters, root mean square error (RMSE), reduced Chi-square (χ^2) and mean bias error (MBE) were calculated to evaluate the fitting of a model to experimental data (Hayaloglu et al., 2007). The highest the values of R^2_{adj} and the lowest values of RMSE, χ^2 and MBE, the better the models fit experimental data.

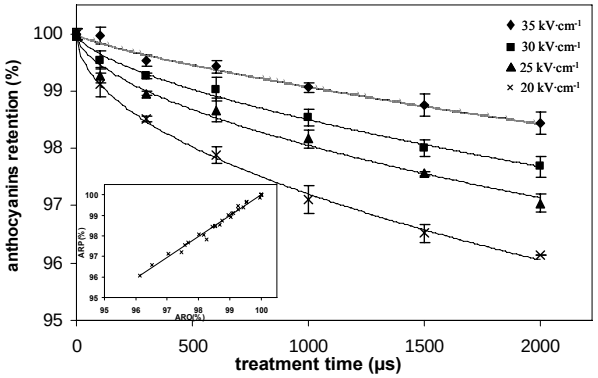
RESULTS AND DISCUSSION

Anthocyanins

The total concentration of anthocyanins, expressed as pelargonidin-3-glucoside, in fresh strawberry juice was 27.3 ± 0.3 mg/100 mL. As can be seen in Figure 1, the total anthocyanins retention ranged between 96.1% and 100.5% in HIPEF-processed juice treated under the studied experimental conditions. Lower retentions (80%-94%) of cyanidin-3-glucoside (cyn-3-glu) in a methanolic solution were obtained after carrying out less intense HIPEF treatment conditions (1.2, 2.2 and 3.0 kV/cm, 300 numbers of pulses, $T^a \leq 47$ ºC) than those reported in the present study (Zhang et al., 2007). Differences in anthocyanins due to HIPEF treatments can be attributed not only to factors intrinsic to the food product, but also to other factors such as HIPEF system, treatment chambers, pulse characteristics and electrical conditions.



(a)



(b)

Figure 1.- Effect of treatment time and electric field strength on the anthocyanin retention of strawberry juice (mean ± SD) as modeled by simple first-order model (**a**), and Weibull approach (**b**). Treatments were performed at 232 Hz and square bipolar pulses of 1-µs. ARO: anthocyanins retention (observed), ARP: anthocyanins retention (predicted).

Anthocyanins content significantly depended on the HIPEF treatment time and electric field strength applied during HIPEF-processing of the samples. The lower the treatment time and the higher the electric field strength, the greater the anthocyanin retention (Figure 1). Contrarily, Zhang et al. (2007) reported that the degradation of cy-3-glu in a methanolic solution increased as the electric field strength rose. Not all anthocyanins appear to react equally regarding their resistance to the degrading effects of various agents (Markakis, 1982). In addition, intensive HIPEF treatments might stimulate the degradation of proanthocyanins to

anthocynins. It has been reported that proanthocyanins are converted into anthocyanins after treatments at high temperature in acidic water-free conditions (Porter et al., 1986; Saint-Cricq de Gaulejac et al., 1999; Simonetti et al., 1997). Although the maximum temperature reached in the sample during HIPEF treatments at 35 kV/cm was 37 ºC, the stress produced by HIPEF-processing at high electric field strength might explain this transformation. Further investigations are still needed to explain the mechanisms that mediate this HIPEF-induced conversion of proanthocyanins to anthocynins.

The kinetic constants estimated by simple first-order and Weibull model as well as the adjusted determination coefficients, RMSE, χ^2 and MBE, of the fitted models at different electric field strengths are shown in Table 1. Based on these coefficients, it can be observed that the two models predicted well the relationship between anthocyanins retention and HIPEF treatment time irrespective of the electric field strength. However, the Weibull approach exhibits the highest R^2_{adj}, the lowest RMSE, χ^2 and MBE. Furthermore, the deviations in the values estimated by a first-order model in relation to the observed values are higher than those estimated by the Weibull model (Figure 1).

Table 1.- Results of the statistical analyses on the kinetic changes of anthocyanins in strawberry juices subjected to high intensity pulsed electric fields.

Model	*E*	Constants[1]	Statistical parameters			
			R^2_{adj}	RMSE	χ^2	MBE
First-order	20	$k_1 = 2.33 \times 10^{-5}$ µs^{-1}	0.771	0.443	0.141	0.097
	25	$k_1 = 1.66 \times 10^{-5}$ µs^{-1}	0.817	0.373	0.134	0.094
	30	$k_1 = 1.30 \times 10^{-5}$ µs^{-1}	0.846	0.310	0.054	0.060
	35	$k_1 = 8.39 \times 10^{-6}$ µs^{-1}	0.731	0.196	0.004	0.017
Weibull	20	$\alpha = 11.33\times10^5$ µs $\gamma = 5.07\times10^{-1}$	0.959	0.248	0.040	0.051
	25	$\alpha = 10.91\times10^5$ µs $\gamma = 5.62\times10^{-1}$	0.930	0.205	0.0005	0.006
	30	$\alpha = 7.56\times10^5$ µs $\gamma = 6.32\times10^{-1}$	0.912	0.161	0.0002	0.004
	35	$\alpha = 4.12\times10^5$ µs $\gamma = 7.76\times10^{-1}$	0.727	0.147	0.0001	0.003

[1]Mean values ***E***: electric field strength (kV/cm)
k_1: first-order rate constant; **α:** scale factor**;** **γ:** shape factor
R^2_{adj}: Adjusted determination coefficient;
RMSE: Root mean square error; **χ^2**: Reduced Chi-square; **MBE**: Mean bias error

A simple first-order model was used by Zhang et al. (2007) to describe degradation of cy-3-glu in a methanolic solution. The authors obtained *k*-values ranging from 4.2×10^{-7} to 1.1×10^{-6} µs^{-1} for cy-3-glu using electric fields strength between 1.2 and 3.0 kV/cm and HIPEF treatment times up to 140 µs. In our study, kinetic rates defined by the simple first-order model ranged from 8.39×10^{-6} to 2.33×10^{-5} µs^{-1}, which suggests that the resistance of anthocyanins to HIPEF processing is quite similar when treating strawberry juice or a methanolic solution.

The scale parameter (α) and the shape parameter (γ) of the Weibull model were obtained by fitting Equation 3 to the experimental data. The γ parameter took values of 5.07×10^{-1} to 7.76×10^{-1}, and the electric field strength dependency was found to be exponential (R^2_{adj} = 0.972) in the range of the applied conditions (Equation 5). Values of the γ below 1 could be regarded as evidence that a great amount of anthocyanins were degraded at relatively fast rate leaving behind anthocyanins with higher resistance to HIPEF treatment. This trend is in accordance with the fact that anthocyanins stability is influenced by the composition of the food matrix, as certain compounds such as flavonoids and phenolic acids can react with them enhancing their resistance to different stresses (Brouillard, 1982). On the other hand, estimated α values decreased when the electric field strength increased following a trend that could be described with good accuracy (R^2_{adj} = 0.873) using a simple first-order model (Equation 6). According to our results, the studies performed with enzymes showed that the plots of the shape and scale factor obtained from Weibull model adjustment versus the applied electric field strength matched an exponential trend with good agreement (Soliva-Fortuny et al., 2006). Mean time (t_m) can be defined as the mean processing time to completely destroy the anthocyanin content of strawberry juice. The values of t_m were calculated from Equation 4 and varied from 959 to 3368 ms. Therefore, it may be assumed that total anthocyanins degradation by HIPEF treatments in the range of the studied electric field strength will not be reached since these prolonged treatments are not used in HIPEF processing.

$$\alpha = \left[3569110 \cdot exp\left(-0.0536 \cdot E\right)\right] \qquad (5)$$

$$\gamma = \left[0.2738 \cdot exp\left(0.0292 \cdot E\right)\right] \qquad (6)$$

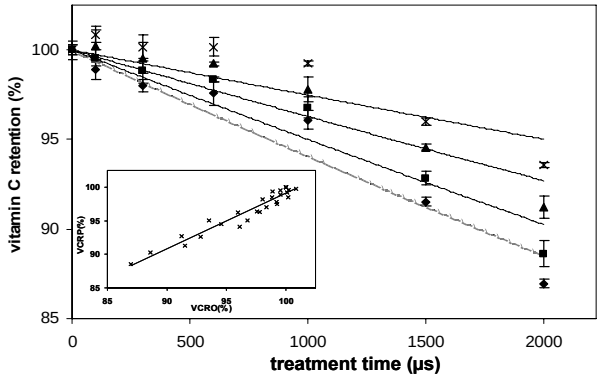
The combined effect of treatment time and electric field strength on total anthocyanins of strawberry juice (AR) is described by Equation 7, where α and γ in Weibull model have been replaced by Equation 5 and Equation 6, respectively.

$$AR(\%) = 100\left[-\left(\frac{t}{\left[3569110 \cdot exp\left(-0.0536 \cdot E\right)\right]}\right)^{\left[0.2738 \cdot exp\left(0.0292 \cdot E\right)\right]}\right] \qquad (7)$$

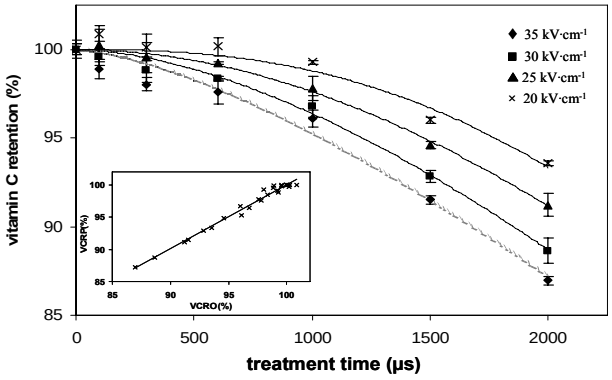
The good fitting of the model is confirmed by the statistical parameters (RMSE = 0.389, χ^2 = 0.024, MBE = 0.389) and the high adjusted determination coefficient (R^2_{adj} = 0.937).

Vitamin C

The vitamin C content of the untreated strawberry juice was 48.6 ± 0.4 mg/100 mL. The effect of HIPEF processing parameters on the concentration of vitamin C in strawberry juice is shown in Figure 2.



(a)



(b)

Figure 2.- Effect of treatment time and electric field strength on the vitamin C retention of strawberry juice (mean ± SD) as modeled by simple first-order model (**a**), and Weibull approach (**b**). Treatments were performed at 232 Hz and square bipolar pulses of 1-µs. VCRO: vitamin C retention (observed), VCRP: vitamin C retention (predicted).

High vitamin C retention (≥ 87%) with respect to the fresh juice was observed after the applied HIPEF-treatments. Sanchez-Moreno et al. (2005) reported a vitamin C retention of 93% after processing orange juice at 35 kV/cm during 750 µs with bipolar pulses of 4-µs and

800 Hz. In this way, Evrendilek et al. (2000) did not observe vitamin C degradation in apple juice subjected to a HIPEF treatment at 35 kV/cm for 94 μs with 1.92 μs monopolar pulses at 952 Hz. Vitamin C content significantly depended on the HIPEF treatment time and electric field strength. The lower the treatment time and electric field strength, the greater the vitamin C retention (Figure 2). Consistently, Elez-Martínez and Martín-Belloso (2007) and Torregrosa et al. (2006) reported an increase of vitamin C degradation when electric field strengths and treatment time rise in HIPEF-treated orange and orange-carrot juice, respectively. Oxidation of ascorbic acid occurs mainly during processing of juices and depends upon many factors such as oxygen presence, heat and light (Robertson and Samaniego, 1986). Ascorbic acid is an unstable compound which under less desirable conditions decomposes easily, thus the milder the treatment, the better the vitamin C retention in juices (Burdulu et al., 2006).

The calculated first-order rate, Weibull parameters and regression coefficients at different electric field strength are shown in Table 2. Up to now, several authors have studied the kinetics of vitamin C thermal degradation in several juices under pasteurization conditions and have stated that this vitamin depletion can be approach by simple first-order models (Johson et al., 1995; Vieira et al., 2000). However, in this study, the fitting performance of the simple first-order kinetic model was high for treatments with an electric field strength between 25 and 35 kV/cm (R^2_{adj} = 0.869-0.920) but dramatically decreased for treatments conducted at 20 kV/cm (R^2_{adj} = 0.752).

Table 2.- Results of the statistical analyses on the kinetic changes of vitamin C in strawberry juices subjected to high intensity pulsed electric fields.

Model	*E*	Constants[1]	Statistical parameters			
			R^2_{adj}	RMSE	χ^2	MBE
First-order	**20**	$k_1 = 2.55\times10^{-5}$ μs^{-1}	0.752	0.717	1.102	-0.270
	25	$k_1 = 3.78\times10^{-5}$ μs^{-1}	0.869	0.647	0.650	-0.207
	30	$k_1 = 5.12\times10^{-5}$ μs^{-1}	0.909	0.625	0.374	-0.157
	35	$k_1 = 6.08\times10^{-5}$ μs^{-1}	0.920	0.635	0.106	-0.083
Weibull	**20**	$\alpha = 7.49\times10^{3}$ μs $\gamma = 1.50$	0.955	0.461	0.196	-0.114
	25	$\alpha = 6.94\times10^{3}$ μs $\gamma = 1.70$	0.974	0.282	0.003	-0.014
	30	$\alpha = 6.81\times10^{3}$ μs $\gamma = 1.94$	0.969	0.344	0.056	0.061
	35	$\alpha = 5.83\times10^{3}$ μs $\gamma = 2.50$	0.934	0.493	0.218	0.120

[1]Mean values; ***E:*** electric field strength (kV/cm)
k_1: first-order rate constant; **α:** scale factor**;** **γ:** shape factor
R^2_{adj}: Adjusted determination coefficient;
RMSE: Root mean square error; **χ^2**: Reduced Chi-square; **MBE**: Mean bias error

The ascorbic acid degradation rates defined by the simple first-order model were statistically influenced by the electric field strength and took values in the range of 2.55×10^{-5} to 6.08×10^{-5} μs^{-1} for field strength between 20 and 35 kV/cm. Torregrosa et al. (2006) obtained first-order rate constants from 9×10^{-3} to 2.2×10^{-2} μs^{-1} when modeling vitamin C changes in orange-carrot juice after HIPEF treatments of up to 340 μs and electric field strengths from 25

to 40 kV/cm. Thus, HIPEF treatments were found to achieve lower rates of vitamin C destruction in strawberry juice than in orange-carrot juice exposed to less intense conditions than those reported in the present study. As can be seen in Table 2, Weibull distribution seemed to be most suitable model to predict kinetic degradation of vitamin C regardless of the treatment intensity. The high determination coefficients (0.934-0.974) and the low RMSE, χ^2 and MBE indicate that Weibull model can be useful to relate vitamin C retention to HIPEF treatment time (Table 2). This good accuracy of the Weibull approach is confirmed by the small deviation in the values estimated by the model and the experimental data to the line of equivalence (Figure 2). The scale parameter (α) and the shape parameter (γ) of the Weibull distribution were obtained by fitting Equation 3 to the experimental data. The γ parameter ranged from 1.5 to 2.5, suggesting that this vitamin became increasingly destroyed overtime. This behavior is in accordance with that observed by Odriozola-Serrano et al. (2008) who obtained γ-values higher than 1 modeling vitamin C degradation in tomato juice affected by HIPEF processing at similar conditions to those reported in the present work. In addition, γ parameter increased with electric field strength following a trend that fitted well an exponential equation (R^2_{adj} = 0. 952) in the range of the applied conditions (Equation 8). On the other hand, α was statistically dependent on the electric field strength and took values from 5834 to 7489 μs. A simple first-order model was found to describe the electric field strength dependency of α (R^2_{adj} = 0.852) (Equation 9).

$$\alpha = \left[4479 \cdot exp\left(0.0149 \cdot E\right)\right] \quad (8)$$

$$\gamma = \left[4.879 \cdot exp\left(-0.0348 \cdot E\right)\right] \quad (9)$$

Moreover, substitution of α and γ by into the Weibull distribution (Equation 3), transforms the equation into a function dependent on both the electric field strength (E) and treatment time (t) (Eq. 10). The model performed well under the whole range of applied conditions, with high determination coefficients (R^2 = 0.948) and good accuracy (RMSE = 0.635, χ^2 = 0.0005, MBE = -0.0037).

$$VCR(\%) = 100 \cdot exp\left[-\left(\frac{t}{\left[4479 \cdot exp\left(0.0149 \cdot E\right)\right]}\right)^{\left[4.879 \cdot exp\left(-0.0348 \cdot E\right)\right]}\right] \quad (10)$$

It has been reported that vitamin C is a typical heat sensitive nutrient, so that its retention is often considered as a significant marker of overall nutrient recovery (Jung et al., 1995). To establish HIPEF processing time to maximal destruction of vitamin C, t_m was calculated from Eq. 4 for the different electric field strengths. Values of t_m ranged from 8175 and 12481 μs, decreasing with electric field strength. These values are similar to those obtained by Odriozola-Serrano et al. (2008), who reported t_m-values varying from 7939 to 11252 μs for vitamin C in tomato juice treated by applying similar HIPEF conditions to those of the present work. These small differences in vitamin C stability between HIPEF-treated juices may be due to the lower

pH of strawberry juice in comparison to tomato juice, as more acidic conditions are known to stabilize vitamin C (Tannenbaum et al., 1985).

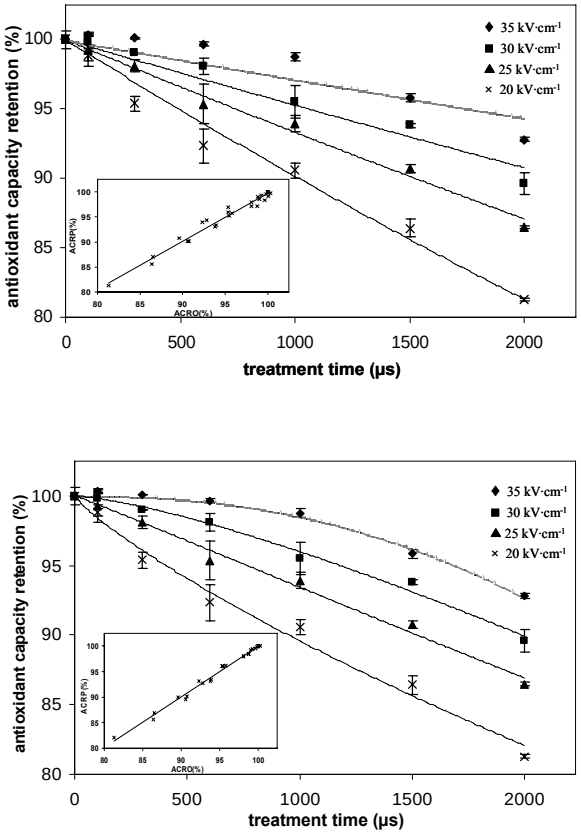


Figure 3.- Effect of treatment time and electric field strength on the antioxidant capacity retention of strawberry juice (mean ± SD) as modeled by simple first-order model (**a**), and Weibull approach (**b**). Treatments were performed at 232 Hz and square bipolar pulses of 1-µs. ACRO: antioxidant capacity retention (observed), ACRP: antioxidant capacity retention (predicted).

Antioxidant Capacity

The DPPH method has been proposed as an easy and accurate method for measuring the antioxidant activity of juice samples (Gil et al., 2000). In addition, DPPH assay is not specific to any particular antioxidant component, thus determining the overall antioxidant capacity of the sample. The effect of HIPEF treatment conducted at 232 Hz with 1 µs bipolar pulses on the antioxidant capacity of strawberry juice at the assayed electric field strength and treatment time

is shown in Figure 3. Antioxidant capacity of fresh strawberry juice was 39.3 ± 0.6 % of DPPH inhibition. Antioxidant capacity of strawberry juice decreased significantly as electric field strength and treatment time increased (Figure 3). HIPEF treatment carried out at 20 kV/cm and 2000 μs led to the maximum antioxidant capacity degradation (18.7%). The results of the antioxidant capacity retention after HIPEF processing are in the range of those reported in the literature working with similar operation conditions but for different fruit juices (Elez-Martínez and Martín-Belloso, 2007; Odriozola-Serrano et al., 2008).

Table 3.- Results of the statistical analyses on the kinetic changes of antioxidant capacity in strawberry juices subjected to high intensity pulsed electric fields.

Model	*E*	Constants[1]	Statistical parameters			
			R^2_{adj}	RMSE	χ^2	MBE
First-order	20	$k_1 = 1.04\times10^{-4}$ μs^{-1}	0.973	0.572	0.351	0.153
	25	$k_1 = 6.91\times10^{-5}$ μs^{-1}	0.990	0.428	0.001	-0.010
	30	$k_1 = 4.84\times10^{-5}$ μs^{-1}	0.948	0.534	0.206	-0.117
	35	$k_1 = 2.91\times10^{-5}$ μs^{-1}	0.814	0.667	0.765	-0.225
Weibull	20	$\alpha = 1.36\times10^{4}$ μs $\gamma = 8.46\times10^{-1}$	0.981	0.562	0.002	0.010
	25	$\alpha = 1.30\times10^{4}$ μs $\gamma = 1.05$	0.989	0.412	0.003	0.013
	30	$\alpha = 1.02\times10^{4}$ μs $\gamma = 1.38$	0.973	0.362	0.002	0.012
	35	$\alpha = 6.06\times10^{3}$ μs $\gamma = 2.34$	0.972	0.314	0.034	0.048

[1]Mean values; ***E***: electric field strength (kV/cm)
k_1: first-order rate constant; **α:** scale factor**;** **γ:** shape factor
R^2_{adj}: Adjusted determination coefficient;
RMSE: Root mean square error; **χ^2**: Reduced Chi-square; **MBE**: Mean bias error

The kinetic parameters of simple first-order and Weibull model at different electric field strength are shown in Table 3. The adjusted regression coefficients depending upon the electric field strength were greater than 0.814; thus, there is a good correlation between antioxidant capacity degradation and HIPEF treatment time. However, Weibull model exhibited lower RMSE, χ^2 and MBE values than the first-order model (Table 3). Furthermore, the deviations in the values estimated by the simple first-order model in relation to the observed values are higher than those obtained by the Weibull model (Figure 3). Therefore, from a quantitative point of view, data were best described by the Weibull function. The antioxidant capacity degradation rate was inversely influenced by the electric field strength, taking values between 2.91×10^{-5} and 1.04×10^{-4} μs^{-1}. In this way, both α and γ resulted to be significantly dependent on electric field strength. The higher the electric field strength, the greater the shape factor and the lower the scale factor.Values of mean time to completely lose of antioxidant capacity (t_m) were calculated according to Equation 4 and varied from 8666 to 29662 μs. As no references have been found reporting the use of Weibull equation to describe antioxidant capacity retention in HIPEF-treated products, Weibull parameters were compared with those reported in other

studies for microorganisms (Sampedro et al., 2006) and enzymes (Elez-Martínez et al., 2006) to have an idea of the resistance of the antioxidant properties to HIPEF treatments. Comparing t_m-values and according to Weibull model, strawberry juice antioxidant capacity is shown to be less sensitive to HIPEF treatments than microorganisms, but more than enzymes.

The electric field strength dependency of α and γ estimated by the Weibull approach can be described by simple first-order equations. This model adequately fitted α (Equation 11) and γ (Equation 12) explaining 76.41% and 90.25% of the variability, respectively.

$$\alpha = \left[35052 \cdot exp\left(-0.0441 \cdot E\right)\right] \tag{11}$$

$$\gamma = \left[0.158 \cdot exp\left(0.0758 \cdot E\right)\right] \tag{12}$$

To estimate the antioxidant capacity retention (ACR) after HIPEF treatments of a given electric field strength (E) and treatment time (t) in the range of the applied conditions, a secondary model was developed by introducing Equation 11 and 12 in Equation 3 (Equation 13). The good accuracy of the secondary model was confirmed by the high adjusted determination coefficients (R^2_{adj} = 0.874) and the statistical parameters (RMSE = -0.428, χ^2 = 6.25, MBE = 1.01).

$$ACR(\%) = 100 \cdot exp\left[-\left(\frac{t}{\left[35052 \cdot exp\left(-0.044 \cdot E\right)\right]}\right)^{\left[0.158 \cdot exp\left(0.0758 \cdot E\right)\right]}\right] \tag{13}$$

As a conclusion, the proposed mathematical models can be applied for engineering design to evaluate and optimize HIPEF processing conditions to produce strawberry juices with a high retention of bioactive compounds. The variation of the antioxidant potential of strawberry juice as affected by key parameters involved in HIPEF treatments can be described with good accuracy by secondary models.

ACKNOWLEDGEMENTS

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REFERENCES

Aguiló-Aguayo, I., Sobrino-López, A., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric fields processing on lipoxygenase and β-glucosidase activities in strawberry juice. *Innovative Food Science and Emerging Technologies*, 94(4), 455-462.

Ahmed, J., Shivhare, U.S., Raghavan G.S.V. (2004). Thermal degradation kinetics of anthocyanin and visual colour of plum puree. *European Food Research and Technology*, 218, 525-528.

Bendicho, S., Espachs, E., Arántegui, J., Martín, O. (2002). Effect of high intensity pulsed electric fields and heat treatments on vitamins of milk. *Journal of Dairy Research*, 69, 116-123.

Brouillard, R. (1982). Chemical structure of anthocyanins. In: Markakis, P. Anthocyanins as food colors. Food Science and Technology. Academic Press, London, UK. pp. 1-40.

Burdurlu, H.S., Koca, N., Karadeniz F. (2006). Degradation of vitamin C in citrus juice concentrates during storage. *Journal of Food Engineering*, 74, 211-216.

Cordenunsi, B.R., Genovese, M.I., Nascimiento, J.R.O., Hassimotto, N.M.A., Santos, R.J., Lajolo, F.M. (2005). Effects of temperature on the chemical composition and antioxidant capacity of three strawberry cultivars. *Food Chemistry*, 91, 113-121.

Davey, M.W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science and Food Agriculture,* 80, 825-860.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R.C., Martín-Belloso, O. (2004). Inactivation of *Saccharomyces cerevisiae* suspended in orange juice using high-intensity pulsed electric fields. *Journal of Food Protection*, 67, 2596-2602.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R.C., Martín-Belloso, O. (2005). Inactivation of *Lactobacillus brevis* in orange juice by high-intensity pulsed electric fields. *Food Microbiology*, 22, 311-319.

Elez-Martínez, P., Aguiló-Aguayo, I., Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of the Science and Food Agriculture*, 86, 71-81.

Elez-Martínez, P., Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102, 201-209.

Evrendilek, G.A., Jin, Z.T., Ruhlman, K.T., Qiu, X., Zhang, Q.H., Richter, E.R. (2000). Microbial safety and shelf-life of apple juice and cider processed by bench and pilot scale PEF systems. *Innovative Food Science and Emerging Technology*, 1, 77-86.

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, M., Kader, A. (2000). Antioxidant activity of pomegrate juice and its relationship with phenolic composition and processing. *Journal of Agriculture and Food Chemistry*, 48, 4581-4589.

Giner, J., Gimeno, V., Espachs, A., Elez, P., Barbosa-Cánovas, G.V., Martín, O. (2000). Inhibition of tomato (Licopersicon esculentum Mill.) pectin methylesterase by pulsed electric fields. *Innovative Food Science and Emerging Technologies*, 1, 57-67.

Giner, J., Grouberman, P., Gimeno, V., Martín, O. (2005). Reduction of pectinesterase activity in a commercial enzyme preparation by pulsed electric fields: Comparison of inactivation kinetic models. *Journal of the Science and Food Agriculture,* 85, 1613-1621.

Hayaloglu, A.A., Karabulut, I., Alpaslan, M., Kelbaliyev, G. (2007). Mathematical modeling of drying characteristics of strained yoghurt in a convective type tray-dryer. *Journal of Food Engineering,* 78, 109-117.

Jackman, R.L., Smith, J.L. (1997). Anthocyanins and betalains. Natural Food colorants. In: Hendry, G.A.F., Houghton, J.D. Natural Food Colorants. Blackie Academic & Professional, Chapman & Hall, London, UK. pp. 244-309.

Johnson, J.R., Braddock, R.J., Chen, C.S. (1995). Kinetics of ascorbic acid loss and nonenzymatic browning in orange juice serum: experimental rate constants. *Journal of Food Science*, 60, 502-505.

Jung, M.Y., Kim, S.K., Kim, S.Y. (1995). Riboflavin-sensitized photodynamic UV spectrophotometry for ascorbic acid assay in beverages. *Journal of Food Science*, 60, 360-363.

Kırca, A., Cemeroğlu, B. (2003). Thermal degradation of blood orange anthocyanins. *Food Chem*istry, 81, 583–587.

Linneman, A.R., Meerdink, G., Meulenberg, M.T.C., Jongen, W.M.F. (1999). Consumer-oriented technology development. *Trends in Food Science and Technology*, 9, 409-414.

Markakis, P. (1982). Anthocyanins as food colors. Food Science and Technology. Academic Press, London, UK.

Mazza, G., Miniati, E. (1993). Anthocyanins in fruits and vegetables and grains. CRCPress, Boca-Raton, Florida.

Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agriculture and Food Chemistry*, 51, 6887-6892.

Mosqueda-Melgar, J. (2007). Aplicación de pulsos eléctricos de alta intensidad de campo en combinación con sustancias antimicrobinas para garantizar la calidad e inocuidad microbiológica de zumos de frutas. Doctora thesis, University of Lleida, Spain.

Nicoli, M.C., Anese, M., Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science and Technology*, 10, 94-100.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits. *Food Chemistry,* 105, 1151-1158.

Odriozola-Serrano, I., Soliva-Fortuny, R., Gimeno-Añó, V., Martín-Belloso, O. (2008). Modeling changes in health-related compounds of tomato juice treated by high-intensity pulsed electric fields. *Journal of Food Engineering*, 89, 210-216.

Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT-Food Science and Technology,* DOI: 10.1016/j.lwt.2008.05.008.

Porter L.J., Hrstich, L.N., Chan, B.G. (1986). The conversion of proanthocyanidins and prodelphinidins to cyanidin and delpinidin. *Phytochemistry*, 25, 223-230.

Robertson, G.L., Samaniego, C.M.L. (1986). Effect of Initial Dissolved Oxygen Levels on the Degradation of Ascorbic Acid and the Browning of Lemon Juice during Storage. *Journal of Food Science*, 51, 184-187.

Rodrigo, D., Martínez, A., Harte, F., Barbosa-Cánovas, G.V., Rodrigo, M. (2001). Study of inactivation *Lactobacillus plantarum* in orange-carrot juice by means of pulsed electric fields. *Journal of Food Protectection*, 64, 259-263.

Rodrigo, D., Barbosa-Cánovas, G.V., Martínez, A., Rodrigo, M. (2003). Pectin methyl esterase and natural microflora of fresh mixed orange and carrot juice treated with pulsed electric fields. *Journal of Food Protection*, 66, 2336-2342.

Saint-Cricq de Gaulejac, N. Glories, Y., Vivas, N. (1999). Free radical scavenging effect of anthocynins in red wines. *Food Research International*, 32, 327-333.

Sampedro, F., Rivas, A., Rodrigo, D., Martínez, A., Rodrigo, M. (2006). Effect of temperature and substrate on Pef inactivation of Lactobacillus plantarum in an orange juice-milk beverage. *European Food Research and Technology,* 223, 30-34.

Sánchez-Moreno, P., Cano, M.P., De Ancos, B., Plaza, L., Olmedilla, B., Granado, F., Elez-Martínez, P., Martín-Belloso, O., Martín, A. (2005). Intake of Mediterranean soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *International Journal of Food Science and Nutrition*, 56, 115-124.

Simonetti, P., Pietta, P., Testolin, G. (1997). Polyphenol content and total antioxidant potential of selected Italian wines. *Journal of Agriculture and Food Chemistry***,** 45, 1152-1155.

Soliva-Fortuny, R., Bendicho-Porta, S., Martín-Belloso, O. (2006). Modeling high-intensity pulsed electric fields inactivation of a lipase from *Pseudomonas fluoresecens*. *Journal of Dairy Science*, 89, 4096-4104

Tannenbaum, S.R., Archer, M.C., Young, V.R. (1985). Vitamins and minerals. In: Fennema O.R.Food Chemistry. Marcel Dekker Inc, New York. pp. 488-493.

Torregrosa, F., Cortés, C., Esteve, M.J., Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry*, 53, 9519-9525.

Torregrosa, F., Esteve, M.J., Frígola, A., Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*, 73, 339-345.

Vieira, M.C., Teixeira, A.A., Silva, C.L.M. (2000). Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuaçu (theobroma grandiflorum) nectar. *Journal of Food Engineering*, 43, 1-7.

Vikram, V.B., Ramesh, M.N., Prapulla, S.G. (2005). Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. *Journal of Food Engineering*, 69, 31-40.

Wang, W.D., Xu, S.Y. (2007). Degradation kinetics of anthocyanins in blackberry juice and concentrate. *Journal of Food Engineering*, 82, 271-275.

Zhang, Y., Liao, X. J., Ni, Y.Y. , Wu, J.H., Hu, X.S., Wang, Z.F., Chen, F. (2007). Kinetic analysis of the degradation and its color change of cyanidin-3-glucoside exposed to pulsed electric field. *European Food Research and Technology*, 224, 597-603.

Capítulo VII

Phenolic acids, flavonoids, vitamin C and antioxidant capacity of strawberry juices processed by high intensity pulsed electric fields or heat treatments

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ABSTRACT
The effect of high intensity pulsed electric fields (HIPEF) processing (35 kV/cm for 1700 μs in bipolar 4-μs pulses at 100 Hz) on individual phenolic compounds (phenolic acids and flavonoids), vitamin C and antioxidant capacity of strawberry juice was evaluated and compared to heat (90 ºC for 60 s or 30 s) and fresh juice as a reference. Although strawberry juice underwent a substantial depletion of health-related compounds with storage time irrespective of the treatment conducted, ellagic acid was enhanced. HIPEF-treated strawberry juice maintained higher amounts of phenolic acids (ellagic and *p*-coumaric acid) and total anthocyanins than the thermally treated juices during the storage period. Regarding the antioxidant capacity, similar DPPH and ABTS values were obtained so that differences among pasteurized juices were non significant. HIPEF processing may be a technology as effective as thermal treatments not only to achieve safe and stable juices, but also to obtain juices with a high content of antioxidant compounds.

Key words: strawberry juice, phenolic compounds, vitamin C, antioxidant capacity, thermal treatment, high intensity pulsed electric fields.

INTRODUCTION

The beneficial properties of fruits and vegetables have been partially attributed to their content in antioxidant compounds. Antioxidants can scavenge free radical and nonradical reactive oxygen species, which have been associated with several cellular toxic processes including oxidative damage to protein and DNA, membrane lipid oxidation, enzyme inactivation, and gene mutation that may lead to carcinogenesis (Eberhardt, 2000). Ascorbic acid is commonly recognized as one of the major, naturally occurring antioxidants in fruits with protective effects against various oxidative stress-related diseases (Omaye and Zhang, 1998). Recently, other substances present in relatively high amounts in plants have attracted increasing attention, such as flavonols, anthocyanins and other phenolic compounds. Many of these substances are potent antioxidants which may stimulate carcinogen-detoxifying enzymes and counteract inflammatory process (Parr and Bolwell, 2000).

Strawberry and its derivates are of great interest because they are rich in vitamin C and a good source of phenolic compounds such as flavonoids and phenolic acids (Olsson et al., 2004). Citrus and apple juices have dominated the fruit juice market for many years, but within the past decade, juices from other fruits, including strawberry, have come onto the market. Currently, heat processing is the most commonly used technology for inactivating microorganisms and enzymes of juices. However, during thermal processing, in addition to the inactivation of microorganism, sensory and nutritional compounds of juices were reduced (Braddock, 1999). In this context, non-thermal technologies such as high-intensity pulsed electric fields (HIPEF) have received increasing attention because of their ability to preserve juices while avoiding the negative effects of heat pasteurization (Deliza et al., 2003). Microbial inactivation by HIPEF has been shown to reach values as high as those achieved with heat treatments (Elez-Martínez et al., 2006). In addition, the enzymes commonly present in fruit juices are partially or totally inactivated (Marsellés-Fontanet et al., 2007). The extent of microbial and enzyme inactivation in HIPEF-treated juices has been reported to be affected by several factors such as electric field strength, treatment time, pulse width, frequency, and polarity mode, among others (Elez-Martínez et al., 2004; Aguiló-Aguayo et al., et al., 2008). The literature on the effects of HIPEF technology on health-related compounds is limited and information about the impact of HIPEF-processing on the antioxidant food properties is scarce. When compared to heat processing, HIPEF treatments led to orange-carrot, orange and tomato juices with higher amounts of vitamin C (Torregrosa et al., 2006; Elez-Martínez et al., 2007; Odriozola-Serrano et al., 2008). As far as we know, studies on how HIPEF treatments can alter or modify the individual phenolic profile in processed vegetable foods have been not been yet published. Moreover, little is known about the variation of the antioxidant compounds during the commercial shelf-life of HIPEF-processed juices. Therefore, the aim of the present work was to evaluate and compare the effects of HIPEF and heat pasteurization on individual phenolic compounds and vitamin C of strawberry juice. In addition, the effect of storage time (4 ºC) in the concentration of these bioactive compounds was investigated. Antioxidant capacity measured through the evaluation of the free radical-scavenging effect on two different radical (DPPH and ABTS) was correlated to the presence of individual health-related compounds.

MATERIALS AND METHODS

Strawberry juice

Strawberry fruits (*Fragaria ananassa* Duch, cultivar Camarosa) were purchased from a local supermarket (Lleida, Spain). The fruits were washed, drained and chopped. Then, the squeezed strawberry juice was centrifuged at 24.000×g for 15 min and the supernatant was filtered using a steel sieve with a mesh of 2 mm. Electric conductivity (Testo 240 conductivimeter ; Testo GmBh & Co, Lenzkirch, Germany), colorimetric CIELAB values (Macbeth-Kollmorgen inst. Corp., Newburg, NY) using a D_{75} light source and observer angle at 10º, pH (crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain) and soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan) of non-treated and just treated strawberry juice were determined (Table 1).

Table 1.-Analytical characteristics of non-treated and treated strawberry juices

parameters[a]		strawberry juice			
		before treatment	after treatments		
			HIPEF	T 90 ºC-30 s	T 90 ºC-60 s
pH		3.39 ± 0.05	3.38 ± 0.03	3.41 ± 0.02	3.40 ± 0.02
soluble solids (ºBrix)		7.2 ± 0.2	7.1 ± 0.1	7.1 ± 0.1	7.0 ± 0.2
Color	**L***	17.7 ± 0.3	21.4 ± 0.5	19.1 ± 0.3	19.9 ± 0.2
	a*	4.1 ± 0.2	4.4 ± 0.1	5.8 ± 0.3	5.7 ± 0.5
	b*	2.5 ± 0.2	2.4 ± 0.3	3.4 ± 0.1	3.7 ± 0.3
Electrical conductivity (S/m)		0.38 ± 0.02	0.41 ± 0.05	0.40 ± 0.02	0.39 ± 0.03

[a]Results are the mean ± SD of three measurement

Pulsed electric fields equipment

A continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that holds bipolar squared wave pulses was used to treat strawberry juice samples. The juice was pumped at a flow rate of 60 mL/min through a system of eight collinear chambers connected in series. Each chamber had a treatment volume of 0.012 cm^3 that was delimited by two stainless steel electrodes separated by a gap of 0.29 cm. The flow was controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). Treated strawberry juice was passed through a cooling coil connected between each pair

of chambers and submerged in an ice-water shaking bath. Thermocouples were attached to the surface of the stainless-steel coils, 2.5 cm away from the HIPEF zones along the flow direction. The thermocouples were connected to temperature readers and isolated from the atmosphere with an insulation tape. The temperatures of the inlet and outlet of each pair of chambers were recorded every 0.1 s during HIPEF treatment and the samples never exceeded 40 ºC. Strawberry juice was subjected to HIPEF treatment consisted of bipolar square-wave pulses of 4 μs, with a frequency of 100 Hz, and 35 kV/cm field strength during 1700 μs to achieve safe strawberry juices (Mosqueda-Melgar, 2008).

Thermal treatment

Fruit juice industry usually conducts treatments at 90 ºC for 60 s to ensure the safety of juices (Nagy et al., 1993). However, heat treatments carried out at 90 ºC for 30 s can also achieve pasteurization levels. In this study, the impact of both thermal treatments on the antioxidant properties of the juices was compared. In addition, thermal pasteurization treatments at 90 ºC for 60 and 30 s were used as a reference that allows evaluating the effectiveness of HIPEF treatments on bioactive compounds and antioxidant capacity. Strawberry juice was thermally processed in a tubular heat exchanger. A gear pump was used to maintain the juice flow rate through a stainless steel heat exchange coil immersed in a hot water shaking bath (Universitat de Lleida, Lleida, Spain).

Sample packaging and storage

HIPEF and thermal fluid handling system was disinfected first with 4% NaOH and then with 10% chlorine and 20% ethanol solutions prior to processing. Water was pumped through the system after each disinfectant solution (NaOH, chlorine and ethanol). The first 200 mL of treated liquid were discarded to ensure stationary treatment conditions. Polypropylene bottles of 100 mL previously sterilized at 121 ºC for 30 min were used to store strawberry juice. The juice was bottled directly from the treatment system, leaving the minimum amount of headspace volume. Once filled, the receptacle was tightly closed and stored, up to analysis, under refrigeration at 4 ºC in darkness. Mosqueda-Melgar et al. (2008) reported a microbial shelf-life above 91 days for strawberry juices treated by heat (90 ºC- 60 s) or HIPEF (35 kV/cm for 1700 μs in bipolar 4-μs pulses at 100 Hz) and stored at 4 ºC. However, heat treated strawberry juice (90 ºC, 30s) spoiled after 56 days of refrigerated storage. Therefore, the determinations of the antioxidant potential of strawberry juices were performed for 56 days to be able to compare changes of health-related compounds among safe treated juices.

Phenolic compounds

Extraction and Hydrolysis of phenolic acids and flavonols

The extraction was done following the method validated by Hertog et al. (1992). A portion of 40 mL of 62.5% aqueous methanol (2g/L TBHQ) and 10 mL of 6 M HCl were carefully mixed with 0.50 g of freeze-dried strawberry juice. After refluxing at 90 ºC for 2 h with regular swirling, the extract was allowed to cool and was subsequently made up to 100 mL with

methanol and sonicated for 5 min. The extract was then passed through a 0.45 μm filter prior to injection.

Extraction of anthocynins

The extraction was carried following the method proposed by Gómez-Plaza et al. (2008) with some modifications. Approximately 100 mg of freeze-dried strawberry juice was mixed with 2 mL of methanol. After vigorous mixing for 1 min, the mixture was sonicated for 20 min. Then, the suspension was centrifuged at 3400×g for 10 min. Prior to HPLC analysis the samples were passed through a syringe filter 0.45 μm.

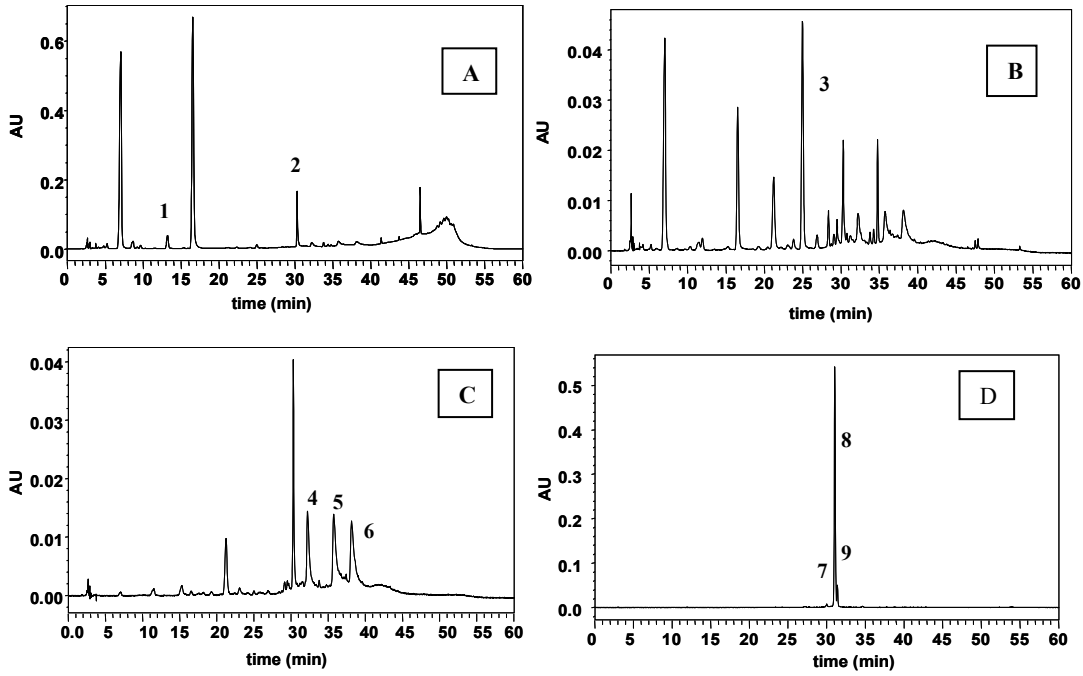

Figure 1.- HPLC chromatogram of the strawberry juice. Detection: 260nm (A), 320 nm (B), 360 nm (C) and 520 nm (D). Peaks: 1, *p*-hydroxybenzoic acid; 2, ellagic acid; 3, *p*-coumaric acid; 4, myricetin; 5, quercetin; 6, kaempferol; 7, cyanidin-3-glucoside; 8, pelargonidin-3-glucoside; 9, pelargonidin-3-rutinoside.

High-Performance Liquid Chromatography

HPLC system was equipped with a 600 Controller and a diode array detector (Waters, Milford, MA) which was set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6mm x 250mm) operating at room temperature with a flow rate of 1 mL/min. A gradient elution was employed with a solvent mixture of 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B) as follows: linear gradient from 5% to 13% B, 0-15 min; linear gradient from 13% to 15% B, 15-20 min; linear gradient from 15% to 30% B, 20-25 min; isocratic elution 30% B, 25-28 min; linear gradient from 30% to 45% B, 28-32 min; isocratic elution 45% B, 32-35 min; linear gradient 45% to 90% B, 35-40 min; isocratic elution 90% B, 40-45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection. Components were identified by comparison of their retention times to those of the standards. *p*-hydroxybenzoic acid, *p*-coumaric acid, ellagic acid, cyanidin-3-glucoside, pelargonidin-3-rutinoside and pelargonidin-3-glucoside., kaempferol, quercetin and myricetin were used to prepare calibration curves. Typical HPLC chromatograms of flavonoids, phenolic acid and anthocyanins of strawberry juice are shown in Figure 1. Results were expressed as mg of each individual compound per 100 g of fresh strawberry juice

Vitamin C

Vitamin C content of strawberry juice was analysed by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (2007). A sample of 25 g of juice was mixed with 25 mL of a solution containing 45 g of metaphosphoric acid and 7.2 g of DL-1,4-dithiotreitol per litre. The mixture was centrifuged at 22100 x g for 15 min at 4 ºC. The supernatant was vacuum-filtered through Whatman No. 1 paper. The sample was then passed through a Millipore 0.45 μm membrane. An aliquot of 20 μs was injected into the HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm x 250 cm). The mobile phase was a 0.01 % solution of sulphuric acid adjusted to pH = 2.6. The flow rate was fixed at 1.0 mL/min at room temperature. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm (Figure 2). Results were expressed as mg of vitamin C per 100 g of strawberry juice.

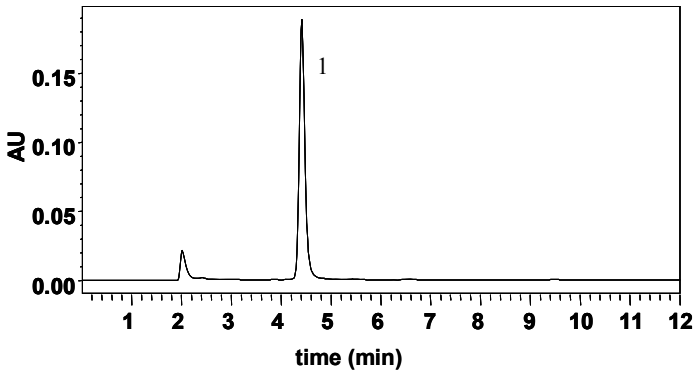

Figure 2.-HPLC chromatogram of the vitamin C (peak 1) of strawberry juice at 245nm

Antioxidant capacity

The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radical according to the procedure described by Odriozola-Serrano et al. (2008). In addition, the ABTS assay, based on the ability of the antioxidants to scavenge the blue-green radical cation 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonate ($ABTS^{\cdot+}$) was conducted according to the method described by Re et al. (1999). Samples of strawberry juice were centrifuged at 6000 x g for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and aliquots of 0.01 mL of the supernatant were mixed with 3.9 mL of methanolic DPPH or ABTS solutions and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 60 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm for DPPH assay or at 734 nm for ABTS assay. The percentage of inhibition of the radicals ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$) was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH and ABTS values were calculated using a regression equation between the Trolox concentration and the percentage of inhibition. Results were expressed as micromol of Trolox equivalents in 100 mL of strawberry juice.

Statistical analysis

Treatments were conducted in duplicate and two replicate analyses were carried out for each sample in order to obtain the mean value. Significance of the results and statistical differences were analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md). Analysis of variance (ANOVA) was performed to compare treatment mean values. The least significant difference test was employed to determine differences between means at a 5 % significance level. Correlations between antioxidant capacity and the studied heath-related compounds were evaluated with Pearson's test.

RESULTS AND DISCUSSION

Phenolic compounds

Total phenolic compounds were calculated as a result of the sum of each individual phenolic compound quantified by HPLC. The effects of processing and storage time on total phenolic content of strawberry juices are shown in Figure 3. Fresh strawberry juice had the highest amount of phenolic compounds (47.3 mg/100 mL). These values are within the range of those observed in fresh strawberries, varying from 21.5 to 53 mg/100g [20, 21]. Heat treated (90 ºC for 60 s) strawberry juices had lower total phenolic content (43.6 mg/100 mL) than those fresh or processed by other treatments (Figure 3). The loss of phenolic content over the storage time in HIPEF and thermally processed strawberry juices was in the range of 21.5 to 24.1 mg/100 mL after 56 days at 4 ºC (Figure 3).

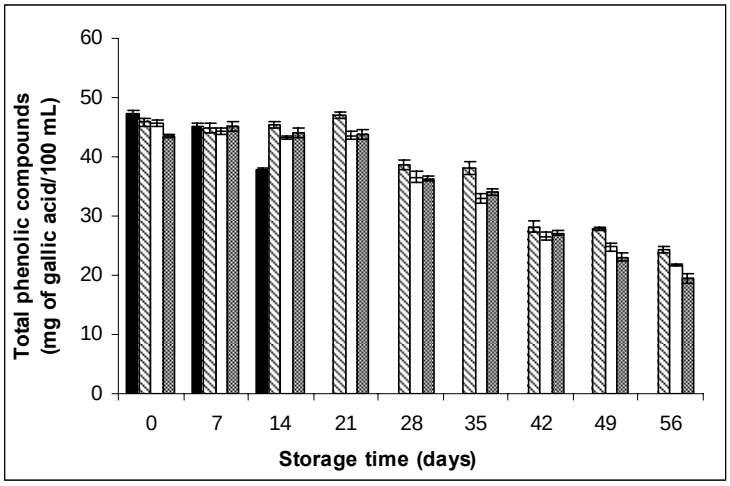

Figure 3.- Effect of HIPEF and heat processing on total phenolic compounds of strawberry juices throughout storage at 4 ºC. Strawberry juices: (■) untreated, (▧) HIPEF treated at 35 kV/cm for 1700 μs in bipolar 4-μs pulses and 100 Hz, (□) heat treated at 90 ºC for 30 s and (▨) heat treated at 90 ºC for 60 s. Data shown are mean ± standard deviation. Total phenolic compounds were calculated as a result of the sum of each phenolic compound quantified by HPLC.

Phenolic acids

Among phenolic acids, ellagic acid was found in the highest concentration (4.1-4.5 mg/100 mL) followed by *p*-coumaric acid (1.69-1.70 mg /100 mL) and *p*-hydroxybenzoic (0.39 to 0.43 mg /100 mL) (Table 2). Studies carried out by Häkkinen et al. (1999) confirm our observations that ellagic acid and *p*-coumaric acid are the two predominant acids in strawberry fruits. As can be seen in Table 2, the kind of treatment applied had a significant effect on the amount of individual phenolic acids. *p*-hydroxybenzoic content was enhanced slightly but significantly after thermal or HIPEF processing compared to the untreated juice, whereas ellagic acid was substantially reduced when the heat treatment was conducted for 60 s. Knowledge of the mechanism involved in the biosynthesis of hydroxybenzoic acids is rather limited, particularly with regard to fruits and vegetables, and thus the conversion of other minor phenolic compounds into *p*-hydroxybenzoic acid should be studied. On the other hand, changes in the amounts of individual phenolic acids over time were observed, irrespective of the treatment applied (Table 2). *p*-coumaric and *p*-hydroxybenzoic acid depleted substantially throughout the storage of strawberry juices, thus attaining values of 1.33-1.49 and 0.23-0.26 mg/100 mL, respectively, after 56 days at 4 ºC (Table 2). On the other hand, an increase in the amount of

ellagic acid was observed during the first weeks of storage in treated strawberry juices stored for 28 days. Beyond this day, the ellagic acid content decreased but residual amounts were kept above those initially obtained for fresh and just-processed strawberry juices. HIPEF-treated strawberry juice maintained phenolic acids better than heat processing during the storage period (Table 2). Higher amounts of ellagic and *p*-coumaric acid over the time (56 days) were observed in HIPEF-treated strawberry juice compared to the thermally processed juices.

Table 2.- Effects of high-intensity pulsed electric fields and heat pasteurization on phenolic acid of strawberry juice throughout storage at 4 ºC.

storage time (days)	process	Individual phenolic acid (mg/100 mL)		
		***p*-OH-benzoic acid**	***p*-coumaric acid**	**ellagic acid**
0	untreated	0.39[b]	1.69[a]	4.5[a]
	HIPEF	0.43[a]	1.70[a]	4.4[a]
	T 90 ºC-30 s	0.43[a]	1.69[a]	4.4[a]
	T 90 ºC-60 s	0.42[a]	1.70[a]	4.1[b]
7	untreated	0.38[b]	1.65[b]	5.7[a]
	HIPEF	0.42[a]	1.69[a]	5.1[c]
	T 90 ºC-30 s	0.44[a]	1.70[a]	5.4[b]
	T 90 ºC-60 s	0.42[a]	1.70[a]	5.0[c]
14	untreated	0.34[b]	1.63[b]	6.1[a]
	HIPEF	0.40[a]	1.69[a]	5.9[a]
	T 90 ºC-30 s	0.42[a]	1.70[a]	6.0[a]
	T 90 ºC-60 s	0.42[a]	1.71[a]	5.4[b]
21	HIPEF	0.39[a]	1.70[a]	6.2[a]
	T 90 ºC-30 s	0.40[a]	1.64[b]	5.9[b]
	T 90 ºC-60 s	0.36[b]	1.62[b]	5.9[b]
28	HIPEF	0.38[a]	1.65[a]	6.4[a]
	T 90 ºC-30 s	0.35[b]	1.55[b]	5.9[b]
	T 90 ºC-60 s	0.34[b]	1.53[b]	6.0[b]
35	HIPEF	0.35[a]	1.60[a]	5.9[a]
	T 90 ºC-30 s	0.34[a]	1.48[b]	5.7[b]
	T 90 ºC-60 s	0.32[b]	1.43[b]	5.7[b]
42	HIPEF	0.32[a]	1.54[a]	6.0[a]
	T 90 ºC-30 s	0.31[a]	1.47[b]	6.1[a]
	T 90 ºC-60 s	0.28[b]	1.45[b]	5.6[b]
49	HIPEF	0.30[a]	1.51[a]	5.9[a]
	T 90 ºC-30 s	0.31[a]	1.38[b]	5.9[a]
	T 90 ºC-60 s	0.27[b]	1.37[b]	5.6[b]
56	HIPEF	0.26[a]	1.49[a]	5.3[a]
	T 90 ºC-30 s	0.26[a]	1.40[b]	5.0[b]
	T 90 ºC-60 s	0.23[b]	1.33[c]	4.9[b]

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1700 µs; bipolar 4-µs pulses at 100 Hz
T 90 ºC-60 s: thermal treatment at 90 ºC for 60 s ; **T 90 ºC-30 s**: thermal treatment at 90 ºC for 30 s
[a]Different letters in the same raw indicate significant differences ($p<0.05$)

Flavonoids

Flavonoids are a widespread group of secondary plant phenolics with low molecular weight, which are characterized by flavan nucleus. This group comprises, among others, anthocyanins, flavonols, flavan-3-ols, flavonones and flavononols (Macheix et al., 1990). The effects of HIPEF and heat treatments on the concentration of anthocyanins in strawberry juices as well as changes in these health-related compounds during storage at 4 ºC are shown in Table 3. Total anthocyanins contents in HIPEF (38.1 mg/100 mL) and thermally processed (36.2-38.0 mg/100 mL) strawberry juices were slightly but significantly lower than in the untreated juice (39.5 mg/100 mL) (Table 3). An equilibrium between four anthocyanin species, including quinonoidal bases, flavylium cations, pseudobases or carbitols, and chalcones have been reported in fresh juices. Increasing temperature reverses the equilibrium towards the formation of chalcones (colourless), diminishing quinoidal, flavylium and carbitol species (Brouillard, 1982). These chalcones can be degraded to brown-colour polymeric structures when exposed to high temperatures for prolonged time, causing a decrease of total anthocyanin content (Jackman and Smith, 1997). Dangles and Brouillard (1992) reported that heat treatments lead to dissociation of the complexes and a release of the flavylium cation that is hydrated to the colorless and labile hemiacetal base, causing a decrease in total anthocyanins. Therefore, the high temperatures reached during heat treatments (90 ºC) can greatly affect the rates of anthocyanins degradation. Pelargonidin-3-glucoside (plg-3-glu), cyanindin-3-glucoside (cy-3-glu) and pelargonidin-3-rutinoside (plg-3-rut) are reported to be the main anthocyanins in strawberry products, accounting for their bright red colour (Sadilova et al., 2007). As observed for total anthocyanins, plg-3-glu and cy-3-glu were affected by processing treatments. Higher reductions in both anthocyanins were observed in processed juices compared to the untreated (Table 3). Zhang et al. (2007) observed that after treating a cy-3-glu methanolic solution with 300 pulses of 1.2-3 kV/cm ($T^a \leq 47$ ºC), the anthocyanin was degraded and the formation of colourless anthocyanin species, particularly chalcones took place. The stability of anthocyanins is influenced by pH, storage temperature, presence of enzymes and oxygen, light and processing conditions (Rein and Heinonen, 2004). Plg-3-glu and plg-3-rut content and, in turn, total anthocyanins of juices, depleted with storage time irrespective of the treatment applied. On the contrary, a significant increase in cy-3-glu was observed during the first 21 days of storage in processed juices, and afterwards, the content decreased up to values of 1.12-1.47 mg/100mL (Table 3). These changes in anthocyanins content throughout storage might be associated to the enhancement of enzymatic activities as a consequence of processing. Aguiló-Aguayo et al. (2008) observed a 10% increment in β-glucosidase activity of strawberry juice subjected to a HIPEF treatment at similar conditions to those reported in the present work. Thermal treatments conducted at 90 ºC led to a 30% increase in the β-glucosidase activity in strawberries fruits (Orruño et al., 2001). Lower anthocyanins concentration was obtained in strawberry juices treated by heat compared to those HIPEF processed (Table 3). On the other hand, no differences were observed in the cy-3-glu and plg-3-rut content of HIPEF- and heat-treated juices.

Table 3.- Effects of high-intensity pulsed electric fields and heat pasteurization on flavonoids of strawberry juice throughout storage at 4ºC

ST	process	Individual flavonoids (mg/100mL)						TA[1]
		flavonols			anthocyanins			
		kaemp.	quercet.	myr.	cy-3-glu	plg-3-glu	plg-3-rut	
0	untreated	0.57[a]	0.39[a]	0.21[a]	3.38[a]	34.6[a]	1.50[a]	39.5[a]
	HIPEF	0.60[a]	0.40[a]	0.21[a]	3.07[b]	33.6[b]	1.46[a]	38.1[b]
	T 90 ºC-30 s	0.60[a]	0.39[a]	0.20[a]	3.18[ab]	33.3[b]	1.46[a]	38.0[b]
	T 90 ºC-60 s	0.58[a]	0.38[a]	0.19[a]	2.94[b]	31.8[c]	1.51[a]	36.2[c]
7	untreated	0.57[b]	0.37[a]	0.16[b]	4.06[a]	30.9[b]	1.41[b]	36.4[a]
	HIPEF	0.57[b]	0.38[a]	0.18[a]	3.67[b]	31.5[ab]	1.48[a]	36.6 [a]
	T 90 ºC-30 s	0.52[c]	0.38[a]	0.19[a]	3.33[c]	31.0[b]	1.40[b]	35.7[a]
	T 90 ºC-60 s	0.61[a]	0.37[a]	0.10[c]	3.27[c]	32.2[a]	1.41[b]	36.9[a]
14	untreated	0.54[b]	0.37[a]	0.06[b]	3.98[a]	23.6[c]	1.08[b]	28.7[c]
	HIPEF	0.54[b]	0.38[a]	0.13[a]	3.32[c]	31.6[a]	1.45[a]	36.4[a]
	T 90 ºC-30 s	0.52[b]	0.35[b]	0.12[a]	3.19[c]	29.6[b]	1.43[a]	34.2[b]
	T 90 ºC-60 s	0.59[a]	0.37[a]	0.08[b]	3.60[b]	30.4[b]	1.39[a]	35.4[ab]
21	HIPEF	0.54[a]	0.40[a]	0.12[a]	4.98[a]	31.1[a]	1.62[a]	37.7[a]
	T 90 ºC-30 s	0.56[a]	0.39[a]	0.09[b]	4.73[a]	28.3[b]	1.58[a]	34.6[b]
	T 90 ºC-60 s	0.48[b]	0.39[a]	nd	5.03[a]	28.5[b]	1.46[b]	35.0[b]
28	HIPEF	0.54[a]	0.38[a]	nd	3.49[b]	24.3[a]	1.40[a]	29.2[a]
	T 90ºC-30s	0.56[a]	0.36[a]	nd	3.77[b]	22.6[b]	1.49[a]	27.9[b]
	T 90ºC-60s	0.54[a]	0.36[a]	nd	4.14[a]	22.0[b]	1.44[a]	27.6[b]
35	HIPEF	0.50[a]	0.34[a]	nd	2.33[a]	25.6[a]	1.38[a]	29.3[a]
	T 90 ºC-30 s	0.54[a]	0.35[a]	nd	2.07[b]	21.2[b]	1.32[a]	24.6[c]
	T 90 ºC-60 s	0.51[a]	0.36[a]	nd	2.06[b]	22.3[b]	1.28[b]	25.6[b]
42	HIPEF	0.51[a]	0.32[a]	nd	2.23[a]	16.4[a]	0.89[a]	19.5[a]
	T 90 ºC-30 s	0.52[a]	0.30[a]	nd	2.15[a]	15.0[b]	0.82[b]	18.0[b]
	T 90 ºC-60 s	0.53[a]	0.30[a]	nd	1.98[a]	16.2[a]	0.80[b]	19.0[a]
49	HIPEF	0.50[a]	0.29[a]	nd	1.38[b]	17.0[a]	0.93[a]	19.3[a]
	T 90 ºC-30 s	0.53[a]	0.23[b]	nd	2.46[a]	14.2[b]	0.78[b]	17.4[b]
	T 90 ºC-60 s	0.49[a]	0.26[ab]	nd	1.54[b]	12.7[b]	0.85[ab]	15.1[b]
56	HIPEF	0.51[b]	0.17[b]	nd	1.12[b]	14.6[a]	0.86[a]	16.6[a]
	T 90 ºC-30 s	0.50[a]	0.21[a]	nd	1.47[a]	12.0[b]	0.80[a]	14.3[b]
	T 90 ºC-60 s	0.47[b]	0.21[a]	nd	1.29[b]	10.3[c]	0.71[b]	12.3[c]

nd: not detected; **kaemp**: kaempferol; **quercet**: quercetin ; **myr**: myricetin; **ST**: storage time (days)
HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1700 µs; bipolar 4-µs pulses at 100 Hz
T 90 ºC-60 s: thermal treatment at 90 ºC for 60 s **T 90 ºC-30 s**: thermal treatment at 90 ºC for 30 s
[1]Calculated as a result of the sum of each anthocyanin quantified by HPLC
[a]Different letters in the same raw indicate significant differences ($p<0.05$)

Regarding the flavonol content, kaempferol was found to be the main flavonol in strawberry juices with values varying from 0.57 to 0.60 mg/100 mL (Table 3). Amounts of flavonols were found in lower concentration than other phenolic compounds such as phenolic acids and anthocyanins. The initial concentrations of quercetin in the studied strawberry juices were 0.38-0.40 mg/100 mL, whereas myricetin was found at concentrations of 0.19-0.21 mg /100 mL just after processing (Table 3). The amounts of flavonoids in strawberry and strawberry based products varied according to the strawberry cultivar, geographical origin, harvesting seasons, growing condition as well as to different extraction/hydrolysis and analytical methods used (Kosar et al., 2004). No significant differences in flavonol content were observed between fresh and treated strawberry juices; thus, these phenolic compounds were not affected by processing (Table 3). In addition, kaempferol, quercetin and myricetin content depleted significantly throughout the storage of strawberry juices irrespective of the treatment conducted. Myricetin content decreased much more considerably than the other flavonols throughout the storage period, leading to non-detectable levels at 21-28 days of storage. In general, HIPEF-processed strawberry juices exhibited similar amounts of quercetin and kaempferol than those treated by heat for 56 days at 4 ºC (Table 3).

Vitamin C

The concentration of vitamin C varied from 61.6 mg/100 mL (heat pasteurized at 90 ºC-60 s) to 65.2 mg/100 mL (untreated) (Figure 4). These values are within the range observed in other studies. Klopotek et al. (2005) reported vitamin C contents between 37 to 69 mg/100g in different strawberry juices. As can be seen in Figure 4, HIPEF-processing resulted into significantly greater vitamin C retention (98%) than thermal treatments (94-95%). Different studies have proved the effectiveness of HIPEF in achieving higher vitamin C retention in comparison to heat treatments. For instance, Odriozola-Serrano et al. (2008) observed that vitamin C retention just after treatment in heat-processed tomato juice was significantly lower (79.2-80.4%) than that found in a juice treated at 35 kV/cm for 1500 μs in bipolar 4-μs pulses at 100 Hz (86.5%). Vitamin C is a heat-sensitive health-related compound in the presence of oxygen. However, low pH conditions, as it is the case of strawberry juice (pH 3.39), are known to stabilize vitamin C (Davey et al., 2000). Therefore, lower processing temperatures reached through HIPEF-processing (T < 40 ºC) would explain the higher retention of vitamin C in HIPEF-treated strawberry juice compared to the thermally processed samples. The concentration of vitamin C in thermally processed, HIPEF-processed and untreated juices decreased gradually with storage time (Figure 4). Although during the first 21 days of storage the concentration of vitamin C was similar among processed strawberry juices, beyond this day, juices subjected to thermal treatment for 60 s exhibited lower vitamin C content compared to the other treated juices.

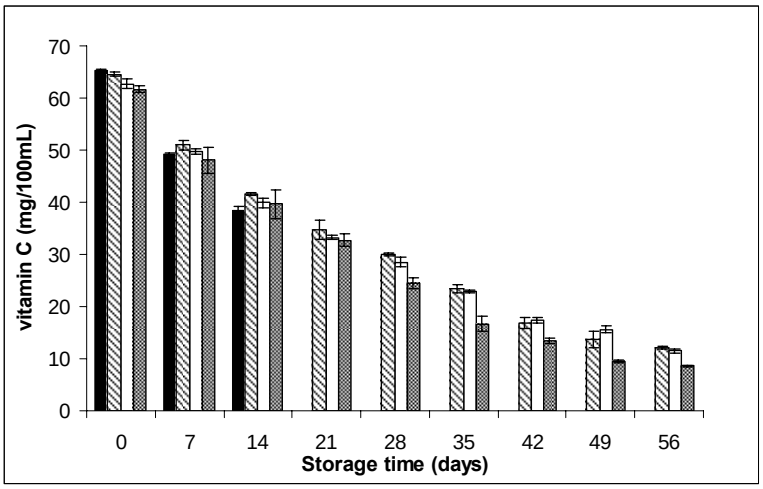

Figure 4.- Effect of HIPEF and heat processing on vitamin C content of strawberry juices throughout storage at 4 ºC. Strawberry juices: (■) untreated, (▨) HIPEF treated at 35 kV/cm for 1700 µs in bipolar 4-µs pulses and 100 Hz, (□) heat treated at 90 ºC for 30 s and (▩) heat treated at 90 ºC for 60 s. Data shown are mean ± standard deviation.

Recommended daily intake (RDI) of vitamin C is currently revised but should be never below 60 mg, as established by the U.S. Food and Drug Administration (FDA, 1999). According to this recommendation, a strawberry juice 250 mL serving size should contain 24 mg/100 mL in order to contribute to the 100% of the RDI. Vitamin C content of juice processed with either HIPEF or heat at 90 ºC for 30 s felt below the RDI at 35 days of storage. The concentration of vitamin C in juices treated at 90 ºC for 60 s was reduced below 24 mg/100 mL after 28 days of storage at 4 ºC.

Antioxidant Capacity

Antioxidant capacities of strawberry juices measured through the DPPH and ABTS methods are shown in Table 4. The free-radical DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The ABTS radical has been used to screen the relative radical-scavenging abilities of flavonoids and phenolic through their properties as electron- or H-donating agents (Miller et al., 2000). According to both methods, the antioxidant capacity of fresh juice was significantly higher than those of pasteurized strawberry juices immediately after the treatments. However, no significant differences in antioxidant capacity were observed between HIPEF and thermally treated strawberry juices (Table 4). In this way, Elez-Martínez y Martín-Belloso (2007) reported similar levels of antioxidant capacity in orange juices treated by HIPEF (15 or 35 kV/cm for 1000 µs at 200 Hz, 4-µs bipolar pulses) and heat (90 ºC, 1min).

Table 4.-Effect of high-intensity pulsed electric fields and heat pasteurization on the antioxidant capacity of strawberry juice throughout storage at 4ºC

Storage time (days)	Process			
	Untreated	**HIPEF**	**T 90 ºC-30 s**	**T 90 ºC-60 s**
	DPPH assay (µmol Trolox/100 mL fw)			
0	414[a]	406[b]	398[b]	403[b]
7	339[a]	331[a]	342[a]	310[b]
14	311[c]	338[b]	349[a]	307[c]
21	-----	300[a]	306[a]	311[a]
28	-----	289[b]	315[a]	283[b]
35	-----	287[a]	280[a]	284[a]
42	-----	279[a]	277[a]	280[a]
49	-----	280[a]	280[a]	281[a]
56	-----	289[a]	275[a]	283[a]
	ABTS assay (µmol Trolox/100 mL fw)			
0	487[a]	463[b]	464[b]	461[b]
7	398[a]	376[b]	384[b]	375[b]
14	326[c]	363[b]	385[a]	371[b]
21	-----	395[a]	348[b]	336[b]
28	-----	314[b]	359[a]	298[b]
35	-----	281[b]	328[a]	290[b]
42	-----	310[a]	306[a]	294[a]
49	-----	309[a]	310[a]	285[b]
56	-----	307[a]	295[a]	288[a]

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1700 µs; bipolar 4-µs pulses at 100 Hz
T 90 ºC-60 s: thermal treatment at 90 ºC for 60 s **T 90 ºC-30 s**: thermal treatment at 90 ºC for 30 s
[a]Different letters in the same raw indicate significant differences ($p<0.05$)

Antioxidant capacity of fruits and vegetables is known to depend on a wide number of compounds. Eberhardt et al. (2000) indicated that most of the antioxidant capacity comes from the natural combination of different phytochemicals. Strawberry juices underwent a substantial loss in total anthocyanins and vitamin C due to pasteurization treatments, which could account for the depletion in antioxidant capacity found in the treated samples in relation to the fresh juices. The antioxidant capacity of strawberry juice depleted with storage time at 4 ºC irrespective of the treatment applied (Table 4). Although fresh strawberry juice had initially the highest antioxidant capacity, greater DPPH and ABTS values were obtained in treated juices compared to the fresh juice beyond the 7 day at 4 ºC. Therefore, processing not only plays a relevant role in obtaining safe and stable juices but also helps to maintain their antioxidant potential. Antioxidant capacity of pasteurized strawberry juices felt considerably during the first weeks of storage, and was then kept almost constant, with values ranging from 275 to 307 µmol Trolox/100 mL after 56 days of storage. Consistently, Odriozola-Serrano et al. (2008) observed a marked depletion in the antioxidant capacity of tomato juices subjected to HIPEF or heat treatment during the first 14 days of storage up to levels above 50 % of the initial DPPH value. Regarding the results obtained in this research, the changes observed in antioxidant capacity over time might be associated to the variations of the individual health-related

compounds. DPPH and ABTS values correlated outstandingly ($R^2 = 0.9480$), and in turn, were highly related to vitamin C levels ($R^2 \geq 0.8843$), which demonstrates that vitamin C is one of the main antioxidant compounds in strawberry juices. Antioxidant capacity and total anthocyanins, and thus plg-3-glu, were also found to be well correlated ($R^2 \geq 0.7281$), suggesting that in this study the variation in antioxidant capacity over time is modulated by anthocyanins. Therefore, the changes in antioxidant capacity could mainly be attributed to vitamin C and anthocyanins rather than to the other studied compounds. Plaza et al. (2006) concluded that the decrease of antioxidant capacity in different orange juices treated by emerging technologies (HIPEF or high pressure) during refrigerated storage could be associated to vitamin C losses. However, the slight relation between flavonols and phenolic acid with the antioxidant capacity of strawberry juice might be explained by the fact that during the phenolic extraction some compounds change their structure, enhancing the concentration of free aglycons. Thus, the structure of flavonols and phenolic acids analyzed may not correspond to that of the actual compounds in the juice.

CONCLUSIONS

HIPEF processing is appropriate for obtaining juices with high content of antioxidant compounds. HIPEF-treated (35 kV/cm for 1700 μs with 4-μs bipolar pulses at 100 Hz) strawberry juice contained higher amounts of anthocyanins, ellagic and *p*-coumaric acids over the storage time than thermally treated (90 ºC-30 s and 90 ºC-60 s) strawberry juices. This greater content of health-related compounds in strawberry juices processed by HIPEF compared to those heat processed juices is not reflected in a high antioxidant capacity. Strawberry juices underwent a substantial depletion of total phenolic compounds, total anthocyanins and vitamin C through storage time, whereas a rise in ellagic acid was observed irrespective of the applied treatment.

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REFERENCES

Aguiló-Aguayo, I., Sobrino-López, A., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric fields processing on lipoxygenase and β-glucosidase activities in strawberry juice. *Innovative Food Science and Emerging Technologies*, 9(4) 455-462.

Aguiló-Aguayo, I., Odriozola-Serrano, I., Quintão-Teixeira, L.J., Martín-Belloso O. (2008). Inactivation of tomato juice peroxidase by high-intensity pulsed electric fields as affected by process conditions. *Food Chemistry*, 107,949-955.

Allende, A., Luo, Y., McEvoy, J.L, Artés, F., Wang, CY. (2004). Microbial and quality changes in minimally processed baby spinach leaves stored under superatmospheric oxygen and modified atmosphere conditions. *Postharvest Biology and Technology*, 33, 51-59.

Braddock, R.J. (1999). Handbook of citrus by-products and processing technology. & Sons. New York, John Wiley.

Brouillard, R. (1982). Chemical structure of anthocyanins. In: Markakis, P. Anthocyanins as food colors. Food Science and Technology. Academic Press, London, UK. pp. 1-40.

Dangles, O., Brouillard. R. (1992). A spectroscopic method based on the anthocyanins copigmentation interaction and applied to the quantitative study of molecular complexes. *Journal of Chemical Society Perkin Transactions*, 2, 247–257.

Davey, M.W., Van Montagu, M., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science of Food and Agriculture,* 80, 825-860.

Deliza, R., Rosenthal, A., Silva, A.L.S. (2003). Consumer attitude towards information on non conventional technology. *Trends in Food Science and Technology*, 14(1-2), 43-49.

Eberhardt, M.V., Lee, C.Y., Liu, R.H. (2000). Antioxidant activity of fresh apples. *Nature*, 405(6789), 903-904.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R.C., Martín-Belloso, O. (2004). Inactivation of *Saccharomyces cerevisiae* suspended in orange juice using high-intensity pulsed electric fields. *Journal of Food Protection,* 67, 2596-2602.

Elez-Martínez, P., Soliva-Fortuny, R.C., Martín-Belloso, O. (2006). Comparative study on shelf-life of orange juice processed by high intensity pulsed electric fields or heat treatments. *European Food Research Technology*, 222, 321-329.

Elez-Martínez, P., Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102, 201-209.

FDA. (1999). Nutritional labelling manual: a guide for developing and using databases. Center for Food Safety and Applied Nutrition: Washington D.C.

Gómez-Plaza, E., Gil-Muñoz, R., Hernández-Jiménez, A., López-Roca, J.M., Ortega-Regules, A., & Martínez-Cutillas, A. (2008). Studies on anthocyanins profile of Vitis Vinifera intraspecific hybrids (Monastrell × Cabernet Sauvignon). *European Food Research and Technology*, 227, 479-484.

Häkkinen, S.H., Heinonen, M., Kärenlampi, S., Mykkänen, H., Ruuskanen, J., Törrönen, R. (1999). Screening of selected flavonoids and phenolic acids in 19 berries**.** *Food Research International*, 32**,** 345-353.

Hernanz, D., Recamales, A,F., Meléndez-Martínez, A.J., González-Miret, M.L., Heredia, F.J. (2007). Assessment of the differences in the phenolic composition of five strawberry cultivars (Fragaria × ananassa duch.) grown in two different soilless systems. *Journal of Agricultural and Food Chemistry*, 55, 1846-1852.

Hertog, M., Hollman, P., Venema, D. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, 40, 1591-1598.

Jackman, R.L., Smith, J.L. (1997). Anthocyanins and betalains. Natural Food colorants. In: Hendry, G.A.F., Houghton J.D. Natural Food Colorants. Blackie Academic & Professional, Chapman & Hall, London. pp.244-309.

Klopotek, Y., Otto, K., Böhm, V. (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 53, 5640-5646.

Kosar, M., Kafkas, E., Paydas, S., Baser, K.H. (2004). Phenolic composition of strawberry genotypes at different maturation stages. *Journal of Agricultural and Food Chemistry*, 52, 1586–1589.

Macheix, J.J., Fleuriet, A., Billot, J. (1990). Fruit phenolics. CRC Press, Boca Raton, Florida.

Marsellés-Fontanet, A.R., Martín-Belloso, O. (2007). Optimization and validation of PEF processing conditions to inactivate enzymes of grape juices. *Journal of Food Engineering*, 83, 452-462.

Miller, H.E., Rigelhof, F., Marquart, L., Prakash, A., Kanter, M. (2000). Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of the American College of Nutrition*, 19, 312S–319S.

Mosqueda-Melgar, J., Raybaudi, R.M., Martín-Belloso, O. (2008). Non-thermal pasteurization of fruits juices by combining high-intensity pulsed electric fields with natural antimicrobials. *Innovative Food Science and Emerging Technologies*, 9, 328-340.

Nagy, S., Chen, C.S., Shaw, P.E. (1993). Fruit juice processing technology. Auburndale, Agscience, Florida.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits. *Food Chemistry,* 105, 1151-1158.

Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. *Innovative Food Science and Emerging Technologies,* 9, 272-279.

Olsson, M.E., Ekvall, J., Gustavsson, K.E., Nilsson, J., Pillai, D., Sjöholm, I., Svensson, U., Åkesson, B., Nyman, M.G.L. (2004). Antioxidants, Low Molecular Weight Carbohydrates, and Total Antioxidant Capacity in Strawberries (*Fragaria × ananassa*): Effects of Cultivar, Ripening, and Storage. *Journal of Agricultural and Food Chemistry*, 52, 2490-2498.

Omaye, S.T., Zhang, P. (1998). Phytochemical interactions: β-carotene, tocopherol and ascorbic acid. In: Bidlack, W.R., Omaye, S.T., Meskin, M.S., Jahner, D. Phytochemicals, a new paradigm. Technomic, Lancaster. pp. 53-75.

Orruño, E., Apenten, R.O., Zabetakis, I. (2001). The role of β-glucosidase in the biosynthesis of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in strawberry (*Fragaria* × *ananassa* cv. Elsanta). *Flavour and Fragrance Journal*, 16, 81–84.

Parr, A.J., Bolwell, G.P. (2000). Phenols in plant and in man. Te potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science and Food Agriculture*, 80, 985-1012.

Plaza, L., Sánchez-Moreno, C., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., Cano, M.P. (2006). Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. *European of Food Research and Technology*, 223, 487-493.

Re, R., Pellegrin, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free Radical Biology and Medicine*, 26, 1231-1237.

Rein M.J., Heinonen M. (2004). Stability and enhancement of berry juice color *Journal of Agricultural and Food Chemistry*, 52, 3106-3114.

Sadilova, E., Carle, R., Stintzing, F.C. (2007). Thermal degradation of anthocyanins and its impact on color and in vitro antioxidant capacity. *Molecular Nutrition and Food Research*, 51, 1461-1471.

Torregrosa, F., Esteve, M.J., Frígola, A., Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*, 73, 339-345.

Zhang, Y., Liao, X. J., Ni, Y.Y., Wu, J.H., Hu, X.S., Wang, Z.F., Chen, F. (2007). Kinetic analysis of the degradation and its color change of cyanidin-3-glucoside exposed to pulsed electric field. *European Food Research and Technology*, 224, 597-603.

Capítulo VIII

Effect of minimal processing on bioactive compounds and color attributes of fresh-cut tomatoes

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ABSTRACT

The feasibility of minimal processing and modified atmosphere packaging (5%O_2 +5%CO_2) to preserve color attributes and bioactive compounds of fresh-cut tomato from different cultivars (Rambo, Durinta, Bodar, Pitenza, Cencara and Bola) was evaluated through storage under refrigeration. The phenolic compounds and vitamin C content of the six cultivars varied between 187.4 and 335.9 mg/kg fw and from 69.6 and to 212.3 mg/kg fw, respectively. The highest content of lycopene was found in Bodar tomatoes (80.5 mg/kg fw) while the concentration in the other cultivars ranged between 20.0 and 43.1 mg/kg fw. Antioxidant capacity, measured on the basis of the DPPH stable radical, was higher than 9.8% of DPPH inhibition. Neither the content of health-related compounds (lycopene, vitamin C and phenolic compounds) nor the antioxidant capacity changed significantly between whole and just-processed fresh-cut tomatoes. Furthermore the initial colors of fresh-cut tomatoes as well as vitamin C were maintained for three weeks under cold storage. The antioxidant capacity was well correlated with vitamin C and phenolic content, whereas lycopene was directly related to color measurements (a*, L* and hº). Minimal processing maintains the main antioxidant compounds and colour parameters of slices tomatoes for 21 days at 4ºC, thus preserving their initial nutritional value.

Key words: fresh-cut tomato, minimal processing, bioactive compounds, antioxidant capacity and color

INTRODUCTION

The protection provided by fruits and vegetables against diseases, including cancer and cardio- and cerebrovascular diseases, has been attributed to the antioxidants they contain (Ames, 1983). Tomatoes are rich in health-related compounds as they are good sources of vitamins, carotenoids and phenolic compounds (Giovanelli et al., 1999). Regarding vitamins, tomatoes have a remarkable concentration of vitamin C. Experimental studies have shown that vitamin C plays an important role in human health, including effects on immune system and the risk of Alzheimer diseases (Sánchez-Moreno et al., 2003). On the other hand, tomatoes constitute the main available source of lycopene, a carotenoid with a high oxygen-radical scavenging and quenching capacities (Beecher, 1998). Phenolic compounds, although present in lesser amounts, could also contribute to the beneficial effects of tomato products. This group of substances is known to show high antioxidant activity, owing to their diversity and extensive distribution. Phenolics posses biological and chemical properties in common: reducing character, capacity of sequestering reactive oxygen species (ROS) and several electrophiles, for chelating metallic ions, tendency to self-oxidation and capacity to modulate the activity of some cell enzymes (Robards et al., 1999).

The increase in the consumers' awareness of the health benefits of fruits and vegetables and the emerging need for convenience due to a fast-paced lifestyle is leading to an increasing demand of minimally processed fruits and vegetables. Minimal processing has been defined as a combination of procedures, such as washing, sorting trimming, peeling and slicing or chopping, that do not affect the fresh-like quality of the food (Wiley, 1994). Consequently, fresh-cut fruit and vegetables should offer consumers highly nutritious, convenient and healthful food while still maintaining the desired freshness. However, as a result of peeling, cutting and preparation of ready-to-eat fruits, a large number of physiological phenomena such as biochemical changes and microbiological spoilage take place and may result in degradation of color, texture, and flavor (Martín-Belloso et al., 2006). Passive and active modified atmosphere packages (MAP) with different concentrations of CO_2 and O_2 have been used to maintain the quality of fresh-cut tomato. Artés et al. (1999) observed that shelf-life of tomato slices could be maintained for 10 days at 2ºC under both active and passive MAP (7.5%O_2 and 0% CO_2). Furthermore, Aguayo et al. (2004) obtained good quality, appearance and texture when fresh-cut tomato was stored at 5ºC during 14 days in MAP (3kPa O_2 + 4 kPa CO_2, with N_2 as balance gas). Nevertheless, as far as we know, little information is available about the content of health-related compounds in different tomato cultivars. Moreover, the available studies report neither the influence of minimal processing on bioactive compounds of fresh-cut tomato nor the effect of cultivar on the evolution of these compounds throughout storage time.

Therefore the aim of the present work was to determine the content of lycopene, vitamin C and phenolic as well as the antioxidant capacity in six different cultivars of tomato (Rambo, Durinta, Bodar, Pitenza, Cencara and Bola). In addition, the effect of minimal processing and postharvest storage (4ºC) on the content of bioactive compounds in the different cultivars was investigated. Color was also considered as a quality parameter related to those compounds and correlation studies were conducted.

MATERIALS AND METHODS

Plant material

Tomatoes *(Lycopersium esculentum* Mill*.)* from different cultivars: Rambo, Durinta, Bodar, Pitenza, Cencara, and Bola, grown in Alicante (Spain), were purchased in a local supermarket (Lleida, Spain) at commercial maturity and stored at 4±1ºC. Analytical characteristics of whole tomatoes of the selected varieties are shown in Table 1. pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), titratable acidity, soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Tokyo, Japan), color measurement (spectrophotocolorimeter Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan), texture (TA-XT2 Texture Analyzer; Stable Micro Systems Ltd, Surrey, UK) and respiration quotient of whole tomato were measured (Soliva-Fortuny et al., 2005).

Parameters		Tomato cultivars					
		Rambo	**Durinta**	**Bodar**	**Pitenza**	**Cencara**	**Bola**
pH		4.34±0.01^{a}	4.37±0.02^{b}	4.46±0.01^{c}	4.44±0.01^{c}	4.34±0.00^{a}	4.33±0.00^{a}
Titrable acidity (g CA /100 g)		0.50±0.02^{a}	0.41±0.03^{b}	0.34±0.01^{c}	0.56±0.01^{de}	0.54±0.01^{d}	0.59±0.04^{e}
Soluble solids (ºBrix)		4.35±0.07^{ab}	4.25±0.07^{a}	4.55±0.07^{b}	5.15±0.07^{c}	5.45±0.21^{d}	6.30±0.00^{e}
Color	**L***	42.0±1.3^{a}	47.9±3.2^{b}	42.2±1.8^{a}	37.5±2.1^{c}	40.0±0.5^{ac}	43.1±2.0^{a}
	a*	13.7±2.6^{a}	11.9±2.4^{a}	21.8±2.5^{b}	17.8±2.6^{b}	19.5±1.8^{b}	17.9±3.2^{b}
	b*	21.6±1.2^{a}	33.1±4.3^{b}	25.1±1.9^{a}	22.8±2.6^{a}	23.4±1.7^{a}	27.1±3.6^{a}
Texture (N. s)		141.7±20.3^{a}	80.2±21.5^{b}	77.0±17.6^{c}	189.5±24.5^{d}	83.9±16.4^{b}	116.7±19.2^{e}
Respiration quotient		0.64±0.05^{a}	0.52±0.08^{ab}	0.77±0.06^{c}	0.23±0.03^{d}	0.68±0.01^{ac}	0.49 ±0.04^{b}

Table 1.- Physicochemical properties of whole tomatoes.

Values are expressed as mean ± SD; CA = citric acid

[a]Different letters in the same line indicate significant differences ($p<0.05$)

Sample preparation

Tomatoes were sanitized for 2 min in chlorinated water (0.2 mg free chroline/l) at 4ºC, rinsed with tap water and gently dried by hand. The fruits were cut into 7 mm-thick slices from the stem-end portion perpendicularly to the long axis of the fruit with a commercial slicing machine (Food Slicer-6128; Toastmaster Corp., Elgin, USA). Five tomato slices (100g) were packaged in polypropylene trays containing absorbent paper on the bottom to avoid juice accumulation. The modification of package atmosphere was carried out by flushing a mixture of 5kPa CO_2 + 5kPaO_2 (N_2 balanced) in a ratio product/gas mixture 1:2. The trays were sealed using a thermosealing system with a digitally-controlled compensated vacuum packing machine (ILPRA Food Pack Basic V/6, ILPRA Systems. CP., Vigevono, Italia). ILPRA The O_2 and CO_2 permeabilities were 110 $cm^3/m^2*day^1*bar^1$ and 500 $cm^3/m^2*day^1bar^1$ at 23 ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain).

Headspace gases analysis

A Micro-GC CP 2002 gas analyzer (Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector was used to analyze the composition of the package headspace. An aliquot of 1.7 ml was withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection module. Sample volumes of 0.25 and 0.33 µl were injected for O_2 and CO_2 determination respectively. The oxygen content was analyzed with a CP-Molsieve 5 Å packed column (4mx 0.32 mm, df = 10 µm) at 55 ºC and 90 kPa. On the other hand, a Pora-PLOT Q column (10 m x 0.32mm, df = 10 µm) was held at 75 ºC and 140 kPa used for carbon dioxide quantification.

Lycopene

Total lycopene content was measured spectrophotometrically following the method proposed by Davis et al. (2003). Approximately 0.6 g of sample were weighed from each cultivar and added to a mixture consisting of 5ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml of 95% USP grade ethanol and 10 ml of hexane. The homogenate was centrifuged at 320xg for 15 min on ice. After shaking, 3 ml of deionized water were added. The vials were then agitated for 5 min and left at room temperature to allow phase separation. The absorbance of the upper, hexane layer, was measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample was estimated using the absorbance at 503 nm and the sample weight (Fish et al., 2002). Results were expressed as mg of lycopene per kg of fresh-cut tomato.

Vitamin C

The extraction procedure was based on a method proposed by Sanchez-Mata et al. (2000). A portion of 25 g of fruit was added to 25 ml of a 4.5% metaphosforic solution with 10% of DTT (20mg/mL) as reducing agent. The mixture was crushed, homogenized and centrifuged at

22100xg for 15 min at 4 ºC. The supernatant was vacuum-filtered through Whatman No. 1. The sample was then passed through a Millipore 0.45 µm membrane and injected into the HPLC system.

Chromatographical conditions

A Waters 600E multisolvent delivery system was used for the analysis. Samples were introduced onto the column via a manual injector equipped with a sample loop (20 µl). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6mm x 250cm). The mobile phase was a 0.01% solution of sulphuric acid adjusted to pH=2.6. The flow rate was fixed at 1.0 ml/min. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Identification of the ascorbic acid was carried out by HPLC comparing the retention time and UV-visible absorption spectrum with those of the standards.

Total Phenolic compounds

Total phenols were determined by the colorimetric method of Singleton et al. (1999) using the Folin-Ciocalteu reagent. Samples of tomato were centrifuged at 6000xg for 15 minutes at 4ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and filtered through Whatman No 1 filter paper. Afterwards, 0.5 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 10 ml of saturated Na_2CO_3 solution. Samples were allowed to stand for 1 h at room temperature before the absorbance at 725 nm was measured. Concentrations were determined by comparing the absorbance of the samples with standards. Results were expressed as milligrams of gallic acid in a kg of fresh-cut tomato.

Antioxidant capacity

The antioxidant capacity was studied through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. This determination was based on the method proposed by de Ancos et al. (2002). Samples of tomato were centrifuged at 6000xg for 15 minutes at 4ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and aliquots of 0.01ml of the supernatant were mixed with 3.9 ml of methanolic DPPH (0.025g/l) and 0.090ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured on a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm against blank of methanol without DPPH. Results were expressed as percentage decrease with respect to the absorption value of a reference DPPH solution.

Color

The color of fresh-cut tomatoes was directly measured with a spectrophotocolorimeter Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan). The CIE L* (lightness), CIE a* (red-green) and CIE b* (yellow-blue) were read using a D_{75} light source and the observer angle

at 10º. Chroma and Hue angle were calculated being Chroma = $(a^{*2}+ b^{*2})^{1/2}$ and Hue = $\tan^{-1}$ (b*/a*).

Statistical analysis

Two trays were taken at each time to perform the analysis and two replicates analyses were carried out for each tray in order to obtain the mean value. Significance of the results and statistical differences were analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md). Analysis of variance (ANOVA) was performed to compare cultivar mean values. The least significant difference test was employed to determine differences between means at a 5% significance level. The means obtained for each variable were subjected to principal component analysis (PCA) to evaluate relationships among the studied parameters. This method was also used to detect sample similarities and differences. As variables were measured in different units, there were large differences between them with respect to the mean, variance and standard deviation. To solve this problem, the data were centered and weighted before applying PC models.

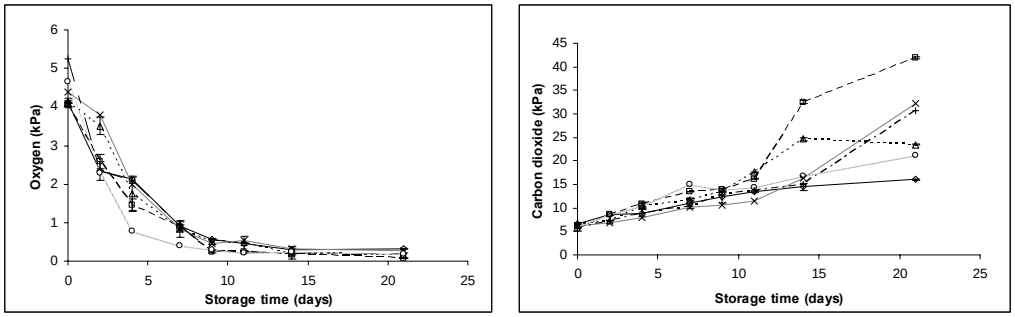
RESULTS AND DISCUSSION

Evolution of modified atmospheres during storage

The O_2 concentration in the headspace of fresh-cut tomato packages decreased dramatically during the first week of storage to values below 1 kPa (Figure 1a). O_2 decreased from 4-5 kPa to 0.085-0.33 after 21 days, without significant differences among cultivars. This trend was also reported by Aguayo et al. (2004) who observed a high reduction in the package O_2 concentration of fresh-cut Calibra tomato throughout the first 7 days of storage at 5ºC. On the contrary, CO_2 production raised dramatically reaching values between 16 to 42 kPa after 21 days of storage (Figure 1b). Gorny et al. (2002) reported severe injury caused by 20 kPa CO_2 atmospheres, similar to damage observed in whole fruit. Contrarily to the O_2 evolution, the CO_2 composition differed significantly among cultivars over 11 days. The highest CO_2 levels were observed in fresh-cut Durinta tomatoes, followed by those from cv. Pitenza and cv. Cencara. Fresh-cut Rambo tomatoes showed the lowest CO_2 levels at 21 days of storage. However, no significant differences were found in CO_2 evolution between Rambo and Bola fresh-cut tomatoes.

Respiration rate is inversely associated with product shelf-life (Kader, 1987). Consequently, cultivars that maintain low respirations rates may have longer potential shelf-life. Roming (1995) discussed the importance of an appropriate selection and development of fruit and vegetable cultivars for being used in fresh-cut products. The cultivar selection has a direct influence on the initial physiology and quality of the fresh fruit and, in turn, it greatly impacts the acceptability of fresh-cut products in the retail market. On the other hand, atmospheres of high CO_2 combined with too low O_2 levels may induce production of fermentative metabolites and impart off-flavors in the headspace atmosphere, indicating fermentation that could be the cause of the undesirable sensory changes (Martín-Belloso et al., 2006). The accumulation of fermentation products in response to high CO_2 can be influenced

by the fruit cultivar (Toivonen and DeEll, 2002). In this case, the presence of off-flavors in the headspace atmosphere was not detectable during 21 days in fresh-cut tomatoes for any cultivar.



(a) **(b)**

Figure 1.- Evolution of headspace oxygen (a) and carbon dioxide (b) concentration in fresh-cut tomato packages throughout storage at 4º C. Tomato cultivars: Rambo (◊), Durinta (□), Bodar (Δ), Pitenza (×), Cencara (+) and Bola (○).

Bioactive compounds

Lycopene

Lycopene content varied widely among the studied cultivars (Table 2). The highest content of lycopene was found in Bodar tomatoes (80.5 mg/kg fw) while the concentration in the other cultivars ranged between 20.0 and 43.1 mg/kg fw. Lycopene concentration was significantly different among cultivar except for Pitenza (40.2 mg/kg fw) and Cencara (43.1 mg/kg fw) tomatoes, which presented similar content of this carotenoid. The results obtained in the present work for lycopene content in different tomato cultivars were in the range of those published in the literature which varied from 18 to 170 mg/kg fw (Martínez-Valverde et al., 2002; Spagna et al., 2005; George et al., 2004). The lycopene content of tomatoes varied considerably among cultivars, stages of maturity and growing condition (Sahlin et al., 2004). Harvest maturity, soil fertilization, irrigation, light intensity and day/night temperatures could also affect lycopene formation in tomatoes (Heinonen et al., 1989). On the other hand, lycopene content was well maintained after processing. No significant differences were found between fresh-cut and whole tomatoes (Table 2).

Table 2.- Effect of minimally processing on the health-related compounds of tomato cultivars

TOMATO CULTIVARS		PARAMETERS			
		Lycopene (mg/kg)	Vitamin C (mg/kg)	Total phenolic (mg/kg)	AC (% of DPPH inh)
Rambo	*Whole*	33.0 ± 2.4[a]	69.6 ± 2.4[a]	187.4 ± 9.1[a]	9.8± 1.25[a]
	Fresh-Cut	35.1 ± 0.1[a]	73.3 ± 7.7[a]	170.1± 1.7[a]	11.0± 0.2[a]
Durinta	*Whole*	20.0 ± 1.5[b]	212.3 ± 1.5[b]	326.0 ± 7.0[b]	17.6 ± 0.6[b]
	Fresh-Cut	18.2 ± 1.8[b]	204.8 ± 9.0[b]	314.1 ± 4.0[b]	16.4 ± 0.5[b]
Bodar	*Whole*	80.5 ± 2.2[c]	151.1 ± 3.5[c]	251.4 ± 1.3[c]	13.3 ± 0.6[c]
	Fresh-Cut	77.9 ± 0.6[c]	139.9 ± 9.3[c]	260.3 ± 10.7[c]	13.8 ± 0.4[c]
Pitenza	*Whole*	40.2 ± 2.3[d]	94.5 ± 0.2[d]	226.5 ± 6.0[cd]	10.8 ± 0.6[a]
	Fresh-Cut	42.0 ± 0.1[d]	108.1 ± 5.3[d]	236.2 ± 12.0[cd]	10.8 ± 0.7[a]
Cencara	*Whole*	43.1 ± 0.7[d]	81.3 ± 3.0[a]	214.5 ± 5.7[d]	10.4 ± 0.1[a]
	Fresh-Cut	39.4± 5.7[d]	74.3± 14.4[a]	230.5 ± 16.7[d]	10.9 ± 0.1[a]
Bola	*Whole*	28.0 ± 0.7[e]	129.2 ± 5.1[e]	282.5 ± 7.8[e]	26.3 ± 0.6[d]
	Fresh-Cut	28.1± 0.4[e]	143.8 ± 2.6[e]	302.3 ± 12.0[e]	26.6 ± 0.5[d]

Values are expressed as mean ± SD

[a]Different letters in the same raw indicate significant differences ($p<0.05$)

As can been seen in Figure 2, fresh-cut tomatoes retained their initial lycopene content for a period of 21 days at 4ºC, except for cv. Rambo and cv. Bodar. Fresh-cut Rambo tomatoes kept their lycopene content for 14 days and then, concentration decreased reaching values of 29.3 mg/kg fw at 21 days. On the contrary, lycopene content in fresh-cut Bodar tomatoes depleted slightly but continuously throughout storage from 77.9 to 42.0 mg/kg fw. As far as we know, there are no published works quantifying lycopene content throughout storage of fresh-cut tomatoes. However, insignificant changes in lycopene were reported by Perkins-Veazie and Collins (2004) for fresh-cut watermelon stored for 7 days at 2ºC under similar conditions to those studied in the present work. Mencarelli and Salveit (1988) reported that fruits biosynthesize carotenoids during ripening throughout the storage time. On the other hand, Shi and Le Maguer (2000) observed that carotenoids are susceptible to oxidation in the presence of light, oxygen and low pH. Consequently, the maintenance of lycopene might be due to the synthesis of lycopene induced by ripening and the low oxidation of this carotenoid as a result of low availability of O_2 in the package headspace.

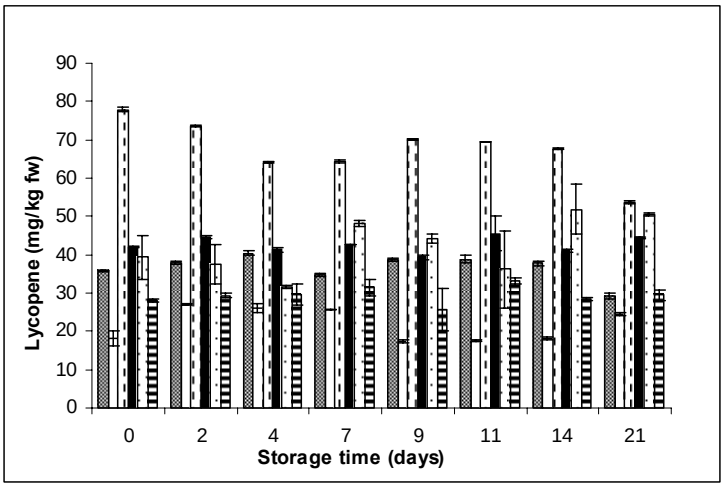


Figure 2.- Evolution of lycopene content in fresh-cut tomato packages throughout storage at 4ºC. Tomato cultivars: Rambo ▨, Durinta □, Bodar ⊡, Pitenza ■, Cencara ⊡ and Bola ▤.

Vitamin C

Vitamin C ranged from 69.6 to 212.3 mg/kg fw in the tomato cultivars studied. Durinta tomatoes showed the highest vitamin C value, while the lowest values were found in Rambo tomatoes. No differences in vitamin C content were observed between whole and the fresh-cut tomato irrespective of the studied cultivar (Table 2). Hence no effect of slicing and packaging was observed on vitamin C content. Consistently, Gil et al. (2006) reported non significant decrease of vitamin C due to cutting in fruits such as mango, strawberry and watermelon. Vitamin C concentration showed no substantial variations through the storage time (Figure 3). This trend is in concordance with those observed by other authors. Wring and Kader (1997) reported no significant losses of vitamin C in fresh-cut strawberries (pH=3.5) over 8 days of study. The maintenance of vitamin C concentration in fresh-cut tomatoes may be explained through the low presence of O_2 inside the trays (Figure 1a). Soliva-Fortuny et al. (2002) reported that the magnitude of vitamin C degradation can be related to the O_2 concentrations inside the packages. Hence, the higher amount of O_2 in the bags headspace the greater decrease in vitamin C content. Vitamin C rapidly disappeared when fresh-cut spinach was stored in air compared to modified atmosphere (Gil et al., 1999). The combination of modified atmosphere with low temperatures may reduce the degradation of vitamin C in fresh-cut fruits. In general, low O_2 atmospheres and CO_2 atmospheres up to 10% reduce deterioration and losses of ascorbic acid in fresh fruits (Kader, 1987). Differences in vitamin C content among cultivar appeared to be significant during the storage period. The highest vitamin C content throughout the storage time was found in fresh-cut Durinta tomatoes leading to levels of 196.1 mg/kg fw at 21 days of storage. On the other hand, fresh-cut Bola tomatoes contained much higher levels of vitamin C during the storage time when compared to fresh-cut Bodar and Pitenza tomatoes. In

contrast, fresh-cut Cencara and Rambo tomatoes did not show significant differences in vitamin C content throughout the cold storage.

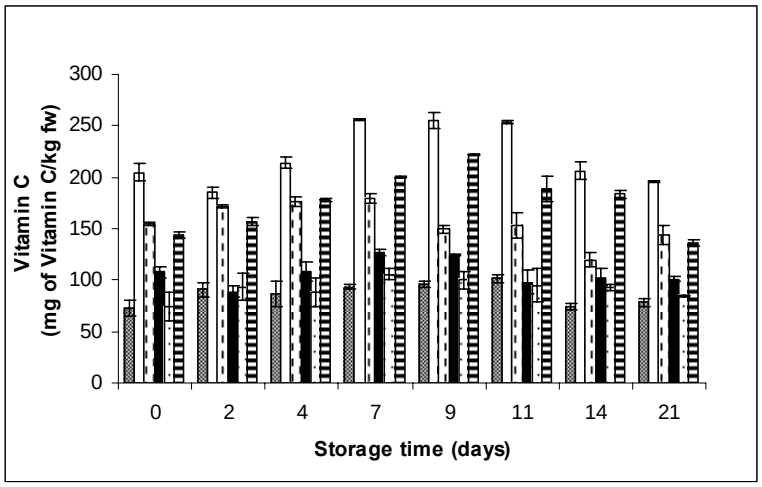


Figure 3.- Evolution of vitamin C content in fresh-cut tomato packages throughout storage at 4ºC. Tomato cultivars: Rambo, Durinta, Bodar, Pitenza, Cencara and Bola.

Total Phenolic Compounds

The concentration of total phenolic compounds in samples of the six cultivars studied varied from 187.4 (Rambo) to 335.9 mg/kg fw (Durinta) (Table 2). These values are within the range observed in other studies. Martínez-Valverde et al. (2002) reported a concentration of phenolic compounds between 272.6 to 498.6 mg/kg fw in different cultivars of tomatoes. On the other hand, phenolic content was not significantly affected by minimal processing operations just after processing (Table 2). As shown in Figure 4, total phenolic compounds of fresh-cut tomato did not change significantly during the first 14 days of storage. However, a significant increase was observed beyond this day, reaching maximum values of 347.5 mg gallic acid/kg fw for fresh-cut Durinta tomatoes, followed by those of cv. Bola (337.1 mg gallic acid/kg fw) and cv. Pitenza (275.9 mg gallic acid/kg fw). This increase in phenolic compounds could be directly associated with the wounding response. Plants respond to wounding generating phenolic compounds involved in the repair of wound damage and in defense against microbial invasion (Dixon & Paiva, 1995). Wounding stimulates the cells adjacent to the injury to produce more phenolics in an attempt to initiate repair process (Toivonen et al., 2002).

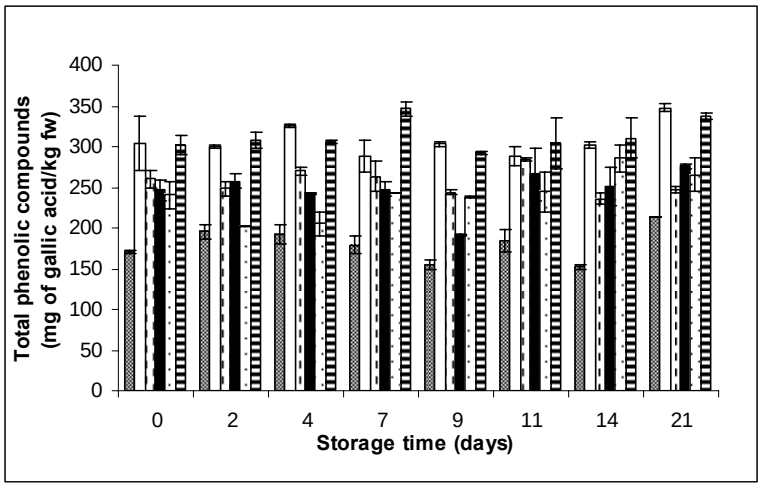


Figure 4.- Evolution of total phenolic compounds content in fresh-cut tomato packages throughout storage at 4ºC. Tomato cultivars: Rambo ▨, Durinta □, Bodar ⊡, Pitenza ■, Cencara ⁘ and Bola ▤.

Antioxidant Capacity

The antioxidant capacity, measured on the basis of the DPPH stable radical, of the six cultivars ranged from 9.8 to 26.3% of DPPH inhibition (Table 2). Bola tomatoes showed the significantly highest antioxidant capacity, whereas no significant differences were observed among tomatoes cv. Rambo (9.8% of DPPH inhibition), cv. Cencara (10.4% of DPPH inhibition) and cv. Pitenza (10.8 % of DPPH inhibition). In our study, minimal processing operations did not affect the DPPH inhibition of fresh-cut tomatoes versus the fresh fruit. Nevertheless, in recent works, significant differences on antioxidant capacity were observed as a consequence of processing. A decrease in the antioxidant capacity after processing was observed in fresh-cut spinach (Gil et al., 1999) and fresh-cut mandarin (Piga et al., 2002). Lana and Tijskens (2006) reported that fresh-cut tissues are primarily subjected to oxidative stress, presumably causing membrane damage and altering the composition and content of antioxidant compounds, resulting in changes in the total antioxidant activity of the tissue. Antioxidant capacity of vegetables is known to depend on a wide number of compounds. Eberhardt et al. (2002) indicated that most of the antioxidant capacity comes from the natural combination of different phytochemicals. Regarding the results obtained in this research, no variation in antioxidant compounds (lycopene, vitamin C and phenolic compounds) was induced during minimal processing operations. Consequently, no changes in antioxidant capacity might be due to the maintenance of the health-related compounds just after cutting and packaging. On the other hand, the antioxidant capacity of fresh-cut tomatoes depleted with storage time irrespective of the cultivar (Figure 5). Fresh-cut Bola tomatoes had the significantly highest

antioxidant capacity over the study period. Durinta tomatoes were the second cultivar with highest antioxidant capacity just after processing, but showed the lowest free-radical scavenging (4.7% DPPH inhibition) at 21 days. The changes in free-radical scavenging capacity might be related to the physicochemical response to the stress induced by wounding (Lana et al., 2006).

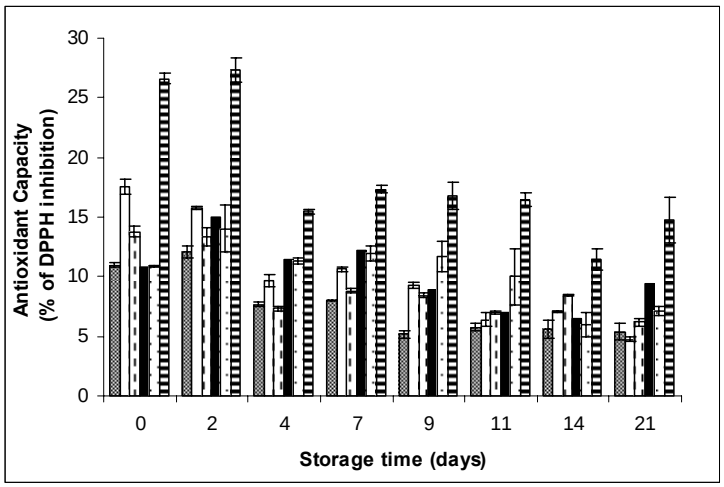


Figure 5.- Evolution of antioxidant capacity in fresh-cut tomato packages throughout storage at 4ºC. Tomato cultivars: Rambo , Durinta , Bodar , Pitenza , Cencara and Bola

A principal components analysis (PCA) was used to explore relationships among data, thus reducing the amount of studied variables. Two principal components (PC1 and PC2) were calculated. They account for 69.2% of the variability in the original data (Figure 6). Results show that antioxidant capacity could not be associated with lycopene. However, there was an outstanding correlation among the DPPH radical scavenging capacity, phenolic compounds and vitamin C. Thus, the higher the vitamin C and phenolic content in tomatoes the greater antioxidant capacity. These correlations were also observed by other authors in tomato fruits (Toor, Savage & Lister, 2006).

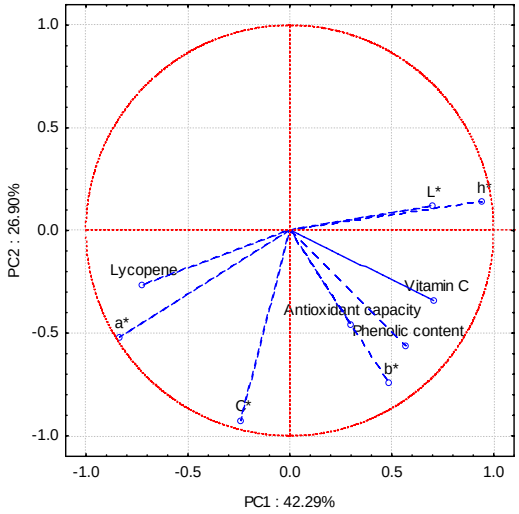


Figure 6.-Principal compounds weights of the bioactive compounds, antioxidant capacity and colour parameters.

Color measurements

CIELab parameters were measured in the different tomato cultivars (Table 1). Differences in these parameters among cultivars appeared to be significant. Durinta tomatoes exhibited the lowest a* (11.9) value and the highest b* (33.1), indicating the highest amount of green pigments among the studied cultivars. This cultivar also was shown to have the highest lightness values of the mesocarpic tissue (47.9). In general, the assayed tomatoes cultivars presented CIE Lab parameters in agreement with values reported by Thompson, Marshall, Sims, Wei, Sargent and Scott (2000). Table 3 shows the CIE Lab parameters of fresh-cut tomato cultivars through the storage time. Neither CIE a* nor CIE b* shifted significantly throughout time in the tested conditions. These results are comparable with those reported by other authors for tomato Durinta slices stored under similar conditions (Artés et al., 1999). Furthermore, lightness (L*), hue angle (hº) and chroma (C*) did not vary significantly during the storage time.

Table 3.- Changes in a*, b*, L*, hue (hº) and chroma (C*)in fresh-cut tomato from different cultivars

		Time (days)[1]							
		0	**2**	**4**	**7**	**9**	**11**	**14**	**21**
a*	Rambo	10.9^{a}	12.2^{a}	12.0^{a}	11.3^{a}	14.3^{b}	12.8^{ab}	12.9^{a}	10.2^{a}
	Durinta	10.0^{a}	11.6^{a}	11.3^{a}	11.6^{a}	10.2^{a}	9.6^{a}	9.7^{b}	9.5^{a}
	Bodar	14.8^{bc}	17.4^{b}	17.5^{bc}	18.1^{d}	16.6^{c}	17.0^{c}	18.8^{d}	16.6^{c}
	Pitenza	14.7^{bc}	15.2^{bc}	13.9^{ab}	16.2^{c}	16.1^{c}	14.7^{bc}	15.2^{c}	15.3^{bc}
	Cencara	18.1^{c}	17.5^{c}	15.9^{b}	16.5^{c}	16.2^{c}	14.0^{bc}	15.4^{c}	16.3^{c}
	Bola	11.5^{b}	15.9^{c}	12.0^{ab}	15.2^{b}	12.5^{b}	11.7^{ab}	14.3^{bc}	14.2^{b}
b*	Rambo	17.6^{a}	18.8^{a}	18.8^{a}	18.7^{a}	18.0^{a}	18.0^{a}	20.6^{a}	18.8^{ab}
	Durinta	20.4^{a}	18.9^{a}	22.5^{a}	21.1^{bc}	21.0^{b}	20.8^{a}	22.8^{a}	19.9^{bc}
	Bodar	21.0^{a}	20.2^{a}	20.4^{a}	19.4^{ab}	19.5^{b}	19.9^{a}	21.1^{a}	22.0^{cd}
	Pitenza	21.9^{a}	20.2^{a}	22.1^{a}	19.1^{ab}	21.4^{b}	20.4^{a}	20.0^{a}	22.4^{d}
	Cencara	20.8^{a}	19.4^{a}	19.5^{a}	19.0^{ab}	18.4^{ab}	17.2^{a}	18.1^{a}	16.8^{a}
	Bola	21.2^{a}	23.0^{b}	21.1^{a}	23.1^{c}	21.3^{b}	22.0^{a}	22.5^{a}	22.4^{d}
L*	Rambo	47.0^{a}	48.4^{c}	47.3^{bc}	48.1^{b}	39.1^{a}	42.1^{a}	46.5^{a}	44.6^{b}
	Durinta	48.6^{a}	50.1^{c}	51.9^{c}	47.1^{b}	46.0^{a}	51.4^{a}	49.1^{a}	45.4^{b}
	Bodar	48.7^{a}	40.3^{ab}	45.1^{ab}	44.5^{b}	41.4^{a}	46.5^{a}	46.7^{a}	43.7^{b}
	Pitenza	39.4^{a}	38.1^{a}	40.7^{a}	37.3^{a}	38.6^{a}	38.7^{a}	37.5^{b}	41.0^{b}
	Cencara	36.7^{a}	35.6^{a}	39.6^{a}	37.1^{a}	36.3^{a}	40.5^{a}	35.9^{b}	34.8^{a}
	Bola	47.4^{a}	45.9^{ab}	43.5^{ab}	43.3^{b}	46.3^{a}	42.4^{a}	40.6^{b}	41.0^{b}
hº	Rambo	58.1^{a}	57.1^{bc}	57.4^{b}	58.8^{c}	51.5^{a}	54.5^{ab}	57.9^{b}	61.5^{de}
	Durinta	63.8^{a}	58.4^{c}	63.4^{b}	61.3^{d}	64.0^{b}	65.2^{c}	66.9^{c}	64.5^{e}
	Bodar	54.8^{a}	49.3^{a}	49.4^{a}	46.9^{a}	49.5^{a}	49.5^{a}	48.3^{a}	53.0^{b}
	Pitenza	56.0^{a}	53.0^{ab}	57.8^{b}	49.6^{b}	53.1^{a}	54.2^{ab}	52.8^{ab}	55.7^{bc}
	Cencara	48.9^{a}	48.0^{a}	50.8^{a}	49.1^{ab}	48.8^{a}	50.5^{ab}	49.5^{a}	45.9a
	Bola	61.6^{a}	55.3^{bc}	60.2^{b}	56.8^{c}	59.7^{b}	62.0^{bc}	57.5^{b}	57.6^{cd}
C*	Rambo	20.8^{a}	22.4^{a}	22.3^{a}	21.8^{a}	23.0^{a}	22.1^{a}	24.3^{a}	21.4^{a}
	Durinta	22.7^{ab}	22.2^{a}	25.2^{a}	24.1^{ab}	23.3^{ab}	22.9^{a}	24.8^{a}	22.1^{ab}
	Bodar	25.7^{cd}	26.7^{b}	26.9^{a}	26.5^{bc}	25.6^{bc}	26.2^{a}	28.3^{a}	27.5^{c}
	Pitenza	26.4^{cd}	25.3^{b}	26.1^{a}	25.1^{bc}	26.8^{c}	25.2^{a}	25.1^{a}	27.1^{c}
	Cencara	27.6^{d}	26.1^{b}	25.2^{a}	25.2^{bc}	24.5^{bc}	22.1^{a}	23.7^{a}	23.4^{b}
	Bola	24.1^{bc}	27.9^{b}	24.3^{a}	27.7^{c}	24.7^{bc}	24.9^{a}	26.6^{a}	26.5^{c}

[1]Different letters in the same raw for each parameters indicate significant differences ($p<0.05$)

*No differences throughout the storage time were found for ach parameter and cultivar

As can be seen in figure 6, a* is well correlated with lycopene content, which is consistent with the fact that an intense red color should be indicative of a higher lycopene content. Moreover, a negative correlation was found between lycopene concentrations and L* and hº. Perkins-Veazie et al. (2004) observed that lycopene levels and L* values were negatively correlated in fresh-cut watermelon. In addition, Thompson et al. (2000) reported that the hue value is a good indicator of lycopene content. On the other hand, PC2 correlates moderately well with C* and b*, showing that these color parameters are weakly related to lycopene. Nevertheless, b* was well related to vitamin C, phenolic compounds and antioxidant capacity. Thus, tomatoes with high values of b* should have greater values of vitamin C, phenolic

compounds and free-radical scavenging capacity. The score plot of PC1 versus PC2 from the full-data PCA model is presented in Figure 7. Six different groups were observed, indicating the six studied cultivar. Durinta and Bola cultivar tomatoes are located in the right part of the score plot, consequently these samples are well correlated with L*, hº and b* values, vitamin C, phenolic content and antioxidant capacity. On the other hand, Rambo and Cencara fresh-cut tomatoes samples appeared on the left-hand side showing the highest a* values and lycopene content.

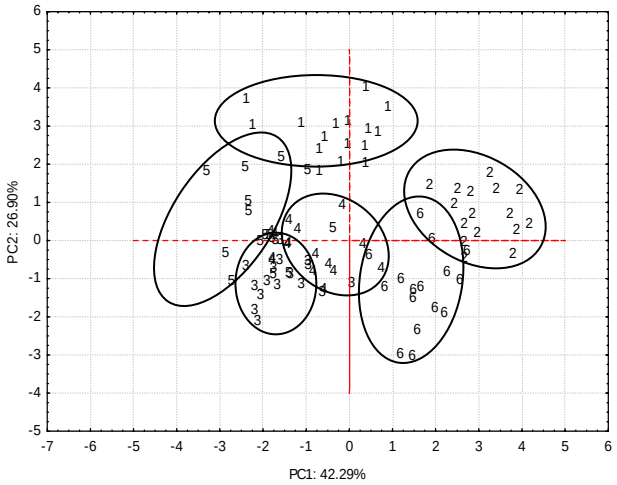


Figure 7.- Principal compounds weights of the samples from different cultivar: Rambo(1), Durinta (2), Bodar (3), Pitenza (4), Cencara (5) and Bola (6).

CONCLUSIONS

Health-related compounds varied greatly among tomato cultivars. Durinta tomatoes had the highest vitamin C and phenolic content; however, this cultivar showed the lowest lycopene levels. The results of this study indicate that the main bioactive compounds (lycopene, vitamin C and phenolic compounds) as well as the antioxidant capacity of just-processed fresh-cut tomatoes do not differ significantly from the values found in the non-processed fruits. However, fresh-cut tomatoes underwent an increase in respiration as a consequence of cutting and packaging. In general, fresh-cut tomatoes maintained the main antioxidant compounds and colour parameters for 21 days at 4ºC. Moreover, some health-related compounds such as phenolics increased after 14 days of storage at 4ºC irrespective of the studied cultivar. Thus, minimal processing offers a potential additional marketing opportunity for this vegetable.

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REFERENCES

Aguayo, E., Escalona, V., Artés, F. (2004). Quality of fresh-cut tomato as affected by type of cut, packaging, temperature and storage time. *European Food Research and Technology*, 219, 492-499.

Ames, B.M. (1983). Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science*, 221, 1256-1263.

Artés, F., Conesa, M.A., Hernández, S., Gil, M.I. (1999). Keeping quality of fresh-cut tomato. *Postharvest Biology and Technology*, 17, 153-162.

Beecher, G.R. (1998). Nutrient content of tomatoes and tomato products. *Proceedings of the Society for Experimental Biology and Medicine*, 218(2), 98-100.

Davis, A.R., Fish, W.W., Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biology and Technology* 28, 425-430.

de Ancos, B., Sgroppo, S., Plaza, L., Cano, M.P. (2002). Posible nutricional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science of Food and Agriculture*, 82, 790-796.

Dixon, R.A., Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell*, 7, 1085-1097.

Eberhardt, M.V., Lee, C.Y. & Liu, R.H. (2000). Antioxidant activity of fresh apples. *Nature*, 405(6789), 903-904.

Fish, W.W., Perkins-Veazie, P., Collins, J.K. (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of Food Composition and Analysis*, 15(3), 309-317.

George, B., Kaur, C., Khurdiya, D.S., Kapoor, H.C. (2004). Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. *Food Chemistry*, 84, 45-51.

Gil, M.I., Ferreres, F., Tomás-Barberán F.A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal Agricultural and Food Chemistry*, 47, 2213-2217.

Gil, M.I., Aguayo, E., Kader, A.A. (2006). Quality and nutrient retention in fresh-cut versus whole fruit during storage. *Journal Agricultural and Food Chemistry*, 54, 4284-4296.

Giovanelli, G., Lavelli, V., Peri, C., Nobili, S. (1999). Variation in antioxidant compounds of tomato during vine and post-harvest ripening. *Journal of the Science of Food and Agriculture,* 79, 1583-1588.

Gorny, J.R., Hess-Pierce, B., Cifuentes, R.A., Kader, A.A. (2002). Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology,* 24, 271-278.

Heinonen, M.I., Ollilainen, V., Linkola, E.K., Varo, P.T., Koivistoinen, P.E. (1989). Carotenoids in Finnish food: vegetables, fruits and berries. *Journal Agricultural and Food Chemistry,* 37, 655-659.

Kader, A.A. 1987. Respiration and gas exchange of vegetables. In: Weichmann, J. Postharvest Physiology of vegetables. Marcel Dekker, New York. (pp. 25-43).

Lana, M.M., Tijskens, L.M.M. (2006). Effect of cutting and maturity on antioxidant activity of fresh-cut tomatoes. *Food Chemistry*, 97, 203-211.

Martín-Belloso, O., Soliva-Fortuny, R., Oms-Oliu, G. (2006). Fresh-Cut Fruits. In: Hui, Y.H.,. Handbook of fruits and fruit processing Blackwell Publishing, Oxford. pp. 129-144.

Martínez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant capacity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture*, 82, 323-330.

Mencarelli, F., Salveit, M.E. (1988). Ripening of mature-green tomato fruit slices. *Journal of the American Society for Horticultural Science*, 113(5), 742-745.

Perkins-Veazie, P., Collins, J.K. (2004). Flesh quality and lycopene stability o fresh-cut watermelon. *Postharvest Biology and Technology*, 31, 159-166.

Piga, A., Agabbio, M., Gambella, F., Nicoli, M.C. (2002). Retention of antioxidant activity in minimally processed mandarin and satuma fruits. *LWT-Food Science and Technology*, 35, 344-347.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P., Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, 66, 401-436.

Roming, W.R. (1995). Selection of cultivars for lightly processed fruit and vegetables. *HortScience*, 30, 38-40.

Sánchez-Mata, M.C., Cámara-Hurtado, M., Díez-Marqués, C., Torija-Isasa, M.E. (2000). Comparison of high-performance liquid chromatography and spectrofluorimetry for vitamin C analysis of green beans (*Phaseolus vulgaris* L.). *European Food Research and Technology*, 210(3), 220-225.

Sánchez-Moreno, C., Cano, M.P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F. Martin, A. (2003). Effect of orange juice intake on vitamin C concentrations and biomarkers of antioxidant status in humans. *American Journal of Clinical Nutrition* 78, 454-460.

Sahlin, E., Savage, G.P., Lister, C.E. (2004). Investigation of the antioxidant properties of tomatoes after processing. *Journal of Food Composition and Analysis*, 17, 635-647.

Shi, J., Le Maguer, M. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Technology*, 40, 1-42.

Singleton, V.L., Orthofer, R.M., Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.

Soliva-Fortuny, R.C., Oms-Oliu, G., Martín-Belloso, O. (2002). Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67(5), 312-317.

Soliva-Fortuny, R.C., Ricart-Coll, M., Martín-Belloso, O. (2005). Sensory quality and internal atmosphere of fresh-cut Golden Delicious apples. *International Journal of Food Science and Technology*, 40, 369-375.

Spagna, G., Barbagallo, R.N., Chisari, M., Branca, F. (2005). Characterization of a tomato polyphenol oxidase and its role in browning and lycopene content. *Journal of Agricultural and Food Chemistry*, 53(6), 2032-2038.

Thompson, K.A., Marshall, M.R., Sims, C.A., Wei, C.I., Sargent, S.A., Scott, J.W. (2000). Cultivar, maturity, and heat treatments on lycopene content in tomatoes. *Journal of Food Science*, 65(5), 503-506.

Toor, R.K., Savage, G.P., Lister, C.E. (2006). Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. *Journal of Food Composition and Analysis,* 19, 1-10.

Toivonen P.M.A., DeEll, J.R. (2002). Physiology of fresh-cut fruits and vegetables. In: Lamikanra, O. Fresh-cut fruits and vegetables CRC Press, New York. pp. 91-123.

Wiley, R.C. (1994). Introduction to minimally processed refrigerated fruits and vegetables. In: Wiley, R.C. Minimally processed refrigerated fruits and vegetables Chapman & Hall, New-York. pp.1-14.

Wright, K.P., Kader, A.A. (1997). Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. *Postharvest Biology and Technology*, 10, 39-48.

Capítulo IX

Antioxidant properties and shelf-life extension in fresh-cut tomatoes stored at different temperatures

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ABSTRACT
The feasibility of modified atmosphere packaging (5 kPa O_2 + 5 kPa CO_2) to maintain the antioxidant properties during shelf-life of fresh-cut tomato was determined through storage at different temperatures (5, 10, 15 and 20 ºC). Health-related compounds, antioxidant capacity, microbiological counts, physicochemical parameters and in-packaged atmosphere of tomato slices were determined. The initial lycopene, vitamin C and phenolic content as well as physicochemical parameters of tomato slices were well maintained for 14 days at 5 ºC. Lycopene and total phenolic contents were enhanced over the time in tomato slices stored at 15 ºC and 20 ºC. However, this increase in the antioxidant compounds of fresh-cut tomatoes throughout storage may be associated with excessive amounts of CO_2 (R^2 = 0.5679-0.7328) inside the trays due to microbial growth. Although keeping tomato slices at temperatures over 10 ºC increase their antioxidant content, the shelf-life of the product is reduced for up 4 days. A storage temperature of 5 ºC, appropriate for maintaining the microbiological shelf-life of fresh-cut tomatoes for 14 days, let also keeping the antioxidant properties of tomato slices over this period, reducing wounding stress and deteriorative changes.

Key words: fresh-cut tomato, minimal processing, antioxidant properties, health-related compounds, antioxidant capacity, shelf-life.

INTRODUCTION

A diet rich in fresh fruits and vegetables has been associated with a high number of health benefits including the prevention of cancer and chronic diseases (Weisburger, 2002). These beneficial effects are believed to be due, at least partially, to the action of antioxidant compounds, which reduce oxidative damage in the body. Among fruits and vegetables, tomatoes are rich in health-related compounds as they are good sources of vitamins, carotenoids and phenolic compounds (Giovanelli, et al., 1999). On the other hand, there is a consumer's increasing demand for "fresh-like", convenient and healthy fruit commodities. Therefore, fresh-cut produce have been a rapidly growing segment in the food service and retail markets. Fresh-cut fruits are generally more perishable than whole fruits because they have been subjected to physiological stresses caused by physical damage or wounding that can limit their shelf-life (Varoquaux and Wiley, 1997) Modified atmosphere packaging (MAP) is a good supplement to cold storage in extending shelf-life of fresh-cut tomato (Mencarelli and Salveit, 1988). Nevertheless, temperature control is the most common and important factor to minimize the effects of cutting in fruit and vegetables (Brecht, 1995). Although temperatures slightly above 0 ºC are usually the desirable temperature for most fresh-cut products, many of them are shipped and marketed at 5 ºC and sometimes at temperatures as high as 10 ºC (Watada et al., 1996). Overfilled shelves, blocked return airflow, or even the position of the product in the shelf may significantly impact the product temperature (Martín-Belloso et al., 1996). Refrigeration may account for more than 50% of the annual electric energy costs of retails. Therefore, small temperature changes are commercially significant because supermarkets operate on a narrow profit margin and increased energy costs impact their competitiveness. On the other hand, consumers are sometimes not aware of the storage requirements of these products and the chill chain is not kept. Abusive temperatures (over 10 ºC) can also be handed at home to maintain fresh-cut products. There is evidence that this period of time could be critical for maintaining quality and shelf-life of plant tissues subjected to wounding stress (Toivonen and DeEll, 2002). Some studies on quality changes and safety of tomato fresh-cut affected by storage temperature have been reported. Aguayo et al. (2004) observed that tomato pieces stored at 0 ºC showed better aroma, appearance, overall quality and microbial stability than those stored at 5 ºC after 7 days of storage. However, as far as we know, no information is currently available about how physicochemical parameters, shelf-life and bioactive compounds of fresh-cut tomatoes are influenced by abusive storage temperatures. Therefore, the objective of this study was to determine the effect of the storage temperature (5 ºC, 10 ºC, 15 ºC and 20 ºC) on the antioxidant potential and microbial stability of fresh-cut tomato stored under MAP conditions (5 kPa O_2 + 5 kPa CO_2, with N_2 as balance gas). Moreover the packages headspace atmosphere was evaluated to determine any possible influence on bioactive compounds. Color, soluble solids and acidity were also considered as physicochemical parameters related to those compounds and correlation studies were conducted.

MATERIALS AND METHODS

Plant material

Tomatoes (*Lycopersium esculentum* Mill. cv. Bola) grown in Alicante (Spain), were purchased in a local supermarket (Lleida, Spain) at commercial maturity and stored at 4 ºC ± 1 ºC. Analytical characteristics of whole tomatoes are shown in Table 1. pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), titratable acidity, soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Tokyo, Japan), color (spectrophotocolorimeter Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan), texture (TA-XT2 Texture Analyzer; Stable Micro Systems Ltd, Surrey, UK) and respiration quotient of whole tomato were measured (Soliva-Fortuny et al., 2005).

Table 1.-Physicochemical characteristics of fresh-tomato before processing

pH		4.48 ± 0.42
Titratable acidity (g citric acid 100 g^{-1})		0.61 ± 0.3
Soluble solids (ºBrix)		4.8 ± 0.5
Surface Color	L*	45.7 ± 1.2
	a*	18.3 ± 0.7
	b*	25.2 ± 2.1
Texture (N s)		108.1 ± 8.3
Respiration quotient		0.51 ± 0.02

Values are expressed as mean ± SD

Sample preparation

Tomatoes were sanitized for 2 min in chlorinated water (0.2 mg free chlorine l^{-1}) at 4 ºC ± 1 ºC, rinsed with tap water and gently dried by hand. The fruits were cut into 7 mm-thick slices from the stem-end portion perpendicularly to the long axis of the fruit with a commercial slicing machine (Food Slicer-6128; Toastmaster Corp., Illinois, Elgin, USA). Five tomato slices (100 g) were packaged in polypropylene trays containing absorbent paper on the bottom

to avoid juice accumulation. The modification of package atmosphere was carried out by flushing a mixture of 5 kPa CO_2 + 5 kPaO_2 (N_2 balanced) in a ratio product/gas mixture 1:2. The trays were sealed using a thermosealing system with a digitally-controlled compensated vacuum packaging machine (ILPRA Food Pack Basic V/6, ILPRA Systems. CP., Vigevono, Italia). The O_2 and CO_2 permeabilities were $5.2419 \cdot 10^{-13}$ mol CO_2 m^{-2} s^{-1} Pa^{-1} and $2.3825 \cdot 10^{-12}$ mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23 ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). Twenty-one packages were stored at each temperature (5 ºC, 10 ºC, 15 ºC and 20 ºC). Initial analyses were determined within the next 4 h after packaging, thus corresponding to values at time 0. Then, three trays were taken at days 2, 4, 7, 9, 11 and 14 to perform the analysis and duplicate analyses were carried out for each tray in order to obtain mean values.

Headspace gases analysis

A Micro-GC CP 2002 gas analyzer (Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector was used to analyze the composition of the package headspace. An aliquot of 1.7 ml was withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection module. Sample volumes of 0.25 and 0.33 µL were injected for O_2 and CO_2 determination respectively. The oxygen content was analyzed with a CP-Molsieve 5 Å packed column (4mx 0.32 mm, df = 10 µm) at 55 ºC and 90 kPa. On the other hand, a Pora-PLOT Q column (10 m x 0.32mm, df = 10 µm) was held at 75 ºC and 140 kPa used for carbon dioxide and ethylene quantification.

Microbiological stability

The microbial stability of tomato slices was evaluated through the determination of total mesophilic aerobic bacteria and yeast and mould populations. A sample of 10 g tomato was homogenized for 2 min with 90 ml of 0.1% sterile peptone solution with a Stomacher Lab Blender 400 (Seward Medical, London, UK). Serial dilutions of fruit homogenates were plated on plate-count agar (PCA) at 30 ºC ± 1 ºC for 72 h ± 3 h for mesophilic bacteria counts (ISO 4833, 1991) and chloroamphenicol glucose agar (GCA) at 25 ºC ± 1 ºC for 5 days for yeast and mould counts (ISO 7954, 1998).

Physicochemical parameters

Soluble solids and pH

The pH and soluble solids content of the juice obtained from a 40 g sample were determined with a CRISON 2001 pH-meter (Crison, Barcelona, Spain) and with a temperature-compensated refractometer Atago RX-1000 (Atago Company Ltd., Tokyo, Japan), respectively.

Color

The color of fresh-cut tomatoes was directly measured with a Minolta CR-400 spectrophotocolorimeter (Konica Minolta Sensing, Inc., Osaka, Japan). The CIE L* (lightness),

CIE a* (red-green) and CIE b* (yellow-blue) were read using a D_{75} light source and setting the observer angle at 10º. The hue angle (hº) was calculated as $h^o = \tan^{-1}$ (b*/a*).

Bioactive compounds

Lycopene

Total lycopene content was measured spectrophotometrically (Davis et al., 2003) Approximately 0.6 g of sample was weighed and added to a mixture consisting of 5 ml 0.05 % (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml 95 % USP grade ethanol and 10 ml hexane. The homogenate was centrifuged at 320xg for 15 min at 4 ºC. Afterwards, 3 ml of deionized water were added. The vials were then agitated for 5 min and left at room temperature to allow phase separation. The absorbance of the upper, hexane layer, was measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample was estimated at 503 nm and the sample weight (Fish et al., 2002) Results were expressed as mg of lycopene per kg of fresh-cut tomato.

Vitamin C

The extraction procedure was based on a previous validated method (Odriozola-Serrano, et al., 2007). A portion of 25 g of fruit was added to 25 ml of a 4.5% metaphosforic solution with 7.2 g l^{-1} DTT as reducing agent. The mixture was crushed, homogenized and centrifuged at 22100xg for 15 min at 4 ºC. The supernatant was vacuum-filtered through Whatman No. 1. The sample was then passed through a Millipore 0.45 µm membrane and injected into the HPLC system. A Waters 600E multisolvent delivery system was used for the analysis. Samples were introduced onto the column via a manual injector equipped with a sample loop (20 µl). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6mm x 250cm). The mobile phase was a 0.01% solution of sulphuric acid adjusted to pH=2.6. The flow rate was fixed at 1.0 ml min^{-1}. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Vitamin C was quantified throughout a calibration curve built with ascorbic acid pure standards and results were expressed as milligrams of vitamin C in a kg of fresh-cut tomato.

Total Phenolic compounds

Total phenols were determined following the colorimetric method which uses the Folin-Ciocalteu reagent (Singleton et al., 1999). Samples of tomato were centrifuged at 6000xg for 15 mi at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and filtered through Whatman No 1 filter paper. Afterwards, 0.5 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 10 ml of saturated Na_2CO_3 solution. Samples were allowed to stand for 1 h at room temperature before the absorbance at 725 nm was read. Concentrations were determined by comparing the absorbance of the samples with standards of different concentration of gallic acid. Results were expressed as milligrams of gallic acid in a kg of fresh-cut tomato.

Antioxidant capacity

The antioxidant capacity was studied through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. DPPH assay is not specific to any particular antioxidant component, thus applying to the overall antioxidant capacity of the sample. This determination was based on the method described previously (De Ancos et al., 2002). Samples of tomato were centrifuged at 6000xg for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH (0.025g/L) and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm against a blank of methanol without DPPH. Results were expressed as percentage decrease with respect to the absorption value of a reference DPPH solution.

Statistical analysis

Significance of the results and statistical differences were analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md). Analysis of variance (ANOVA) of data was performed to compare mean values for each variable under different storage temperature. The least significant difference test (LSD) was employed to determine differences between means at a 5% significance level. Correlations among parameters were evaluated with Pearson's test.

RESULTS AND DISCUSSION

Atmosphere composition in packages of fresh-cut tomato

The concentrations of oxygen and carbon dioxide in trays headspace of fresh-cut tomatoes packaged under 5 kPa O_2 + 5 kPa CO_2 are shown in Fig 1A and B. The O_2 contents decreased dramatically in all the assayed conditions during the first few days of storage up to levels below 0.3 kPa, when values stabilized. In addition, the oxygen levels in packages of fresh-cut tomato were influenced by the storage conditions (Fig 1a). A slight but significantly lower reduction in O_2 concentration in package headspace was observed over time when fresh-cut tomatoes were preserved at 5 ºC, compared to those stored under elevated temperatures. On the other hand, CO_2 production raised dramatically reaching values between 12 and 69 kPa after 14 days of storage (Fig 1b). The storage temperature had an important effect on CO_2 accumulation inside the packages. An increment of CO_2 accumulation in the trays headspace was observed when increasing storage temperature. Consistently, Aguayo et al. (2004) reported increased production of CO_2 and higher levels of O_2 consumption of fresh-cut tomatoes with rising storage temperature. The high CO_2 availability inside packages might affect the antioxidant properties of tomato slices during the storage period. Excessively low O_2 and/or excessive amounts of CO_2 in the packages headspace are often detrimental to the fruit shelf-life because anaerobic respiration is induced, leading to fermentation processes, and the subsequent production of undesirable metabolites (Soliva-Fortuny et al., 2002). Signs of fermentative processes and objectionable aromas were detected after 7 days in the samples stored at 10-20 ºC, whereas tomatoes stored at 5 ºC preserved their initial flavor during all the storage time.

Fig 1c shows ethylene evolution of fresh-cut tomatoes stored at different temperatures. Ethylene production increased immediately after cutting irrespective of the storage temperature

(Fig 1C). Nevertheless, ethylene concentration gradually decreased after 2-4 days of storage in fresh-cut tomatoes at temperatures between 10 and 20 ºC, probably due to the effect of the excessive CO_2 and low amount of accumulated O_2 during this period. Tomato slices stored at 5 ºC exhibited a gradual increase in ethylene production during the first 7 days and then, concentration remained unchanged, reaching values of 13 µl l^{-1} at 14 days (Fig 1C). The inhibition of ethylene production under high CO_2 conditions has been reported by many authors. Hong and Gross (2001) concluded that Ethylene accumulation in packages of fresh-cut tomatoes was partially inhibited by high CO_2 concentration. Buescher (1979) suggested that high CO_2 levels inhibited autocatalytic production of ethylene in tomato fruit. Moreover, it has been demonstrated that low O_2 concentrations reduce ethylene biosynthesis, proving that O_2 participates in the conversion of 1-amino-cyclopropane-1-carboxylic acid to ethylene (Yang, 1981).

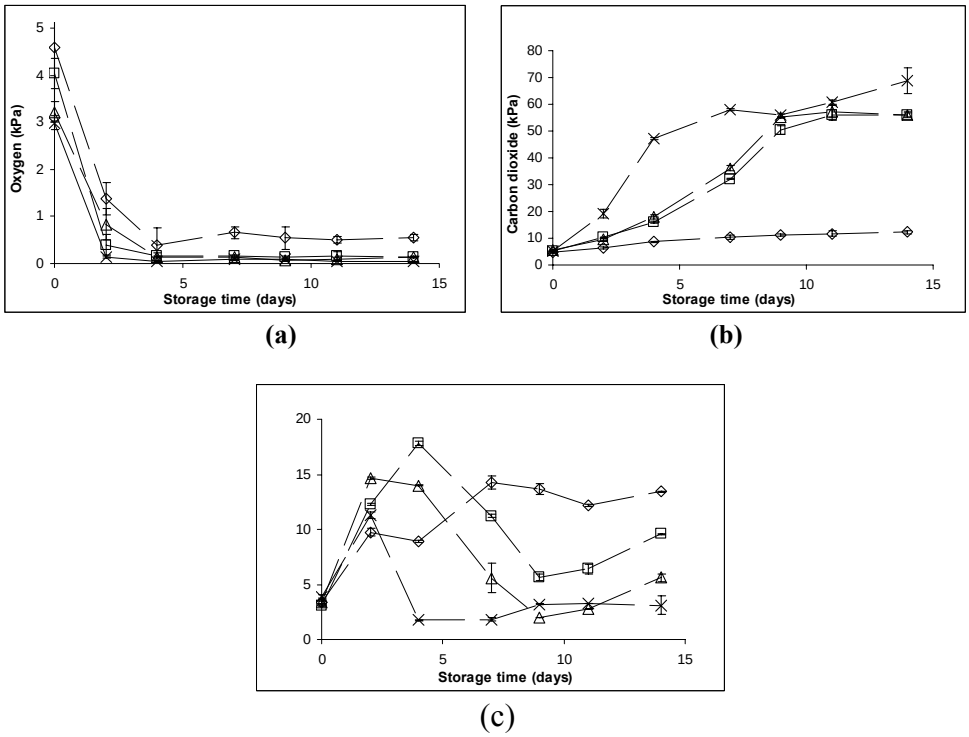

Figure 1.- Effect of storage temperature on (a) headspace oxygen, (b) carbon dioxide concentration and (c) ethylene concentration in fresh-cut tomato packages throughout storage at 5 ºC (◊), 10 ºC (□), 15 ºC (Δ) and 20 ºC (×) for 14 days. Data shown are mean ± standard deviation.

Microbial stability

Initial counts of aerobic mesophilic microorganisms as well as yeast and moulds on just processed tomatoes were lower than 1 log (cfu g^{-1}) (Table 2). Our results are in accordance with those reported by Aguayo et al. (2004) for 'Calibra' tomato slices stored under similar MAP conditions. Storage temperature affected significantly to microbiological counts throughout the storage time. The higher the temperature, the greater the microbiological counts (Table 2). Although modified packaging extends the shelf-life of fresh-cut products by inhibiting the growth of spoilage microorganisms, seriously abusive temperatures can stimulate their proliferation (Nguyen and Carlin, 1994). Tomatoes slices stored at temperatures higher than 10 ºC exceeded 6 log counts (cfu g^{-1}) after 4 of storage. In this way, aerobic mesophilic counts of about 6 log (cfu g^{-1}) were attained beyond the day 7 in fresh-cut tomatoes stored at 10 ºC. The United States and most European countries have regulations relative to fresh-cut produce, which limit the counts of aerobic microorganisms to 6 log (cfu g^{-1}) at expiration day (Martín-Belloso et al., 2006). Counts of aerobic mesophilic microorganisms as well as yeast and moulds were lower than 6 log (cfu g-1) over time in fresh-cut tomatoes stored at 5 ºC (Table 2).

Table 2.- Microbial stability (log cfu g^{-1}) of fresh-cut tomatoes stored under modified packaging at different temperatures for 14 days.

Days	Temperature							
	5 ºC		10 ºC		15 ºC		20 ºC	
	AM	YM	AM	YM	AM	YM	AM	YM
0	< 1	<1	<1	<1	<1	<1	<1	<1
2	2.0	<1	2.0	1.0	3.9	2.4	4.8	3.3
4	3.1	2	2.2	1.0	5.2	2.6	5.8	4.0
7	3.5	2	5.7	2.2	>6	3.8	> 6	4.5
9	3.7	2.2	>6	3.4	>6	5.5	>6	>6
11	3.9	2.3	>6	5.0	>6	>6	>6	>6
14	4.2	2.8	>6	>6	>6	>6	>6	>6

AM = aerobic mesophilic microorganism **YM** = yeast and molds

Physicochemical changes

Soluble solids content and pH of tomatoes were not significantly affected by minimal processing operations just after processing (Table 1 and 3). The pH of fresh-cut tomatoes stored at temperatures between 10-20 ºC, rose slightly but continuously throughout the storage period reaching values from 4.64 and 4.75 at 14 days, whereas it was kept similar to the initial values in tomato slices stored at 5 ºC (Table 3). This increase in pH through storage suggests that the amount of organic acid, and in turn the pH, could be affected by the microbial deterioration of the cut fruit. In addition, the enhancement in the physiological activity would play an important role in the degradation of organic acids in fresh-cut products (Buta, et al., 1999). On the other hand, soluble solids content of tomato slices stored at 10-20 ºC underwent

a substantial decrease throughout storage from 4.7-4.9 to 2.5-3.0 ºBrix after 14 days of storage (Table 3). However, the initial soluble solids content was kept for 11 days in fresh-cut tomatoes stored at 5 ºC and then, concentration increased, suggesting that fruits continue the ripening process during the storage period.

Table 3.- Changes in pH and solid soluble content (ss) in fresh-cut tomato stored under modified packaging storage at different temperatures.

Days	Temperature							
	5 ºC		10 ºC		15 ºC		20 ºC	
	pH	ss	pH	ss	pH	ss	pH	ss
0	4.45^{aA}	4.8^{aA}	4.46^{aA}	4.9^{aD}	4.49^{aAB}	4.8^{aD}	4.46^{aA}	4.7^{aE}
2	4.41^{aA}	4.9^{bA}	4.52^{cB}	4.8^{bD}	4.50^{aB}	4.8^{bD}	4.48^{aA}	4.6^{aE}
4	4.42^{aA}	4.9^{bA}	4.54^{cBC}	4.8^{bD}	4.48^{bA}	4.7^{bD}	4.49^{bA}	3.3^{aD}
7	4.41^{aA}	4.8^{cA}	4.52^{bB}	3.8^{bC}	4.52^{bC}	3.9^{bC}	4.64^{cB}	3.0^{aC}
9	4.43^{aA}	4.9^{cA}	4.56^{bD}	3.4^{bB}	4.58^{bcD}	3.1^{aB}	4.61^{cB}	2.8^{aB}
11	4.43^{aA}	4.8^{dA}	4.55^{bCD}	3.1^{cA}	4.61^{cE}	2.8^{bA}	4.62^{cB}	2.5^{aA}
14	4.44^{aA}	5.5^{cB}	4.64^{bE}	3.0^{bA}	4.68^{cF}	2.7^{aA}	4.75^{dC}	2.5^{aA}

Values within the same line followed by the same lower letter indicate that means are not significantly different by LSD test ($P < 0.05$).

Values within the same column followed by the same capital letter indicate that means are not significantly different by LSD test ($P < 0.05$).

The depletion of soluble solid content in tomato slices preserved at temperatures over 5 ºC might be attributed to the rise in the respiration after cutting, provided that soluble solids are known to be depleted by reactions involved in respiration and also to the action of microorganisms (Table 2). An outstanding correlation between the production of CO_2 and the consumption of soluble solids was observed ($R^2 = -0.97$), meaning that the more accumulation of CO_2 inside the trays, the lower content of soluble solids in tomato slices (Table 4). Neither temperature nor storage time had a significant effect on lightness (L*) Nevertheless, the hue value (hº) was significantly affected by the storage time and temperature (Table 5). Stored temperatures over 10 ºC led to a decline of hº values throughout the storage period, whereas the initial hº values were kept for a period of 14 days in fresh-cut tomatoes preserved at both 5 ºC and 10 ºC. No significant differences in hº values over storage time (15 days) was observed when studying fresh-cut tomatoes packaged under air conditions (Ayala-Zavala, et al., 2008).

Table 4.- Correlation coefficients among in-packaged atmosphere, health-related compounds,

	O_2	CO_2	Ethyl	Lyc	VitC	TP	AC	pH	ss	L*	hº
O_2	------	-0.58	-0.23	-0.48	0.29	-0.25	0.90	-0.41	0.46	-0.06	0.26
CO_2	-0.57	------	-0.53	0.73	-0.57	0.73	-0.61	0.87	-0.97	0.38	-0.62
Ethyl	-0.24	-0.53	------	-0.36	0.33	-0.51	-0.14	-0.45	0.62	-0.47	-0.50
Lyc	-0.47	0.73	-0.36	------	-0.85	0.77	-0.54	0.74	-0.73	0.33	-0.83
VitC	0.29	-0.57	0.33	-0.85	------	-0.74	0.31	-0.70	0.56	-0.18	0.72
TP	-0.25	0.73	-0.51	0.77	-0.74	------	-0.32	0.78	-0.75	0.30	-0.75
AC	0.90	-0.61	-0.14	-0.54	0.31	-0.32	------	-0.48	0.53	-0.14	0.37
pH	-0.41	0.87	-0.45	0.74	-0.70	0.78	-0.48	------	-0.86	0.30	-0.69
ss	0.46	-0.97	0.62	-0.73	0.56	0.75	0.53	-0.86	------	-0.42	0.67
L*	-0.06	0.38	-0.47	0.33	-0.18	0.30	-0.14	0.30	-0.42	------	-0.48
h*	0.26	-0.62	-0.50	-0.83	0.72	-0.75	0.37	-0.69	0.67	-0.48	------

antioxidant capacity and physicochemical parameters of fresh-cut tomato stored at different temperatures for 14 days

Ethyl: ethylene; **Lyc**: lycopene; **VitC**: vitamin C, **TP**: total phenolic compounds; **AC**: antioxidant capacity; **ss**: soluble solids content;

Table 5.- Changes in L* and hº in fresh-cut tomato storage stored under modified packaging at different temperatures

Days	Temperature[1]							
	5 ºC		10 ºC		15 ºC		20 ºC	
	L*	hº	L*	hº	L*	hº	L*	hº
0	49.2	63.7 ^{cA}	49.9	61.3 ^{bA}	52.1	59.3 ^{aA}	51.5	60.2 ^{abA}
2	49.1	64.1 ^{dA}	48.2	61.7 ^{cA}	49.4	58.9 ^{bA}	51.2	57.1 ^{aB}
4	48.1	64.2 ^{bA}	49.3	61.8 ^{bA}	51.8	59.1 ^{aA}	54.3	58.0 ^{aC}
7	49.2	63.8 ^{bA}	49.9	62.9 ^{bA}	51.6	59.3 ^{aA}	51.6	58.7 ^{aD}
9	49.1	63.7 ^{cA}	49.2	62.5 ^{cA}	51.1	59.0 ^{bA}	53.3	55.0 ^{aE}
11	50.0	64.1 ^{dA}	50.6	61.7 ^{cA}	52.0	56.9 ^{bB}	52.0	51.0 ^{aF}
14	49.0	63.7 ^{cA}	49.6	62.8 ^{cA}	51.0	57.0 ^{bB}	50.6	51.6 ^{aF}

No differences during the storage time and temperature were found for L* throughout LSD test ($P<0.05$).

Values within the same line followed by the same lower letter indicate that means are not significantly different by LSD test ($P < 0.05$).

Values within the same column followed by the same capital letter indicate that means are not significantly different by LSD test ($P < 0.05$).

Effect of temperature on bioactive compounds

Lycopene

Lycopene is an isoprenoid compound which provides red color to fruits and vegetables (Britton and Hornero-Méndez, 2004). As can been seen in Fig 2, fresh-cut tomatoes retained their initial lycopene content for a period of 14 days at temperatures up to 10 ºC. On the contrary, the concentration of lycopene in fresh-cut tomatoes stored at 15 ºC increased slightly but continuously throughout storage from 24.7 to 34.6 mg kg^{-1} fw. In this way, the amount of lycopene in fresh-cut tomatoes at 20 ºC underwent a substantial rise from 22.1 to 46.7 mg kg^{-1} fw during the two weeks of storage (Fig 2). Odriozola-Serrano et al. (2008) reported no significant changes in lycopene content over storage time (21 days) at 5 ºC in fresh-cut tomatoes packaged under similar MAP conditions to those studied in this work. Based on the present results it seems that temperature has an important effect on lycopene content. Mencarelli and Salveit (1988) suggested that fruits biosynthesize carotenoids during ripening. However, it has been shown that the formation of lycopene depends on temperature and takes place between 12 and 32 ºC (Leoni, 1992). Turk et al. (1994) found that the optimal temperature for lycopene synthesis in tomatoes was between 16 ºC to 20 ºC. In addition, it has been demonstrated that a large number of bacteria and moulds can synthesize carotenoids during the second growth phase, from their precursor, mevalonic acid pyrophosphate (Shukolyukov and Saakov, 2001). Thus, high amounts of aerobic mesophilic microorganisms or yeast and moulds in fresh-cut tomatoes might enhance their carotenoids content.

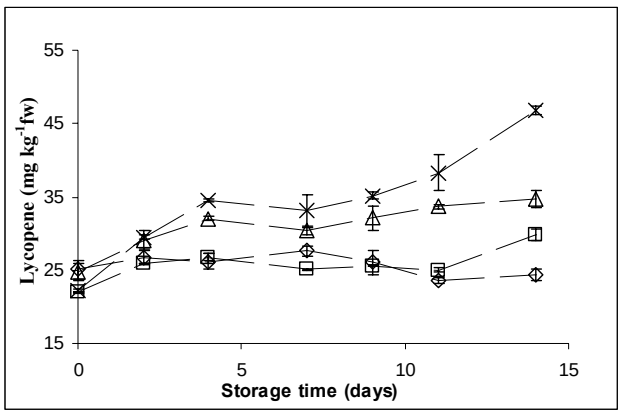

Figure 2.-Effect of storage temperature on lycopene content in fresh-cut tomato packages throughout storage at 5 ºC (◊), 10 ºC (□), 15 ºC (Δ) and 20 ºC (×) for 14 days. Data shown are mean ± standard deviation.

On the other hand, lycopene is susceptible to oxidation in the presence on light, oxygen and low pH (Shi and Le Maguer, 200). Rodriguez-Amaya (1993) found that the stability of lycopene in foods greatly depends on oxygen availability and packaging conditions. In our study, although changes in lycopene through the storage time could not be associated with O_2 content inside the trays (R^2 = -0.47), there was a good correlation between CO_2 concentrations and lycopene content (R^2 = 0.73) (Table 4). High accumulation of CO_2 and limited amounts of O_2 inside packages enhance the synthesis of lycopene throughout the storage in fresh-cut tomatoes. In addition, an inverse correlation between lycopene content and hº value was observed (R^2= -0.83). The same pattern was observed by Hernández et al. (2007) who suggested that hº value is a good indicator of the amount of lycopene in tomato fruits.

Vitamin C

Vitamin C content of fresh-cut tomatoes ranged from 118 to 124 mg kg^{-1} fw (Fig 3). No substantial variations of vitamin C over the storage time were observed neither in fresh-cut tomatoes stored at 5 ºC nor in those preserved at 10 ºC (Fig 3). Consistently, Odriozola-Serrano et al (2008) observed no significant loss of vitamin C in fresh-cut tomatoes packaged under MAP conditions (5 kPa O_2 + 5 kPa CO_2) over 21 days of study at 4 ºC. The combination of MAP with low temperatures may reduce the degradation of vitamin C in fresh-cut fruits (Lee and Kader, 2000). Fresh-cut tomatoes stored at 15 ºC kept their initial vitamin C content (118.6 mg kg^{-1} fw) for 11 days and then, concentration decreased reaching values of 76.8 mg kg^{-1} fw by the end of the storage. In the same way, vitamin C content of fresh-cut tomatoes stored at 20 ºC depleted dramatically throughout the storage from 124.1 to 17.3 mg kg^{-1} fw after 14 days. Thus, the higher the storage temperature, the more substantial variation in ascorbic acid content of tomato slices. Adisa et al. (1986) observed that fruits and vegetables show a gradual depletion in ascorbic acid content as temperature increases. This decrease of vitamin C at high storage temperatures might be due to the wounding response. Plant tissue cells enclose natural antioxidant systems to regulate the production of reactive oxygen species (ROS) resulting from a cascade of reactions during normal cellular metabolism or induced by stress. From the many antioxidants present in the cell, ascorbic acid rapidly reacts with radical species to protect cell integrity upon redox imbalances (Mittler, 2002). Consequently, the large decrease in vitamin C in fresh-cut tomatoes stored at temperatures higher than 10 ºC might be due to the protection of cells from redox imbalances caused by wounding. In addition, it has been demonstrated that high CO_2 concentrations cause degradation of vitamin C in fresh-cut fruits. A significant correlation (R^2 = -0.5679) was observed between the concentration of vitamin C and the CO_2 levels inside headspace packages of fresh-cut tomatoes (Table 4). Agar et al. (1999) reported accelerated losses of vitamin C in kiwifruit slices stored under elevated CO_2 atmospheres. High CO_2 levels might provoke a cytoplasm acidification with the consequent impairment of mitochondrial function that could result in oxidative damage, which in turn could be overcome by ascorbate peroxidase (Pinto et al., 2001).

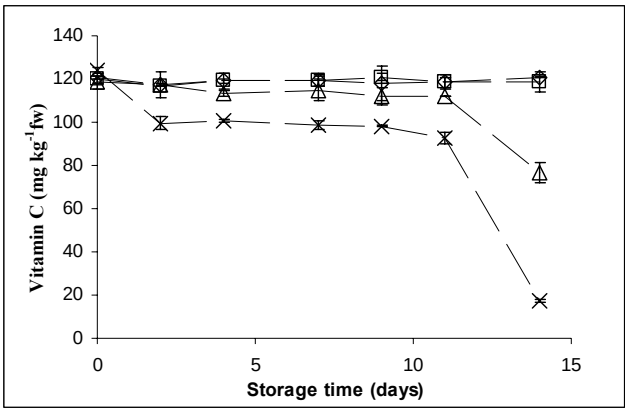

Figure 3.-Effect of storage temperature on vitamin C content in fresh-cut tomato packages throughout storage at 5 ºC (◊), 10 ºC (□), 15 ºC (Δ) and 20 ºC (×) for 14 days. Data shown are mean ± standard deviation.

Total Phenolic Compounds

Fig 4 shows the effect of temperature conditions on total phenolic compounds of fresh-cut tomatoes during storage. Storage temperature of 20 ºC induced a higher production of phenolic compounds than other temperatures. Total phenolic content of fresh-cut tomatoes did not change significantly during the first 4 days of storage irrespective of temperature. However, phenolic content in tomato slices stored at 20 ºC underwent a marked increase during the subsequent days, reaching maximum values of 444.7 mg gallic acid kg^{-1} fw at 14 days. A slight but significant rise in phenolic content was observed in fresh-cut tomatoes preserved at 10 ºC and 15 ºC after 4 days. This increase in phenolic compounds could be directly related to an enhanced oxidative stress induced by too low O_2 and high CO_2 concentration (R^2 = 0.7276) inside packages (Table 4). High CO_2 levels have shown to increase phenylalanine lyase (PAL) activity, which is the key enzyme that uses phenylalanine to synthesize phenolic compounds (Ke and Salveit, 1989). On the other hand, Dixon and Paiva (1995) reported that plants respond to wounding with a rise in phenolic compounds involved in the repair of wound damage and the defense against microbial invasion. In our study, the enhancement of phenolic content in fresh-cut tomatoes after 4 days coincided with an evident growth of spoilage microorganisms (Table 2). In addition, Reyes et al. (2007) suggested that tissues with low levels of ascorbic acid could synthesize phenolic compounds to partly regulate the production of ROS resulting from the cascade of reactions induced by wounding stress. In this way, there was a negative correlation (R^2 = -0.74) between phenolic and vitamin C content, indicating that under wounding conditions fruits with low amounts of vitamin C produce phenolic compounds to prevent cells from ROS.

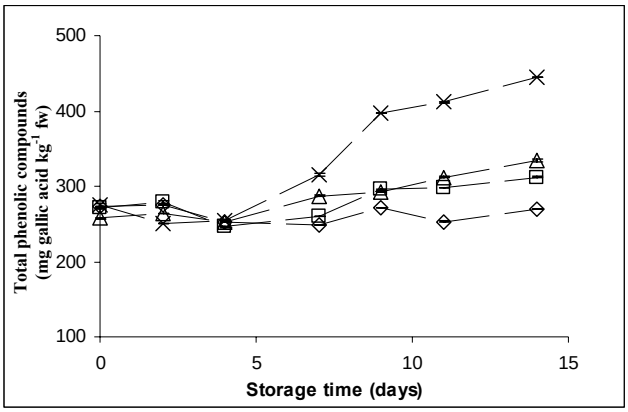

Figure 4.-Effect of storage temperature on phenolic content in fresh-cut tomato packages throughout storage at 5 ºC (◊), 10 ºC (□), 15 ºC (Δ) and 20 ºC (×) for 14 days. Data shown are mean ± standard deviation.

Effect of temperature on antioxidant capacity

The effect of storage temperature on the antioxidant capacity, measured on the basis of the DPPH stable radical, of fresh-cut tomatoes is shown in Fig 5. No significant differences in antioxidant capacity were obtained among just-processed fresh-cut tomatoes. Antioxidant capacity of tomato slices underwent a substantial depletion during the first 2 days of storage, but then was maintained or slightly decreased reaching values from 12.3 to 15.5% of DPPH inhibition at 14 of storage. Fresh-cut tomatoes stored at temperatures up to 10 ºC had greater antioxidant capacity than those preserved at temperatures of 15 ºC and 20 ºC. Fresh-cut tissues are primarily subjected to oxidative stress, presumably causing membrane damage and altering the composition and content of antioxidant compounds, thus resulting in changes in the total antioxidant activity of the tissue (Lana and Tijskens, 2006). Eberhardt et al. (2000) indicated that most of the antioxidant capacity comes from the natural combination of different phytochemicals. In this regard, Chu et al. (2000) suggested that several phytochemicals, such as flavonoids, phenolic acid, aminoacids, ascorbic acid tocopherols and pigments, might contribute to the total antioxidant capacity. Vitamin C and phenols are reported to be the major antioxidant components in tomato (Takeoka et al., 2001). However, the magnitude of the changes in the antioxidant capacity of fresh-cut tomatoes could not be related to neither the total phenolic compounds (R^2 = -0.32) nor to vitamin C (R^2 = 0.31) during storage. Wang et al. (1996) reported that the contribution of vitamin C to the total antioxidant activity of a fruit is less than 15%. In addition, results show that antioxidant capacity is significant but weak correlated with lycopene content (R^2 = -0.54) (Table 4). Martinez-Valverde et al. (2002) have reported a low correlation between the amount of lycopene in tomato products and their antioxidant capacity.

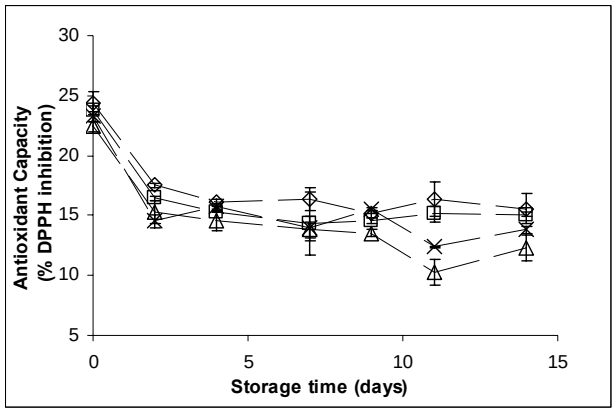

Figure 5.-Effect of storage temperature on antioxidant capacity in fresh-cut tomato packages throughout storage at 5 ºC (◊), 10 ºC (□), 15 ºC (Δ) and 20 ºC (×) for 14 days. Data shown are mean ± standard deviation.

CONCLUSIONS

Temperature affects the shelf-life, antioxidant properties and physicochemical parameters of tomato slices during postharvest storage. A rise in lycopene and phenolic concentration as well as a decrease in vitamin C content is observed in tomato slices stored at abusive temperatures (over 10 ºC). However, the increment of the antioxidant compounds throughout storage may be associated with excessive amounts of CO_2 inside the trays as a consequence of microbial growth. The results of this study indicate that fresh-cut tomatoes stored at temperatures 15-20 ºC retain quality and safety for up 4 days. Although, maintaining tomato slices at temperatures of 10 ºC kept the initial content of health-related compounds for 14 days, the microbial shelf-life was reduced to 7 days. A storage temperature of 5 ºC was adequate for ensuring safety of tomato slices and maintaining the health-related compounds for 14 days, without the appearance of undesirable off-flavors inside packages.

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REFERENCES

Adisa, V.A. (1986). The influence of molds and storage factors on the ascorbic content of orange and pineapple fruits. *Food Chemistry,* 22, 139-146.

Agar, I.T., Massantini, R., Hess-Pierce, B., Kader, A.A. (1999). Postharvest CO_2 and ethylene production and quality maintenance of fresh-cut kiwifruit slices. *Journal of Food Science,* 64, 433-440.

Aguayo, E., Escalona, V., Artés, F. (2004).Quality of fresh-cut tomato as affected by type of cut, packaging, temperature and storage time. *European Food Research and Technology,* 219**,** 492-499.

Ayala-Zavala, J.F., Oms-Oliu, G., Odriozola-Serrano, I., González-Aguilar, G.A., Álvarez-Parrilla, E., Martín-Belloso, O. (2008) Bio-preservation of fresh-cut tomatoes using natural antimicrobials. *European Food Research and Technology,* 226,1047-1055

Brecht, J.K. (1995). Physiology of lightly processed fruits and vegetables. *HortScience*, 30, 18–22.

Britton, G., Hornero-Méndez, D. (2004). Carotenoids and colour in fruit and vegetables. In: Britton G., Liaaen-Jensen, S., Pfander, H. Carotenoids Handbook. Birkhauser Verlag, Basle, Switzerland. pp. 11-27.

Buescher, R.W. (1979). Influence of carbon dioxide on post-harvest ripening and deterioration of tomatoes. *Journal of the American Society for Horticultural Science*, 104, 545-547.

Buta, J.G., Moline, E.H., Spaulding, D.W., Wang, C.Y. (1999). Extending the shelf-life of fresh-cut apples using natural products and their derivates. *Journal of Agricultural and Food Chemistry,* 47, 1-6 (1999).

Chu, Y.H., Chang, C.L., Hsu, H.F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture,* 80,561-566.

Davis, A.R., Fish, W.W., Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biology and Technology,* 28, 425-430.

De Ancos, B., Sgroppo, S., Plaza, L., Cano, M.P. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science of Food and Agriculture,* 82,790-796.

Dixon, R.A., Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell* **7**:085-1097.

Eberhardt, M.V., Lee, C.Y., Liu, R.H. (2000). Antioxidant activity of fresh-cut apples. *Nature,* 405(6789), 903-904.

Fish, W.W., Perkins-Veazie, P., Collins, J.K. (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvent. *Journal of Food Composition and Analysis* 15(3), 309-317 (2002).

Giovanelli, G., Lavelli, V., Peri, C., Nobili, S. (1999). Variation in antioxidant compounds of tomato during vine and post-harvest ripening. *Journal of the Science and Food Agriculture,* 79, 1583-1588.

Hernández, M., Rodríguez, E., Díaz, C. (2007). Free hydroxycinnamic acids, lycopene and color parameters in tomato cultivars. *Journal of Agricultural and Food Chemistry,* 55, 8604-8615 (2007).

Hong, J.H., Gross, K.C. (2001). Maintaining quality of fresh-cut tomato slices trough modified atmosphere packaging and low temperature, *Journal of Food Science,* 66(7), 960-965 (2001).

ISO, 4833. (1991). Methods for microbiological examination of food and animal feeding stuffs. Geneva.

ISO, 7954. (1988). General guidance for enumeration of yeast and moulds. Geneva.

Ke, D., Salveit, M.E. (1989). Carbon dioxide-induced brown stain development as related to phenolic metabolism in Iceberg lettuce. *Journal of the American Society for Horticultural Science,* 114, 789-794.

Lana, M.M., Tijskens, L.M.M. (2006). Effect of cutting and maturity on antioxidant activity of fresh-cut tomatoes. *Food Chemistry,* 97, 203-211.

Lee, S.K., Kader, A.A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology,* 20,207-220.

Leoni, C. (1992). Industrial quality as influenced by crop management. *Acta Horticulturae*, 301, 177-184.

Martín-Belloso, O., Soliva-Fortuny, R., Oms-Oliu, G. (2006). Fresh-Cut Fruits. In: Hui, Y.H. Handbook of fruits and fruit processing. Blackwell Publishing, Oxford. pp. 129-144.

Martínez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant capacity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science and Food Agriculture,* 82, 323-330.

Mencarelli, F., Salveit, M.E. (1988). Ripening of mature-green tomato fruit slices. *Journal of the American Society for Horticultural Science,* 113, 742-745.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science,* **7**, 405-410.

Nguyen, C., Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *Critical Review in Food Science and Nutrition,* 34, 371–401.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007). Comparative study of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry,* 105, 1151-1158.

Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Effect on minimally processing on bioactive compounds and color attributes of fresh-cut tomatoes. *LWT-Food Science and Technology*, 41, 217-226.

Pinto, A., Lentheric, I., Vendrell, M., Larrigaudiere, C. (2001). Role of fermentative and antioxidant metabolism in the induction of core browning in controlled-atmosphere stored pears. *Journal of the Science and Food Agriculture,* 81, 364-370.

Reyes, L.F., Villarreal, J.E., Cisneros-Zavallos, L. (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chemistry,* 101, 1254-1262.

Rodriguez-Amaya, D.B. (1993). Stability of carotenoids during the storage of foods. In: Charalambous, G. Shelf-life studies of foods and beverages. Elsevier, New-York. pp. 591-628.

Singleton, V.L., Orthofer, R.M., Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology,* 299, 152-178.

Shi, J., Le Maguer, M. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition,* 40, 1-42.

Shukolyukov, S.A., Saakov, V.S. (2001). American Cockroach (Periplaneta americana) Synthesizes Carotenoids from the Precursor [14C] Mevalonic Acid Pyrophosphate. *Biochemistry (Moscow)*, 66, 535-540.

Soliva-Fortuny, R.C., Oms-Oliu, G., Martín-Belloso O. (2002). Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science,* 67(5):312-317.

Soliva-Fortuny, R.C., Ricart-Coll, M., Martin-Belloso, O. (2005). Sensory quality and internal atmosphere of fresh-cut Golden Delicious apples. *International Journal Food Science and Technology,* 40, 369-375.

Takeoka, G.R., Dao, L., Flessa, S., Gillespie, D.M., Jewell, W.T., Huebner, B., Bertow, D., Ebeler, S.E. (2001). Processing effects on lycopene content and antioxidant activity of tomatoes. *Journal of Agricultural and Food Chemistry,* 49, 3713-3717.

Toivonen, P.M.A., DeEll, J.R. (2002). Physiology of fresh-cut fruits and vegetables. In: Lamikanra, O. Fresh-cut fruits and vegetables: science, technology and market. CRC Press, Boca Raton, FL. pp. 91-123.

Turk, R., Seniz, V., Ozdemir, N., Suzen, M.A. (1994). Changes in the chlorophyll carotenoid and lycopene content in relation to temperature. *Acta Horticulturae,* 368, 856-862.

Varoquaux, P., Willey, R.C. (1997). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In: Wiley, R.C. Minimally processed refrigerated fruits & vegetables. Chapman and Hall, New York. pp. 226–268.

Wang, H., Cao, G., Prior, R.L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry,* 44, 701-705.

Watada, A.E., Ko, N.P., Minott, D.A. (1996). Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology,* 9, 115-125.

Weisburger, J.H. (2002). Lycopene and tomato products in health promotion. *Experimental Biology and Medicine,* 227, 924-927.

Yang, S.F. (1981). Biosynthesis of ethylene and its regulation. In: Friend J., Rhodes M.J.C. Recent advances in the biochemistry of fruit and vegetables. Academic Press, London. pp. 89-106.

Capítulo X

Effect of high-oxygen atmospheres on the antioxidant potential of fresh-cut tomatoes compared to low-oxygen and passive atmospheres

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Postharvest Biology and Technology, *enviado*

ABSTRACT
The effect of different initial in-package O_2 and CO_2 concentrations (2.5 kPa O_2 + 5 kPa CO_2, 10 kPa O_2 + 5 kPa CO_2, 21 kPa O_2, 60 kPa O_2 and 80 kPa O_2) on the antioxidant potential of fresh-cut tomatoes was investigated. Changes in individual phenolic compounds, individual carotenoids, vitamin C and antioxidant capacity (measured through DPPH and ABTS radicals) as well as in O_2, CO_2 and ethylene headspace concentrations inside packages were assessed for 21 days at 4 ºC. High oxygen and passive atmospheres induced higher production of carotenoids and phenolic compounds, which may be related to an enhanced oxidative stress due to ethylene accumulation inside packages. In this way, the concentration of β-carotene in tomato slices under 10 O_2 kPa + 5 CO_2 kPa underwent a substantial increase over time, whereas vitamin C was considerably decreased. The degradation of the initial content of vitamin C was highly promoted by the presence of oxygen. Regarding DPPH, lower hydrophilic antioxidant capacity was obtained in tomato slices stored under 80 kPa O_2, whereas the antioxidant capacity of the lipophilic fraction was enhanced with oxygen availability inside headspace packages. Therefore, 2.5 kPa O_2 + 5 kPa CO_2 atmospheres not only prevented wounding stress and reduced the formation of phenylpropanoids, but also maintained vitamin C in fresh-cut tomatoes.

Key words: fresh-cut tomato, minimal processing, antioxidant potential, carotenoids, phenolic compounds, vitamin C.

INTRODUCTION

Epidemiological studies have suggested that the consumption of tomato reduces the risk of some types of cancer and heart diseases (Clinton, 1998). This beneficial effect is believed to be due, at least partially, to the action of tomato antioxidant compounds, which could reduce oxidative damage in the body. Tomatoes are the predominant source of lycopene in human diet, which exhibits high oxygen-radical scavenging and quenching capacities, and β-carotene, which is the main carotenoid with provitamin A activity (Beecher, 1998). However, carotenoids are highly unsaturated compounds with an extensive conjugated double-bonds system and are therefore susceptible to oxidation, isomerisation and other chemical changes during processing and storage (Shi and Le Maguer, 2000). In addition to carotenoids, other antioxidant compounds such as phenolics also contribute to the beneficial effects of tomato. Phenolics possess reducing character, capacity of sequestering reactive oxygen species (ROS) and several electrophiles, tendency to self-oxidation and capacity to modulate the activity of some cell enzymes (Robards et al., 1999). On the other hand, tomatoes have a remarkable concentration of vitamin C. Experimental studies have shown that vitamin C plays an important role in human health, including effects on immune system and the risk of Alzheimer diseases (Sánchez-Moreno et al., 2003).

Fresh-cut fruits are generally more perishable than whole fruits because they have been subjected to physiological stresses caused by physical damage or wounding (Varoquaux and Willey, 1997). Slicing operations induce a rapid rise in CO_2 and ethylene production associated to the wounding response which can reduce the quality of tomato slices (Artés et al. 1999). Modified atmosphere packaging (MAP) effectively extends shelf-life of fresh-cut tomato (Mencarelli and Salveit, 1988). In this way, Artés et al. (1999) observed that shelf-life of tomato slices could be maintained for 10 days at 2 ºC under both passive and active MAP conditions (7.5% O_2 and 0% CO_2). Furthermore, Aguayo et al. (2004) obtained fresh-cut tomatoes with good quality, appearance and texture when storing them at 5 ºC during 14 days in MAP conditions (3 kPa O_2 + 4 kPa CO_2, with N_2 as balance gas). However, problems associated with the development of off-odours, physiological and microbial decay, browning and softening may appear when O_2 level is too low and CO_2 is accumulated in packages (Allende et al., 2004). Thus, high O_2 atmospheres have been suggested as an effective method to inhibit the growth of microorganisms and prevent undesired anoxic fermentation (Amanitidou et al., 1999; Van der Steen et al., 2002). Previous works have focused on assessing the effects of different modified atmosphere packaging systems on quality of fresh-cut fruits (Jacxsens et al., 2001; Gil et al., 2002). However, the impact of different atmosphere compositions on nutritional and antioxidant properties has been neglected up to date and scarce information is available, especially in fresh-cut tomato. Therefore, the aim of the present work was to determine the effect of high-O_2 atmospheres on individual carotenoids, phenolics, vitamin C and antioxidant capacity of fresh-cut tomato, as well as, to compare the results with those obtained under conventional low-O_2 and passive atmospheres. Ethylene, O_2 and CO_2 headspace concentrations were also evaluated during 14 days of storage at 4 ºC.

MATERIALS AND METHODS

Quality characterization

Tomatoes (*Lycopersicum esculentum* Mill. cv. Bola) harvested in Alicante (Spain), were purchased from a local supermarket (Lleida, Spain) at commercial maturity and stored at 4 ºC ± 1 ºC. pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), titratable acidity, soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Tokyo, Japan), colour (spectrophotocolorimeter Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan) and firmness (Mechanical Fruit Firmness Tester, QA Supplies, LLC, Norfolk, Virginia, USA). Analytical characteristics of whole tomatoes were: pH = 4.30 ± 0.21, titratable acidity = 0.51 ± 0.2, soluble solids = 5.9 ± 0.1 ºBrix, colour L* = 50.0 ± 2.6, a* = 13.9 ± 2.1 and b* = 16.4 ± 1.3, firmness = 48.4 ± 3.7 N.

Sample preparation

Tomatoes were sanitized in a 200 µL L^{-1} NaClO solution for 2 min, rinsed with tap water and dried prior to cutting operations. The fruits were cut into 7 mm-thick slices from the stem-end portion perpendicularly to the long axis of the fruit with a commercial slicing machine (Food Slicer-6128; Toastmaster Corp., Elgin, USA). Five tomato slices (100 g) were packaged in each polypropylene tray. The active modification of package atmosphere was carried out by flushing a mixture of 2.5 kPa O_2 + 5 kPa CO_2, 10 kPa O_2+ 5 kPa CO_2, 60 kPa O_2 , 80 kPa O_2 balance with N_2 , before sealing the trays, using a digitally controlled compensated vacuum (ILPRA Food Pack Basic V/6, ILPRA Systems. CP., Vigevono, Italia). For a passive modification of package atmospheres (PA), the trays were sealed without fluxing gas mixture. The relationship between the amount of product and the headspace gas was 1:2 (v/v). The O_2 and CO_2 permeance of the 64 µm thick polypropylene sealing film were $5.2419 \cdot 10^{-13}$ mol O_2 m^{-2} s^{-1} Pa^{-1} and $2.3825 \cdot 10^{-12}$ mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23 ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The packages were stored at 4 ºC ± 1 ºC in darkness until random withdrawal for analysis. Three trays were taken at each rime to perform the analysis, and two readings were carried out for each package.

Headspace gases analysis

The gas composition of the package headspace was determined with a gas analyzer (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. An aliquot of 1.7 mL was automatically withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection system. The determination of O_2 concentrations was carried out by injecting a sample of 0.25 µL to a CP-Molsieve 5 Å packed column (4m x 0.32 mm, d.f. = 10 µm) at 55 ºC and 90 kPa, whereas a sample of 0.33 µL was injected to a Pora-PLOT Q column (10 m x 0.32mm, d.f. = 10 µm) at 75 ºC and 140 kPa for carbon dioxide and ethylene quantification.

Determination of carotenoids

The extraction method was based on a procedure proposed by Tonucci et al. (1995). First, a 2.5 g of magnesium carbonate and 2.5 g of Celite, used as filter aid, were added to 25 g of tomato with 0.5 mg of internal standard (β-apo-8'-carotenal). The mixture was blended for 20 min in an Omni Mixer with 25 mL of tetrahydrofuran (THF) and then filtered through Whatman No. 1 filter paper using a Büchner funnel. The solid material was extracted two or three more times until it was devoid of red/orange colour. The THF extracts were combined, and the volume was reduced by about two-thirds under vacuum at 35 ºC with a rotary evaporator. Fractions of the combined extract were portioned into 250 mL of methylene chloride and 150 mL of saturated sodium chloride aqueous solution in a separatory funnel. The water layer was washed with methylene chloride until carotenoids were completely removed. The methylene chloride layer containing carotenoids was dried over anhydrous sodium sulfate and filtered through Whatman No. 42 filter paper. The volume of the filtrate was reduced under vacuum to approximately 25 mL and brought up to 50 mL with methylene chloride. Then the extracts were passed through a Millipore 0.45 μm membrane and injected into the HPLC system. Conditions for the HPLC separations were those reported by Khachik et al. (1992). The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 470 nm. Samples were introduced into a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6mm x 250mm) through a manual injector equipped with a sample loop (20 μL). The flow rate was 0.7 mL min^{-1} at room temperature. An isocratic elution of 85% acetonitrile: 10% methanol: 3% methylene chloride: 2% hexane was maintained from 0 to 10 min, followed by a linear gradient to 45% acetonitrile: 10% methanol: 23% methylene chloride: 22% hexane from 10 to 40 min. At the end of the gradient, the column was equilibrated under the initial conditions for 20 min. Carotenoids were quantified by comparison with external standards of lycopene, lutein, neurosporene, γ-carotene, δ-carotene, β-carotene and phytofluene. Results were expressed as milligrams of carotenoids per kg of tomato.

***Vitamin A*:** Vitamin A values were expressed as retinol equivalents (RE). To calculate RE on the basis of carotenoids, the following conversion was employed: RE = μg of β-carotene/6 + μg of γ-carotene/12 (Trumbo et al., 2003).

Determination of phenolic compounds

A high-performance liquid chromatography method was used for the analysis of individual phenolic compounds. The extraction was carried out following the method validated by Hertog et al. (1992). A sample of 0.5 g of previously freeze-dried tomato tissue was carefully mixed with 40 mL of 62.5% methanol (2 g/L of tert-bytlhydroquinon) and 10 mL of 6 M HCl. After refluxing at 90 ºC for 2 h with regular swirling, the extract was cooled and subsequently made up to 100 mL with methanol and sonicated for 5 min. The extract was then passed through a 0.45 μm filter prior to injection. HPLC system was equipped with a 600 Controller and a diode array detector (Waters, Milford, MA) which was set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column

(4.6mm x 250mm) at room temperature with a flow rate of 1 mL min^{-1}. A gradient elution was employed with a solvent mixture of 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B) as follows: linear gradient from 5 to 13% B, 0-15 min; linear gradient from 13 to 15% B, 15-20 min; linear gradient from 15 to 30% B, 20-25 min; isocratic elution 30% B, 25-28 min; linear gradient from 30 to 45% B, 28-32 min; isocratic elution 45% B, 32-35 min; linear gradient 45 to 90% B, 35-40 min; isocratic elution 90% B, 40-45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection. Individual phenolics were quantified by comparison with external standards of chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, kaempferol and quercetin. The results were expressed as milligrams of phenolic compounds per kg of tomato.

Determination of vitamin C

The extraction procedure was based on a previously validated method (Odriozola-Serrano et al., 2007). A portion of 25 g of fruit was added to 25 mL of a 4.5% metaphosforic solution with 7.2 g L^{-1} DTT as reducing agent. The mixture was crushed, homogenized and centrifuged at 22100xg for 15 min at 4 ºC. The supernatant was vacuum-filtered through Whatman No. 1. The sample was then passed through a Millipore 0.45 µm membrane and injected into the HPLC system. A Waters 600E multisolvent delivery system was used for the analysis. Samples were introduced onto the column via a manual injector equipped with a sample loop (20 µl). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6mm x 250cm). The mobile phase was a 0.01% solution of sulphuric acid adjusted to pH=2.6. The flow rate was fixed at 1.0 mL min^{-1}. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Vitamin C was quantified throughout a calibration curve built with pure ascorbic acid standards and results were expressed as milligrams of vitamin C per kg of fresh-cut tomato.

Determination of Antioxidant Capacity

Antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radical according to the procedure described by Odriozola-Serrano et al. (2008). In addition, the ABTS assay, based on the ability of the antioxidants to scavenge the blue-green radical cation 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonate ($ABTS^{\cdot+}$) was conducted according to the method described by Re et al. (1999). Two fractions (hydrophilic and lipophilic) were prepared from fresh-cut tomatoes and used in the antioxidant assay following the method proposed by Lenucci et al. (2006). About 5 g of sample were mixed with 25 mL of absolute methanol or hexane and then were centrifuged at 6000 x g for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) to obtain the hydrophilic and lipophilic extracts, respectively. Aliquots of 0.1 mL of the hydrophilic or lipophilic supernatants were mixed with 3.9 mL of methanolic DPPH or ABTS solutions. The homogenate was shaken vigorously and kept in darkness for 60 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm for the DPPH assay or at 734 nm for the ABTS assay. The percentage of inhibition of the radicals ($DPPH^{\cdot}$ and $ABTS^{+}$) was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH and ABTS values were calculated using a regression equation between the Trolox concentration and the

percentage of DPPH or ABTS inhibition. Results were expressed as millimol of Trolox equivalents per kg of fresh-cut tomato.

Statistical analysis

Statistical analysis was performed using the Statgraphics Plus v.5.1 Windows package (Manugistics, Inc., Rockville, MA, USA). Data were analyzed by multifactor analysis of variance and a Duncan multiple-range test was employed to find differences among means, with a level of significance of 0.05. Principal component analysis (PCA) was carried out to obtain relationships among variables. PCA is a multivariate statistical technique based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be reduced to a set of new variables (PCs). A correlation matrix is used to standardize the variables which are not measured on the same scale. The loadings plot summarises the main relationship between variables and principal components and also highlights relationships between different variables themselves. Variables that appear close together in this plot correlated positively. On the other hand, the score plot represents the projection of each sample into PC, defining different groups.

RESULTS AND DISCUSSION

Changes in carotenoids profile

Changes in total and individual carotenoids of fresh-cut tomato stored under different initial in-packages conditions are shown in Table 1. Initial carotenoids content in fresh-cut tomato ranged from 113.3 to 116.6 mg kg^{-1}. A marked decrease in total carotenoids over time (21 days) was observed in samples stored under 2.5 kPa O_2 + 5 kPa CO_2 or 10 kPa O_2 + 5 kPa CO_2 despite the restriction in O_2 concentrations. This trend may be explained by the fact that modified atmospheres including either reduced O_2 or elevated CO_2 are generally considered to inhibit the biosynthesis of carotenoids (Kader et al., 1989). In contrast, exposure to $\geq$ 60 kPa O_2 induced a higher production of carotenoids, which may be related to the oxidative stress induced by high ethylene content inside packages (Figure 1C). Consistently, Kader and Ben-Yehoshua (2000) reported that ripening of tomatoes is accelerated under 40-50 kPa O_2 compared with air. The presence of O_2 rapidly induces enzymatic activities related to the ethylene biosynthetic pathway, resulting in a large burst of ethylene synthesis (Abeles et al., 1992). This enhancement of ethylene promotes physiological changes, being the most important the initiation of ripening in climacteric fruits such as tomato (Li et al., 1992).

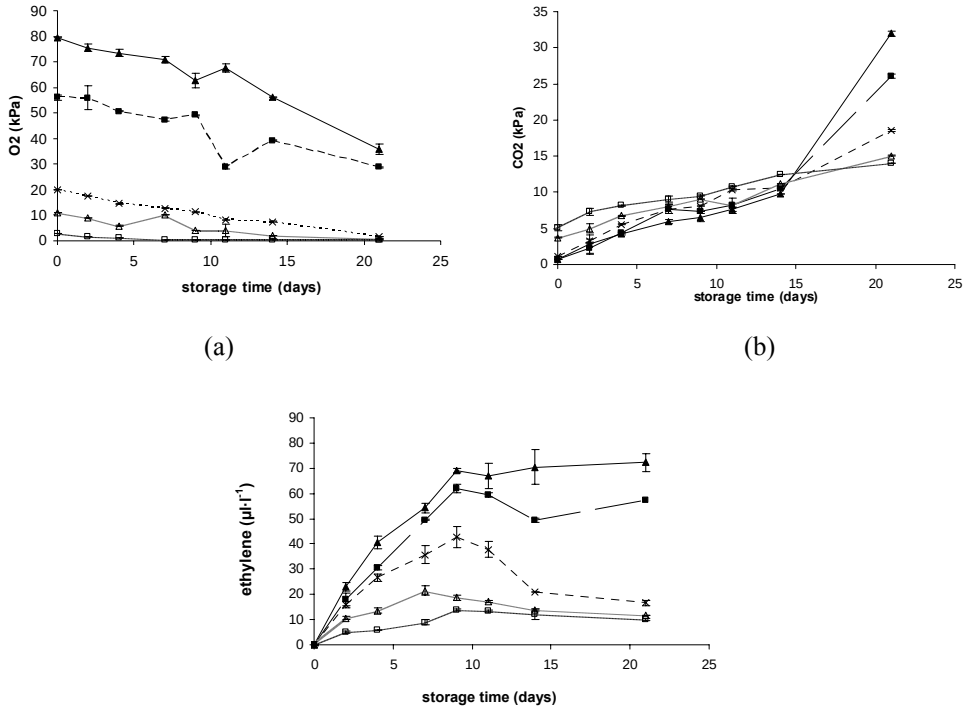

Figure 1.-Oxygen (a) carbon dioxide (b) and ethylene (c) headspace composition in trays of fresh-cut tomato stored during 21 days at 4 ºC under different packaging condition: (▲) 80 kPa O_2, (■) 60 kPa O_2, (×) 21 kPa O_2, (Δ) 10 kPa O_2 + 5 kPa CO_2, (□) 2.5 kPa O_2 + 7 kPa CO_2. Data shown are mean ± standard deviation.

Regarding the individual carotenoids, lycopene was the main carotenoid found in fresh-cut tomatoes, accounting for 28% of the total carotenoids content. The initial lycopene content of tomato slices ranged from 31.4 to 33.7 mg kg^{-1} (Table 1). Previous studies demonstrated that the tomato used in the present work had lower lycopene content than that observed in other cultivars (Odriozola-Serrano et al., 2008). Other individual carotenoids which are lycopene precursors such as phytofluene (5.0-5.1 mg kg^{-1}) and neurosporene (27.1-28.5 mg kg^{-1}) were also found in tomato slices.

Table 1.- Carotenoid content of fresh-cut tomatoes stored during 21 days at 4 ºC under different packaging conditions.

	ST (days)	Individual carotenoids (mg kg^{-1})							TC (mg kg^{-1})
		lutein	lycopene	neu	γ-carotene	δ-carotene	β-carotene	phyto	
T1	0	6.7^{b}	33.7^{e}	27.6^{a}	20.0^{a}	4.2^{a}	19.3^{f}	5.1^{a}	116.6^{c}
	2	5.5^{c}	38.3^{d}	26.4^{a}	15.3^{b}	3.7^{b}	28.9^{e}	3.8^{b}	121.9^{c}
	4	4.9^{c}	39.4^{d}	15.3^{b}	13.3^{c}	3.2^{b}	49.7^{d}	3.2^{c}	129.0^{b}
	7	10.1^{a}	43.5^{c}	6.9^{d}	10.8^{d}	3.3^{b}	55.8^{d}	2.6^{d}	133.0^{a}
	9	10.1^{a}	42.5^{c}	6.9^{d}	9.2^{e}	3.2^{b}	61.1^{b}	1.9^{e}	134.9^{a}
	11	6.9^{b}	45.4^{bc}	8.3^{c}	8.3^{f}	3.5^{b}	53.3^{c}	1.7^{e}	127.4^{b}
	14	6.6^{b}	47.7^{b}	nd	4.3^{h}	2.1^{c}	54.5^{c}	2.8^{d}	118.0^{c}
	21	7.1^{b}	50.4^{a}	nd	7.1^{g}	1.9^{c}	65.9^{a}	4.1^{b}	136.5^{a}
T2	0	6.7^{d}	32.3^{f}	27.6^{a}	18.6^{b}	4.1^{a}	19.0^{e}	5.0^{a}	113.3^{c}
	2	6.3^{d}	35.5^{e}	24.6^{b}	19.2^{a}	4.1^{a}	24.6^{d}	4.5^{b}	118.8^{b}
	4	7.2^{d}	35.6^{e}	17.2^{c}	18.5^{b}	3.9^{a}	42.8^{b}	3.8^{c}	129.0^{a}
	7	13.5^{a}	41.4^{d}	6.7^{e}	19.3^{a}	3.2^{b}	39.2^{bc}	3.1^{d}	126.4^{a}
	9	11.2^{b}	43.5^{e}	7.3^{e}	10.3^{d}	3.5^{ab}	37.6^{c}	2.8^{d}	116.2^{bc}
	11	9.1^{c}	44.5^{c}	7.0^{e}	11.4^{c}	3.0^{b}	42.8^{b}	2.7^{d}	120.5^{b}
	14	4.6^{e}	46.4^{b}	8.3^{d}	11.7^{bc}	2.3^{c}	37.2^{c}	3.4^{cd}	113.9^{c}
	21	4.5^{e}	50.0^{a}	nd	11.0^{cd}	2.2^{c}	47.9^{a}	4.2^{b}	119.8^{b}
T3	0	6.3^{d}	31.4^{e}	27.1^{a}	20.1^{a}	4.1^{a}	19.4^{e}	5.1^{a}	113.5^{c}
	2	6.0^{d}	33.1^{d}	28.9^{a}	19.5^{a}	3.9^{a}	18.6^{e}	4.5^{b}	114.5^{c}
	4	5.8^{d}	35.7^{c}	12.9^{b}	16.7^{b}	3.5^{ab}	31.0^{d}	3.4^{c}	109.0^{c}
	7	13.5^{a}	38.8^{b}	12.9^{b}	17.7^{b}	3.1^{b}	45.2^{b}	3.0^{d}	134.2^{a}
	9	10.8^{b}	38.9^{b}	11.9^{b}	11.5^{c}	2.7^{b}	45.2^{b}	3.0^{d}	124.0^{b}
	11	7.3^{c}	39.9^{b}	7.5^{c}	8.7^{d}	3.1^{b}	41.1^{c}	2.5^{e}	110.1^{c}
	14	4.9^{e}	44.9^{a}	7.3^{c}	10.8^{c}	2.7^{b}	38.9^{c}	1.8^{f}	111.3^{c}
	21	4.3^{e}	44.5^{a}	7.6^{c}	9.3^{d}	1.9^{c}	50.7^{a}	2.1^{ef}	120.4^{b}
T4	0	6.5^{d}	32.4^{c}	28.5^{a}	18.7^{b}	4.3^{a}	18.3^{f}	5.0^{a}	113.7^{a}
	2	5.4^{d}	29.5^{d}	27.0^{b}	20.9^{a}	3.9^{b}	24.2^{d}	4.2^{b}	115.1^{a}
	4	10.6^{b}	29.7^{d}	14.3^{c}	14.5^{c}	3.3^{c}	21.7^{e}	4.1^{b}	98.2^{c}
	7	12.3^{a}	27.5^{e}	10.9^{d}	13.9^{c}	2.2^{e}	37.3^{a}	3.2^{c}	107.3^{b}
	9	9.4^{c}	37.0^{ab}	9.8^{d}	8.8^{d}	2.5^{de}	36.5^{a}	3.5^{c}	107.5^{b}
	11	9.3^{c}	35.7^{b}	7.9^{e}	6.5^{ef}	2.8^{d}	30.5^{c}	3.1^{c}	95.8^{c}
	14	10.4^{b}	39.0^{a}	9.8^{d}	5.0^{f}	1.8^{f}	31.6^{c}	2.1	99.7^{c}
	21	11.1^{b}	36.2^{b}	4.5^{f}	7.2^{e}	2.2^{e}	35.1^{b}	2.5^{d}	98.8^{c}
T5	0	7.0^{d}	32.2^{a}	27.6^{a}	20.5^{a}	4.2^{a}	19.7^{e}	5.1^{a}	116.3^{a}
	2	5.6^{e}	31.0^{ab}	27.2^{b}	19.0^{a}	2.7^{b}	20.6^{d}	4.2^{b}	110.3^{b}
	4	11.5^{b}	30.1^{ab}	21.8^{b}	11.0^{b}	2.2^{c}	21.2^{d}	3.0^{c}	100.8^{c}
	7	13.8^{a}	29.0^{b}	11.1^{c}	10.3^{b}	2.3^{c}	26.5^{a}	3.2^{c}	96.2^{c}
	9	9.7^{c}	28.5^{b}	11.9^{b}	5.4^{c}	2.0^{c}	22.8^{c}	3.1^{c}	83.4^{d}
	11	10.4^{c}	29.8^{ab}	12.1^{a}	5.6^{c}	1.9^{c}	24.2^{b}	2.7^{cd}	86.7^{d}
	14	10.8^{bc}	30.3^{ab}	9.7^{b}	5.1^{c}	2.0^{c}	22.8^{c}	2.3^{d}	83.0^{d}
	21	14.5^{a}	29.7^{ab}	9.6^{b}	4.2^{d}	2.1^{c}	20.8^{d}	2.1^{d}	83.0^{d}

[a]Different letters in the same column for each in-package condition indicate significant differences among days ($p<0.05$).
TC: total carotenoids calculated by the sum of carotenoids determined by HPLC;
T1: 80 kPa O_2, **T2**: 60 kPa O_2, **T3**: 21 kPa O_2, **T4**: 10 kPa O_2 + 5 kPa CO_2, **T5**: 2.5 kPa O_2 + 5 kPa CO_2
ST: storge time; **neu**: neurosporene; **phyto**: phytofluene; **nd**: not detected

The increase in lycopene in fresh-cut tomatoes under ≥ 60 kPa O_2 was greater over time than that observed under lower initial O_2 concentrations. On the contrary, neurosporene content decreased considerably throughout the storage period, leading to non-detectable levels at 14-21 days of storage in fresh-cut tomatoes stored under ≤ 10 kPa O_2. The initial content of phytofluene was also decreased through the first 11 days of storage in fresh-cut tomato irrespective of the initial atmosphere, and then the values rose in tomato slices stored under high-O_2 atmospheres, reaching values of 4.1-4.2 mg kg^{-1} at the end of storage. Thus, initial atmospheres rich in oxygen might stimulate the transformation of some carotenoids into lycopene. Phytofluene undergoes a series of desaturation reactions, each of which creates a new double bond and extends the chromophore by two conjugated double bonds; the end product is lycopene, produced via the intermediate neurosporene (Britton and Hornero-Méndez, 1997). However, little is know about the mechanisms involved in carotenoid biosynthesis in plants. The biosynthetic enzymes are encoded by nuclear genes, and precursor proteins are post-translationally imported into plastids where carotenoid biosynthesis occurs. Disruption of tissue by wounding, followed by exposure to various atmospheres, could promote the transcription of the genes or the transport of the mRNA (Bartley and Scolnik, 1995). β-carotene and γ-carotene were found in tomato slices in amounts ranging from 18.3 to 19.7 mg kg^{-1} and from 18.6 to 20.5 mg kg^{-1}, respectively. Biochemical reactions related to the production of β-carotene seemed to increase in tomato slices stored under ≥ 10 kPa O_2 atmospheres. As can be seen in Table 1, β-carotene content was higher in fresh-cut tomatoes under 80 kPa O_2 than that obtained in other packaging conditions. Fresh-cut tomatoes underwent a substantial depletion of γ-carotenoid during the storage period in all the studied samples, which may be a consequence of their conversion to β-carotene. Hence, the γ-carotene may undergo cyclization to form six membered rings at one end of the molecule, giving β-carotene as a product (Britton and Hornero-Méndez, 1997).

Tomatoes have been found to be a rich source of vitamin A which is related to the amount of β-carotene and γ-carotene in the samples (Rodriguez-Amaya, 1997). The initial content of vitamin A in fresh-cut tomatoes was 4609-4995 RE kg^{-1}. The amount of vitamin A in tomato slices stored under ≥ 10 kPa O_2 remarkably increased over time (Figure 2). Based on the present results, it seems that ethylene production and in turn, oxygen availability has an important effect on carotenoids with provitamin A activity (Figure 2). The greater increase of vitamin A observed in fresh-cut tomatoes could be associated with the acceleration of ripening and enhancement of senescence. Changes in other minor individual carotenoids such as lutein (6.3-7.0 mg kg^{-1}) and δ-carotene (4.1-4.3 mg kg^{-1}) are also shown in Table 1. The main losses of δ-carotene throughout the first 14 days of storage were found in fresh-cut tomatoes under high-O_2 atmospheres, whereas lutein was enhanced in tomato slices stored under these packaging conditions (Table 1).

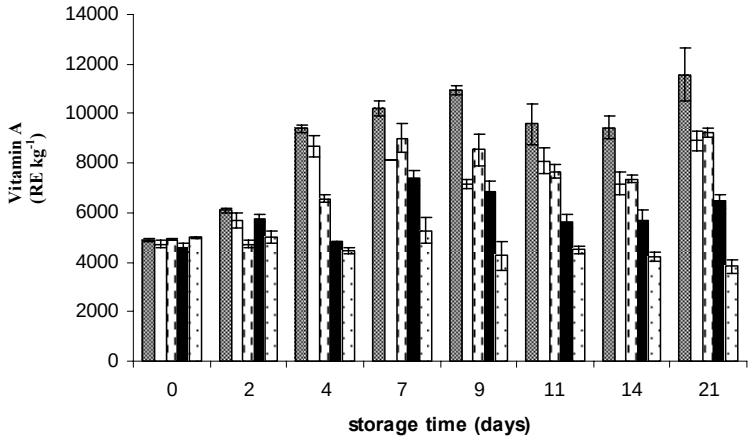

Figure 2.- Vitamin A content of fresh-cut tomatoes stored during 21 days at 4 ºC under different packaging conditions: 80 kPa O_2, 60 kPa O_2, 21 kPa O_2, 10 kPa O_2 + 5 kPa CO_2, 2.5 kPa O_2 + 5 kPa CO_2. Data shown are mean ± standard deviation.

Changes in phenolic compounds profile

The initial total phenolic content of fresh-cut tomatoes calculated as the sum of each phenolic compound measured by HPLC was approximately 45.0-45.7 mg kg^{-1} (Table 2). Chlorogenic acid was the main hydroxycinnamic acid derivative in tomato slices, obtained in concentrations of 21.2-22.0 mg kg^{-1}. Tomato slices under ≥ 60 kPa O_2 underwent the highest increase in chlorogenic acid through storage up to values of 26.7-30.4 mg kg^{-1} at 21 days. In turn, chlorogenic acid was further increased in fresh-cut tomatoes storage under passive atmospheres in comparison with those stored under low-O_2 atmospheres during 11 days (Table 2). Consistently, Alasalvar et al. (2005) reported that low O_2 and CO_2 levels inside headspace packaging prevent the accumulation of phenolic compounds in ready-to-eat shredded orange and purple carrots. Low O_2 levels can inhibit the biosynthesis of phenolic compounds in fresh-cut products which are induced usually in response to cutting damage (Artés et al., 2007). Wounding may stimulate phenylalanine ammonio lyase activity (PAL) during minimal processing with consequent further production of phenolic compounds (Alasalvar et al., 2005). The PAL activation of phenylpropanoid metabolisms could be elicited through induced reactive oxygen species (Reyes et al., 2006).

Table 2.- Phenolic content of fresh-cut tomatoes stored during 21 days at 4 ºC under different packaging conditions.

	ST (days)	Individual phenolic compounds (mg kg^{-1})						TP (mg kg^{-1})
		Chlorogenic acid	ferulic acid	*p*-coumaric acid	caffeic acid	quercetin	kaempferol	
T1	0	21.6^{d}	4.0^{b}	3.6^{a}	2.5^{a}	9.0^{e}	4.3^{c}	45.0^{c}
	2	20.8^{d}	4.0^{b}	3.6^{a}	1.9^{b}	11.5^{d}	5.9^{ab}	47.7^{c}
	4	30.6^{a}	4.1^{b}	3.3^{a}	1.7^{b}	11.4^{d}	5.5^{b}	56.6^{a}
	7	27.6^{b}	3.5^{c}	2.4^{a}	1.8^{b}	11.0^{d}	6.1^{a}	52.4^{b}
	9	25.4^{c}	4.1^{b}	2.0^{a}	nd	11.3^{d}	5.7^{b}	48.5^{bc}
	11	25.9^{c}	4.4^{a}	2.0^{a}	nd	12.2^{c}	5.7^{b}	50.2^{b}
	14	28.4^{b}	4.1^{b}	2.0^{a}	nd	13.1^{b}	6.2^{a}	53.8^{b}
	21	30.4^{a}	4.2^{b}	2.0^{b}	nd	14.8^{a}	6.2^{a}	57.6^{a}
T2	0	21.2^{e}	4.2^{a}	3.8^{a}	2.7^{a}	9.6^{e}	4.2^{d}	45.7^{c}
	2	25.9^{b}	3.9^{b}	3.9^{a}	1.6^{c}	10.4^{d}	5.6^{b}	51.3^{a}
	4	22.8^{d}	3.7^{c}	3.3^{b}	1.7^{c}	10.6^{d}	5.2^{c}	47.3^{b}
	7	22.6^{d}	4.1^{a}	2.5^{c}	2.0^{b}	11.1^{c}	5.6^{b}	47.9^{b}
	9	23.8^{c}	4.1^{a}	2.1^{cd}	1.7^{c}	11.0^{c}	5.2^{c}	47.9^{b}
	11	26.9^{a}	3.6^{c}	1.8^{d}	2.1^{b}	11.9^{b}	5.2^{c}	51.5^{a}
	14	26.3^{ab}	3.8^{bc}	1.9^{d}	nd	12.1^{b}	5.5^{b}	49.6^{ab}
	21	26.7^{a}	3.9^{b}	1.9^{d}	nd	12.9^{a}	5.9^{a}	51.3^{a}
T3	0	22.0^{d}	3.9^{c}	3.6^{a}	2.6^{a}	9.4^{d}	4.2^{d}	45.7^{b}
	2	22.3^{d}	4.1^{bc}	3.5^{a}	2.2^{b}	10.2^{c}	4.5^{d}	46.8^{b}
	4	24.7^{b}	4.3^{b}	3.2^{b}	1.9^{c}	10.9^{dc}	5.4^{c}	50.4^{a}
	7	26.3^{a}	4.6^{a}	2.5^{c}	1.8^{c}	11.1^{b}	5.9^{ab}	52.2^{a}
	9	26.5^{a}	4.1^{bc}	1.9^{d}	1.7^{c}	11.2^{b}	6.2^{a}	51.6^{a}
	11	21.9^{e}	3.9^{c}	1.9^{d}	1.8^{c}	12.3^{a}	5.8^{b}	47.6^{b}
	14	23.7^{c}	4.2^{b}	1.8^{d}	nd	11.8^{ab}	6.0^{ab}	47.5^{b}
	21	23.3^{c}	4.3^{b}	2.0^{d}	nd	11.4^{b}	5.7^{b}	46.7^{b}
T4	0	21.5^{b}	4.2^{a}	3.6^{a}	2.7^{ab}	9.0^{d}	4.1^{c}	45.1^{ab}
	2	23.4^{a}	3.9^{ab}	3.8^{a}	2.7^{ab}	10.0^{c}	4.2^{c}	48.0^{a}
	4	22.9^{ab}	4.0^{a}	3.0^{b}	2.6^{b}	11.5^{a}	5.0^{b}	49.0^{a}
	7	21.8^{b}	3.7^{b}	2.8^{b}	2.8^{a}	10.7^{b}	5.7^{a}	47.5^{a}
	9	23.5^{a}	4.2^{a}	1.8^{c}	3.0^{a}	11.5^{a}	5.6^{a}	49.6^{a}
	11	21.1^{b}	3.8^{b}	1.7^{c}	2.4^{c}	10.8^{b}	5.3^{b}	45.1^{ab}
	14	19.6^{c}	4.0^{a}	1.8^{c}	1.7^{d}	10.6^{b}	5.2^{b}	42.9^{b}
	21	19.7^{c}	4.0^{a}	1.7^{c}	1.7^{d}	11.4^{a}	5.2^{b}	43.7^{b}
T5	0	21.6^{c}	4.1^{b}	3.4^{a}	2.7^{b}	9.4^{d}	4.0^{a}	45.2^{b}
	2	23.6^{a}	3.9^{bc}	3.5^{a}	2.7^{b}	9.9^{c}	4.3^{b}	47.9^{ab}
	4	23.2^{a}	3.8^{c}	3.1^{b}	2.8^{b}	10.9^{ab}	4.4^{b}	48.2^{a}
	7	23.9^{a}	4.1^{b}	3.0^{b}	2.4^{c}	11.5^{a}	4.2^{b}	49.1^{a}
	9	22.5^{b}	3.9^{bc}	1.6^{c}	3.4^{a}	10.9^{ab}	4.7^{a}	47.0^{ab}
	11	22.0^{b}	4.4^{a}	1.6^{c}	2.4^{c}	11.1^{b}	4.8^{a}	46.3^{b}
	14	18.2^{d}	3.7^{c}	1.6^{c}	1.7^{d}	9.8^{c}	4.2^{b}	39.2^{c}
	21	17.0^{e}	3.9^{bc}	1.6^{c}	1.8^{d}	9.3^{d}	4.4^{b}	38.0^{c}

[a]Different letters in the same column for each in-package condition indicate significant differences among days ($p<0.05$).
TP: total phenolic compounds calculated by the sum of each phenolic compounds determined by HPLC; **ST**: storage time
T1: 80 kPa O_2, **T2**: 60 kPa O_2, **T3**: 21 kPa O_2, **T4**: 10 kPa O_2 + 5 kPa CO_2, **T5**: 2.5 kPa O_2 + 5 kPa CO_2; **nd**: not detected

Fresh-cut tomatoes have been found to be a rich source of flavonols such as quercetin and kaempferol (Stewart et al., 2000). Initial quercetin concentrations in tomato slices were 9.0-9.6 mg kg^{-1}, whereas kaempferol was found at concentrations of 4.0-4.3 mg kg^{-1}. In this way, Martínez-Valverde et al. (2002) reported quercetin and kaempferol concentrations in different tomato cultivars ranging between 7.2 and 43.6 mg kg^{-1} and between 1.2 and 2.0 mg kg^{-1}, respectively. Quercetin and kaempferol content increased significantly throughout storage irrespective of the initial packaging atmosphere. However, such increase was the lowest in tomato slices stored under initial ≤ 10 kPa O_2 atmospheres (Table 2). Zheng et al. (2003) reported that high-O_2 atmospheres enhanced quercetin and kaempferol in blueberries in comparison with air. Quercetin in strawberry fruits stored under 60 and 100 kPa O_2 was also better maintained than in fruits held in air after 14 days of storage (Zheng et al. 2007). Regarding other phenolic acids, ferulic acid (3.9-4.2 mg kg^{-1}), *p*-coumaric acid (3.4-3.8 mg kg^{-1}) and caffeic acid (2.5-2.7 mg kg^{-1}) were found in minor quantities. As can be seen in Table 2, the caffeic acid content in tomato slices substantially depleted through the storage period, regardless of the initial packaging conditions. However, this phenolic acid was best maintained in fresh-cut tomatoes stored under low-O_2 atmospheres for 21 days, since a non-detectable amount of caffeic acid was found in tomatoes under ≥ 21 kPa O_2 beyond 9-14 days. Fresh-cut tomatoes underwent a substantial depletion of *p*-coumaric acid during storage, which may be a consequence of its conversion to flavonols. *p*-coumaric acid is formed in plant products via the action of PAL due to the phenylpropanoid metabolism. Biosynthesis of flavonols starts with the sequential addition of three molecules of malonyl-CoA to suitable hydroxycinnamic acid CoA esters such as *p*-coumaric acid, to form chalcones. The chalcones are then isomerised into (2S)-flavanones which are converted into flavonols by hydroxylations and desaturations (Heller, 1986).

Changes in vitamin C content

Initial vitamin C contents of fresh-cut tomatoes ranged from 108 to 120 mg kg^{-1} (Figure 3). A significant decrease in vitamin C content was observed in tomato slices stored under high-O_2 atmospheres immediately after processing compared to those stored under low O_2 levels. Odriozola-Serrano et al. (2008) reported non-significant decrease in vitamin C due to cutting in fresh-cut tomatoes from different cultivars stored under 5 kPa O_2 + 5 kPa CO_2. Thus, the initial depletion of vitamin C after processing may be due to packaging conditions rather to slicing. Figure 3 shows changes in vitamin C concentrations of fresh-cut tomato through storage. As expected, vitamin C oxidation was greatly favoured by the presence of oxygen. Soliva-Fortuny et al. (2002) reported that the magnitude of vitamin C degradation can be related to the O_2 concentrations inside the packages. Hence, the higher amount of O_2 in the bags headspace the greater decrease in vitamin C content. A substantial variation of vitamin C over the storage time was observed in fresh-cut tomatoes stored under ≥ 10kPa O_2 atmospheres. Thus, the most dramatic decrease in vitamin C was detected in fresh-cut tomatoes stored under 80 kPa O_2, reaching the lowest content (50 mg kg^{-1}) after 21 days of storage at 5 ºC. Fresh-cut tomatoes stored under initial 2.5 kPa + 5 kPa CO_2 atmosphere kept their initial vitamin C content (108-115 mg kg^{-1}) for 21 days. Consistently, Oms-Oliu et al. (2008) reported that 70 kPa O_2 atmospheres induced higher losses of vitamin C in fresh-cut pears in comparison to air and 2.5 kPa O_2 + 5 kPa CO_2 atmospheres.

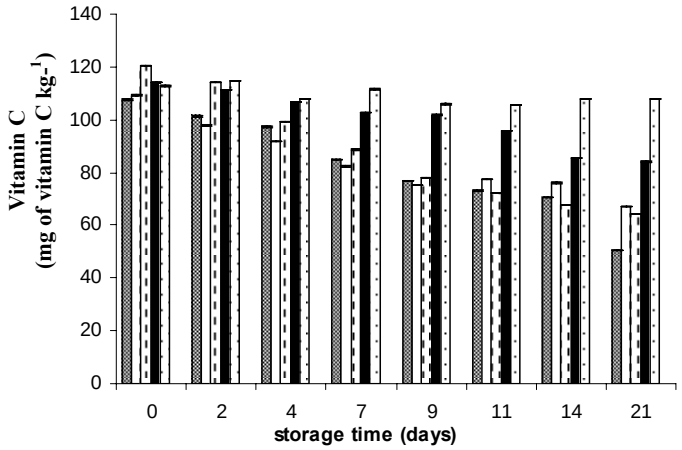

Figure 3.- Vitamin C content of fresh-cut tomatoes stored during 21 days at 4 ºC under different packaging conditions: 80 kPa O_2, 60 kPa O_2, 21 kPa O_2, 10 kPa O_2 + 5 kPa CO_2, 2.5 kPa O_2 + 5 kPa CO_2. Data shown are mean ± standard deviation.

Changes in antioxidant capacity

Antioxidant capacity of fresh-cut tomatoes measured through the DPPH and ABTS methods are shown in Table 3. Regarding the DPPH assay, the hydrophilic antioxidant capacity of fresh-cut tomatoes stored under 2.5 kPa O_2 + 5 kPa CO_2 was significantly higher than those of tomatoes stored under higher O_2 concentrations. According to ABTS values, non significant differences in hydrophilic antioxidant capacity were observed among fresh-cut tomatoes packaged under different initial atmospheres. The highest antioxidant capacity of the lipophilic fraction was obtained in fresh-cut tomatoes stored under ≥ 60 kPa O_2 irrespective of the assay carried out. Several methods have been used to evaluate the antioxidant profile of food products and results may greatly vary depending on the experimental conditions and the specificity of the free radical used (Cao et al., 1993).

Table 3.-Changes in antioxidant capacity of fresh-cut tomato stored during 21 days at 4 ºC at different initial in-package conditions.

Storage time (days)	DPPH assay (mmol kg^{-1})									
	Hydrophilic fraction					Lipophilic fraction				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
0	3.16^{a}	3.20^{a}	3.26^{a}	3.30^{a}	3.30^{a}	1.58^{d}	1.62^{d}	1.62^{b}	1.49^{d}	1.52^{b}
2	3.19^{a}	3.17^{a}	3.33^{a}	3.32^{a}	3.26^{a}	1.57^{d}	1.66^{cd}	1.62^{b}	1.74^{a}	1.66^{a}
4	2.41^{b}	2.34^{b}	2.62^{b}	2.74^{b}	2.77^{b}	1.71^{c}	1.70^{c}	1.63^{b}	1.59^{bc}	1.61^{a}
7	2.27^{c}	22.6^{c}	2.39^{c}	2.59^{c}	2.68^{c}	1.52^{d}	1.63^{d}	1.60^{b}	1.54^{c}	1.55^{b}
9	2.31^{c}	2.32^{b}	2.26^{d}	2.40^{d}	2.68^{c}	1.92^{a}	1.89^{a}	1.78^{a}	1.59^{bc}	1.51^{b}
11	1.77^{d}	1.83^{c}	1.96^{e}	2.28^{e}	2.64^{c}	1.73^{c}	1.80^{b}	1.66^{b}	1.64^{b}	1.38^{c}
14	1.71^{d}	1.85^{c}	2.00^{e}	2.31^{e}	2.58^{d}	1.84^{b}	1.73^{c}	1.58^{b}	1.47^{d}	1.36^{c}
21	1.77^{d}	1.71^{d}	2.01^{e}	2.16^{e}	2.56^{d}	1.80^{b}	1.73^{c}	1.59^{b}	1.54^{c}	1.33^{c}
Storage time (days)	ABTS assay (mmol kg^{-1})									
	Hydrophilic fraction					Lipophilic fraction				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
0	2.15^{a}	2.05^{a}	1.97^{a}	1.99^{a}	2.09^{a}	2.02^{a}	2.00^{a}	2.07^{a}	2.02^{a}	1.89^{a}
2	2.04^{b}	1.90^{b}	2.00^{a}	1.99^{a}	1.90^{b}	1.76^{b}	1.75^{b}	1.63^{b}	1.62^{b}	1.66^{b}
4	1.75^{c}	1.88^{b}	1.80^{b}	1.71^{b}	1.78^{c}	1.44^{c}	1.65^{c}	1.43^{c}	1.54^{c}	1.42^{c}
7	1.80^{c}	1.73^{c}	1.68^{c}	1.64^{c}	1.68^{c}	1.47^{c}	1.42^{d}	1.17^{d}	1.29^{e}	1.24^{d}
9	1.70^{c}	1.71^{c}	1.55^{d}	1.66^{c}	1.75^{c}	1.25^{d}	1.06^{e}	1.17^{d}	1.26^{e}	1.29^{d}
11	1.05^{d}	1.08^{d}	0.92^{e}	0.96^{d}	0.93^{d}	1.47^{c}	1.05^{e}	0.99^{e}	1.31^{d}	1.05^{e}
14	1.00^{d}	0.96^{d}	0.81^{f}	0.90^{d}	0.93^{d}	1.06^{e}	0.93^{f}	1.15^{d}	0.92^{f}	0.87^{f}
21	0.83^{e}	0.88^{e}	0.76^{f}	0.96^{d}	0.83^{e}	0.74^{f}	0.76^{g}	0.68^{f}	0.84^{f}	0.76^{g}

[a]Different letters in the same column indicate significant differences among days ($p<0.05$).
T1: 80 kPa O_2, **T2**: 60 kPa O_2, **T3**: 21 kPa O_2, **T4**: 10 kPa O_2 + 5 kPa CO_2, **T5**: 2.5 kPa O_2 + 5 kPa CO_2.

A principal components analysis (PCA) was used to determine relationships among antioxidant compounds. Two principal components (PC1 and PC2) were calculated. They account for 72.8% of the variability in the original data (Figure 4). As can be seen in Figure 4, there is a close relationship between DPPH hydrophilic values and vitamin C content. Therefore hydrophilic antioxidant capacity of fresh-cut tomatoes determined through the DPPH assay could be mainly attributed to vitamin C rather than to phenolic compounds. The free-radical DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. DPPH assay is not specific to any particular antioxidant component, thus applying to the overall antioxidant capacity of the sample (Gil et al., 2000). In addition, the statistical analysis reveals an outstanding correlation among the DPPH radical scavenging capacity of the lipophilic fraction, lycopene and β-carotene, whereas changes in phytofluene and neurosporene were well associated to the variation in the lipophilic ABTS antioxidant capacity (Figure 4). The ABTS radical has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their properties as electron- or H-donating agents (Miller et al., 2000). However, there was a slight relationship between flavonols, phenolic acid and the hydrophilic antioxidant capacity determined through the ABTS radical.

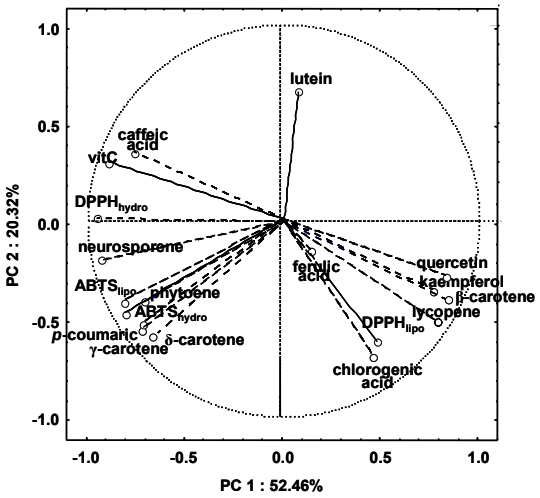

Figure 4.- Principal components plot of fresh-cut tomato stored for 21 days at 4 ºC under different packaging conditions.

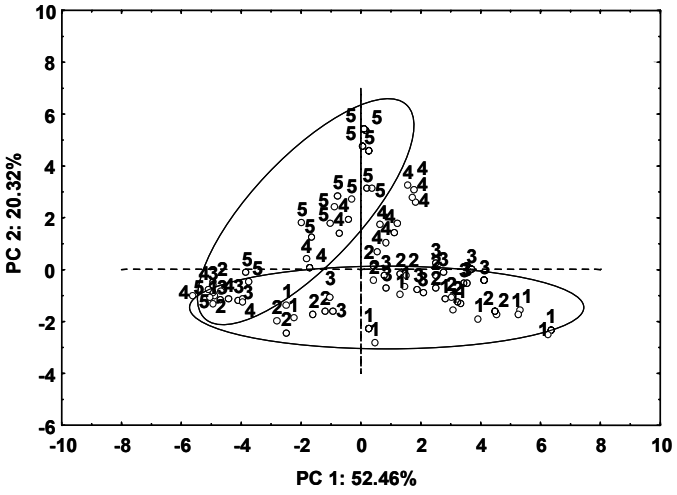

Figure 5.- Score plot of PC1 vs. PC2 of fresh-cut tomato juices stored during 21 days at 4 ºC under different packaging condition: (1) 80 kPa O_2, (2) 60 kPa O_2, (3) 21 kPa O_2, (4) 10 kPa O_2 + 5 kPa CO_2, (5) 2.5 kPa O_2 + 5 kPa CO_2.

The score plot of PC1 versus PC2 from the full-data PCA model plotted in Figure 5 describes differences between fresh-cut tomatoes stored under different in-package conditions. It can be observed that the most of the samples packaged under 2.5 kPa O_2 + 5 kPa CO_2 atmospheres are situated in the upper part of the plot, whereas samples packaged under 80 kPa O_2 were located at the bottom (Figure 5). Therefore, fresh-cut tomatoes stored under 80 kPa O_2 atmospheres scored higher on flavonols, lycopene, β-carotene, chlorogenic acid and DPPH values of the lipophilic fraction than those packaged under lower O_2 concentrations.

CONCLUSIONS

Low-O_2 atmospheres better maintained the hydrophilic antioxidant capacity of tomato slices compared to high-O_2 and passive atmospheres by preserving vitamin C through storage. On the other hand, the amounts of individual phenolic compounds and carotenoids in fresh-cut tomatoes stored under initial 80 kPa O_2 were dramatically enhanced during storage, with the exception of caffeic acid, p-coumaric, δ-carotene, γ-carotene and neurosporene. The DPPH lipophilic antioxidant capacity of fresh-cut tomatoes seems to be mainly attributed to lycopene and β-carotene content, whereas the ABTS values of the lipophilic fraction were associated to neurosporene and phytoene. The results obtained in the present study suggest that storage of fresh-cut tomatoes under ≥ 21 kPa O_2 can induce the formation of phenylpropanoids as a result of physiological stress. On the contrary, 2.5 kPa O_2 + 5 kPa CO_2 storage atmospheres may decrease wounding stress and reduce deteriorative changes related to the acceleration of ripening and enhancement of senescence processes.

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REFERENCES

Abeles, F.B., Morgan, P.W., Saltveit, M.E. (1992). Ethylene in plant biology. Academic Press, New York.

Aguayo, E., Escalona, V., Artés, F. (2004). Quality of fresh-cut tomato as affected by type of cut, packaging, temperature and storage time. *European Food Research and Technology*, 219, 492-499.

Alasalvar, C., Al-Farsi, M., Quantick, P.C., Shahidi, F., Wiktorowicz, R. (2005). Effect of chill stoarge and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, pehnolics and sensory quality of ready-to-eat shredded orange and purple carrots. *Food Chemistry*, 89, 69-76.

Allende, A., Luo, Y., McEvoy, J.L, Artés, F., Wang, CY. (2004). Microbial and quality changes in minimally processed baby spinach leaves stored under superatmospheric oxygen and modified atmosphere conditions. *Postharvest Biology and Technology*, 33, 51-59.

Amanitidou, A., Smid, E.J., Gorris, L.G.M. (1999). Effect of elevated oxygen and carbon dioxide on the surface growth of associated microorganisms. *Journal of Applied Microbiology* 86, 429-438.

Artés, F., Conesa, M.A., Hernández, S., Gil, M.I. (1999). Keeping quality of fresh-cut tomato. *Postharvest Biology and Technology*, 17, 153-162.

Artés, F., Gómez, P.A., Artés-Hernández, F (2007). Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. *Food Science and Technology International*, 13, 177-188.

Bartley, G.E., Scolnik, P.A. (1995). Plant carotenoids: pigments for photoprotection, visual attraction, and human health. *Plant Cell*, 7, 1027-1038.

-Beecher, G.R. (1998). Nutrient content of tomatoes and tomato products. Proc. Soc. Exp. Biol. Med. 218, 98-100.

-Britton, G., Hornero-Mendez, D. (1997). Carotenoids and colour in fruit and vegetables. In: Tomas-Barberán, F.A., Robins R.J. Phytochemistry of fruit and vegetables. Science Publications, Oxford. pp. 11–27.

Cao, G., Alessio, H.M., Cutler, R.G. (1993).Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine*, 14, 303-311.

Clinton, S.K. (1998). Lycopene: chemistry, biology, and implications for human health and disease. *Nutrition Reviews*, 56, 35-51.

Gil, M.I., Conesa, M.A., Artés, F. (2002). Quality changes in fresh-cut tomato as affected by modified atmosphere packaging. *Postharvest Biology and Technology*, 25, 199-207.

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, M., Kader, A. (2000). Antioxidant activity of pomegrate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, 48, 4581-4589.

Heller, W. (1986). Flavonoid biosynthesis: an overview. In: Cody, V., Middleton, E, Harbone, J.B. Plant flavonoids in biology and medicine: biochemical, pharmacological, and structure-activity relationships. Alan R. Liss, New York. pp. 25-42.

Hertog, M., Hollman, P., Venema, D. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, 40, 1591-1598.

Jacxsens, L., Devlieghere, F, Van der Steen, C, Debevere, J. (2001). Effect oh high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71, 197-210.

Kader, A.A., Zagory, D., Kerbel, E.L. (1989). Modified atmosphere packaging of fruits and vegetables. *Critical Reviews in Food Chemistry*, 35, 187-195.

Kader, A.A., Ben-Yehoshua, S. (2000). Effect of superatmospheric oxygen levels on postharvest physiology and quality on postharvest physiology and quality of fresh-cut fruits and vegetables. *Postharvest Biology and Technology*, 20, 1-13.

Khachik, F., Goli, M.B., Beecher, G., Holden, J., Lusby, W.R., Tenorio, M.D., Barrera, M.R. (1992). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *Journal Agricultural and Food Chemistry*, 40, 390-398.

Lenucci, M.S., Codinu, D., Taurina, M., Piro, G., Dalessandro, G. (2006). Antioxidant composition in cherry and high-pigment tomato cultivar. *Journal of Agricultural and Food Chemistry*, 54, 2606-2613.

Li, N., Parsons, B.L., Liu, D. and Mattoo, A.K. (1992). Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. *Plant Molecular Biology*, 18, 477-487.

Martínez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant capacity in commercial varieties of tomato (*Lycopersicum esculentum*). *Journal of the Science and Food Agriculture*, 82, 323-330.

Mencarelli, F., Salveit, M.E. (1988). Ripening of mature-green tomato fruit slices. *Journal of the American Society for Horticultural Science*, 113, 742-745.

Miller, H.E., Rigelhof, F., Marquart, L., Prakash, A., Kanter, M. (2000). Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of the American College of Nutrition*, 19, 312S–319S.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso O. (2007). Comparative study of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105, 1151-1158.

Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Effect on minimally processing on bioactive compounds and color attributes of fresh-cut tomatoes. *LWT-Food Science and Technology*, 41, 217-226.

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Antioxidant content of fresh-cut pears stored in high-O_2 active packages compared with conventional low-O_2 atmospheres and passive modified atmosphere packaging. *Journal of Agricultural and Food Chemistry*, 56, 932-940.

Re, R., Pellegrin, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free Radical Biology and Medicine*, 26, 1231-1237.

Reyes, L.F., Villarreal, J.E., Cisneros-Zevallos, L.C. (2006). The increase in antioxidant capcity alter wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 101, 1254-1262.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P., Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, 66, 401-436.

Rodriguez-Amaya, D.B. (1997). Carotenoids and food preservation: the retention of provitamin A carotenoids in prepared, processed and stored food. USAID, OMNI Project.

Sánchez-Moreno, C., Cano, M.P., de Ancos, B., Plaza L., Olmedilla, B., Granado, F., Martin, A. (2003). Effect of orange juice intake on vitamin C concentrations and biomarkers of antioxidant status in humans. *American Journal of Clinical Nutrition*, 78, 454-460.

Shi, J., Le Maguer, M. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition*, 40, 1-42.

Soliva-Fortuny, R.C., Oms-Oliu, G., Martín-Belloso, O. (2002). Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67, 312-317.

Stewart, A.J., Bozonnet, S., Mullen, W., Jenkins, G.I., Lean, M.E.J., Crozier, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *Journal of Agricultural and Food Chemistry*, 48, 2663-2669.

Tonucci, L.H., Holden, J.M., Beecher, G.R., Khachik, F., Davis, C.S., Mulokozi, G. (1995). Carotenoid content of thermally processed tomato-based food products. *Journal of Agricultural and Food Chemistry*, 43, 579-586.

Trumbo, P.R., Yates, A.A., Schlicker-Renfro, S., Suitor C. (2003). Dietary Reference Intakes: revised nutritional equivalents for folate, vitamin E and provitamin A carotenoids. *Journal of Food Composition and Analysis*, 16, 379-382.

Van der Steen, C., Jacxsens, L., Devlieghere, F., Debevere, J. (2002). Combining high oxygen atmosphere with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberry. *Postharvest Biology and Technology*, 26, 49-58.

Varoquaux, P., Wiley, R.C. (1997). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In: Wiley, R.C. Minimally processed refrigerated fruits & vegetables. Chapman and Hall, New York. pp. 226–268.

Zheng, Y., Wang, C.Y., Wang, S.Y., Zheng, W. (2003). Effect of high-oxygen atmospheres on blueberry phenolics anthocynins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 51, 7162-7169.

Zheng, Y., Wang, S.Y., Wang, C.Y., Zheng, W. 2007. Changes in strawberry phenolics, anthocynins, an antioxidant capacity in response to high oxygen treatments. *LWT- Food Science and Technology*, 40, 49-57.

Capítulo XI

Kinetics of anthocyanins, vitamin C and antioxidant capacity changes in fresh-cut strawberries during storage at different temperatures

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Journal of Food Science, *enviado*

ABSTRACT

Changes in the main antioxidant properties of fresh-cut strawberries stored under high oxygen atmospheres were studied at selected temperatures (5-20 ºC). The suitability of zero and first-order kinetics as well as a model based on Weibull distribution function to describe changes in experimental data is discussed. An Arrhenius approach was use to determine the temperature dependence of the estimated rate constants. A Weibull kinetic model most accurately ($R^2_{adj} \geq$ 0.800) predicted changes in anthocyanins, vitamin C and antioxidant capacity of fresh-cut strawberries through the storage period. The temperature dependency of the Weibull kinetic rate constants for each antioxidant property was successfully predicted ($R^2_{adj} \geq 0.874$) through the Arrhenius approach. The activation energies obtained for anthocyanins, vitamin C and antioxidant capacity degradation at 5-20ºC were 101.8 kJ/mol, 33.8 kJ/mol and 119.7 kJ/mol, respectively, indicating high susceptibility of these properties to temperature increases. These findings will help to predict and describe the variation of the antioxidant potential of fresh-cut strawberries upon storage time and temperature.

Key words: kinetics, fresh-cut strawberries, anthocyanins, vitamin C, antioxidant capacity.

INTRODUCTION

Fresh-cut produce is a rapid growing segment in the food service and the retail market because of the demand for healthy and ready-to-eat products. Fresh-cut fruits are generally more perishable than whole fruits because they have been subjected to physiological stresses caused by wounding (Varoquaux and Willey 1997). Respiration increases when fruits are wounded, causing accelerated consumption of sugars, lipids, and organic acids, and an increase in ethylene production, which induces ripening and causes senescence (Kays, 1991). Many factors may affect the physiological response of the fruit to wounding-stress, including the cultivar, maturity stage, temperature or packaging conditions (Rocculi et al., 2006). The combination of different preservation methods such as modified atmosphere packaging (MAP), low-temperature storage or the addition of preservatives may be an excellent way to preserve the original quality attributes of these products (Alzamora et al., 1998). Up to now, the studies evaluating the influence of storage conditions on fresh-cut products have demonstrated that storage temperature is an important variable to be controlled in order to extend the shelf-life and quality of these commodities (Aguayo et al., 2004; Lavelli et al., 2006; Saavedra del Aguila et al., 2006; Artés-Henández et al., 2007). Therefore, the evaluation of the influence of storage temperature and time on the antioxidant properties of fresh-cut products may provide useful information for consumers, in order to determine their nutritional value.

Diets rich in fruits and vegetables have been associated with several health benefits, including the prevention of cancer and cardiovascular disease, due to the action of the antioxidants they contain (Ames, 1993). Among fruits, strawberry is one of the most consumed berries and it is an important source of fiber and health-related compounds, in particular they are rich in vitamin C and anthocyanins (Tulipani et al., 2008). It is well known that anthocyanins impart to strawberries their appealing, bright red color. Anthocyanins can be decolorized and degraded by many factors such as temperature, light, pH, oxygen, enzymes, the presence of copigments and metallic ions, ascorbic acid, sulphur dioxide and sugars (Jackman and Smith 1996). Vitamin C is a thermolabile vitamin, which is oxidized to a non antioxidant substance in the presence of oxygen (Davey et al., 2000). Some models have been used to predict shelf-life and quality changes of different fresh-cut vegetables through the storage (Riva et al., 2001; Piagentini et al., 2005). Several authors reported the kinetic rates of anthocyanins and vitamin C degradation during the storage of juices and nectars (Cemeroğlu et al., 1994; Johnson et al., 1995; Lee and Chen 1998; Iversen, 1999; Kırca and Cemeroğlu, 2003; Kırca et al., 2006). However, little information is currently available on modeling antioxidant properties of fresh-cut commodities as a function of storage time and temperature. Therefore, the objective of this study was to propose kinetic approaches to properly describe changes in the antioxidant properties of fresh-cut strawberries stored at different storage temperatures.

MATERIALS AND METHODS

Plant material

Strawberry fruits (*Fragaria ananassa* Duch, cultivar Camarosa) were purchased from a local wholesaler (Lleida, Spain) and stored at 4 ºC ± 1 ºC. Soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Tokyo, Japan), pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), color (spectrophotocolorimeter Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan) and texture (TA-XT2 Texture Analyzer; Stable Micro Systems Ltd, Surrey, UK). Analytical characteristics of strawberries were, pH of 3.45 ± 0.12, soluble solids of 6.5 ± 0.5 ºBrix, color L* = 31.2 ± 2.2, a* = 38.4 ± 2.3 and b* = 18.8 ± 3.5, and firmness of 19.4 ± 3.2 N. (results are expressed as the mean ± standard deviation).

Sample preparation

Strawberries were sanitized in a solution containing 200 µL L^{-1} NaClO for 2 min, rinsed with tap water and dried. Then, the calyx was cut off from the whole strawberry and fruits were cut longitudinally with a sharp knife into four wedges. After that, wedges (100 g) were packaged in polypropylene trays. The modification of package atmosphere was carried out by flushing a 80 kPa O_2 balance with N_2 before sealing the trays, using a digitally controlled compensated vacuum (ILPRA Food Pack Basic V/6, ILPRA Systems. CP., Vigevono, Italia). High O_2 atmospheres have been suggested as effective to inhibit the growth of moulds and maintain the fresh-like sensory properties of strawberries for 14 days at 5 ºC (Van der Steen and others 2002). The relationship between the amount of product and the injected gas mixture was 1:2 (v/v). The O_2 and CO_2 permeance of the 64 µm thick polypropylene sealing film were 110 and 500 cm^3/m^2 day bar at 23 ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The packages were stored at 4 ºC ± 1 ºC in darkness until random withdrawal for analysis. Three trays were taken at each rime to perform the analysis, and two readings were carried out for each package.

Anthocyanins

The anthocyanins content of fresh-cut strawberries was determined with a modified pH differential method described by Meyers et al. (2003), using two buffer systems: potassium chloride 0.025 M at pH 1, and sodium acetate 0.4 M at pH 4.5. A sample of 25 mg was mixed with 25 mL of methanol. The homogenate was centrifuged at 22100x*g* for 15 min at 4 ºC (Centrifuge Avanti™ J-25, Beckman Instruments Inc., Fullerton, CA, USA). Briefly, 10 mL of the supernatant were transferred to a 50 mL volumetric flask and made up with each buffer. The absorbance of the mixtures at pH 1 and 4.5 was then measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 510 and 700 nm against a blank of distilled water.

The anthocyanins content was calculated according to Equation 1 and expressed as pelargonidin-3-glucoside per kg:

$$TA = \frac{\left[\left(A_{510} - A_{700}\right)_{pH1.0} - \left(A_{510} - A_{700}\right)_{pH4.5}\right] \times MW \times DF \times 1000}{\varepsilon \times L} \qquad (1)$$

where MW is the molecular weight of pelargonidin-3-glucoside (433.0 g/mol), DF is the dilution factor, L is the pathlength in cm, and ε is the molar extinction coefficient for pelargonidin-3-glucoside (22400 L/mol·cm). Results were expressed as pelargonidin-3-glucoside retention compared to the content in just-processed strawberry wedges.

Vitamin C

Vitamin C content of strawberry wedges was analyzed by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (2007). A sample of 25 mg was mixed with 25 mL of a solution containing 45 g/L metaphosphoric acid and 7.2 g/L of DTT. The homogenate was centrifuged at 22100x*g* for 15 min at 4 ºC (Centrifuge Avanti^{TM} J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vacuum-filtered through Whatman No. 1 paper. Then, the samples were filtered with a Millipore 0.45-µm membrane. An aliquot of 20 µL was injected into a HPLC system consisting of a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6 mm x 250 cm) and a 486 Absorbance Detector (Waters, Milford, MA). A 0.01% solution of sulphuric acid adjusted to pH = 2.6 was used as eluent. The flow was isocratic at a rate of 1 mL/min at room temperature. Detection was performed at 245 nm. Identification of ascorbic acid was carried out by comparing retention times and UV-visible absorption spectrum of the juice samples with those of the standards. Results were expressed as vitamin C retention related to the content in just-processed strawberry wedges.

Antioxidant capacity

The antioxidant capacity of fresh-cut strawberries was studied through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), according to the method described by Odriozola-Serrano et al. (2008). Strawberry samples were centrifuged at 6000xg for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain) and aliquots of 0.01 mL of the supernatant were mixed with 3.9 mL of methanolic DPPH (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 60 min. Absorption of the samples was measured with a spectrophotometer at 515 nm against a blank of methanol without DPPH. Results were expressed as antioxidant capacity retention related to the just-processed strawberry wedges.

Kinetic models

The fitting of several kinetic models to experimental data was evaluated in order to properly relate and understand the changes in the antioxidant potential of fresh-cut strawberries to storage time at different temperatures.

Zero-order model (Equation 2) has shown to properly fit the degradation of heath-related compounds in fruits and juices (Özhan et al., 2004; Koca et al., 2007). A simple first-order model (Equation 3) has also been commonly suggested as the best model to fit degradation of anthocyanins and vitamin C in juices and nectars (Cemeroğlu et al., 1994; Kırca et al., 2006; Wang and Xu, 2007) as well as in frozen (Giannakourou and Taoukis, 2003) and dried fruits (Uddin et al., 2002) during storage at different temperatures. Lana and Tijskens (2006) proposed a first-order model to describe changes in the antioxidant capacity of fresh-cut tomatoes at three different stages of maturity through storage.

$$RA = RA_0 - k_0 \cdot t \qquad (2)$$

$$RA = RA_0 \cdot exp(-k_1 \cdot t) \qquad (3)$$

where RA (%) is the relative antioxidant content after storage time t (days), RA_0 (%) is the intercept of the curve and k_0 is the zero-order kinetic constant (%/days^{-1}) and k_1 is the first-order kinetic constant (days^{-1}).

The Weibull model (Equation 4) is extremely flexible owning to the inclusion of a shape constant in addition to the rate constant. It has been proved to have an interesting potential for describing microbial, enzymatic and chemical degradation kinetics (Cunha et al., 1998). In this way, Manso et al. (2001) used the Weibull distribution functions to describe the thermal degradation (Tª 20-45 ºC) of ascorbic acid in orange juice. The use of Weibull distribution function to fit the retention of the antioxidant properties in fresh-cut products as a function of storage time has not been reported yet.

$$RA = RA_0 \, exp\left[-\left(t \cdot k_\alpha\right)^\gamma\right] \qquad (4)$$

where RA (%) is the relative antioxidant property, RA_0 (%) is the intercept of the curve, t (days) is the storage time, k_α (days^{-1}) is the kinetic constant, which is the inverse of the scale factor (α) (Corradini and Peleg, 2004), and γ is the shape parameter. The γ parameter indicates concavity (tail-forming) or convexity (shoulder-forming) of the curve when it takes values below and above 1, respectively. In general, γ may be expected to be temperature-independent, within a limited range of temperatures (Manso et al., 2001).

Derived from the Weibull distribution function parameters (α, γ), t_m is defined as the mean storage time to achieve complete destruction of the antioxidant property (Equation 5):

$$t_m = \left(\frac{1}{k_\alpha}\right) \cdot \Gamma\left(1 + \frac{1}{\gamma}\right) \quad (5)$$

where k_α and γ are the parameters of the Weibull distribution and Γ is the gamma function.

Effect of temperature

The temperature dependence of the kinetic rate constants (k) obtained for each antioxidant property was then modeled by the Arrhenius equation (Eq. 6).

$$k = A_o \, exp\left(\frac{-Ea}{R \cdot T}\right) \quad (6)$$

where A_o (days^{-1}) is the Arrhenius constant (frequency factor), E_a is the activation energy (J/mol) of the chemical reaction and R is the universal gas constant (8.32 J/mol·K).

Statistical analysis

The analysis of variance was carried out with Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, MD). The least significant difference test was used to determine differences between treatments at a 5% significance level. The models were fitted to experimental data by nonlinear regression procedures, using the Statgraphics Plus v.5.1 Windows package. The adjusted regression coefficients (R^2_{adj}) and the statistical parameters, root mean square error (RMSE), reduced Chi-square (χ^2) and mean bias error (MBE) were calculated to evaluate the fitting of a model to experimental data (Hayaloglu et al., 2007). The highest the values of R^2_{adj} and the lowest the values of RMSE, χ^2 and MBE, the better the fitting of the models to experimental data was.

RESULTS AND DISCUSSION

Anthocyanins

The initial concentrations of total anthocyanins in the strawberry fruits varied from 402 to 416 mg/kg, which agrees well with the reports of other authors for strawberries (Ayala-Zavala et al., 2004; Cordenunsi et al., 2005). As can be seen in Figure 1, anthocyanins content

significantly depended on the storage time and temperature. The longer the time and the higher the storage temperature, the greater the degradation of anthocyanins was.

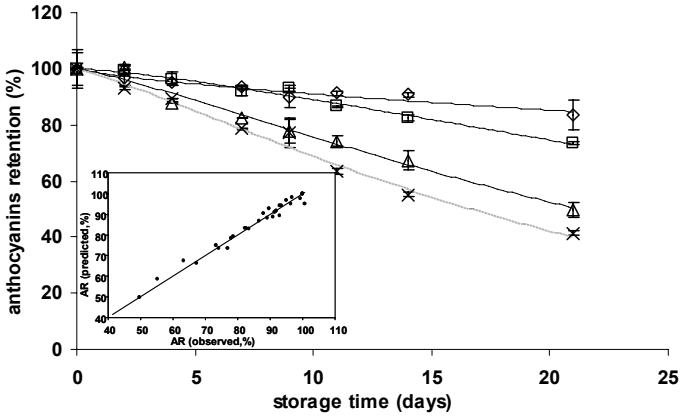

Figure 1.- Effect of storage time and temperature on the anthocyanins retention of fresh-cut strawberry (mean ± SD) as modeled by Weibull approach. Temperatures: 5 ºC (◊), 10 ºC (□), 15 ºC (Δ) and 20 ºC (×). Plotted lines correspond to the values estimated from the Weibull model. AR: anthocyanins retention

The kinetic constants estimated by zero-order, first-order and Weibull models as well as the adjusted determination coefficients, RMSE, χ^2 and MBE, of the fitted models at different storage temperatures are shown in Table 1. Although zero and first-order models predicted well the changes in anthocyanins, Weibull distribution seemed to be the most suitable because of the highest determination coefficients (R^2_{adj} = 0.800-0.973) and the lowest RMSE, χ^2 and MBE (Table 1). Furthermore, the deviations in the values estimated by Weibull model in relation to the observed values are small (Figure 1). A simple first-order model was used by Kırca and Cemeroğlu (2003) and Wang and Xu (2007) to describe anthocyanins degradation in blood orange and blackberry juices and concentrates during storage at different temperatures. The authors obtained *k*-values ranging from 2.0×10^{-3} to 3.3×10^{-1} day^{-1} for anthocyanins in juices and concentrates stored between 5 and 37 ºC. In our study, kinetic rates defined by the simple first-order model ranged from 8.7×10^{-3} to 3.9×10^{-2} $days^{-1}$, suggesting that anthocyanins in fresh-cut products had similar stability during the storage period than in juices or concentrates. The kinetic constant (k_α) and the shape parameter (γ) of the Weibull model were obtained by fitting Equation 4 to the experimental data. The γ below 1 obtained for fresh-cut strawberries stored at 5 ºC could indicate that a great amount of anthocyanins were degraded during the first days of storage, but then the content was almost maintained. On the contrary, anthocyanins in fresh-cut strawberries stored between 10 and 20 ºC were increasingly destroyed overtime as indicated by the γ values above 1. The k_α constants, which varied from 4.4×10^{-3} and 4.4×10^{-2}

days^{-1}, were directly dependent on the storage temperature. Consistently, Shin et al. (2008) reported increased degradation of anthocyanins in strawberries with increasing storage temperature. Although, it has been suggested that the biosynthetic pathway for anthocyanins is still operative after strawberry harvest, elevated CO_2 atmospheres induced a reduction of anthocyanins synthesis compared to fruits stored in air where metabolism is not affected as much as in CO_2-enriched atmospheres (Gil et al., 1997). In addition, CO_2 atmospheres may affect the metabolism of organic acids by dissolution of CO_2, which would cause an increase in the pH of the tissues and, reducing the stability of anthocyanins (Brouillard, 1982).

Table 1.-Kinetic constants of zero-order, first-order and Weibull model of anthocyanins retention in fresh-cut strawberries as a function of the storage temperature

Model	T(ºC)	Constants[1]	Statistical parameters			
			R^2_{adj}	RMSE	χ^2	MBE
Zero-order	5	$k_0 = 8.1\times10^{-1} \pm 5.8\times10^{-2}$	0.772	0.799	1.269	0.273
	10	$k_0 = 1.2 \pm 4.5\times10^{-2}$	0.953	0.758	1.324	-0.278
	15	$k_0 = 2.4 \pm 7.7\times10^{-2}$	0.961	0.819	0.366	-0.146
	20	$k_0 = 2.9 \pm 8.0\times10^{-2}$	0.969	1.043	0.277	0.127
First-order	5	$k_1 = 8.7\times10^{-3} \pm 6.5\times10^{-4}$	0.783	0.770	0.842	0.222
	10	$k_1 = 1.3\times10^{-2} \pm 6.4\times10^{-4}$	0.938	0.840	1.737	-0.319
	15	$k_1 = 2.9\times10^{-2} \pm 1.4\times10^{-3}$	0.947	1.015	2.669	1.015
	20	$k_1 = 3.9\times10^{-2} \pm 1.6\times10^{-3}$	0.961	1.131	1.449	-0.291
Weibull	5	$k_\alpha = 4.4\times10^{-3} \pm 2.1\times10^{-3}$ $\gamma = 7.5\times10^{-1} \pm 1.4\times10^{-1}$	0.800	0.706	0.011	0.024
	10	$k_\alpha = 1.9\times10^{-2} \pm 2.4\times10^{-3}$ $\gamma = 1.3 \pm 9.2\times10^{-2}$	0.970	0.647	0.012	-0.025
	15	$k_\alpha = 3.5\times10^{-2} \pm 3.5\times10^{-3}$ $\gamma = 1.2 \pm 1.0\times10^{-1}$	0.959	0.885	0.124	-0.082
	20	$k_\alpha = 4.4\times10^{-2} \pm 1.8\times10^{-3}$ $\gamma = 1.2 \pm 8.1\times10^{-2}$	0.973	0.915	0.040	0.047

[1]Mean values ± standard error

k_0: zero-order rate constant (days^{-1})**;** **k_1:** first-order rate constant (days^{-1});

k_α: 1/scale factor (days^{-1})**;** **γ:** shape factor

R^2_{adj}: adjusted determination coefficient; **RMSE**: Root mean square error; **χ^2**: Reduced Chi-square; **MBE**: Mean bias error

Mean time (t_m) was calculated from Equation 5 for each storage temperature and values ranged from 41 and 532 days, increasing with storage temperature. Therefore, it can be assumed that anthocyanins will not be completely depleted, at least in the range of the studied storage temperatures, because the shelf-life of fresh-cut strawberries is not greater than 14 days. High oxygen atmospheres (≥ 60 kPa O_2) alleviated tissue injury, reduced microbial growth and maintained quality of strawberries for 14 days at 5 ºC (Van der Steen et al., 2002). The effect of the storage temperature on the Weibull degradation rate constants is shown in Figure 2. Temperature-dependent rate constant obeyed the Arrhenius relationship (Equation 6). The good fitting of the model is confirmed by the statistical parameters (RMSE = 5.5×10^{-1}, χ^2 = 1.3×10^{-5}, MBE = 1.6×10^{-3}) and the high adjusted determination coefficient (R^2_{adj} = 0.877). The

predicted value of the activation energy for changes in anthocyanins in fresh-cut strawberries was 101.8 kJ/mol. In this way, the activation energy for anthocyanins degradation was reported to be 113.0 kJ/mol in whole strawberries (Markakis, 1982), 90 kJ/mol in blackcurrant jam (Viberg et al., 1997) and varied from 65.1 kJ/mol to 80.9 kJ/mol in fruit juices and concentrates (Kırca and Cemeroğlu, 2003; Wang and Xu, 2007). High inactivation energies imply that anthocyanins are more susceptible to temperature elevation in fresh-cut products than in juices, concentrates or jams. Nevertheless, fresh-cut strawberries stored at 80 kPa O_2 are found to be affected by high temperatures in a similar way than the whole fruit.

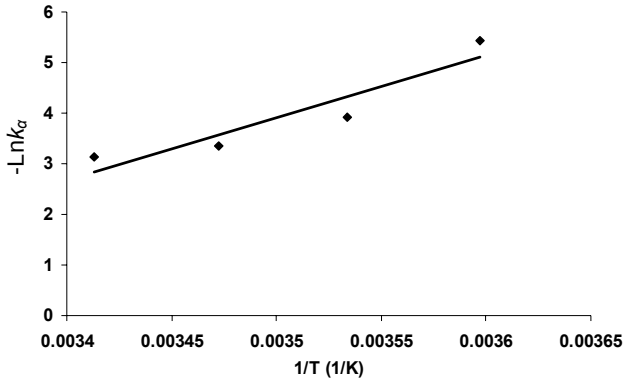

Figure 2.-Effect of temperature on the rate constant (k_α) estimated by fitting Weibull model to anthocyanins of fresh-cut strawberries as affected by storage time.

Vitamin C

The effect of storage time and temperature on the concentration of vitamin C in fresh-cut strawberries is shown in Figure 3. The vitamin C content of the strawberry wedges just after processing ranged from 652 to 667 mg/kg. These values are within the range observed by Davey et al. (2000) and Cordenunsi et al. (2005) who reported vitamin C contents between 400 to 900 mg/kg in different strawberry cultivars. Vitamin C concentration in fresh-cut strawberries underwent a substantial depletion through the storage time irrespective of the storage temperature (Figure 3). Table 2 displays the estimated parameters and the R^2_{adj}, RMSE, MBE and X^2 of the models used to describe vitamin C changes throughout the storage time at different temperatures. The fitting performances of first-order and Weibull models were good irrespective of the storage temperature (R^2_{adj} = 0.982-0.994), but when using zero-order models these coefficients were much lower (R^2_{adj} = 0.633-0.909). The Weibull approach is reduced to a first-order model when the shape factors is close to 1, and thus, R^2_{adj}, RMSE, MBE and X^2 values obtained for first-order and Weibull models were quite similar. The good accuracy of the Weibull approach is confirmed by the small deviation in the values estimated by the model and the experimental data to the line of equivalence (Figure 3). The vitamin C degradation

rates defined by the simple first-order model and the Weibull approach took values in the range of 6.7×10^{-2} to 1.3×10^{-1} $days^{-1}$ for storage temperatures of 5-20ºC. On the other hand, the γ parameter ranged from 1 to 0.85, suggesting that this vitamin became decreasingly destroyed overtime. As can be seen in Table 2, the first-order and Weibull kinetic rate constant were significantly influenced by storage temperature. The ascorbic acid degradation rate in fresh-cut strawberries stored at 20 ºC almost doubled the obtained for strawberries preserved at 5 ºC. Thus, the higher the storage temperature, the more substantial variation in ascorbic acid content. Adisa (1986) observed that fruits and vegetables show a gradual depletion in ascorbic acid content as temperature increases. This greater decrease of vitamin C at high storage temperatures might be due to the wounding response as a consequence of low O_2 and high CO_2 accumulation inside packages (Table 3). Increasing CO_2 concentrations might provoke cytoplasm acidification with the consequent impairment of mitochondrial function, which could result in oxidative damage (Tudela et al., 2002). In addition, from the many antioxidants present in the cell, ascorbic acid rapidly reacts with radical species to protect cell integrity upon redox imbalances (Reyes et al., 2007). It has been reported that vitamin C is a typical heat sensitive nutrient, so that its retention is often considered as a significant marker of overall nutrient retention (Jung et al., 1995). Values of mean time to achieve complete loss of vitamin C were calculated from Equation 5. Values of t_m ranged from 16 and 30 days, suggesting that the lower the storage temperature, the higher the vitamin content in fresh-cut strawberries. Several studies reported that storing fresh-cut products at temperatures ≥ 10 ºC can reduce their microbial shelf-life for up 4-7 days (Artés-Hernández et al., 2007; Odriozola-Serrano et al., 2008). Therefore, strawberry wedges will be discarded due to microbiological spoilage before they reach losses higher than 50% of the initial vitamin C content.

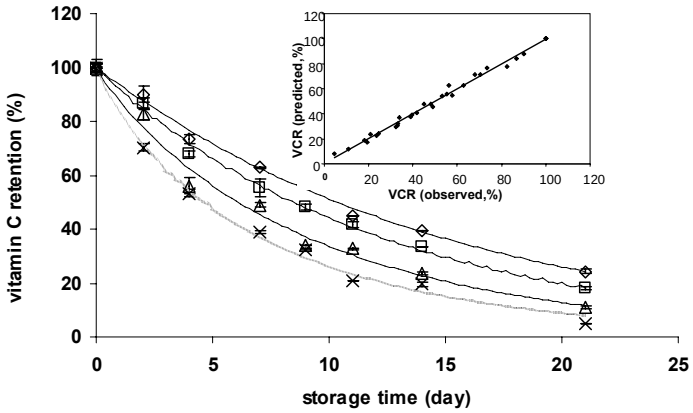

Figure 3.- Effect of storage time and temperature on the vitamin C retention of fresh-cut strawberry (mean ± SD) as modeled by Weibull approach. Temperatures: 5 ºC (◊), 10 ºC (□), 15 ºC (Δ) and 20 ºC (×). Plotted lines correspond to the values estimated from the Weibull model. VCR: vitamin C retention

Table 2.- Kinetic constants of zero-order, first-order and Weibull model of vitamin C retention in fresh-cut strawberries as a function of the storage temperature

Model	T(ºC)	Constants[1]	Statistical parameters			
			R^2_{adj}	RMSE	χ^2	MBE
Zero-order	5	$k_0 = 4.2 \pm 1.7\times10^{-1}$	0.909	1.706	31.93	1.368
	10	$k_0 = 4.6 \pm 2.3\times10^{-1}$	0.856	1.999	68.22	1.999
	15	$k_0 = 5.3 \pm 3.5\times10^{-1}$	0.737	2.427	156.9	3.032
	20	$k_0 = 5.8 \pm 4.3\times10^{-1}$	0.633	2.736	276.5	4.025
First-order	5	$k_1 = 6.7\times10^{-2} \pm 1.4\times10^{-3}$	0.992	0.866	0.036	-0.046
	10	$k_1 = 8.2\times10^{-2} \pm 1.5\times10^{-3}$	0.993	0.790	0.007	0.021
	15	$k_1 = 1.1\times10^{-1} \pm 3.7\times10^{-2}$	0.982	1.192	0.001	-0.009
	20	$k_1 = 1.3\times10^{-1} \pm 4.4\times10^{-3}$	0.986	1.149	0.330	0.139
Weibull	5	$k_\alpha = 6.7\times10^{-2} \pm 2.3\times10^{-3}$ $\gamma = 1.0 \pm 4.1\times10^{-2}$	0.992	0.864	0.021	-0.034
	10	$k_\alpha = 8.1\times10^{-2} \pm 2.3\times10^{-3}$ $\gamma = 9.5\times10^{-1} \pm 3.4\times10^{-2}$	0.994	0.770	0.022	-0.035
	15	$k_\alpha = 1.0\times10^{-1} \pm 5.4\times10^{-3}$ $\gamma = 9.1\times10^{-1} \pm 5.6\times10^{-2}$	0.984	1.161	0.054	-0.054
	20	$k_\alpha = 1.3\times10^{-1} \pm 5.1\times10^{-3}$ $\gamma = 8.5\times10^{-1} \pm 3.6\times10^{-2}$	0.993	0.968	0.207	0.106

[1]Mean values ± standard error
k_0: zero-order rate constant ($days^{-1}$); k_1: first-order rate constant ($days^{-1}$)
k_α: 1/scale factor ($days^{-1}$); γ: shape factor
R^2_{adj}: adjusted determination coefficient;
RMSE: Root mean square error; χ^2: Reduced Chi-square; **MBE**: Mean bias error

Table 3.- O_2 and CO_2 headspace composition in packages of fresh-cut strawberries stored under different temperatures

Storage time (days)	O_2 kPa[1]				CO_2 kPa[1]			
	5 ºC	10 ºC	15 ºC	20 ºC	5 ºC	10 ºC	15 ºC	20 ºC
0	82.5^{aA}	85.6^{aA}	86.7^{aA}	85.4^{aA}	1.4^{aA}	1.1^{aA}	1.2^{aA}	1.1^{aA}
2	69.2^{bA}	63.0^{bA}	47.4^{bB}	38.9^{bC}	12.3^{bD}	18.0^{bC}	30.5^{bB}	43.0^{bA}
4	60.8^{bA}	37.9^{cB}	27.6^{cC}	4.3^{cD}	25.7^{cD}	47.8^{cC}	63.6^{cB}	87.5^{cA}
7	48.6^{cA}	$35.2^{c\,B}$	15.3^{dC}	2.2^{cD}	31.7^{dD}	53.6^{dC}	78.7^{dB}	88.8^{cA}
9	48.8^{cA}	23.9^{dB}	17.8^{dB}	0.4^{cC}	37.5^{eD}	64.2^{eC}	71.2^{dB}	92.2^{cdA}
11	40.6^{dA}	23.5^{dB}	6.6^{eC}	0.6^{cC}	42.4^{fC}	73.2^{fB}	94.0^{eA}	98.9^{dA}
14	36.2^{eA}	18.4^{eB}	5.6^{eC}	0.4^{cD}	44.6^{fD}	68.7^{efC}	91.8^{eB}	97.8^{dA}
21	41.3^{dA}	12.5^{fB}	4.8^{eC}	0.4^{cD}	46.0^{fC}	75.2^{fB}	90.3^{eA}	96.6^{dA}

[1]Values within a column followed by the same lower case letter indicate that the mean values are not significantly different by LSD multiple-range test ($P < 0.05$).
Values within a row for each gas followed by the same capital letter indicate that the mean values are not significantly different by LSD multiple-range test ($P < 0.05$).

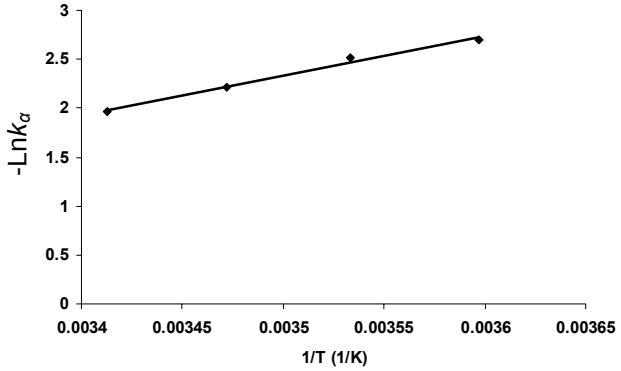

Figure 4.-Effect of temperature on the rate constant (k_α) estimated by fitting Weibull model to vitamin C of fresh-cut strawberries as affected by storage time.

The effect of storage temperature on rate constants obtained by the Weibull model was adequately predicted by the Arrhenius relationship (Figure 4). The activation energy was determined with good accuracy (R^2_{adj} = 9.9×10^{-1}, RMSE = 1.5×10^{-1}, MBE = 2.1×10^{-4}, X^2 = 2.5×10^{-7}) as 33.8 kJ/mol. According to Gainnakourou and Taoukis (2003) the activation energies for vitamin C degradation in frozen vegetables varied from 97.9 to 112 kJ/mol and in orange juice from 13.1 to 18.3 kJ/mol (Polydera et al., 2003). As expected, the high activation energy value reported for frozen vegetables showed that in these products vitamin C losses are more susceptible to temperature rise than in fresh-cut commodities.

Antioxidant Capacity

The DPPH assay is an easy and accurate method to measure the antioxidant capacity of fruits since the radical compound is stable and does not have to be regenerated as in other radical scavenging assays (Sánchez-Moreno, 2002). Figure 5 shows the effect of storage temperature and time on the antioxidant capacity of fresh-cut strawberries. The shorter the time and the lower the storage temperature, the higher the antioxidant capacity of strawberry wedges. Changes in the antioxidant capacity were consistent with the depletion in vitamin C and anthocyanins through the storage time. Fresh-cut strawberries with the highest antioxidant capacity had the greatest anthocyanin and vitamin C contents. In order to adequately relate changes in antioxidant capacity to storage time, zero-order (Equation 2), first-order (Equation 3) and Weibull model (Equation 4) were fitted to the experimental data. Estimated kinetic rates and regression coefficients for the three models obtained for each storage temperature are shown in Table 4. The fitting performance for the zero-order was high for storage temperatures of 10 and 15 ºC ($R^2_{adj} \geq 0.722$) but dramatically decreased when the storage was conducted at 5 ºC and 20 ºC ($R^2_{adj} \leq 0.438$). The first-order regression coefficients were greater than 0.795 for

storage temperatures between 10 ºC and 20 ºC, but significantly decreased when fresh-cut strawberries were stored at 5 ºC (R^2_{adj} = 0.572). In contrast, adequacy of Weibull distribution was consistently good regardless of the storage temperature ($R^2_{adj} \geq 0.912$). In addition, Weibull model exhibited higher R^2_{adj} and lower RMSE, χ^2 and MBE values than the zero and first-order modes (Table 4). The small deviations in the values estimated by the simple Weibull approach in relation to the observed values confirmed the good accuracy of model (Figure 5). The antioxidant capacity degradation rate obtained through Weibull model was directly influenced by storage temperature, taking values between 4.1×10^{-3} and 6.9×10^{-2} $days^{-1}$. Consistently, other authors reported the positive influence of low temperatures in the maintenance of the antioxidant capacity during the storage of fruits and vegetables (Gil et al., 1999; Odriozola-Serrano et al., 2008). High storage temperatures induce a rapid rise in CO_2 production associated to the wounding response which can cause membrane damage and alter the composition and content of antioxidant compounds, thus resulting in changes in the total antioxidant activity of the tissue (Lana and Tijskens 2006). γ-values of the Weibull model ranged from 0.42 to 0.69, suggesting that the highest reduction in antioxidant capacity occurred at the beginning of the storage time. Antioxidant capacity of strawberry wedges underwent a substantial depletion during the first 4-7 days of storage, but was then maintained or slightly decreased reaching values from 34.3 to 68.2% at 21 days of storage.

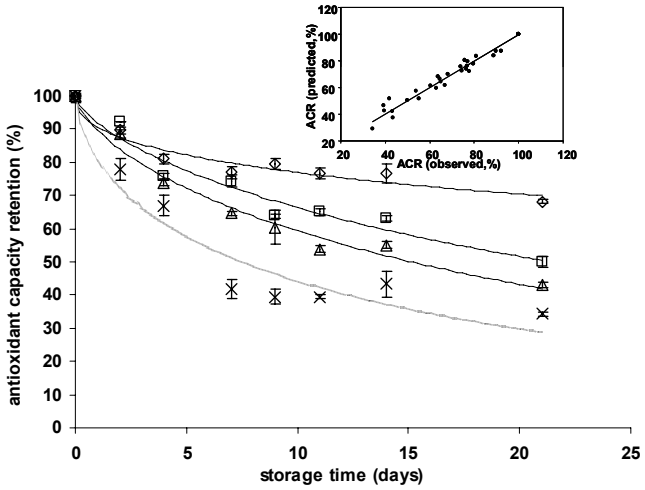

Figure 5.- Effect of storage time and temperature on the antioxidant capacity retention of fresh-cut strawberry (mean ± SD) as modeled by Weibull approach. Temperatures: 5 ºC (◊), 10 ºC (□), 15 ºC (Δ) and 20 ºC (×). Plotted lines correspond to the values estimated from the Weibull model. ACR: antioxidant capacity retention.

Table 4.- Kinetic constants of zero-order, first-order and Weibull model of antioxidant capacity retention in fresh-cut strawberries as a function of the storage temperature

Model	T(ºC)	Constants[1]	Statistical parameters			
			R^2_{adj}	RMSE	χ^2	MBE
Zero-order	5	$k_0 = 1.86 \pm 1.7\times10^{-1}$	0.438	1.671	40.99	1.549
	10	$k_0 = 2.83 \pm 1.8\times10^{-1}$	0.768	1.723	36.41	1.461
	15	$k_0 = 3.37 \pm 2.2\times10^{-1}$	0.722	1.992	64.29	1.941
	20	$k_0 = 4.37 \pm 4.3\times10^{-1}$	0.357	2.749	238.1	3.735
First-order	5	$k_1 = 2.2\times10^{-2} \pm 2.0\times10^{-3}$	0.572	1.534	19.55	1.070
	10	$k_1 = 3.9\times10^{-2} \pm 2.2\times10^{-3}$	0.888	1.362	5.372	0.561
	15	$k_1 = 5.0\times10^{-1} \pm 2.8\times10^{-3}$	0.902	1.503	4.707	0.525
	20	$k_1 = 4.4\times10^{-1} \pm 4.3\times10^{-3}$	0.795	1.984	1.369	0.283
Weibull	5	$k_\alpha = 4.1\times10^{-3} \pm 3.8\times10^{-3}$ $\gamma = 4.2\times10^{-1} \pm 6.5\times10^{-2}$	0.912	0.951	6×10^{-4}	-0.005
	10	$k_\alpha = 2.7\times10^{-2} \pm 5.1\times10^{-3}$ $\gamma = 6.9\times10^{-1} \pm 6.7\times10^{-2}$	0.949	1.128	0.055	0.055
	15	$k_\alpha = 3.8\times10^{-2} \pm 4.7\times10^{-3}$ $\gamma = 6.8\times10^{-1} \pm 5.5\times10^{-2}$	0.966	1.026	0.116	-0.080
	20	$k_\alpha = 6.9\times10^{-2} \pm 1.1\times10^{-2}$ $\gamma = 5.7\times10^{-1} \pm 8.5\times10^{-2}$	0.913	1.612	0.332	-0.135

[1]Mean values ± standard error
k_0: zero-order rate constant ($days^{-1}$); k_1: first-order rate constant ($days^{-1}$)
k_α: 1/scale factor ($days^{-1}$); γ: shape factor
R^2_{adj}: adjusted determination coefficient;
RMSE: Root mean square error; χ^2: Reduced Chi-square; **MBE**: Mean bias error

Dependence of the rate constant of Weibull model obeyed the Arrhenius relationship (Equation 6) with an activation energy value of 119.7 kJ/mol. The good accuracy of the model was confirmed by the high adjusted determination coefficients ($R^2_{adj} = 0.877$) and the statistical parameters (RMSE = 5.6×10^{-1}, $\chi^2 = 7.6\times10^{-6}$, MBE = 3.7×10^{-4}) (Figure 6). As no references have been found reporting the use of Arrhenius equation to describe the kinetic rates dependency in fresh-cut products, the activation energy obtained in the present study was compared with those reported by Suh and others (2004) for the antioxidant capacity of mulberry fruit extracts. These authors obtained activation energies which varied from 41.1 to 82 kJ/mol, indicating that the antioxidant capacity of fresh-cut strawberries is less resistant to an increase in temperature than in mulberry fruit extracts.

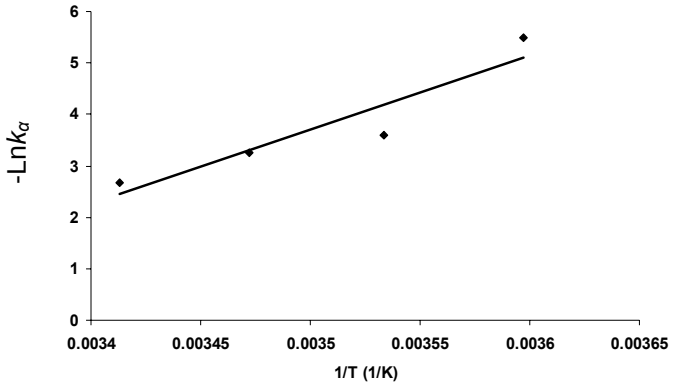

Figure 6.-Effect of temperature on the rate constant (k_a) estimated by fitting Weibull model to antioxidant capacity of fresh-cut strawberries as affected by storage time.

CONCLUSIONS

The evaluation of the effects of storage time and temperature on the antioxidant potential of fresh-cut strawberries contributes to determine the nutritional shelf-life of these products. Temperature directly affects the anthocyanins and vitamin C content as well antioxidant capacity of strawberry wedges during postharvest storage. A storage temperature of 5 ºC is the most appropriate for keeping the antioxidant properties of strawberry wedges for 21 days, reducing wounding stress. The model based on Weibull distribution function is likely to be a useful tool for describing changes in the antioxidant properties of fresh-cut strawberries stored at different temperatures. High activation energies for the degradation of anthocyanins, vitamin C and antioxidant capacity degradation indicated that these antioxidants are highly susceptible to temperature changes. The proposed mathematical models may help to predict the variation of the antioxidant potential of strawberry wedges as affected by storage time at each selected temperature.

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REFERENCES

Adisa. V.A. (1986). The influence of molds and storage factors on the ascorbic content of orange and pineapple fruits. *Food Chemistry*, 22, 139-146.

Aguayo, E., Escalona, V., Artés, F. (2004). Quality of fresh-cut tomato as affected by type of cut, packaging, temperature and storage time. European Food Research and Technology, 219,492-499.

Alzadora, S., Tapia, M.S., Welti, J. (1998). New strategies for minimally processed foods. The role of multitarget preservation. *Food Science and Technology International*, 4,353-361

Ames, B.M. (1983). Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science*, 221, 1256-1263.

Artés-Hernández, F., Rivera-Cabrera, F., Kader, A.A. (2007). Quality retention and potential shelf-life of fresh-cut lemon as affected by cut type and temperature. *Postharvest Biology and Technology*, 43, 245-254.

Ayala-Zavala, J.F., Wang, S.Y., Wang, C.Y., González-Aguilar, G.A. (2004). Effect of storage temperaturas on antioxidant capacity and aroma compounds in strawberry fruit. *LWT-Food Science and Technology*, 37, 687-695.

Brouillard, R. (1982). Chemical structure of anthocyanins. In: Markakis P. Anthocyanins as food colors. Food Science and Technology. Academic Press, London. pp. 1-40.

Cemeroğlu, B., Velioğlu, S., Işık, S. (1994). Degradation kinetics of anthocyanins in sour cherry juice and concentrate. *Journal of Food Science*, 59(6), 1216-1217.

Cordenunsi, B.R., Genovese, M.I., Nascimiento, J.R.O., Hassimotto, N.M.A., Santos, R.J., Lajolo, F.M. (2005). Effects of temperature on the chemical composition and antioxidant capacity of three strawberry cultivars. *Food Chemistry*, 91, 113-121.

Corradini, M.G., Peleg, M. (2004). A model of non-isothermal degradation of nutrients, pigments and enzymes. *Journal of the Science and Food Agriculture*, 84, 217-226.

Cunha, L.M., Oliveira, F.A.R., Oliveira, J.C. (1998). Optimal experimental design for estimating the kinetic parameters of processes described by the Weibull probability distribution function. *Journal of Food Engineering*, 37, 175-191.

Davey, M.W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science and Food Agriculture*, 80, 825-860.

Giannakourou, M.C., Taoukis, P,S. (2003). Kinetic modelling of vitamin C loss in frozen green vegetables under variables storage conditions. *Food Chemistry*, 83, 33-41.

Gil, M.I., Holcroft, D.M., Kader, A.A. (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *Journal of Agricultural and Food Chemistry*, 45, 1662-1667.

Gil, M.I., Ferreres, F., Tomás-Barberán, F.A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry*, 47, 2213-2217.

Hayaloglu, A.A., Karabulut, I., Alpaslan, M., Kelbaliyev, G. (2007). Mathematical modeling of drying characteristics of strained yoghurt in a convective type tray-dryer. *Journal of Food Engineering*, 78, 109-117.

Iversen, C.K. (1999). Black currant nectar: effect of processing and storage on anthocyanin and ascorbic content. *Journal of Food Science*, 64(1):37-41.

Jackman, R.L., Smith, J.L. (1996). Anthocyanins and betalains. Natural Food colorants. In: Hendry GAF, Houghton JD, editors. Natural Food Colorants. Blackie Academic & Professional, Chapman & Hall, London. pp. 244-309.

Johnson, J.R., Braddock, R.J., Chen, C.S. (1995). Kinetics of ascorbic acid loss and nonenzymatic browning in orange juice serum: experimental rate constants. *Journal of Food Science*, 60, 502-505.

Jung, M.Y., Kim, S.K., Kim, S.Y. (1995). Riboflavin-sensitized photodynamic UV spectrophotometry for ascorbic acid assay in beverages. *Journal of Food Science*, 60, 360-363.

Kays, S.J. (1991). Metabolic Processes in Harvested Products. Postharvest Physiology of Perishable Plant Products. Van Nostrand Reinhold, New York. pp. 75–142.

Kırca, A., Cemeroğlu, B. (2003). Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chemistry*, 81, 583-587.

Kırca, A., Özhan, M., Cemeroğlu, B. (2006). Stability of black carrot anthocyanins in various fruit juices and nectars. *Food Chemistry*, 97, 598-605.

Koca, N., Burdurlu, H.S., Karadeniz, F. (2007). Kinetic of colour changes in dehydrated carrots. *Journal of Food Engineering*, 78,449-455.

Lana, M.M., Tijskens, L.M.M. (2006). Effect of cutting and maturity on antioxidant activity of fresh-cut tomatoes. *Food Chemistry*, 97, 203-211.

Lavelli, V., Paglianari, E., Ambrosoli, R., Minati, J.L., Zanoni, B. (2006). Physicochemical, microbial and sensory parameters as indices to evaluate the quality of minimally-processed carrots. *Postharvest Biology and Technology*, 40, 34-40.

Lee, H.S., Chen, C.S. (1998). Rates of vitamin C loss and discoloration in clear orange juice concentrate during storage at temperatures of 4-24 ºC. *Journal of Agricultural and Food Chemistry*, 46, 4723-4727.

Manso, M.C., Oliveira, F.A.R., Oliveira, J.C., Frías, J.M. (2001). Modelling ascorbic acid thermal degradation and browning in orange juice under aerobic conditions. *International Journal of Food Science and Technology*, 36, 303-312.

Markakis, P. (1982). Anthocyanins as food colors. Food Science and Technology. Academic Press, London, UK.

Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry*, 51, 6887-6892.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007). Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits. *Food Chemistry*, 105, 1151-1158.

Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Antioxidant properties and shelf-life extension of fresh-cut tomatoes stored at different temperatures. *Journal of the Science and Food Agriculture*, DOI: 10.1002/jsfa.3368.

Özhan, M., Kırca, A., Cemeroğlu, B. (2004). Effect of hydrogen peroxide on stability of ascorbic acid in various fruits. *Food Chemistry*, 88, 591-597.

Piagentini, A.M., Mendez, J.C., Guemes, D.R., Pirovani, M.E. (2005). Modelling changes of sensory attributes for individual and mixed fresh-cut leafy vegetables. *Postharvest Biology and Technology*, 38, 202-212.

Polydera, A.C., Stoforos, N.G., Taoukis, P.S. (2003). Comparative shelf life and vitamin C loss kinetics and high pressure processed reconstituted orange juice. *Journal of Food Engineering*, 60, 21-29.

Reyes, L.F., Villarreal, J.E., Cisneros-Zavallos, L. (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 101, 1254-1262.

Riva, M., Franzetti, L., Galli, A. (2001). Microbial and quality and shelf-life modelling of ready-to-eat cicorino. *Journal of Food Protection*, 7, 228-234.

Rocculi, P., Del Nobile, M.A., Romani, S., Baiano, A., Dalla, R.M. (2006). Use of simple mathematical model to evaluate dipping and MAP effects on aerobic respiration of minimally processed apples. *Journal of Food Engineering*, 76, 334-340.

Saavedra del Aguila, J., Sasaki, F.F., Heiffig, L.S., Ortega, E.M.M., Jacomino, A.P., Kluge, R.A. (2006). Fresh-cut radish using different cut types and storage temperatures. *Postharvest Biology and Technology*, 40, 149-154.

Sánchez-Moreno C. 2002. Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International*, 8,121-137.

Shin, Y., Ryu, J.A., Liu, R.H., Nock, J.F., Watkins, C.B. (2008). Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. *Postharvest Biology and Technology*, 49, 201-209.

Suh, H.J., Kim, J.M., Lee, H., Lee, S.W., Choi, Y.M. (2004). Thermal kinetics on antiradical capacity of mulberry fruit extract. *European Food Research and Technology*, 219, 80-83.

Tudela, J.A., Espín, J.C., Gil, M.I. (2002). Vitamin C retention in fresh-cut potatoes. *Postharvest Biology and Technology*, 26, 75-84.

Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., Ric de Vos, C.H., Capanoglu, E., Bovy, A., Battino, M. (2008). Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food Chemistry*, 56, 696-704.

Uddin, M.S., Hawlader, M.N.A., Ding, L., Mujumdar, A.S. (2002). Degradation of ascorbic acid in dried guava during storage. *Journal of Food Engineering*, 51, 21-26.

Van der Steen, C., Jacxsens, L., Devlieghere, F., Debevere, J. (2002). Combining high oxygen atmosphere with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberry. *Postharvest Biology and Technology*, 26, 49-58.

Varoquaux, P., Wiley, R.C. (1997). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In: Wiley R.C. Minimally processed refrigerated fruits & vegetables. Chapman and Hall, New York, USA. pp. 226-268.

Viberg, U., Ekström, G., Fredlund, K., Öste, R.E., Sjöholm, I. (1997). A study of some important vitamins and antioxidants in blackcurrant jam with low sugar content and without additives. *International Journal of Food Science and Nutrition*, 4, 57-66.

Wang, W.D., Xu, S.Y. (2007). Degradation kinetics of anthocyanins in blackberry juice and concentrate. *Journal of Food Engineering*, 82, 271-275.

Capítulo XII

Changes in bioactive composition of fresh-cut strawberries stored under superatmospheric oxygen, low-oxygen or passive atmospheres

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ABSTRACT
The effect of different modified atmosphere packaging conditions (2.5 kPa O_2 + 7 kPa CO_2, 10 kPa O_2 + 5 kPa CO_2, 21 kPa O_2, 60 kPa O_2 and 80 kPa O_2) on the antioxidant properties of fresh-cut strawberries was investigated. Changes in phenolic acids, flavonoids (anthocyanins and flavonols), vitamin C and antioxidant capacity were analyzed for 21 days at 4 ºC. O_2 and CO_2 package headspace concentrations were also evaluated. The initial quercetin content was maintained for 11-14 days regardless of the initial in-package atmospheres, but then it was dramatically enhanced in the strawberries stored at ≥ 21 kPa O_2. High O_2 concentrations inside headspace packages promoted greater losses of phenolic acids (p-coumaric, hydroxybenzoic and ellagic acid) and vitamin C during the storage period compared to low O_2 levels. Anthocyanins content increased significantly beyond day 9 in strawberry wedges stored at ≤ 21 kPa O_2, whereas it was almost constant throughout the storage in fresh-cut strawberries at superatmospheric O_2 atmospheres. In general, low-O_2 and passive atmospheres best maintained the initial antioxidant capacity of fresh-cut strawberries through the cold storage. Therefore, 2.5 kPa O_2 + 7 kPa CO_2 atmospheres are proposed to prevent oxidation of the main antioxidant compounds in fresh-cut strawberries.

Key words: fresh-cut strawberries, phenolic acids, flavonols, anthocyanins, vitamin C, antioxidant capacity

INTRODUCTION

Fresh-cut fruits are generally more perishable than whole fruits because they have been subjected to physiological stresses caused by physical damage or wounding (Varoquaux and Willey, 1997). Slicing operations induce a rapid rise in CO_2 and ethylene production associated to the wounding response which can reduce the shelf-life of fresh-cut products (Artés, et al., 2007). Therefore, the combination of different preservation methods such as modified atmosphere packaging (MAP), low-temperature storage or the addition of preservatives may be an excellent way to preserve the original quality attributes of these products (Alzamora, et al., 1998). Carbon dioxide-enriched atmospheres have been used successfully to extend the shelf-life of strawberries by inhibiting fruit softening and decay (Gil, et al., 1997). However, problems associated with the development of off-odours, physiological and microbial decay, browning and softening may appear when O_2 levels are too low and CO_2 is accumulated inside the package (Allende, et al., 2004). In addition, CO_2 enriched atmospheres with low O_2 concentrations can adversely affect ascorbic acid and anthocyanin contents, thus affecting the strawberry colour and its nutritional value (Holcroft and Kader, 1999). Recently, elevated O_2 atmospheres have been used as an alternative to the traditional low O_2 and high CO_2 atmospheres to maintain quality and safety of fresh-cut products (Day, 1996). High O_2 atmospheres have been suggested as an effective method to inhibit the growth of microorganisms and prevent undesired anoxic fermentations (Amanitidou, et al., 1999; Kader and Ben-Yehoshua, 2000). In addition, some authors (Jacxsens, et al., 2001; Van der Steen, et al., 2002) have reported the beneficial effects of high oxygen atmospheres on quality of fresh-cut fruits. However, few studies have been conducted regarding the effect of superatmospheric packaging conditions on nutritional and antioxidant properties of fresh-cut products.

Strawberries are of great interest because they are a good source of vitamin C, anthocyanins and flavonols. Among fruits, they have one of the highest antioxidant activities (Cordenunsi, et al., 2005). Antioxidants can scavenge free radical and nonradical reactive oxygen species, which have been associated with several cellular toxic processes including oxidative damage to proteins and DNA, membrane lipid oxidation, enzyme inactivation, and gene mutation, which may lead to carcinogenesis (Eberhardt, et al., 2000). Therefore, the aim of the present work was to evaluate and compare the effects of different packaging atmospheres on phenolic acids, flavonoids (anthocyanins and flavonols) and vitamin C of strawberry wedges stored for 21 days at 4 ºC. Antioxidant capacity measured through the evaluation of the free radical-scavenging effect on two different radicals (DPPH and ABTS) was correlated to the presence of individual health-related compounds.

MATERIALS AND METHODS

Sample preparation

Strawberry fruits (*Fragaria ananassa* Duch, cultivar Camarosa) were purchased from a local wholesaler (Lleida, Spain) and stored at 4 ºC ± 1 ºC. Strawberries were sanitized in 200 µL L^{-1} NaClO solution for 2 min, rinsed with tap water and dried by hand. Then, the calyx was cut off from the whole strawberries and the fruits were cut vertically with a sharp knife into four wedges. After that, wedges (100 g) were packaged in polypropylene trays. The active modification of package atmosphere was carried out by flushing a gas mixture of 2.5 kPa O_2 +

7 kPa CO_2, 10 kPa O_2+ 5 kPa CO_2, 60 kPa O_2 or 80 kPa O_2 balanced with N_2 before sealing the trays, using a digitally controlled compensated vacuum packaging machine (ILPRA Food Pack Basic V/6, ILPRA Systems. CP., Vigevono, Italia). For passive modification of the package atmospheres, the trays were sealed without flushing gas into package. The relationship between the amount of product and the injected gas mixture was 1:2 (v/v). The O_2 and CO_2 permeance of the 64 µm thick polypropylene sealing film were $5.2419 \cdot 10^{-13}$ mol O_2 m^{-2} s^{-1} Pa^{-1} and $2.3825 \cdot 10^{-12}$ mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23 ºC and 0% RH, respectively (ILPRA Systems España, S.L. Mataró, Spain). The packages were stored at 4 ºC ± 1 ºC in darkness until random withdrawal for analysis. Three trays were taken at each time to perform the analysis, and two readings were carried out for each package.

Headspace gases analysis

The gas composition of the package headspace was determined with a gas analyzer (Micro-GC CP 2002, Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector. An aliquot of 1.7 mL was automatically withdrawn through an adhesive rubber septum with a sampling needle directly connected to the injection system. The determination of O_2 concentrations was carried out by injecting a sample of 0.25 µL to a CP-Molsieve 5 Å packed column (4m x 0.32 mm, d.f. = 10 µm) at 55 ºC and 90 kPa, whereas a sample of 0.33 µL was injected to a Pora-PLOT Q column (10 m x 0.32 mm, d.f. = 10 µm) at 75 ºC and 140 kPa used for carbon dioxide quantification.

Phenolic compounds

Extraction

The extraction of flavonols and phenolic acids was carried out following the method validated by Hertog et al. (1992). A sample of 0.50 g of freeze-dried strawberries was carefully mixed with 62.5% methanol (2g L^{-1} TBHQ) and 10 mL of 6 M HCl. After refluxing at 90 ºC for 2 h with regular swirling, the extract was allowed to cool and subsequently made up to 100 mL with methanol and sonicated for 5 min. prior to injection the extracts were passed through a 0.45-µm filter.

The extraction of anthocyanins was carried following the method proposed by Gómez-Plaza et al. (2008) with some modifications. Approximately 100 mg of freeze-dried strawberry wedges were mixed with 2 mL of methanol. After vigorous mixing for 1 min, the mixture was sonicated for 20 min. Then, the suspension was centrifuged at 3400×g for 10 min. The extracts were passed through a 0.45-µm filter prior to injection.

High-Performance Liquid Chromatography

The HPLC system was equipped with a 600 Controller and a diode array detector (Waters, Milford, MA) which was set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6mm x 250mm) operating at room temperature with a flow rate of 1 mL min^{-1}. A gradient elution was employed with a solvent mixture of 2.5% of formic acid in water (solvent A) and 2.5% of

formic acid in acetonitrile (solvent B) as follows: linear gradient from 5% to 13% B, 0-15 min; linear gradient from 13% to 15% B, 15-20 min; linear gradient from 15% to 30% B, 20-25 min; isocratic elution 30% B, 25-28 min; linear gradient from 30% to 45% B, 28-32 min; isocratic elution 45% B, 32-35 min; linear gradient 45% to 90% B, 35-40 min; isocratic elution 90% B, 40-45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection. Components were identified by comparison of their retention times to those of the standards. *p*-hydroxybenzoic acid, *p*-coumaric acid, ellagic acid, cyanidin-3-glucoside, pelargonidin-3-rutinoside, pelargonidin-3-glucoside, kaempferol, quercetin and myricetin were used to prepare calibration curves. Results were expressed as mg of each individual compound per kg of fresh weight.

Vitamin C

Vitamin C content of fresh-cut strawberries was analysed by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (2007). A sample of 25 g was mixed with 25 mL of a solution containing 45 g L^{-1} metaphosphoric acid and 7.2 g L^{-1} DL-1,4-dithiotreitol. The mixture was centrifuged at 22100 x g for 15 min at 4 ºC. The supernatant was vacuum-filtered through Whatman No. 1 paper. The sample was then passed through a Millipore 0.45-µm membrane. An aliquot of 20 µs was injected into the HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6 mm x 250 cm). The mobile phase was a 0.01 % solution of sulphuric acid adjusted to pH = 2.6. The flow rate was fixed at 1.0 mL/min at room temperature. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Results were expressed as mg of vitamin C per kg of fresh weight.

Antioxidant capacity

The antioxidant capacity was studied through the evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radical, according to the procedure described by Odriozola-Serrano et al. (2008). In addition, the ABTS assay, based on the ability of the antioxidants to scavenge the blue-green radical cation 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonate ($ABTS^{\cdot+}$) was conducted according to the method described by Re et al. (1999). About 5 g of fresh-cut strawberries were mixed with 25 mL of methanol and were then centrifuged at 6000 x g for 15 min at 4 ºC (Centrifuge Medigifer; Select, Barcelona, Spain). Aliquots of 0.01 mL of the supernatant were mixed with 3.9 mL of DPPH or ABTS methanolic solutions and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 60 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm for the DPPH assay or at 734 nm for the ABTS assay. The percentage of inhibition of the radicals ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$) was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH and ABTS values were calculated using a regression equation between the Trolox concentration and the percentage of inhibition. Results were expressed as milligrams of Trolox equivalents per kg of fresh weight.

Statistical analysis

Significance of the results and statistical differences were analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md). Data were analysed by multifactor analysis of variance. The least significant difference test was applied to determine differences among means $p < 0.05$. Correlations between antioxidant capacity and the studied heath-related compounds were evaluated using the Pearson's test.

RESULTS AND DISCUSSION

Headspace gases composition

Table 1 shows the variation of headspace gas partial pressures during the storage of fresh-cut strawberries packaged under different modified atmospheres.

Table 1.- O_2 and CO_2 headspace composition in packages of fresh-cut strawberries stored under different initial atmospheres

Storage time (days)	O_2 kPa[1]					CO_2 kPa[1]				
	1	2	3	4	5	1	2	3	4	5
0	2.4^{a}	9.5^{a}	19.3^{a}	58.9^{a}	80.5^{a}	6.2^{a}	4.3^{a}	1.2^{a}	1.0^{a}	0.9^{a}
2	0.3^{b}	2.6^{b}	11.7^{b}	44.5^{b}	80.3^{a}	13.1^{b}	9.1^{b}	7.2^{b}	9.2^{b}	9.8^{b}
4	0.2^{b}	0.5^{c}	5.5^{c}	36.2^{c}	78.7^{a}	15.3^{c}	16.4^{c}	13.1^{c}	16.6^{c}	16.2^{c}
7	0.2^{b}	0.5^{c}	3.1^{d}	30.1^{d}	60.8^{b}	20.1^{d}	18.7^{c}	22.8^{d}	25.4^{d}	27.0^{d}
9	0.2^{b}	0.5^{c}	2.0^{e}	23.1^{e}	51.6^{c}	24.6^{e}	24.4^{d}	26.3^{e}	30.4^{e}	31.2^{e}
11	0.3^{b}	0.5^{c}	1.4^{f}	18.7^{f}	42.3^{d}	26.1^{f}	25.9^{d}	27.8^{e}	40.7^{f}	37.7^{f}
14	0.3^{b}	0.5^{c}	1.0^{g}	17.2^{f}	38.5^{e}	28.7^{g}	28.0^{e}	31.1^{f}	43.2^{g}	42.4^{g}
21	0.3^{b}	0.5^{c}	0.8^{h}	11.2^{g}	35.7^{f}	28.1^{g}	33.5^{f}	37.3^{g}	48.0^{h}	46.0^{h}

[1]Values within a column followed by the same letter indicate that the mean values are not significantly different by LSD multiple-range test ($P < 0.05$).
1: 2.5 kPa O_2 + 7 kPa CO_2 ; **2**: 10 kPa O_2 + 5 kPa CO_2; **3**: 21 kPa O_2; **4**: 60 kPa CO_2; **5**: 80 kPa O_2

The amount of O_2 inside packages stored under > 60 kPa O_2 decreased continuously during storage, remaining up to 11 kPa at the end of the storage period. In contrast, CO_2 production raised significantly reaching levels between 28 and 46 kPa after 21 days of storage (Table 1). The package headspace of fresh-cut strawberries at 2.5 kPa O_2 + 7 kPa CO_2 exhibited the lowest CO_2 accumulation compared with the other packaging conditions. Consistently, other authors reported significantly lower CO_2 production in other fresh-cut products such as melon stored under low oxygen atmospheres than under air (Oms-Oliu et al. 2008a). Gorny et al. (2002) found that low O_2 (0.25kPa) and elevated CO_2 (20 kPa) acted synergistically to decrease the respiration of fresh-cut peach slices.

Phenolic compounds

Total phenolics were calculated as the sum of each individual phenolic compound quantified by HPLC. Fresh-cut strawberries exhibited high amounts of phenolic compounds, ranging from 639 to 660 mg kg^{-1}. In this way, Da Silva-Pinto et al. (2008) and Tulipani et al. (2008) reported a concentration of total phenolics between 1730 to 3180 mg kg^{-1} in different strawberry cultivars determined by the Folin-Ciocalteu assay. The higher phenolic concentrations found in the above-mentioned studies compared to those obtained in the present work, could be attributed to the analytical method used to determine these compounds. The Folin-Ciocalteu reagent usually overestimates the content of phenolic compounds compared with the sum of the individual phenolics, since other reducing agents present in food, such as ascorbic acid can interfere (Odriozola-Serrano, et al. 2009).

Phenolic acids

Ellagic acid is a naturally occurring polyphenolic secondary metabolite that accumulates in strawberry fruits (Vattem and Shetty, 2005). Ellagic acid was found to be the main phenolic acid (43.3-49.4 mg kg^{-1}) in strawberry wedges, followed by *p*-coumaric acid (12.9-13.2 mg kg^{-1}) and *p*-hydroxybenzoic (4.1-7.9 mg kg^{-1}). Table 2 shows changes in the phenolic acids content of fresh-cut strawberries through storage. Regarding ellagic acid, the initial content in fresh-cut strawberries was maintained for 4 days, but then values rose, reaching maximum accumulation (69.1-78.3 mg kg^{-1}) at 9-11 days of storage. Beyond this day, the amount of ellagic acid was substantially depleted in samples stored at superatmospheric O_2 or passive atmospheres, attaining values about 42.3-52.7 mg kg^{-1} at the end of the storage. A similar trend was observed in ellagic acid content of strawberry fruits stored at air, 60 kPa O_2 or 100 kPa O_2 for 14 days (Zheng et al., 2007). Non significant differences in *p*-hydroxybenzoic acid content were observed among samples during the first 7 days of storage. Afterwards, strawberry wedges stored at 80 kPa O_2 exhibited the lowest amount of this phenolic acid (6.2-10.5 mg kg^{-1}) (Table 2). On the other hand, fresh-cut strawberries underwent a marked depletion in the amount of *p*-coumaric throughout storage, regardless of the initial in-package atmosphere. Significantly higher levels of *p*-coumaric acid were obtained in fresh-cut fruits stored at 2.5 kPa O_2 + 7 kPa CO_2 compared to fruits held in $\geq$ 10 kPa O_2 for 21 days. High O_2 concentrations may induce the accumulation of phenolic compounds in strawberry fruits during the initial storage period, but it may also promote the oxidation of phenolic acids after prolonged time (Zheng et al., 2007). Exposure of fresh-cut apples to air induced the loss of phenolic compounds in comparison to low-O_2 atmospheres (5 kPa O_2 + 5 kPa CO_2) (Cocci et al., 2006). Oms-Oliu et al. (2008b) also reported that high O_2 availability in the packages headspace could have led to stronger degradation of the main phenolic acids of fresh-cut pears than under oxygen-restrictive conditions. However, the reduction of phenolic acids in fresh-cut strawberries over storage time may be due not only to the O_2 levels inside packages, but also to their conversion to flavonoids. Phenolic acids are formed in plant products via the action of phenylalanine ammonio lyase activity (PAL) through the phenylpropanoid metabolism. Biosynthesis of flavonols starts with the sequential addition of three molecules of malonyl-CoA to suitable hydroxycinnamic acid CoA esters, to form chalcones. The chalcones are then isomerised into (2S)-flavanones which are converted into flavonols by hydroxylations and desaturations (Heller, 1986).

Table 2.- Effect of different initial storage atmospheres on phenolic acids content of fresh-cut strawberries stored for 21 days at 4 ºC

storage time (days)	Initial in-package atmospheres				
	1	**2**	**3**	**4**	**5**
	Hydroxybenzoic acid (mg kg^{-1})				
0	7.6^{cA}	7.5^{eA}	7.5^{eA}	7.6^{eA}	7.7^{dA}
2	7.9^{cA}	7.9^{deA}	7.7^{eA}	8.0^{eA}	7.6^{dA}
4	8.0^{cA}	7.5^{eA}	7.5^{eA}	7.9^{eA}	8.0^{dA}
7	12.4^{aA}	12.8^{abA}	12.4^{bA}	12.1^{bA}	12.1^{aA}
9	12.5^{aA}	13.4^{aA}	13.0^{aA}	12.7^{aA}	12.1^{aB}
11	11.1^{abA}	11.7^{bA}	11.3^{cA}	11.3^{cA}	10.5^{bB}
14	10.8^{bA}	10.7^{cA}	10.8^{dA}	10.5^{dA}	9.6^{cB}
21	10.1^{bA}	8.2^{dB}	7.8^{eC}	8.4^{eB}	6.2^{eD}
	***p*-coumaric acid (mg kg^{-1})**				
0	13.2^{aA}	12.7^{aA}	13.2^{aA}	12.4^{aA}	12.4^{aA}
2	11.4^{bA}	5.0^{cB}	5.2^{cB}	5.8^{bB}	4.7^{deB}
4	9.3^{cA}	5.1^{cC}	6.6^{bB}	5.8^{bBC}	5.1^{dC}
7	8.0^{dA}	5.2^{cB}	5.6^{cB}	4.8^{cB}	4.5^{eC}
9	8.6^{dA}	6.0^{bB}	5.7^{cB}	5.8^{bB}	6.2^{bB}
11	7.9^{dA}	5.7^{bcC}	6.8^{bB}	5.6^{bC}	5.5^{cC}
14	8.2^{dA}	4.3^{dC}	6.7^{bB}	4.7^{eC}	4.9^{dC}
21	7.8^{dA}	6.3^{bB}	4.6^{dD}	5.4^{dC}	4.8^{dD}
	Ellagic acid (mg kg^{-1})				
0	46.0^{cA}	44.0^{cA}	46.4^{dA}	45.5^{cA}	46.8^{cA}
2	45.7^{cA}	46.3^{cA}	47.4^{dA}	47.3^{cA}	46.7^{cA}
4	46.4^{cA}	45.5^{cA}	48.8^{dA}	45.7^{cA}	47.7^{cA}
7	49.0^{bC}	55.3^{bB}	51.8^{cC}	59.6^{bA}	58.3^{bA}
9	75.4^{aA}	74.0^{bAB}	77.6^{aA}	73.4^{aB}	69.1^{aB}
11	76.3^{aA}	78.3^{aA}	72.7^{bB}	72.0^{aB}	57.3^{bC}
14	75.4^{aA}	72.6^{bA}	48.1^{dC}	56.4^{bB}	57.3^{bB}
21	73.8^{aA}	70.0^{bA}	52.7^{cB}	42.3^{dC}	43.2^{dC}

[1]Different lower letter within a column for each phenolic acid indicate that the mean values are significantly different by LSD multiple-range test ($P < 0.05$).
Different capital letters in the same row for each day indicate that the mean values are significantly different by LSD multiple-range test ($P < 0.05$).
1: 2.5 kPa O_2 + 7 kPa CO_2 ; **2**: 10 kPa O_2 + 5 kPa CO_2; **3**: 21 kPa O_2; **4**: 60 kPa CO_2; **5**: 80 kPa O_2

Flavonoids

Flavonoids include different groups of flavones, isoflavones, flavonols, flavononones, catechins and anthocyanins (Macheix, et al., 2000). Anthocyanins are responsible for the attractive red colours of strawberries and have been regarded as a natural alternative to replace synthetic food colorants. The effects of different initial in-package atmospheres on the concentration of anthocyanins in strawberry wedges stored at 4 ºC are shown in Figure 1. The initial concentrations of total anthocyanins in the strawberry fruits ranged from 528 to 553 mg kg^{-1}, which agrees well with the reports of other authors for strawberries (Hannun, 2004). Regarding individual anthocyanins, pelargonidin-3-glucoside (plg-3-glu), was the main anthocyanin found in strawberry wedges, accounting for 85-86% of the total amount of anthocyanins. Cyanidin-3-glucoside (cy-3-glu) and pelargonidin-3-rutinoside (plg-3-rut) were also found in fresh-cut strawberries in contents varying from 17.7 to 18.7 mg kg^{-1} and from 56.5 to 61.1 mg kg^{-1}, respectively. Cy-3-glu increased substantially after 2 days of storage in samples stored at 2.5 kPa O_2 + 7 kPa CO_2 and then decreased, but contents remained above the initial values. Fresh-cut strawberries stored at 10 kPa O_2 + 5 kPa CO_2, 21 kPa O_2 or 60 kPa O_2 maintained or slightly reduced the initial content of cy-3-glu for 9 days, after this day, the contents increased up to values of 21.5-26.5 mg kg^{-1}. Cy-3-glu content fluctuated during storage in samples at 80 kPa O_2, keeping values between 15.5 and 20.1 mg kg^{-1} during this period (Figure 1B). Initial plg-3-glu and plg-3-rut contents increased significantly beyond day 9 in strawberry wedges stored at low-O_2 and passive atmospheres, whereas the contents of both anthocyanins were kept almost constant in fresh-cut strawberries stored under superatmospheric O_2 (Figure 1A and 1C). Based in the present results, it seems that anthocyanin contents in fresh-cut strawberries stored at $\leq$ 21 kPa O_2 increased presumably by promotion of their synthesis in tissues. Consistently, Zheng et al. (2007) found that anthocyanins were further increased in strawberries held in air in comparison with those stored under high O_2 environments ($\geq$ 40 kPa O_2) beyond 10 days of storage. It has been reported that preserving strawberry wedges under atmospheres rich in CO_2 induces a reduction in the anthocyanins synthesis compared to air storage where the fruit metabolism is not as much as affected (Gil et al., 1997). Further investigation of this phenomenon indicated that two important enzymes in the biosynthesis pathway of anthocyanins were adversely affected by elevated CO_2 atmospheres (Holcroft and Kader, 1999). The stability of anthocyanins has been reported to be also influenced by pH. In strawberries, equilibrium exists between four anthocyanin species, including quinonoidal base, the flavylium cation (red colour), the pseudobase or carbitol, and the chalcones. High CO_2 atmospheres may affect, by dissolution of CO_2 gas, on the metabolism of organic acid, increasing the pH of the tissues and thus, reversing equilibrium towards the formation of the pseudobase or carbitol (colorless) (Brouillard, 1982).

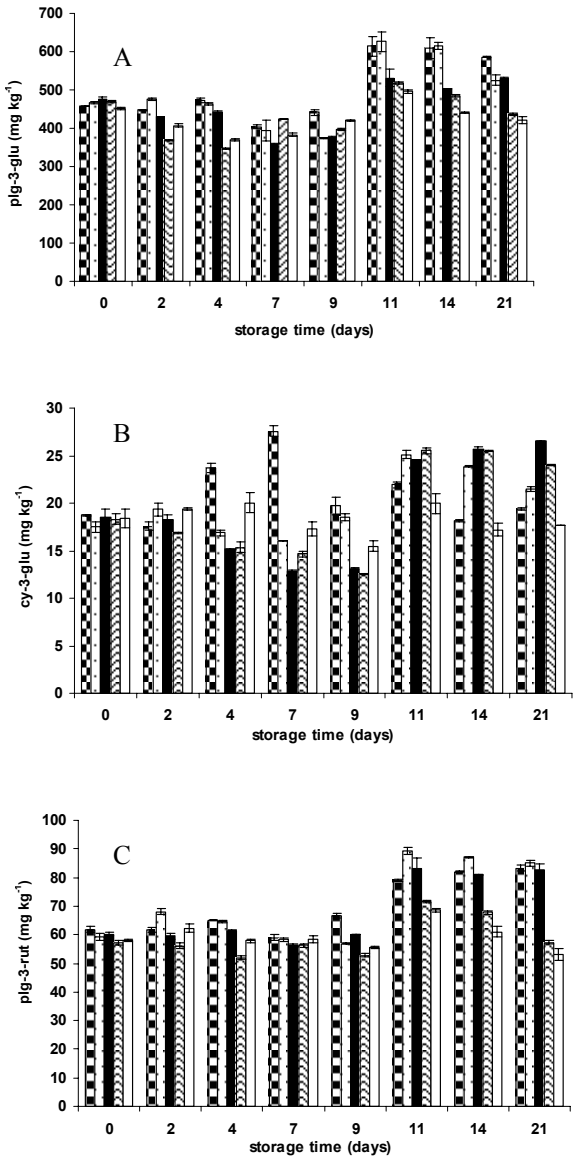

Figure 1.- Effect of different initial storage atmospheres on (A) pelargonidin-3-glucoside (plg-3-glu), (B) cyanidin-3-glucoside (cy-3-glu) and (C) pelargonidin-3-rutinoside (plg-3-rut) in fresh-cut strawberries stored at 4 ºC for 21 days. Initial storage atmospheres: ▩ 2.5 kPa O_2 + 7 kPa CO_2, ▨ 10 kPa O_2 + 5 kPa CO_2, ■ 21 kPa O_2, ▧ 60 kPa O_2, □ 80 kPa O_2. Data shown are mean ± standard deviation.

Among flavonoids, flavonols are found in lower concentration than anthocyanins in strawberry fruits (Macheix et al., 2000). Quercetin was the main flavonol in strawberry wedges with values varying from 34.1 to 38.5 mg kg^{-1}. The initial concentrations of kaempferol in fresh-cut strawberries were 6.5-6.8 mg kg^{-1}, whereas myricetin was found at concentrations of 3.0-3.2 mg kg^{-1} just after processing (Table 3). According to Kosar et al. (2004), the amounts of flavonols in strawberry may vary according to cultivar, geographical origin, harvesting seasons, growing condition as well as to different extraction/hydrolysis and analytical methods used. Flavonol contents were shown to be significantly affected by packaging atmosphere during storage (Table 3). Superatmospheric O_2 concentrations induced a dramatic enhancement of the initial quercetin content in fresh-cut strawberries after 11-14 days of storage, reaching values of 57.7-76.6 mg kg^{-1} at 21 days. Exposure to air and low-oxygen atmospheres during refrigerated storage maintained the initial quercetin content of fresh-cut strawberries during 14 days. Afterwards, quercetin content substantially increased in strawberry wedges stored under 21 kPa O_2 atmospheres. Consistently, Zheng et al. (2007) reported significantly higher quercetin-based flavonol content in strawberries stored at 60 kPa and 100 kPa than in fruits held in air after 14 days of storage. In this way, a substantial rise in quercetin content was observed in fresh-cut pears stored at high-O_2 and passive atmospheres through the storage period (Oms-Oliu et al., 2008b). The increase in quercetin during the storage period could be directly associated with a physiological response to stress conditions. Some authors have demonstrated that wounds stimulate PAL activity with further production of the main phenolic compounds and synthesis of new polyphenolic substances (Tomás-Barberan et al., 1997). Activation of PAL activity has been observed in response to several causes of physiological stress, including CO_2 high levels (Assis et al., 2001). The initial content of myricetin was maintained or increased for 9 days of storage, but then the concentration considerably decreased irrespective of the initial atmosphere packaging, leading to non-detectable levels beyond 11 days. Fresh-cut strawberries initially packaged under superatmospheric O_2 atmospheres exhibited higher amounts of myricetin during the first 7 days of storage compared to those stored under low-O_2 and passive atmospheres (Table 3). Significant differences in kaempferol content were observed among strawberry wedges stored under different initial atmospheres; thus, these phenolic compounds were affected by O_2 and CO_2 availability in trays headspace. Table 3 shows that initial values of kaempferol in fresh-cut strawberries stored under $\geq$ 21 kPa O_2 were substantially higher than the amounts observed at oxygen-restrictive packaging conditions. Our results agree with those reported by Zheng et al. (2003), who found higher levels of kaempferol in blueberries under high oxygen atmospheres than in those stored in air.

Table 3.- Effect of different initial storage atmospheres on flavonols content of fresh-cut strawberries stored for 21 days at 4 ºC

storage time (days)	Initial in-package atmospheres				
	1	2	3	4	5
			Myricetin (mg kg^{-1})		
0	3.2^{bA}	3.2^{cA}	3.2^{cA}	3.1^{cA}	3.0^{cA}
2	3.3^{bB}	2.7^{dC}	2.5^{dC}	3.8^{bA}	3.5^{bA}
4	3.0^{bD}	2.9^{dD}	3.5^{cC}	3.8^{bB}	4.3^{aA}
7	3.3^{bB}	3.5^{bB}	4.1^{bA}	3.9^{bAB}	4.3^{aA}
9	5.2^{aA}	5.0^{aA}	4.7^{aB}	4.4^{aC}	4.3^{aC}
11	nd	nd	nd	nd	nd
14	nd	nd	nd	nd	nd
21	nd	nd	nd	nd	nd
			Quercetin (mg kg^{-1})		
0	36.6^{aA}	34.1^{aA}	38.5^{bA}	35.0^{cA}	35.6^{cA}
2	35.6^{aA}	29.6^{bB}	36.4^{bA}	37.3^{cA}	33.7^{cAB}
4	36.4^{aAB}	30.5^{bB}	40.1^{bA}	45.3^{bA}	42.1^{bA}
7	33.8^{aC}	34.5^{aC}	37.0^{bB}	44.3^{bA}	42.0^{bA}
9	33.4^{aAB}	37.4^{aA}	30.7^{cB}	40.4^{bA}	30.7^{cB}
11	28.2^{bD}	35.0^{aC}	38.0^{bB}	41.4^{bB}	73.5^{aA}
14	30.0^{bC}	30.8^{bC}	38.7^{bB}	60.8^{aA}	68.1^{aA}
21	33.5^{aC}	29.7^{bC}	53.2^{aB}	57.7^{aB}	76.6^{aA}
			Kaempferol (mg kg^{-1})		
0	6.8^{aA}	6.7^{aA}	6.6^{abA}	6.5^{bA}	6.6^{bA}
2	5.6^{bB}	6.7^{aA}	6.9^{aA}	6.6^{bA}	7.0^{aA}
4	4.7^{cC}	6.8^{aA}	6.8^{aA}	7.1^{aA}	6.0^{cB}
7	4.7^{cD}	5.9^{cA}	5.9^{cA}	5.1^{cC}	5.5^{dB}
9	4.4^{dC}	6.3^{bA}	6.2^{bA}	6.2^{bA}	5.7^{dB}
11	4.2^{eD}	5.8^{cC}	7.0^{aA}	6.3^{bB}	6.3^{cB}
14	4.3^{eC}	5.1^{dB}	5.9^{cA}	5.4^{cB}	6.0^{cA}
21	4.0^{eC}	4.7^{eB}	6.5^{abA}	6.5^{bA}	6.8^{aA}

nd: non detected

[1]Different lower letter within a column for each phenolic acid indicate that the mean values are significantly different by LSD multiple-range test ($P < 0.05$).

Different capital letters in the same row for each day indicate that the mean values are significantly different by LSD multiple-range test ($P < 0.05$).

1: 2.5 kPa O_2 + 7 kPa CO_2 ; **2**: 10 kPa O_2 + 5 kPa CO_2; **3**: 21 kPa O_2; **4**: 60 kPa CO_2; **5**: 80 kPa O_2

Vitamin C

The concentration of vitamin C in fresh-cut strawberries varied from 501 to 546 mg kg^{-1} (Figure 2). These values are within the range observed by Davey et al. (2000) and Cordenunsi et al. (2005) who reported vitamin C contents between 400 to 900 mg kg^{-1} in different strawberry cultivars. Vitamin C concentrations in fresh-cut strawberries underwent a substantial depletion through the storage time irrespective of the initial in-package atmosphere

(Figure 2). Oms-Oliu et al, (2008a) also observed significant losses of vitamin C in fresh-cut melon stored at similar conditions to those reported in the present work over 14 days at 5 ºC. The magnitude of vitamin C degradation can be related to the O_2 concentration inside the packages (Soliva-Fortuny, et al., 2002). The strongest depletion in vitamin C was detected in samples packaged at 80 kPa O_2, reaching the lowest content after 21 days of storage (Figure 2). Vitamin C was rapidly reduced when fresh-cut spinach was stored in air compared to modify atmospheres (Gil et al., 1999). In this way, Oms-Oliu et al. (2008b) reported that 80 kPa O_2 atmospheres induced higher losses of vitamin C in fresh-cut pears in comparison to air and 2.5 kPa O_2 + 7 kPa CO_2 atmospheres. However, the marked depletion of vitamin C concentration in strawberry wedges may be explained not only through the low presence of O_2 inside the trays, but also to the high levels of CO_2 (Table 1). High CO_2 concentrations could contribute to increase oxidative stress and, in turn, peroxidase activity and vitamin C oxidation (Oms-Oliu, et al., 2008b). High CO_2 levels could also provoke a cytoplasm acidification with the consequent impairment of mitochondrial function that could result in oxidative damage, which could eventually be overcome by ascorbate peroxidase (Pinto, et al., 2001).

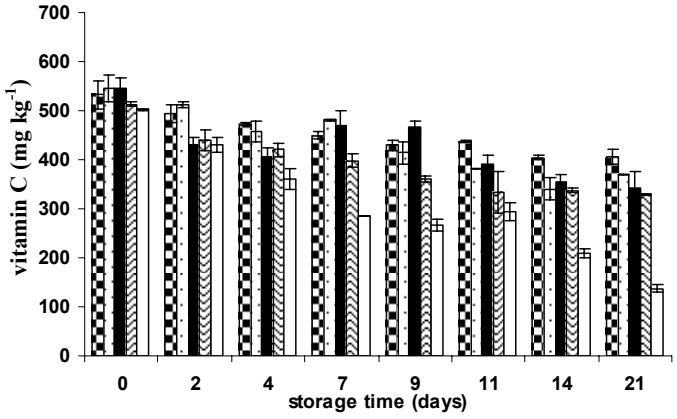

Figure 2.- Effect of different initial storage atmospheres on vitamin C in fresh-cut strawberries stored at 4 ºC for 21 days. Initial storage atmospheres: 2.5 kPa O_2 + 7 kPa CO_2, 10 kPa O_2 + 5 kPa CO_2, 21 kPa O_2, 60 kPa O_2, 80 kPa O_2. Data shown are mean ± standard deviation. Data shown are mean ± standard deviation.

Antioxidant Capacity

Figure 3 shows changes in antioxidant capacity of fresh-cut strawberries measured through the DPPH and ABTS assays. According to both methods, the antioxidant capacity of strawberry wedges stored under low-O_2 or passive atmospheres was significantly higher than those obtained in samples packaged under high-O_2 atmospheres. Other authors have shown a substantial depletion in the antioxidant capacity of fresh-cut melon stored under

superatmospheric O_2 atmospheres compared to other conditions (Oms-Oliu et al., 2008a). The antioxidant capacity through the DPPH and ABTS assays was found to be significantly higher in fresh-cut pears stored at 2.5 kPa O_2 + 7 kPa CO_2 than those stored at 21 kPa O_2 or 70 kPa O_2 (Oms-Oliu et al., 2008b). Regarding DPPH method, the antioxidant capacity of strawberry wedges depleted slightly but continuously through the storage time irrespective of the initial in-package atmospheres.

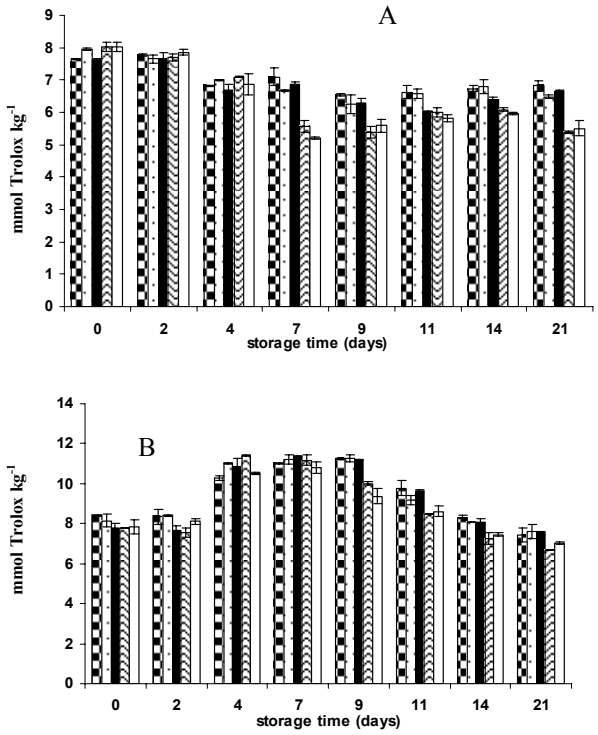

Figure 3.- Effect of different initial storage atmospheres on antioxidant capacity measured through (a) DPPH and (b) ABTS assay in fresh-cut strawberries stored at 4 ºC for 21 days. Initial storage atmospheres: 2.5 kPa O_2 + 7 kPa CO_2, 10 kPa O_2 + 5 kPa CO_2, 21 kPa O_2, 60 kPa O_2, 80 kPa O_2. Data shown are mean ± standard deviation.

The antioxidant capacity of fresh-cut strawberries through the ABTS method was maintained during the first 2 days of storage, then underwent a marked increase at day 4 and finally decreased beyond day 9 under any of the studied packaging conditions. A similar pattern has been reported for the evolution of antioxidant capacity of strawberry fruits stored under air, 60 kPa O_2 or 100 kPa O_2 for 14 days of storage (Zheng et al. 2007). The changes in the antioxidant capacity over time might be associated to the variations of the individual health-related compounds. Antioxidant capacity of fruits and vegetables is known to depend on a wide number of compounds. In this regard, Chu, et al. (2000) have indicated that several phytochemicals, such as flavonoids, phenolic acids, aminoacids, ascorbic acid, tocopherols and pigments, might contribute to the total antioxidant capacity of fruits. The changes of DPPH radical inhibition over time were highly related to vitamin C levels (R^2 = 0.7299), which demonstrates that vitamin C is one of the main antioxidant compounds in fresh-cut strawberry. Del Caro et al. (2004) also reported a good correlation between antioxidant capacity and ascorbic acid content of citrus segments. Although, it has been reported that vitamin C and anthocyanins are the major antioxidant components in strawberries (Hannun, 2004), the magnitude of the changes in values of DPPH and ABTS inhibition was not associated with changes in the anthocyanins during storage. However, results show that antioxidant capacity through the ABTS assay was significantly but weakly correlated with myricetin (R^2 = 0.6275) and hydroxybenzoic acid contents (R^2 = 0.5214).

CONCLUSIONS

Packaging atmospheres affect the content of phenolic acids, anthocyanins, flavonols, vitamin C and antioxidant capacity of fresh-cut strawberries. Vitamin C, phenolic acids and antioxidant capacity of strawberry wedges were better kept through the storage period in oxygen restrictive environments. Fresh-cut strawberries stored under ≤ 21 kPa O_2 underwent a substantial increase in anthocyanins after 9 days of storage. This enhancement was not observed in strawberry wedges preserved under superatmospheric O_2 atmospheres as a consequence of the high CO_2 concentrations accumulated inside packages. The greatest amounts of flavonols were found in fresh-cut strawberries stored under 80 kPa O_2, but this high content may be directly associated with a physiological response to stress. Therefore, low-O_2 atmospheres are more effective to maintain for 21 days the main health-related compounds of fresh-cut strawberries than superatmospheric O_2 and passive atmospheres.

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REFERENCES

Allende, A., Luo, Y., McEvoy, J.L, Artés, F., Wang, CY. (2004). Microbial and quality changes in minimally processed baby spinach leaves stored under superatmospheric oxygen and modified atmosphere conditions. *Postharvest Biology and Technology*, 33, 51-59.

Alzamora, S.M., Tapia, M.S., Welti, J. (1998). New strategies for minimally processed foods. The role of multitarget preservation. *Food Science and Technology Interational*, 4, 353-361.

Amanitidou, A., Smid, E.J., Gorris, L.G.M. (1999). Effect of elevated oxygen and carbon dioxide on the surface growth of associated microorganisms. *Journal of Applied Microbiology*, 86, 429-438.

Artés, F., Gómez, P.A., Artés-Hernández, F. (2007). Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. *Food Science and Technology International*, 13, 177-188.

Assis, J.S., Maldonado, R., Muñoz, T., Escribano, M.I., Merodio, C. (2001). Effect of high carbon dioxide concentration on PAL activity and phenolic contents in ripening cherimoya fruit. *Postharvest Biology and Technology*, 23, 33-39.

Brouillard, R. (1982). Chemical structure of anthocyanins. In: Markakis P. Anthocyanins as food colors. Food Science and Technology. Academic Press, London. pp. 1-40.

Cocci, E., Rocculi, P., Roman, S., Dalla Rosa, M. (2006). Changes in nutritional properties of minimally processed apples during storage. *Postharvest Biology and Technology*, 39, 265-271.

Cordenunsi, B.R., Genovese, M.I., Nascimiento, J.R.O., Hassimotto, N.M.A., Santos, R.J., Lajolo, F.M. (2005). Effects of temperature on the chemical composition and antioxidant capacity of three strawberry cultivars. *Food Chemistry*, 91, 113-121.

Chu, Y.H., Chang, Ch.L., Hsu, H.F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science and Food Agriculture*, 80, 561-566.

Da Silva-Pinto, M., Lajola, F.M., Genovese, M.I. (2008). Bioactive compounds and quantification of total ellagic acid in strawberries (Fragaria x ananassa Duch.). *Food Chemistry*, 107, 1629-1635.

Davey, M.W., Van Montagu, M., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science of Food and Agriculture,* 80, 825-860.

Day, B.P.F. (1996). High oxygen modified atmosphere packaging for fresh prepared produce. *Postharvest News and Information*, 7(3), 1N-34N.

Del Caro, A., Piga, A., Vacca, V., Agabbio, M. (2004). Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. *Food Chemistry*, 99-105.

Eberhardt, M.V., Lee, C.Y. Liu, R.H. (2000). Antioxidant activity of fresh apples. *Nature*, 405(6789), 903-904.

Gil, M.I., Holcroft, D.M., Kader, A.A. (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *Journal of Agricultural and Food Chemistry*, 45, 1662-1667.

Gil, M.I., Ferreres, F. Tomás-Barberán F.A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry*, 47, 2213-2217.

Gómez-Plaza, E., Gil-Muñoz, R., Hernández-Jiménez, A., López-Roca, J.M., Ortega-Regules, A., Martínez-Cutillas, A. (2008). Studies on anthocyanins profile of Vitis Vinifera intraspecific hybrids (Monastrell × Cabernet Sauvignon). *European Food Research and Technology*, 227, 479-484.

Gorny, J.R., Hess-Pierce, B., Cifuentes, R.A., Kader, A.A. (2002). Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24, 271-278.

Hannum, S.M. (2004). Potential impact of strawberries on human health: A review of the science. *Critical Review in Food Science and Nutrition*, 44, 1-17.

Heller, W. (1986). Flavonoid biosynthesis: an overview. In: Cody, V., Middleton, E., Harbone, J.B. Plant flavonoids in biology and medicine: biochemical, pharmacological, and structure-activity relationships. Alan R. Liss, New York, USA. pp. 25-42.

Hertog, M., Hollman, P., Venema, D. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, 40, 1591-1598.

Holcroft, D.M., Kader, A.A. (1999). Controlled atmospheres-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit. *Postharvest Biology and Technology*, 17, 19-32.

Jacxsens, L., Devlieghere, F., Van der Steen, C., Debevere, J. (2001). Effect oh high oxygen modified atmosphere on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology*, 71, 197-210.

Kader, A.A., Ben-Yehoshua, S. (2000). Effect of superatmospheric oxygen levels on postharvest physiology and quality on postharvest physiology and quality of fresh-cut fruits and vegetables. *Postharvest Biology and Technology*, 20, 1-13.

Kosar, M., Kafkas, E., Paydas, S., Baser, K.H. (2004). Phenolic composition of strawberry genotypes at different maturation stages. *Journal of Agricultural and Food Chemistry*, 52, 1586–1589.

Macheix J.J., Fleuriet, A., Billot, J. (2000). Fruit Phenolics. CRC Press, Boca Raton, Florida, USA.

Odriozola-Serrano, I., Hernández-Jover, T., Martín-Belloso, O. (2007). Comparative study of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, 105, 1151-1158.

Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Effect on minimally processing on bioactive compounds and color attributes of fresh-cut tomatoes. *LWT-Food Science and Technology*, 41, 217-226.

Odriozola-Serrano, I., Soliva-Fortuny, R., Hernandez-Jover, T., Martín-Belloso, O. (2009). Carotenoid and phenolic profile of tomato juices processed by high intensity pulsed electric fields compared with conventional thermal treatments. *Food Chemistry*, 112, 258-266.

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008a). The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres. *Food Chemistry*, 106, 1085-1092.

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008b). Antioxidant content of fresh-cut pears stored in high-O_2 active packages compared with conventional low-O_2 atmospheres and passive modified atmosphere packaging. *Journal of Agricultural and Food Chemistry*, 56, 932-940.

Pinto, E., Lentheric, I., Vendrell, M., Larrigaudier, C. (2001). Role of fermentative and antioxidant metabolism in the induction of core browning in controlled-atmosphere stored pears. *Journal of the Science and Food Agricultural*, 81, 364-370.

Re, R., Pellegrin, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free Radical Biology and Medicine*, 26, 1231-1237.

Tomás-Barberán, F.A., Loaiza-Velarde, J., Bonfanti, A., Salveit, M.E. (1997). Early wound- and ethyl-induced changes in phenylpropanoid metabolism in harvested lettuce. *Journal of American Society for Horticultural Science*, 122, 399-404.

Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., Ric de Vos, C.H., Capanoglu, E., Bovy, A., Battino, M. (2008). Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food Chemistry*, 56, 696-704.

Soliva-Fortuny, R.C., Oms-Oliu, G., Martín-Belloso, O. (2002). Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67, 312-317.

Van der Steen, C., Jacxsens, L., Devlieghere, F., Debevere, J. (2002). Combining high oxygen atmosphere with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberry. *Postharvest Biology and Technology*, 26, 49-58.

Varoquaux, P., Wiley, R.C. (1997). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In: Wiley, R.C. Minimally processed refrigerated fruits & vegetables. Chapman and Hall, New York, USA. pp. 226-268.

Vattem, D.A., Shetty, K. (2005). Biological functionality of ellagic acid: a review. *Journal of Food Biochemistry*, 29, 234-266.

Zheng, Y., Wang, C.Y., Wang, S.Y., Zheng, W. (2003). Effect of high-oxygen atmospheres on blueberry phenolics anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 51, 7162-7169.

Zheng, Y., Wang, S.Y., Wang, C.Y., Zheng, W. (2007). Changes in strawberry phenolics, anthocyanins, an antioxidant capacity in response to high oxygen treatments. *LWT- Food Science and Technology*, 40, 49-57.

Discusión General

ZUMOS DE TOMATE Y FRESA TRATADOS POR PULSOS ELECTRICOS DE ALTA INTENSIDAD DE CAMPO

Los pulsos eléctricos de alta intensidad de campo (PEAIC) son una nueva tecnología no térmica de procesado de alimentos, económica y respetuosa con el medio ambiente. Su implantación puede resultar una buena alternativa para conservar los zumos de frutas, ya que además de reducir la carga microbiana y las enzimas responsables del deterioro, mantiene la calidad nutricional del alimento. Hasta ahora, la mayoría de estudios se centran en determinar el efecto que producen los PEAIC sobre microorganismos y enzimas. Sin embargo, existen pocos estudios que evalúen el efecto de los PEAIC sobre el contenido en compuestos bioactivos. Por tanto, en primer lugar, se determinaron las condiciones óptimas de tratamiento por PEAIC en función de la frecuencia, anchura de pulso y polaridad para obtener zumos con alto contenido en compuestos bioactivos. Seguidamente, se llevó a cabo un estudio cinético de los cambios en las propiedades antioxidantes de los zumos tratados por PEAIC a distintas intensidades de campo. Paralelamente, se comparó el efecto de la pasteurización mediante PEAIC con el tratamiento térmico convencional sobre los compuestos bioactivos individuales de zumos de tomate y fresa. El estudio se complementó determinando la evolución del contenido en compuestos bioactivos en los zumos tratados por PEAIC y calor, a lo largo del tiempo de almacenamiento.

TOMATE

Efecto de la frecuencia, anchura de pulso y polaridad sobre las propiedades antioxidantes de zumo de tomate

Se estudió la influencia de las variables frecuencia, anchura de pulso y polaridad en los principales compuestos bioactivos de zumos de tomate (licopeno y vitamina C), así como el efecto de estas tres variables en la capacidad antioxidante. Para ello, se llevaron a cabo una serie de tratamientos mediante PEAIC donde la frecuencia varió entre 50-250 Hz y la anchura de pulso osciló entre 1-7 μs en modo monopolar o bipolar siguiendo un diseño de superficie de respuesta. Los zumos se trataron a 35 kV/cm durante 1000 μs independientemente de la frecuencia, anchura de pulso y polaridad aplicada. Según estudios previos, tratamientos de PEAIC a 35 kV/cm durante 1000 μs alcanzan niveles de pasteurización en zumos de tomate (Mosqueda-Melgar, 2007). Este estudio permitió optimizar las variables de tratamiento mediante PEAIC para obtener zumos de tomate con mínimos cambios en las propiedades antioxidantes.

Vitamina C

El contenido inicial en vitamina C del zumo de tomate fresco fue de 15,2 mg/100 mL. La concentración de vitamina C, expresada como retención de vitamina C después del tratamiento por PEAIC, varió entre 58,2% y 99,0%. Tratamientos con PEAIC aplicados en modo monopolar mantuvieron mejor la vitamina C que los llevados a cabo en modo bipolar para una misma frecuencia y anchura de pulso. La anchura de pulso también afectó en gran medida a la retención de vitamina C en los zumos de tomate. Se observó una diferencia de aproximadamente un 15% entre los tratamientos de PEAIC aplicados a 1 o 7 μs en modo

monopolar a 50 Hz. Por el contrario, se obtuvo mayor variación en la retención de vitamina C (≈30%) cuando los pulsos bipolares oscilaron de 1 a 7 μs a 250 Hz. Por tanto, las diferencias en la retención de vitamina C en zumos de tomate tratados a distintas anchuras de pulso dependieron de la frecuencia y polaridad del tratamiento por PEAIC aplicado. Cuanto más pequeña fue la frecuencia y la anchura de pulso durante el tratamiento de PEAIC, mayor fue el contenido de vitamina C en el zumo de tomate.

El análisis de varianza indicó que un modelo de segundo orden (ecuación 1) puede predecir con buenos resultados (R^2 = 0,84) la retención de vitamina C (VR) en los zumos de tomate después del tratamiento mediante PEAIC en función de la frecuencia (*f*), anchura de pulso (τ) y polaridad.

$$VR(\%) = a - \left(2{,}9 \times 10^{-1}\right) \cdot f - 3{,}7 \cdot \tau + \left(7{,}6 \times 10^{-4}\right) \cdot f^2 \qquad (1)$$

(*monopolar, a = 121; bipolar, a = 115*)

Licopeno

El zumo de tomate fresco presentó un contenido en licopeno de 7,5 mg/100 mL. Los zumos tratados por PEAIC mostraron mayores retenciones de licopeno que los zumos no tratados. El máximo contenido relativo en licopeno (146,2%) se obtuvo en zumos tratados por PEAIC a 35 kV/cm durante 1000 μs con pulsos bipolares de 7 μs a 250 Hz. Otros autores (Torregrosa et al., 2005; Cortés et al., 2006) observaron que los tratamientos mediante PEAIC aumentan significativamente el contenido en carotenos en zumos. Las posibles causas del incremento en licopeno después de los tratamientos mediante PEAIC se discutirá posteriormente.

La polaridad del pulso influyó significativamente en el contenido en licopeno de los zumos. Los tratamientos de PEAIC llevados a cabo con pulsos bipolares dieron lugar a zumos con mayores contenidos en licopeno que cuando se usaron pulsos monopolares. La frecuencia y la anchura de pulso utilizada durante el tratamiento de PEAIC también afectaron considerablemente al contenido en licopeno. Los resultados sugieren que aumentando las dos variables frecuencia y anchura de pulso se consiguen zumos con mayor contenido relativo en licopeno. El incremento simultáneo en las dos variables desde 50 Hz y 1 μs hasta 250 Hz y 7 μs resultó en un incremento del licopeno del 43%, mientras que cuando se aumentó una variable, manteniendo la otra constante, el contenido en licopeno se incrementó entre un 8,4% (50 Hz, 7 μs) y un 26,4% (250 Hz, 1 μs).

Un modelo de segundo orden predijo adecuadamente (R^2_{adj} = 0,94) los cambios en la retención de licopeno (LR) en función de la frecuencia (*f*), anchura de pulso (τ) y polaridad aplicada durante el tratamiento mediante PEAIC (ecuación 2).

$$LR(\%) = a - \left(5{,}1 \times 10^{-2}\right) \cdot f + b \cdot \tau + \left(4{,}8 \times 10^{-4}\right) \cdot f^2 + \left(6{,}8 \times 10^{-3}\right) \cdot (f \cdot \tau) \qquad (2)$$

(*monopolar, a = 98 y b = 1,5; bipolar, a = 108 y b = 1,0*)

Capacidad Antioxidante

La máxima retención de capacidad antioxidante se alcanzó en zumos de tomate tratados mediante PEAIC a 35 kV/cm durante 1000 µs a 150 Hz aplicando pulsos bipolares de 4 µs. Por el contrario, la aplicación de tratamientos de PEAIC combinando frecuencias de 50 Hz y pulsos monopolares de 1 µs dio lugar a zumos de tomate con la menor capacidad antioxidante.

La capacidad antioxidante relativa (CR) de los zumos de tomate se predijo a través de ecuaciones polinomiales (R^2 = 0,86) en función de las variables de PEAIC estudiadas (frecuencia, *f*; anchura de pulso, τ; y polaridad) (ecuación 3).

$$CR(\%) = a + \left(3.9 \times 10^{-1}\right) \cdot f + b \cdot \tau - \left(7.7 \times 10^{-4}\right) \cdot f^2 - \left(5.9 \times 10^{-1}\right) \cdot \tau^2 - \left(3.0 \times 10^{-2}\right) \cdot (f \cdot \tau) \quad (3)$$

(*monopolar, a = 20 y b = 11,4; bipolar, a = 39 y b =9,4*)

La polaridad fue la variable de tratamiento que afectó en mayor grado a la retención de la capacidad antioxidante de los zumos de tomate. Los zumos tratados por PEAIC aplicando pulsos bipolares presentaron mayores retenciones de capacidad antioxidante que aquéllos tratados en modo monopolar. Las máximas retenciones de capacidad antioxidante, cuando se aplicaron tratamientos en modo monopolar, se obtuvieron combinando frecuencias entre 100 y 200 Hz con anchuras de pulso mayores de 4 µs; mientras que, frecuencias mayores de 150 Hz con pulsos menores de 5 µs alcanzaron las máximas retenciones de capacidad antioxidante en modo bipolar.

Optimización de los parámetros de procesado

Se determinaron las condiciones óptimas de procesado para obtener zumos de tomate con un alto contenido en compuestos bioactivos. Los tratamientos de PEAIC llevados a cabo a 35 kV/cm durante 1000 µs con 250 Hz y pulsos bipolares 1 µs conllevaron máximas retenciones en licopeno (131,8%), vitamina C (90,2%) y capacidad antioxidante (89,4%). Además, se validaron los resultados obtenidos durante la optimización de las variables frecuencia, anchura de pulso y polaridad. Para ello, se trataron zumos de tomate mediante PEAIC en las condiciones óptimas seleccionadas. Cuando el zumo de tomate se trató a 250 Hz con pulsos bipolares de 1 µs los valores relativos de licopeno, vitamina C y capacidad antioxidante fueron de 129,3%, 90,2% y 90,1%, respectivamente. Por tanto, no hubo diferencias significativas entre los resultados experimentales y los valores predichos.

Efecto de la intensidad de campo y tiempo de tratamiento sobre las propiedades antioxidantes de zumo de tomate

Una vez seccionadas las condiciones óptimas de procesado por PEAIC en función de la frecuencia, anchura de pulso y polaridad, se procedió al estudio cinético de los cambios en las propiedades antioxidantes para cada una de las intensidades de campo eléctrico estudiadas. Para relacionar adecuadamente los cambios cinéticos en los compuestos bioactivos, los modelos de primer orden, la función de distribución de Weibull y un modelo de extracción sólido-líquido propuesto por Peleg, se ajustaron a los resultados. Una vez obtenidas las

constantes para cada uno de los modelos cinéticos, éstas se ajustaron a modelos de primer orden dependientes de la intensidad de campo eléctrico. El efecto combinado de la intensidad de campo eléctrico y del tiempo de tratamiento en las propiedades antioxidantes de los zumos de tomate se obtuvo sustituyendo cada una de las variables de los modelos cinéticos por su respectiva ecuación de primer orden.

Vitamina C

La retención de vitamina C en los zumos de tomate tratados por PEAIC varió entre 73,1% y 100%. Estos resultados concuerdan con los obtenidos por Torregrosa et al. (2006), quienes observaron retenciones de vitamina C entre 87,5 y 97% en zumo de naranja-zanahoria procesados con diferentes intensidades de campo (25, 30, 35 y 40 kV/cm) durante distintos tiempos de tratamiento (desde 30 a 340 μs) usando pulsos bipolares de 2,5 μs. El contenido en vitamina C dependió significativamente del tiempo de tratamiento y la intensidad de campo aplicado durante el procesado por PEAIC; por tanto, mayores intensidades de campo y tiempos cortos de tratamiento, dieron lugar a mayores contenidos en vitamina C en los zumos. Elez-Martínez y Martín-Belloso (2007) estudiaron la variación de vitamina C después de la aplicación de diferentes tratamientos mediante PEAIC a zumo de naranja y gazpacho, obteniendo resultados similares a los observados en este estudio. La vitamina C es un compuesto inestable que en condiciones no deseadas se destruye fácilmente (Lee y Coates, 1999). La degradación del ácido ascórbico depende de varios factores como pueden ser la presencia de oxígeno y luz, además de la temperatura y el tiempo de procesado. En general, tratamientos de PEAIC suaves mantienen mejor el contenido en vitamina C en los zumos (Davey et al., 2000).

En función de los valores de R^2_{adj}, A_f, RMSE, MBE y χ^2 obtenidos por los modelos, se concluyó que los modelos de primer orden y de Weibull predecían bien los resultados. Sin embargo, el modelo propuesto por Weibull fue más adecuado que el de primer orden porque presentó valores de R^2_{adj} y A_f próximos a la unidad y menores valores de RMSE, MBE y χ^2. Las constantes cinéticas (k_1) obtenidas a través del modelo de primer orden, oscilaron entre $4{,}1\times10^{-5}$ y $1{,}52\times10^{-4}$ μs^{-1}. El parámetro de forma (γ) estimado por el modelo de Weibull varió entre 1,1 y 1,9, dependiendo inversamente de la intensidad de campo eléctrico a través de un modelo de primer orden (R^2_{adj} = 0,802). Valores de γ por encima de la unidad sugieren que la destrucción de la vitamina C se incrementó progresivamente a lo largo del tiempo. Por el contrario, el parámetro de escala (α) no se vio afectado por la intensidad de campo aplicada durante el tratamiento por PEAIC, mostrando valores entre 5741 y 6587 μs. Los valores del tiempo medio (t_m) para la destrucción total de la vitamina C en zumo de tomate variaron entre 7939 y 11.252 μs.

Sustituyendo γ por la ecuación de primer orden y α por el valor medio, calculado a través de los valores obtenidos para cada una de la intensidades de campo aplicadas en la función de distribución de Weibull, se obtuvo una ecuación dependiente de la intensidad de campo (E) y el tiempo de tratamiento (t) capaz de predecir los cambios en el contenido en vitamina C (VR) en función de estas dos variables (ecuación 4) (R^2_{adj} = 0,941, A_f = 1,016, RMSE = 0,781, MBE = 0,042 y χ^2 = 1,202)

$$VR = 100 \cdot exp\left[-\left(\frac{t}{5.65 \cdot 10^{3}} \right)^{(3.6) \cdot exp\left[-\left(3.1 \cdot 10^{-2} \right) \cdot E \right]} \right] \quad (4)$$

Licopeno

El contenido en licopeno aumentó significativamente entre un 3,8% y un 37,7%, respecto zumo fresco, después del tratamiento por PEAIC. La intensidad de campo y el tiempo de tratamiento afectaron significativamente al contenido en licopeno de los zumos. El ajuste cinético llevado acabo por los modelos de Weibull y Peleg fue adecuado independientemente de la intensidad de campo aplicada (R^2 = 0,759-0,992). A pesar de que los valores de A_f para la ecuación de Weibull y Peleg fueron bastante similares (1,007-1,1032), los RMSE, MBE y X^2 alcanzaron valores más próximos a cero, cuando se utilizó el modelo propuesto por Peleg. Por tanto, desde un punto de vista cuantitativo, los resultados se predijeron mejor cuando se aplicó el modelo de Peleg, seguido por el modelo de Weibull. La intensidad de campo influyó en gran medida en los parámetros γ y α obtenidos por el modelo de Weibull. Cuanto más aumenta la intensidad de campo, mayor es γ y más cercano a cero es α. Los valores de γ oscilaron entre $2{,}3\times10^{-1}$ y $5{,}8\times10^{-1}$, evidenciando que la formación de licopeno fue cada vez menos favorecida por el tiempo de tratamiento. La constante cinética del modelo propuesto por Peleg (K_1) no se correlacionó directamente con la intensidad de campo, indicando velocidades similares de formación de licopeno cuando el procesado mediante PEAIC se llevó a cabo durante tiempos de tratamiento cortos ($t = t_o$), independientemente de la intensidad de campo aplicada. Por el contrario, la constante de capacidad (K_2) obtenida también por el modelo de Peleg, la cual está relacionada con la concentración de estabilización después de tiempos de tratamiento largos, aumentó con la intensidad de campo siguiendo una tendencia exponencial que puede ser descrita con buena exactitud por un modelo de primer orden (R^2_{adj} = 0,988). La retención de licopeno después de tiempos prolongados de tratamiento (RC_∞) se calculó a partir de K_2, los resultados variaron entre 105% y 169%. A mayor intensidad de campo, mayor fue la retención de licopeno después de tiempos de procesado por PEAIC largos. El efecto combinado del tiempo de tratamiento (t) y la intensidad de campo (E) en el contenido en licopeno del zumo de tomate se describe por la ecuación 5 donde K_2 del modelo de Peleg se remplazó por el modelo de primer orden obtenido anteriormente y K_1 se sustituyó por el valor medio, calculado a partir de los valores de K_1 obtenidos para cada una de las intensidades de campo estudiadas. Este nuevo modelo predijo con buena exactitud (R^2_{adj} = 0,976, A_f = 1,017, RMSE = 1,004, MBE = 0,561 y χ^2 = 4,493) los cambios en el contenido en licopeno (LR) como consecuencia del tiempo de tratamiento (t) y la intensidad de campo (E) aplicados.

$$LR = 100 + \frac{t}{\left(2.82 \times 10^{1}\right) + \left(\left[1.2 \cdot 10^{1}\right] \cdot exp\left(-\left[2.1 \cdot 10^{-1}\right] \cdot E \right) \cdot t \right)} \quad (5)$$

Capacidad Antioxidante

La retención de la capacidad antioxidante en zumos de tomate dependió de la intensidad de campo y del tiempo de tratamiento. Los zumos de tomate procesados a 20 kV/cm presentaron las máximas retenciones de capacidad antioxidante seguidos por los zumos procesados a 35 kV/cm. No se observaron diferencias significativas en las retenciones de capacidad antioxidante entre los zumos tratados por PEAIC a 25 y 30 kV/cm. Los cambios en la capacidad antioxidante en zumos de tomate coinciden con la variación en compuestos bioactivos durante el procesado. De este modo, la degradación de la vitamina C como resultado de los tratamientos de PEAIC, se refleja en una disminución de la capacidad antioxidante del zumo. Por otro lado, el licopeno es un componente bioactivo que participa en gran proporción en la capacidad antioxidante de los productos derivados del tomate (Gahler et al., 2003). En este estudio, la magnitud de los cambios en la capacidad antioxidante puede estar asociada con la variación en el contenido en licopeno. Los zumos de tomate con el mayor contenido en licopeno, presentaron la segunda mayor capacidad antioxidante. Por tanto, estos resultados sugieren que los cambios en la capacidad antioxidante de los zumos pueden ser debidos principalmente a las variaciones en la concentración de vitamina C, más que en el contenido en licopeno.

El modelo cinético de primer orden predijo bien la retención de la capacidad antioxidante en zumos tratados a intensidades de campo entre 25 y 35 kV/cm, pero los coeficientes de regresión disminuyeron sustancialmente cuando el tratamiento se llevó a cabo a 20 kV/cm (R^2_{adj} = 0,592-0,984). Por el contrario, el modelo de Weibull presentó elevados coeficientes de regresión, independientemente de la intensidad de campo aplicada (R^2_{adj} = 0,912-0,984). A pesar de que los parámetros γ y α estimados mediante el modelo de Weibull variaron con la intensidad de campo, no se observó una clara influencia de esta variable de tratamiento. Los valores de t_m para la destrucción total de la capacidad antioxidante variaron entre 3271 y 15.970 µs.

Por otro lado, Aguiló-Aguayo et al. (2007) concluyeron que el tratamiento mediante PEAIC llevado a cabo a 35 kV/cm durante 1500 µs, alcanzó 5 reducciones logarítmicas de *Lactobacillus brevis*, mientras la actividad de peroxidasa, pectinmetilesterasa y galacturonasa se redujeron un 97%, 82% y 50%, respectivamente. Por tanto, los tratamientos más intensos de PEAIC llevados a cabo en este trabajo dan lugar a zumos seguros, estables y con mínimos cambios en las propiedades antioxidantes. Los zumos de tomate tratados por PEAIC (35 kV/cm durante 1500 µs con pulsos bipolares de 1-µs a 250 Hz) presentaron mayores niveles de licopeno (133%) y concentraciones un poco menores de vitamina C (87%) que los frescos.

Cambios en las propiedades antioxidantes y parámetros colorimétricos de zumo de tomate pasterizado por pulsos eléctricos de alta intensidad de campo o por calor durante el tiempo de almacenamiento

Paralelamente se estudió el efecto de los tratamientos de pasteurización por PEAIC (35 kV/cm durante 1500 µs con pulsos bipolares de 4-µs a 100 Hz, energía aplicada 8269 kJ/L) y por calor (90 ºC durante 30 s o 60 s), en los compuestos bioactivos individuales del zumo de tomate. Las condiciones de pasteurización por PEAIC se seleccionaron de acuerdo con estudios previos (Mosqueda-Melgar, 2007). Además, se estudió la evolución de las propiedades antioxidantes del zumo de tomate procesado mediante PEAIC durante 56 días de

almacenamiento a 4 ºC. Se compararon estos valores con los obtenidos para zumos pasteurizados por calor y zumos no sometido a ningún tipo de tratamiento.

Carotenoides

El zumo de tomate tratado por PEAIC presentó el máximo contenido en carotenos totales (14,7 mg/100 ml), mientras la menor concentración se obtuvo en zumo de tomate fresco (14,1 mg/100 ml). Estas concentraciones concuerdan con las obtenidas en otros estudios, en los cuales el contenido en carotenos de los zumos varió entre 5 y 17 mg/100g (Khachik et al. 2002; Podsędek et al. 2003). Por otro lado, los zumos tratados por PEAIC mantuvieron mejor el contenido en carotenos totales que los tratados térmicamente durante el tiempo. La concentración de carotenos en zumos tratados por calor disminuyó a lo largo del almacenamiento desde 14,4 hasta 7,3 mg/100 ml. Teniendo en cuenta los carotenos individuales de los zumos, el licopeno fue el que se encontró en mayor cantidad, mostrando valores entre 7,13 y 7,84 mg/100 ml. La concentración de licopeno se incremento respecto a los valores obtenidos para el zumo fresco, independientemente del tratamiento de pasterización aplicado. No obstante, otros carotenos individuales precursores del licopeno como fitoeno (1,83-2,07 mg/100ml) y neurosporeno (1,19-1,30 mg/100ml) se redujeron como consecuencia del tratamiento. El contenido en ξ-caroteno (0,19-0,20 mg/100ml) fue similar para zumos frescos y tratados. Según algunos autores, los tratamientos por calor pueden provocar un aumento en los carotenos individuales debido a la degradación enzimática y las pérdidas de agua que pueden concentrar el zumo (Rodríguez-Amaya, 1997). Nguyen y Schwartz (1999) sugirieron que los tratamientos térmicos rompen las membranas celulares y los complejos proteína-caroteno, aumentando la extracción de estos compuestos. Sin embargo, en el presente trabajo, el aumento en el contenido en licopeno coincide con una disminución de los niveles de fitoeno y neurosporeno en los zumos. Por tanto, los tratamientos por PEAIC y calor podrían transformar algunos carotenos en licopeno. Si el fitoeno sufre reacciones de desaturación, cada una de ellas crea un nuevo doble enlace, y se obtiene como producto final licopeno y productos intermedios como fitoflueno, ξ-caroteno y neurosporeno (Britton y Hornero-Mendez, 1997). De acuerdo con nuestros resultados, Torregrosa et al. (2005) observaron que la concentración de algunos carotenos aumentó después de la pasterización por calor y PEAIC de zumo de naranja-zanahoria, concluyendo que la conversión entre algunos carotenos tiene lugar durante los tratamientos. Por otro lado, el contenido en β-caroteno y γ-caroteno en los zumos varió entre 0,29 y 0,40 mg/100 ml y 1,67 y 1,77 mg/100 ml, respectivamente. El contenido en β-caroteno (31-38%) aumentó, mientras la concentración de γ-caroteno (3-6%) disminuyó en los zumos de tomate independientemente del tratamiento aplicado. Una posible explicación de este hecho es que γ-caroteno puede ciclarse transformándose en β-caroteno (Britton y Hornero-Mendez, 1997). El contenido de cada uno de los carotenos individuales disminuyó significativamente durante el almacenamiento, con excepción de β-caroteno y fitoeno cuyas concentraciones se mantuvieron durante 56 días en todos los zumos tratados. La cantidad de licopeno en los zumos disminuyó más que otros carotenos, mostrando niveles de 2,43-2,71 mg/100ml a los 56 días de almacenamiento. La vitamina A de un determinado alimento, expresada como equivalentes de retinol, puede calcularse a partir del contenido en β-caroteno y γ-caroteno del mismo (Rodríguez-Amaya, 1997). Los niveles de vitamina A en los zumos tratados sufren una pequeña disminución, alcanzando valores entre 0,770 y 0,910 RA/ml después de 56 días. Las pérdidas de carotenos durante el almacenamiento están causadas principalmente por la oxidación, ya que estos compuestos poseen varios enlaces insaturados (Kidmose et al., 2002). Estas reacciones de oxidación pueden producir un blanqueo del

producto, como consecuencia de la formación de compuestos incoloros (Gross, 1991). La severidad de las reacciones de oxidación depende de la estructura de cada uno de los carotenos y de las condiciones ambientales (Ramakrishnan y Francis, 1980). Por otro lado, el contenido en carotenos (licopeno, neurosporeno y γ-caroteno), además de la vitamina A, de los zumos tratados mediante PEAIC fue significativamente superior que en zumos tratados térmicamente. En cambio, no existieron diferencias significativas en el contenido en β-caroteno y ξ-caroteno entre zumos tratados por PEAIC y los tratados térmicamente durante su almacenamiento a 4 ºC.

Compuestos fenólicos

El contenido inicial en compuestos fenólicos totales, calculado a partir de la suma de las cantidades de fenoles individuales determinados por HPLC, varió entre 8,9 y 9,1 mg/100 ml. Los tratamientos de pasteurización no afectaron al contenido en fenoles totales de los zumos de tomate. Zumos tratados por PEAIC y calor (90 ºC-30 s) mantuvieron mayores cantidades de fenoles durante el tiempo de almacenamiento que los tratados térmicamente (90 ºC-60 s). Como cabía esperar, el ácido clorogénico fue el ácido fenólico que se encontró en mayor cantidad en los zumos de tomate; su contenido sufrió una gran disminución a lo largo del tiempo desde 4,4 mg/100ml hasta 3,5-3,8 mg/100ml a los 6 días. Tal como sucedió en el caso de fenoles totales, el ácido clorogénico se conservó mejor durante el almacenamiento en los zumos tratados por calor durante 30 s y por PEAIC que en aquellos zumos procesados por calor durante 60 s. El zumo de tomate es un producto con alto contenido en flavonoides entre los que destacan los flavonoles quercitina y kaempferol (Stewart et al. 2000). La concentración inicial de quercitina en los zumos de tomate estudiados osciló entre 1,98-2,05 mg/100 ml, mientras el contenido en kaempferol varió entre 0,56-0,57 mg/100ml justo después del procesado. El contenido en quercitina y kaempferol disminuyó significativamente a través del tiempo de almacenamiento independientemente del tratamiento aplicado, alcanzando valores de 1,32-1,64 mg/100ml y 0,40-0,47/100ml, respectivamente, a los 56 días. El zumo de tomate tratado mediante PEAIC presentó mayores contenidos en quercitina durante el almacenamiento que los zumos tratados térmicamente. Otros ácidos fenólicos se determinaron en el zumo de tomate como el ferúlico (0,86-0,89 mg/100ml), el *p*-cumárico (0,62-0,64 mg/100ml) y el cafeico (0,43-0,45 mg/100ml). El zumo de tomate sufre una disminución importante del contenido en ácido *p*-cumárico durante la conservación hasta valores de 0,32-0,34 mg/100 ml, que puede ser debida a su conversión a ácido cafeico. El contenido en ácido cafeico se vio incrementado durante el almacenamiento, alcanzando valores de entre 0,51 y 0,57 mg/100ml. La hidroxilación del ácido *p*-cumárico a ácido cafeico tiene lugar en los alimentos como consecuencia de la introducción de un segundo grupo hidroxil al ácido *p*-cumárico, probablemente catalizado por la enzima monofenol monoxigenasa (Macheix et al., 1990). Por tanto, el aumento del contenido en cafeico del zumo de tomate a partir de los 28 días de conservación puede asociarse con la actividad residual de las hidrolasas que actúan transformando el ácido *p*-cumárico en cafeico. En la actualidad no existen estudios sobre el efecto de los tratamientos por PEAIC en este tipo de enzimas, por lo que es necesario investigar más en este campo.

Vitamina C

Los contenidos iniciales de vitamina C en zumos frescos y tratados varió entre 10,17 y 12,8 mg/100g. Los resultados obtenidos en este trabajo concuerdan con los encontrados en la bibliografía (Davey et al., 2000; Sánchez-Moreno et al., 2006). El contenido en vitamina C

disminuyó significativamente en los zumos tratados comparado con los frescos. Cabe destacar que después del tratamiento mediante PEAIC el zumo conservó hasta un 86,5% de la vitamina C inicial; en cambio tras el tratamiento térmico durante 60 s y 30 s el zumo de tomate mantuvo el 79,2 y 80,4%, respectivamente. Estos resultados concuerdan con los obtenidos por Elez-Martínez y Martín-Belloso (2007) para gazpacho, cuyo ingrediente mayoritario es el tomate, tratado por PEAIC en condiciones parecidas a las aplicadas en este trabajo. El ácido ascórbico es un compuesto sensible al calor en presencia de oxígeno. Por tanto, las altas temperaturas de procesado pueden acelerar la degradación de vitamina C. La temperatura máxima alcanzada durante el tratamiento mediante PEAIC fue de 40 ºC. Por tanto, la mayor retención en vitamina C de los zumos tratados por PEAIC comparados con los tratados por calor puede ser debida a la menor temperatura alcanzada durante los tratamientos por PEAIC. Otros estudios (Min et al., 2003) han confirmado la efectividad de los tratamientos por PEAIC en conseguir zumos de tomate con mayores contenidos en vitamina C que los procesados por calor.

La concentración de vitamina C disminuyó en zumos tratados y frescos durante el periodo de almacenamiento. Además, se observó que el contenido en vitamina C era significativamente mayor durante los 56 días de almacenamiento en zumo tratado por PEAIC que en el tratado térmicamente. La vitamina C se ha usado como índice de la calidad nutricional de las frutas y se ha considerado que la vida útil de un zumo está finalizando cuando éste ha perdido un 50% de la vitamina C inicial. La concentración de vitamina C en zumos de tomate tratados por PEAIC y por calor durante 30 s fue menor al 50% del contenido inicial, después de 28 días de almacenamiento. Sin embargo, las pérdidas de vitamina C fueron mayores al 50 % después de 21 días en zumos tratados por calor durante 60 s. Estudios similares realizados por Min et al. (2003) en zumo de tomate mostraron que el contenido en vitamina C a los 30 días de envasado era de un 50% respecto la inicial. Según Davey et al. (2000), la vitamina C es un de los constituyentes de los vegetales más vulnerable a las condiciones de conservación. La velocidad de degradación de la vitamina C depende de varios factores entre los que cabe destacar el tiempo y temperatura de almacenamiento, además de la presencia de luz.

Capacidad Antioxidante

La capacidad antioxidante en zumos de tomate fresco y tratados osciló entre 9,8% y 10,4% de inhibición del radical DPPH. No se observaron diferencias significativas en la capacidad antioxidante del zumo de tomate en función del tratamiento aplicado, ni tampoco entre zumos tratados y frescos. Algunos estudios demuestran que el tratamiento térmico no varía la capacidad antioxidante de las frutas y verduras, así como de sus derivados, ya que durante el tratamiento se forman nuevos compuestos como por ejemplo los derivados de las reacciones de Maillard, que también tienen una elevada capacidad antioxidante (Manzocco, et al., 2001). En general, la capacidad antioxidante del zumo de tomate disminuyó a lo largo del tiempo de almacenamiento independientemente del tratamiento aplicado. No se observaron diferencias significativas durante el tiempo de almacenamiento entre los zumos tratados por calor durante 60 s y por PEAIC. La capacidad antioxidante está relacionada con la cantidad y composición de los compuestos bioactivos presentes en los alimentos (Sánchez-Moreno et al., 2005). Eberhardt et al. (2000) indicaron que la capacidad antioxidante de un alimento proviene de la combinación natural de los diferentes compuestos bioactivos. Vitamina C, carotenos y fenoles son los principales compuestos beneficiosos para la salud presentes en el tomate (Takeoka et al., 2001). No obstante, los cambios que se produjeron en el porcentaje de inhibición del DPPH no pudieron asociarse con la variación de los compuestos fenólicos durante el almacenamiento

(R^2 = 0,52). Se observó una buena correlación entre la capacidad antioxidante de los zumos y el contenido en vitamina C (R^2 = 0,86) y licopeno (R^2 = 0,81) de los mismos.

Parámetros colorimétricos

Los cambios de color durante el almacenamiento de zumos de tomate se determinaron mediante la luminosidad (L*) y el tono (hº); éste último parámetro se calculó a partir de los parámetros colorimétricos a* y b*.

El zumo de tomate fresco presentó más brillo y tono que los zumos pasteurizados. Los zumos de tomate procesado mediante PEAIC mantuvieron durante el tiempo mayores valores de luminosidad en comparación con los tratamientos térmicos de pasteurización (90 ºC-60s). Una disminución del valor de L* en zumos tratados por calor se ha asociado con la formación de compuestos oscuros debido a las reacciones no enzimáticas que reducen la aceptabilidad de los mismos (Klim y Nagy, 1988). En zumos frescos y tratados por PEAIC el oscurecimiento del zumo durante su conservación puede ser debida a reacciones enzimáticas. Por otra parte, el parámetro hº aumentó desde 36º-37º hasta 45º-50º durante la conservación de zumos tratados. Por tanto, el color rojo de los zumos procesados disminuyó independientemente del tratamiento de pasteurización aplicado. Sin embargo, esta reducción fue menor en zumos tratados por PEAIC que los tratados por calor a 90 ºC durante 30 s. Además, estos cambios en el parámetro hº durante el periodo de conservación se relacionaron con las variaciones del contenido en licopeno en los zumos de tomate.

FRESA

Efecto de la frecuencia, anchura de pulso y polaridad sobre las propiedades antioxidantes de zumo de fresa

Tal como se procedió para el zumo de tomate, inicialmente se realizó un estudio del efecto del procesado por PEAIC (frecuencia, anchura de pulso y polaridad) en las propiedades antioxidantes del zumo de fresa.

Vitamina C

La retención de vitamina C después de los tratamientos por PEAIC en zumo de fresa osciló entre 91,5% y 100,3%. Si comparamos estos resultados con los obtenidos en los zumos de tomate (58-99%) después de la aplicación de tratamientos de PEAIC similares, se observa que la retención fue mayor en zumos de fresa. Estas diferencias con respecto al zumo de tomate son atribuibles, en parte, al bajo pH de las fresas comparado con el del tomate, ya que condiciones ácidas podrían estabilizar la vitamina C de los zumos (Tannenbaum et al., 1985). La polaridad utilizada durante los diferentes tratamientos de PEAIC afectó significativamente el contenido en vitamina C. Tal como se observó en zumo de tomate, los tratamientos aplicados en modo bipolar disminuyeron más el contenido en vitamina C que los llevados a cabo con pulsos monopolares. No obstante, cabe destacar que cuando los tratamientos de PEAIC se aplicaron a 1 μs, la polaridad del pulso no influyó significativamente en la retención de vitamina C. La concentración de la vitamina C en los zumos de fresa dependió también de la frecuencia y anchura de pulso aplicados. En general, los contenidos en vitamina C disminuyeron cuando se aplicaron tratamientos con altas frecuencias y anchuras de pulso. En cambio, se observó que

aumentando la frecuencia por encima de 200 Hz (monopolar) y 175 Hz (bipolar) la retención de vitamina C disminuyó más, que cuando se aplicaron menores frecuencias. Teniendo en cuenta solamente los parámetros que afectaron significativamente al contenido en vitamina C en los zumos de fresa, las variaciones de vitamina C (VR) como consecuencia de los tratamientos por PEAIC en función de la frecuencia (*f*) y anchura de pulso (τ) pueden ser modelizadas mediante la ecuación 6 ($R^2_{adj} = 0,83$).

$$VR(\%) = a - \left(1,4 \times 10^{-2}\right) \cdot f - \left(1,7 \times 10^{-1}\right) \cdot \tau + \left(4,1 \times 10^{-5}\right) \cdot f^2 - \left(2,6 \times 10^{-3}\right) \cdot (f \cdot \tau) \quad (6)$$

(*monopolar, a = 101; bipolar, a = 100*)

Antocianinas

El contenido en antocianinas, expresado como valor relativo, varió entre 83% y 102% en los zumos procesados por PEAIC. La estabilidad de las antocianinas depende de varios factores entre los que destaca el pH, la presencia de enzimas y luz, además de la temperatura de procesado y almacenamiento (Rein y Heinonen, 2004). En un zumo fresco, existe equilibrio entre cuatro especies de antocianinas, las bases quinoidales, cationes flavilio, bases carbinol y chalconas. Si se aplican altas temperaturas se puede invertir el equilibrio hacía la formación de chalconas a partir de las bases quinoidal, cationes flavilio y bases carbinol, dando lugar a compuestos incoloros (Brouillard, 1982). Las chalconas pueden degradarse dando lugar a estructuras poliméricas marrones cuando se aplican tratamientos a temperaturas altas y tiempos de tratamiento largos (Jackman y Smith, 1997). Zhang et al. (2007) observaron que tratando la cianidina-3-glucósido en una solución metabólica con PEAIC (a 1,2, 2,2 y 3,0 kV/cm con 300 pulsos, $T^a \leq 47$ ºC), las antocianinas se degradaban y se formaban especies incoloras (chalconas). Dentro de las variables de tratamiento, la polaridad afectó significativamente al contenido en antocianinas. En general, los zumos de fresa tratados con pulsos bipolares presentaron mayores retenciones de antocianinas que los tratados con pulsos monopolares. Además, la anchura de pulso y frecuencia de procesado influyeron en el contenido en antocianinas del zumo de fresa. El contenido máximo de antocianinas se obtuvo en zumos tratados con pulsos bipolares menores de 4 μs y frecuencias entre 175 y 200 Hz. Los mismos resultados se obtuvieron cuando los tratamientos se llevaron a cabo con pulsos bipolares por encima de 6 μs y frecuencias entre 100 y 175 Hz. Además de las altas temperaturas, otros factores como la presencia de ácido ascórbico, metales, enzimas, luz, oxígeno y largos tiempos de procesado aceleran la destrucción de las antocianinas durante el tratamiento (Wrolstad, 2000). Los zumos de fresa tratados con bajas frecuencias y anchuras de pulso estrechas mostraron los menores contenidos en antocianinas. Esta disminución en la concentración de antocianinas podría ser debida, no sólo al propio tratamiento por PEAIC, sino también a la baja inactivación enzimática de β-glucosidasa y peroxidasa después del procesado de los zumos de fresa con tratamientos de PEAIC suaves. Aguiló-Aguayo et al. (2008) mostraron una actividad residual de 113% de β-glucosidasa en zumo de fresa tratado a 50 Hz y pulsos monopolares de 1 μs a 35 kV/cm durante 1000 μs. El análisis de varianza indicó que a través de un modelo de segundo orden (ecuación 7) se pueden predecir los cambios en el contenido en antocianinas (AR) en función de la frecuencia (*f*), anchura de pulso (*τ*) y polaridad ($R^2 = 0,74$).

$$AR(\%) = a - \left(2{,}1 \times 10^{-1}\right) \cdot f + 3{,}3 \cdot \tau - \left(3{,}3 \times 10^{-4}\right) \cdot f^2 - \left(1{,}7 \times 10^{-2}\right) \cdot \left(f \cdot \tau\right) \quad (7)$$

(*monopolar, a = 69; bipolar, a = 73*)

Capacidad Antioxidante

La capacidad antioxidante del zumo de fresa tratado por PEAIC varió entre 75 y 100% de inhibición del radical DPPH. La capacidad antioxidante de los zumos dependió de la polaridad del pulso, frecuencia y anchura de pulso aplicados. Tal como sucedió para el zumo de tomate, los zumos de fresa tratados con pulsos bipolares mostraron mayores capacidades antioxidantes que los zumos tratados con pulsos monopolares. Por otra parte, los tratamientos de PEAIC llevados a cabo entre 180 y 205 Hz con pulsos monopolares por debajo de 1,1 μs, además de frecuencias entre 145 y 240 Hz con pulsos bipolares menores de 3,7 μs no afectaron a la capacidad antioxidante de los zumos. La magnitud de los cambios producidos en la capacidad antioxidante se asoció con la variación en compuestos bioactivos debido al tratamiento por PEAIC. Los zumos de fresa que presentaron mayor capacidad antioxidante después del tratamiento fueron los que mostraron mayores retenciones de antocianinas y niveles altos de vitamina C. En cambio, zumos tratados por PEAIC con frecuencias bajas y anchuras de pulso cortas mostraron menores capacidades antioxidantes a pesar de que contenían altos niveles de vitamina C. Por tanto, estos resultados indican que la capacidad antioxidante del zumo de fresa está asociada principalmente al contenido en antocianinas, más que a la concentración de vitamina C presentes en los mismos. Las variaciones de la capacidad antioxidante (CR) como consecuencia de los diferentes tratamientos de PEAIC se modelizaron a través de la ecuación 8 con buenos resultados ($R^2 = 0{,}93$).

$$CR(\%) = a - \left(5{,}6 \times 10^{-1}\right) \cdot f + 3{,}1 \cdot \tau - \left(1{,}4 \times 10^{-3}\right) \cdot f^2 - \left(2{,}4 \times 10^{-2}\right) \cdot \left(f \cdot \tau\right) \quad (8)$$

(*monopolar, a = 47; bipolar, a = 50*)

Optimización de los parámetros de procesado

Se determinaron las condiciones de tratamiento por PEAIC, en función de la frecuencia, anchura de pulso y polaridad, que permiten obtener zumos de fresa con máxima retención en vitamina C, antocianinas y capacidad antioxidante. Los mayores valores se obtuvieron después de tratamientos de PEAIC con frecuencias altas y anchuras de pulso cortas en modo bipolar. En concreto el tratamiento llevado a cabo a 35 kV/cm durante 1000 μs con pulsos bipolares de 1 μs a 232 Hz alcanzó las máximas retenciones de vitamina C (98%), antocianinas (102%) y capacidad antioxidante (100%).

Para completar el estudio se validaron las diferentes expresiones obtenidas para modelizar las variaciones de las propiedades antioxidantes en función de las distintas variables de procesado por PEAIC. Los coeficientes de correlación entre los valores observados y los valores predichos por los modelos de segundo orden demostraron que éstos últimos son adecuados para describir los cambios con buena exactitud.

Efecto de la anchura de pulso y tiempo de tratamiento sobre las propiedades antioxidantes de zumo de fresa

Después de establecer las condiciones óptimas de procesado (frecuencia, anchura de pulso y polaridad) para obtener zumos de fresa con máximo potencial antioxidante, se pasó a realizar un estudio cinético de los cambios de las principales propiedades antioxidantes de los zumos de fresa para cada uno de las intensidades de campo estudiadas. Para ello se trató zumo de fresa a diferentes tiempos de tratamiento (hasta 2000 μs) e intensidades de campo eléctrico entre 20 y 35 kV/cm.

Vitamina C

Después de los tratamientos por PEAIC, los zumos de fresa presentaron elevadas retenciones en vitamina C (≥87%). El tiempo de tratamiento y la intensidad de campo aplicada afectaron significativamente a la cantidad de vitamina C de los zumos. En general, se obtuvieron mayores retenciones de vitamina C en los zumos de fresa cuando se aplicaron tiempos cortos e intensidades de campo bajas. Otros autores han observado la misma tendencia para la vitamina C presente en zumo de naranja (Elez-Martínez y Martín-Belloso, 2007) y en zumo de naranja-zanahoria (Torregrosa et al., 2006). Hasta ahora, la destrucción de la vitamina C en función del tiempo de tratamiento ha sido descrita a través de modelos de primer orden (Jonson et al., 1995; Vieira et al., 2000). En el presente estudio, los coeficiente de regresión obtenidos al ajustar los datos a modelos de primer orden fueron elevados cuando se aplicaron intensidades de campo entre 25 y 35 kV/cm ($R^2_{adj} \geq 0.869$), pero disminuyeron cuando el tratamiento de PEAIC se llevó a cado a 20 kV/cm. El valor de las constantes cinéticas dependió de la intensidad de campo eléctrico aplicada, variando entre $2{,}55\times10^{-5}$ y $6{,}08\times10^{-5}$ μs^{-1}. Torregrosa et al. (2006) obtuvieron constantes cinéticas de primer orden desde 9×10^{-3} hasta $2{,}2\times10^{-2}$ μs^{-1} cuando modelizaron los cambios en el contenido en vitamina C en zumo de naranja-zanahoria tratado a intensidades de campo de entre 25 y 40 kV/cm durante un máximo de 340 μs. Comparando las constantes cinéticas, se puede concluir que la vitamina C en zumo de fresa se destruye a menor velocidad que en zumos de naranja-zanahoria. Al contrario que el modelo de primer orden, la distribución de Weibull presentó elevados coeficientes de determinación (0,934-0,974) y bajos valores de RMSE, X^2 y MBE independientemente de la intensidad de campo aplicada; por tanto, este modelo se seleccionó para predecir los cambios cinéticos de la vitamina C en los zumos de fresa. Tal como se observó en el caso de zumo de tomate, los valores de γ estimados por el modelo de Weibull tomaron valores mayores que 1, indicando que a medida que aumenta el tiempo de tratamiento la velocidad de destrucción de vitamina C es mayor. Ambas constantes, obtenidas a través del modelo de Weibull (α y γ) se relacionaron con la intensidad de campo eléctrico mediante ecuaciones de primer orden. Sustituyendo estas constantes por sus respectivas ecuaciones en el modelo de Weibull, se obtuvo una ecuación de segundo orden capaz de predecir las variaciones de vitamina C (VR)

después de los diferentes tratamientos por PEAIC (ecuación 9) (R^2_{adj} = 0,948, RMSE = 0,635, MBE = -0,0037 y χ^2 = 0,0005).

$$VR(\%) = 100 \cdot exp\left[-\left(\frac{t}{\left[4479 \cdot exp(0.0149 \cdot E)\right]} \right)^{\left[4.879 \cdot exp(-0.0348 \cdot E)\right]} \right] \qquad (9)$$

La vitamina C se considera una vitamina sensible al calor y su retención después del procesado es indicativa de la calidad nutricional media del alimento (Jung et al., 1995). Para establecer el tiempo de destrucción total de la vitamina C se calculó el valor de t_m, que varió entre 8175 y 12481 µs. Estos resultados fueron superiores a los obtenidos para zumo de tomate (7939-11252 µs). Esta mayor resistencia de la vitamina C a los tratamientos por PEAIC podría ser debida a que el zumo de fresa presenta menor pH que el de tomate. Se ha comprobado que condiciones ácidas estabilizan la vitamina C evitando su destrucción (Tannenbaum, et al., 1985).

Antocianinas

La retención de antocianinas después de los diferentes tratamientos de PEAIC varió entre 96,1% y 100,5%. El contenido de antocianinas en los zumos de fresa dependió del tiempo de tratamiento y la intensidad de capo eléctrico aplicado. En general, cuanto más largo es el tiempo y menor es la intensidad de campo eléctrico, mayor es la concentración de antocianinas en los zumos. En cambio, Zhang et al. (2007) observaron que la degradación de cyanidin-3-glucoside en una solución metanólica aumentó con el incremento de la intensidad de campo. Por tanto, parece que no todas las antocianinas reaccionan de la misma manera, siendo algunas más resistentes que otras a los diferentes factores de degradación (Markakis, 1982). Tratamientos de PEAIC intensos podrían estimular la transformación de las proantocianinas en antocianinas. Las proantocianinas se rompen dando lugar a antocianinas después de tratamientos con altas temperaturas en condiciones ácidas (Porter et al., 1986; Saint-Cricq de Gaulejac et al., 1999; Simonetti, et al., 1997). Aunque la temperatura máxima de procesado mediante PEAIC a 35 kV/cm fue de 37 ºC, el estrés producido por estas altas intensidades de campo podría explicar esta transformación. Sin embargo, es necesario ampliar los estudios para poder aclarar como los tratamientos por PEAIC inducen la conversión de proantocianinas en antocianinas.

De los dos modelos ajustados a los valores obtenidos, la distribución de Weibull presentó mayores valores de R^2_{adj} y menores de RMSE, X^2 y MBE que el modelo de primer orden. El parámetro γ estimado por el modelo de Weibull varió entre $5{,}07\times10^{-1}$ y $7{,}76\times10^{-1}$, dependiendo exponencialmente de la intensidad de campo aplicada (R^2_{adj} = 0,972). El parámetro α disminuyó al aumentar la intensidad de campo siguiendo un modelo de primer orden (R^2_{adj} = 0,873). Los valores de γ por debajo de 1 indican que al empezar el tratamiento existe una gran degradación de antocianinas que disminuye progresivamente a lo largo del tiempo. Esta tendencia concuerda con el hecho de que la estabilidad de las antocianinas está influida por la matriz del alimento, ya que algunos compuestos como los flavonoles y ácidos fenólicos pueden reaccionar con las antocianinas aumentando su resistencia a diferentes estreses (Brouillard, 1982). También se calculó el tiempo medio de procesado para que exista una completa

destrucción de las antocianinas, éste tiempo varió entre 959 y 3368 ms. Por tanto, se puede concluir que mediante los diferentes tratamientos de PEAIC estudiados no se llegará a destruir totalmente las antocianinas, ya que nunca se aplicaran tratamientos de PEAIC tan largos. El efecto combinado del tiempo de tratamiento (t) y la intensidad de campo eléctrico (E) sobre el contenido en antocianinas (AR) se describió mediante la ecuación 10, en la cual los dos parámetros de Weibull han sido sustituidos por sus respectivas ecuaciones de primer orden (R^2_{adj} = 0,937, RMSE = 0,389, MBE = 0,389 y χ^2 = 0,024).

$$AR(\%) = 100 \cdot exp\left[-\left(\frac{t}{\left[3569110 \cdot exp(-0.0536 \cdot E)\right]} \right)^{[0.2738 \cdot exp(0.0292 \cdot E)]} \right] \quad (10)$$

Capacidad Antioxidante

La retención de la capacidad antioxidante en los zumos de fresa fue significativamente menor cuando el tiempo de tratamiento aumentó y la intensidad de campo eléctrico aplicada disminuyó. Los tratamientos de PEAIC a 20 kV/cm durante 2000 µs dieron lugar a zumos de fresa con las menores retenciones de capacidad antioxidante. Tal como se observó es el estudio anterior, la capacidad antioxidante del zumo de fresa se asocia principalmente al contenido en antocianinas, más que a la concentración de vitamina C presentes en los mismos.

Tanto el modelo de primer orden como el de Weibull relacionaron bien los cambios de la capacidad antioxidante para cada una de las intensidades de campo estudiadas; no obstante, el modelo de Weibull mostró mayor exactitud. Los parámetros γ y α de la distribución de Weibull se relacionaron con la intensidad de campo eléctrico a partir de ecuaciones de primer orden. Tal como sucedió para la vitamina C y antocianinas, sustituyendo en la distribución de Weibull los parámetros γ y α por sus respectivas ecuaciones de primer orden se obtuvo un modelo secundario capaz de relacionar la variación de la capacidad antioxidante (CR) con el tiempo de tratamiento (t) y la intensidad de campo (E) (ecuación 11) (R^2_{adj} = 0,874, RMSE = -0,428, MBE = 1,01 y χ^2 = 6,25).

$$CR(\%) = 100 \cdot exp\left[-\left(\frac{t}{\left[35052 \cdot exp(-0.044 \cdot E)\right]} \right)^{[0.158 \cdot exp(0.0758 \cdot E)]} \right] \quad (11)$$

Cambios en las propiedades antioxidantes de zumo de fresa pasterizado por pulsos eléctricos de alta intensidad de campo o por calor durante el tiempo de almacenamiento

Para completar el estudio, se comparó el efecto del tratamiento de pasteurización por PEAIC con los de pasterización térmica. El procesado por PEAIC se llevó a cabo a 35 kV/cm durante 1700 µs con pulsos bipolares de 4-µs a 100 Hz siguiendo las condiciones de pasteurización mediante PEAIC propuestas por Mosqueda-Melgar (2007). Además, se determinó la evolución de las principales propiedades antioxidantes del zumo de fresa a lo largo del tiempo de almacenamiento a 4 ºC durante 56 días.

Compuestos fenólicos

El contenido en compuestos fenólicos totales se calculó a partir de la suma de cada uno de los compuestos fenólicos individuales obtenidos por HPLC. Los zumos de fresa tratados térmicamente durante 60 s (43,6 mg/100ml) presentaron menores cantidades de fenoles totales que el fresco (47,3 mg/100ml) y que los procesados con otros tratamientos. La pérdida del contenido en compuestos fenólicos totales durante el tiempo de conservación fue de 21,5-24,1 mg/100ml después de 56 días.

Entre los ácidos fenólicos estudiados, el ácido elágico (4,1-4,5 mg/100ml) fue el que se encontró en mayor proporción en los zumos de fresa, seguido por el *p*-cumárico (1,69-1,70 mg/100 ml) y el *p*-hidroxibenzoico (0,39-0,43 mg/100 ml). El tratamiento de pasteurización aplicado afectó al contenido en ácidos fenólicos de los zumos. La concentración de ácido *p*-hidroxibenzoico aumentó considerablemente después de los diferentes tratamientos en comparación con el zumo fresco; en cambio, el ácido elágico se redujo después del tratamiento térmico (90 ºC-60 s). Este aumento del contenido en ácido *p*-hidroxibenzoico podría estar relacionado con la transformación de otros compuestos fenólicos. Sin embargo, los mecanismos implicados en la biosíntesis de los ácidos hidroxibenzoicos apenas han sido estudiados, particularmente en el caso de frutas y verduras. Por otra parte, el contenido en *p*-cumárico y *p*-hidroxibenzoico disminuyó sustancialmente a lo largo del tiempo alcanzando valores de 1,33-1,49 y 0,23-0,26 mg/100 ml a los 56 días. Por el contrario, la concentración de ácido elágico aumentó durante los primeros 28 días de conservación, para reducirse posteriormente, pero siempre manteniéndose por encima de los contenidos iniciales. Se obtuvieron mayores concentraciones de ácido *p*-cumárico y elágico durante el tiempo en zumos de fresa tratados por PEAIC que en los tratados por calor.

El contenido total de antocianinas en zumos tratados mediante PEAIC (38,1 mg/100 ml) y calor (36,2-38,0 mg/100 ml) fue un poco menor que el obtenido para el zumo fresco (39,5 mg/100ml). Estas reducciones pueden asociarse con altas temperaturas de tratamiento que favorecen la formación de compuestos poliméricos marrones a partir de la degradación de las antocianinas. Por tanto la temperatura alcanzada (90 ºC) durante los tratamientos térmicos podría acelerar la degradación de estos flavonoides. Las principales antocianinas analizadas en los zumos de fresa fueron pelargonidina-3-glucósido, cianidina-3-glucósido y pelargonidina-3-rutinósido. Tal como se observó en antocianinas totales, la concentración de pelargonidina-3-glucósido y cianidina-3-glucósido disminuyó respecto al zumo fresco después de los distintos tratamientos de pasteurización. Los niveles de cianidina-3-glucósido en los zumos de fresa aumentaron durante los primeros 21 días de conservación, disminuyendo posteriormente hasta niveles de 1,12-1,47mg/100ml a los 56 días. Por el contrario, el contenido en pelargonidina-3-glucósido y pelargonidina-3-rutinósido se redujo progresivamente durante todo el

almacenamiento, encontrándose mayores niveles de ambas antocianinas en zumos tratados por PEAIC. Estas variaciones del contenido en antocianinas podrían estar asociadas al aumento de la actividad residual de algunas enzimas después de la pasteurización de los zumos, tal como se ha discutido anteriormente.

El contenido en flavonoles analizados en los zumos fue menor que el de otros compuestos fenólicos como las antocianinas y ácidos fenólicos. El kaempferol fue el flavonol encontrado en mayor cantidad (0,57-0,60 mg/100 ml) en los zumos. La concentración inicial de quercitina varió entre 0,38 y 0,40 mg/100ml, mientras que el contenido de miricetina osciló entre 0,19-0,21 mg/100ml. La cantidad de flavonoles en fresas y sus derivados varía en función de la variedad, origen geográfico, condiciones de crecimiento y recolección, además del método utilizado para la extracción/hidrólisis y análisis utilizados (Kosar et al., 2004). El procesado del zumo de fresa no afectó su contenido en flavonoles. En cambio, este contenido se redujo durante el almacenamiento independientemente del proceso de pasterización aplicado. Los niveles de miricetina diminuyeron considerablemente durante la conservación de los zumos de fresa, alcanzando niveles por debajo del límite de detección a partir de 21-28 días de almacenamiento. No se observaron diferencias significativas en el contenido en flavonoles entre los zumos tratados por PEAIC por calor.

Vitamina C

La concentración de vitamina C en los zumos de fresa varió entre 61,6 mg/100 ml (térmico a 90 ºC-60s) hasta 65,2 mg/100 ml (fresco). Estos resultados concuerdan con los obtenidos por Klopotek (2005) quienes obtuvieron niveles de vitamina C entre 37 y 69 mg/100 ml en diferentes zumos de fresa. El tratamiento por PEAIC dio lugar a zumos con mayor contenido en vitamina C (98%) que los tratamientos térmicos (94-95%). Estas diferencias en la retención de vitamina C después de los tratamientos pueden ser debidas a las elevadas temperaturas alcanzadas durante el procesado. Pese a que la ingesta diaria recomendada (IDR) de vitamina C está siendo actualmente revisada en múltiples estudios, la cantidad mínima recomendada establecida por la FDA corresponde a 60 mg (FDA, 1999). Tomando este valor como referencia, la concentración de vitamina C en una ración de 250 ml de zumo de fresa debería de ser de 24 mg/100 ml para aportar el 100% de la IDR. En el zumo procesado mediante PEAIC o por calor (90 ºC-30 s) la concentración de vitamina C cayó por debajo del IDR a los 35 días de conservación, mientras que cuando el zumo se trató a 90 ºC durante 60 s éste conservó la cantidad necesaria para aportar la IDR durante un máximo de 28 días. Por tanto, aunque no se observaron diferencias significativas en el contenido de vitamina C de los zumos tratados durante los primeros 21 días de almacenamiento, a partir de ese día, el zumo de fresa tratados a 90 ºC-60 s presentó menores retenciones que los tratados mediante PEAIC o 90 ºC-30 s. Cabe destacar que la concentración de vitamina C en zumos de fresa se mantuvo por encima del 50% del contenido inicial durante 21 días, independientemente del tratamiento de pasteurización aplicado.

Capacidad Antioxidante

De acuerdo con los métodos del DPPH y del ABTS, la capacidad antioxidante del zumo fresco fue mayor que la obtenida para los zumos de fresa pasteurizados, inmediatamente después del procesado. De igual manera, Elez-Martínez y Martín-Belloso (2007) no encontraron diferencias en la capacidad antioxidante de zumos de naranja tratados por PEAIC (15 o 35 kV/cm durante 1000 µs a 200 Hz y pulsos bipolares de 4-µs) y calor (90 ºC, 1 min).

Los zumos de fresa sufrieron una disminución importante del contenido total de antocianinas y de vitamina C durante los diferentes tratamientos de pasteurización, lo que podría explicar la disminución de la capacidad antioxidante después del procesado. Los zumos tratados presentaron mayores capacidades antioxidantes que los frescos a partir de los 7 días de conservación. Por tanto, la pasteurización del zumo de fresa no sólo permite obtener productos sanos, si no que también ayuda a mantener el potencial antioxidante de los mismos. La capacidad antioxidante disminuyó sustancialmente durante la primera semana de conservación, para luego permanecer casi inalterable mostrado valores entre 275 y 307 μmol Trolox/100 ml a los 56 días. Los valores obtenidos de DPPH y ABTS se correlacionaron bien, tanto con los contenidos en vitamina C, como con las concentraciones de antocianinas.

TOMATES Y FRESAS FRESCOS CORTADOS

Las dificultades en la comercialización de fruta fresca cortada son debidas principalmente a su inherente característica de materia viva. Las operaciones durante el procesado provocan un fuerte estrés fisiológico, las consecuencias finales del cual serán oxidación, reblandecimiento de los tejidos y alteración de la calidad final del producto. La modificación de la atmósfera de envasado de frutas frescas cortadas permite limitar la actividad respiratoria y los procesos de anaerobiosis, además de obtener un producto microbiológicamente estable que conserve sus propiedades físico-químicas y organolépticas. En base a este planteamiento, se evaluaron distintas alternativas de envasado en atmósferas modificada para la conservación de las propiedades antioxidantes de tomate y fresa frescos cortados.

En primer lugar, se determinó la influencia de la variedad en el mantenimiento de las principales propiedades antioxidantes de los tomates frescos cortados durante la vida útil. Seguidamente, se llevo a cabo el estudio del efecto de distintas temperaturas de conservación en el potencial antioxidante de tomate y fresa frescos cortados. Finalmente, se procedió a la evaluación de los efectos de distintas atmósferas de envasado en los compuestos bioactivos individuales de tomate y fresa frescos cortados. Se estudió el uso de concentraciones de O_2 altas como alternativa al envasado en concentraciones de O_2 bajas o aire.

TOMATE

Influencia de la variedad en el mantenimiento de las propiedades antioxidantes de tomate fresco cortado durante el tiempo de almacenamiento

Varios estudios sugieren que atmósferas con concentraciones de O_2 reducidas y elevadas de CO_2 mantienen la calidad microbiológica y organoléptica en tomate fresco cortado almacenado a 5 ºC durante 14 días (Artés et al., 1999; Hong y Gross, 2001; Aguayo et al., 2004). Por tanto, los tomates frescos cortados de las diferentes variedades (Rambo, Durinta, Bodar, Pitenza, Cencara y Bola) se envasaron en atmósferas modificadas de 5 kPa O_2 y 5 kPa CO_2 y se conservaron durante 21 días a 4 ºC.

Licopeno

El contenido en licopeno en los tomates frescos cortados varió según la variedad procesada. El mayor contenido en licopeno se determinó en tomates de la variedad Bodar (80,5 mg/kg), mientras que en las otras variedades la concentración varió entre 20,0 y 43,1 mg/kg. Los tomates de las variedades Pitenza (40,2 mg/kg) y Cencara (43,1 mg/kg) presentaron niveles similares de licopeno. Los resultados obtenidos concuerdan con los niveles de licopeno determinados en otros estudios (Martínez-Valverde et al., 2002; George, et al., 2004; Spagna et al., 2005). El punto de maduración durante la recolección, la fertilización del suelo, la intensidad de la luz y la temperatura día/noche pueden afectar a la formación de licopeno en los tomates (Heinonen et al., 1989).

La concentración de licopeno en las diferentes variedades de tomate se mantuvo durante los 21 días de almacenamiento a 4 ºC, con excepción de la variedad Rambo y Bodar. En la variedad Rambo el contenido en licopeno permaneció constante hasta el día 14 y a partir de ese día fue significativamente inferior. En cambio, el contenido en licopeno de la variedad Bodar diminuyó progresivamente a lo largo del tiempo de almacenamiento pasando de valores iniciales de 77,9 mg/kg hasta valores de 42,0 mg/kg el día 21. Estudios similares realizados en tomate fresco cortado (Lana, 2005) concluyeron que durante el almacenamiento se forma licopeno como consecuencia de la maduración del fruto, sin embargo, parte de este licopeno se destruye debido a la oxidación. Según Wright y Kader (1997a), la conservación en atmósfera modificada y temperaturas bajas (5 ºC), mantiene prácticamente inalterada la concentración de carotenoides en fruta fresca cortada. En condiciones adecuadas, la vida útil comercial de estos productos finaliza antes de que se produzca una pérdida significativa de carotenoides (Cano et al., 2005).

Vitamina C

El contenido en vitamina C varió entre 69,6 y 212,3 mg/kg en las distintas variedades de tomates estudiadas. La variedad Durinta presentó la mayor concentración de vitamina C, mientras que los menores niveles se dieron en tomates de la variedad Rambo. No se detectaron diferencias significativas en el contenido de vitamina C de tomate de las distintas variedades durante el almacenamiento. Según Cano et al. (2005), los tejidos vegetales con pH ácido ejercen un efecto protector frente a la degradación de la vitamina C. Además, la combinación de atmósferas adecuadas y bajas temperaturas reduce la degradación de esta vitamina en frutas cortadas (Kader, 1987). Los niveles reducidos de oxígeno en la atmósfera modifican el metabolismo celular e inhiben la reducción del ácido ascórbico. Estudios realizados en otros productos frescos cortados (Gil et al., 1999; Soliva-Fortuny et al., 2002) demuestran que la magnitud de la degradación de la vitamina C está relacionada con la cantidad de oxígeno en el envase.

Fenoles totales

Los niveles de fenoles totales en las seis variedades estudiadas de tomates variaron entre 187,4 mg ácido gálico/kg (Rambo) a 335,9 mg ácido gálico/kg (Durinta). La concentración de compuestos fenólicos se mantuvo casi invariable durante los primeros 14 días de almacenamiento. No obstante, el contenido en compuestos fenólicos aumentó significativamente a partir de ese día alcanzando valores máximos de 347,5 mg ácido gálico/kg en la variedad Durinta. Este aumento puede ser debido a las condiciones de hipoxia que se producen en el interior del envase juntamente con el aumento del dióxido de carbono. Según

Salomos (1994), en productos mínimamente procesados, la hipoxia en combinación con altas concentraciones de dióxido de carbono inhiben la síntesis de aquellas enzimas que ejercen efectos nocivos sobre la calidad de los frutos y esta inhibición incrementa la cantidad de compuestos fenólicos en los tejidos. Factores bioticos y abióticos como estrés, bajas temperaturas, presencia de heridas así como de microorganismos inducen a los vegetales a la síntesis de compuestos fenólicos (Dixon y Paiva, 1995).

Capacidad Antioxidante

Los tomates de la variedad Bola presentaron los máximos valores de capacidad antioxidante (26,3 % de inhibición del radical DPPH). A pesar de que los compuestos bioactivos presentes en los tomates cortados se mantuvieron o aumentaron durante el tiempo de almacenamiento, la actividad antioxidante del tomate fresco cortado de las diferentes variedades disminuyó significativamente a lo largo del tiempo. Según Lana y Tijskens (2006), esta disminución es debida a un cambio en la composición de los compuestos antioxidantes debida a la maduración del fruto durante el envasado. Los resultados del presente estudio indican que existe una buena correlación entre el contenido en vitamina C de los tomates frescos cortados y la capacidad antioxidante de los mismos.

Efecto del tiempo y temperatura de almacenamiento sobre la vida útil y propiedades antioxidantes de tomate fresco cortado

Tomate fresco cortado de la variedad Bola se almacenó en atmósferas reducidas de O_2 y altas de CO_2 durante 14 días a distintas temperaturas (5, 10, 15 y 20 ºC) para estudiar el efecto del tiempo y temperatura de almacenamiento en la vida útil y propiedades antioxidantes de los mismos.

Estabilidad microbiológica

Los recuentos iniciales de aerobios mesófilos así como los de levaduras en tomate fresco cortado fueron inferiores a 10 ufc/g. La temperatura de almacenamiento influyó significativamente en el crecimiento microbiano durante el periodo de conservación. La velocidad de crecimiento fue mayor en tomates cortados almacenados a 15 y 20 ºC que en los conservadas a 5 o 10 ºC. Las atmósferas modificadas alargan la vida útil de los productos frescos cortados inhibiendo el crecimiento microbiano, no obstante, el almacenamiento de estos productos a elevadas temperaturas estimula la proliferación de la flora microbiana inicial (Nguyen y Carlin, 1994). Así, se observaron recuentos totales por encima de 10^7 ufc/g a los 4 días de almacenamiento en tomates frescos cortados conservados a temperaturas superiores a 10 ºC. De la misma manera, los tomates frescos cortados almacenados a 10 ºC alcanzaron recuentos de 10^7 ufc/g después de 7 días, siendo fácilmente visible el deterioro del producto. Los recuentos de microorganismos aerobios mesófilos se mantuvieron por debajo de 5×10^7 ufc/g durante los 14 días de almacenamiento a 5 ºC, nivel máximo aceptable de acuerdo con la legislación española que regula este tipo de productos (BOE, 2000).

Licopeno

El contenido en licopeno se mantuvo durante los 14 de almacenamiento en tomates frescos cortados almacenados a temperaturas por debajo de los 10 ºC. En cambio, la concentración de licopeno en las rodajas conservadas a 15 y 20 ºC aumentó durante el periodo de conservación

desde niveles de 22,1-24,7 mg/kg hasta 34,6-46,7 mg/kg a los 14 días. Mencarelli y Salveit (1988) sugirieron que durante la maduración del fruto se sintetizan carotenos, entre ellos el licopeno, sin embargo esta síntesis tiene lugar entre 12 y 32 ºC. Además, gran cantidad de bacterias y mohos pueden formar carotenos durante su fase secundaria a partir de ácido mevalónico (Shukolyukov et al., 2001). De hecho, los altos recuentos microbianos en tomates frescos cortados podrían estar relacionados con estos incrementos en licopeno. Por otro lado, el licopeno se oxida fácilmente en presencia de oxígeno y luz; además su oxidación está favorecida a pH bajo (Shi y Le Maguer, 2000). Rodríguez-Amaya (1993) observó que la estabilidad del licopeno durante el almacenamiento depende de la disponibilidad de oxígeno y de las condiciones de envasado. En el presente trabajo, las variaciones de licopeno durante el almacenamiento no se pudieron asociar con los niveles de O_2 en los envases. En cambio, se observó una buena correlación entre el contenido en licopeno y la concentración de CO_2 en los envases.

Vitamina C

No se observaron cambios sustanciales en el contenido en vitamina C de los tomates frescos cortados almacenados a 5 y 10 ºC. Otros autores (Lee y Kader, 2000) han observado la eficacia de las atmósferas modificadas en combinación con bajas temperaturas de refrigeración para mantener la vitamina C en productos frescos cortados. Por otra parte, la concentración inicial de vitamina C de las rodajas de tomate conservadas a 15 ºC se mantuvo constante durante los primeros 11 días de almacenamiento; después de ese día el contenido disminuyó significativamente, alcanzándose valores de 76,8 mg/kg a los 14 días. En tomates frescos cortados almacenados a 20 ºC el contenido en vitamina C disminuyó considerablemente de 124,1 mg/kg hasta 17,3 mg/kg a los 14 días de conservación. Elevadas temperaturas de conservación asociadas con concentraciones excesivamente reducidas de O_2 y altas de CO_2 podrían provocar un aumento del estrés oxidativo que explicaría la activación de enzimas que catalizan la oxidación del ácido ascórbico como la ascorbato POD (Pinto et al., 2001; Tudela, et al., 2002). El daño oxidativo asociado a la acumulación de especies de O_2 reactivas como el superóxido (O_2^-) y peróxido de hidrógeno (H_2O_2), el cual actúa como sustrato de las reacciones catalizadas por la POD, puede causar la peroxidación de lípidos de las membranas celulares con la subsiguiente pérdida de su integridad.

Fenoles totales

La concentración inicial de compuestos fenólicos de las rodajas de tomate se mantuvo constante durante los primeros 4 días de almacenamiento en todas las muestras analizadas. No obstante, el contenido en estos compuestos aumentó significativamente a partir de este período en tomates cortados conservados en temperaturas entre 10 y 20 ºC, alcanzándose valores máximos de 444,7 mg ácido gálico/kg a los 14 días de almacenamiento a 20 ºC. Se ha demostrado que la síntesis y acumulación de fenilpropanoides en diferentes tejidos es una respuesta a distintas situaciones de estrés (Kang y Salveit, 2002). Por tanto, esta acumulación de compuestos fenólicos en rodajas de tomate podría ser respuesta del daño fisiológico, favorecido por altas temperaturas de conservación y concentraciones excesivamente bajas de O_2 y altas de CO_2. Dichas condiciones podrían estimular la actividad de la enzima fenilalanina amonio-liasa (PAL) con la consecuente producción de compuestos fenólicos (Salveit, 1997). Este incremento de la actividad enzimática podría producirse a través de la formación de especies de O_2 reactivas como consecuencia del estrés oxidativo (Reyes et al., 2007).

Capacidad Antioxidante

La actividad antioxidante del tomate fresco cortado disminuyó significativamente a partir del 2º día de almacenamiento independientemente de la temperatura. Sin embargo, a partir de ese día no se observaron variaciones importantes en la capacidad antioxidante de las rodajas de tomate. El almacenamiento de las muestras a 5 y 10 ºC conllevó una menor reducción de la capacidad antioxidante que los conservados a temperaturas mayores de 10 ºC. En el presente estudio, la capacidad antioxidante no se relacionó bien ni con el contenido en vitamina C, ni con el de compuestos fenólicos. De hecho, un gran número de sustancias como flavonoides, ácidos fenólicos, aminoácidos, tocoferoles y pigmentos contribuyen a la capacidad antioxidante de frutas y vegetales (Chu et al., 2000).

Por tanto, los resultados muestran que el almacenamiento del tomate fresco cortado a 5 ºC no sólo alarga la vida útil del producto desde un punto de vista microbiológico, sino que también contribuye al mantenimiento del potencial antioxidante del mismo.

Efecto de la atmósfera de envasado sobre las propiedades antioxidantes de tomates frescos cortados

Se estudió y comparó el efecto de la aplicación de distintas atmósferas de envasado (2,5 kPa O_2 + 5 kPa CO_2, 10 kPa O_2 + 5 kPa CO_2, 21 kPa O_2, 60 kPa O_2, 80 kPa O_2) en el contenido en carotenoides, vitamina C, compuestos fenólicos y capacidad antioxidante de tomate fresco cortado variedad Bola almacenado durante 21 días a 4 º C.

Carotenoides

Los tomates frescos cortados envasados en atmósferas ricas en oxígeno presentaron mayores concentraciones de carotenos que los envasados en condiciones de hipoxia durante el tiempo de almacenamiento (21 días). En cambio, el contenido en carotenos totales de las rodajas de tomates envasados en 2,5 kPa O_2 + 5 kPa CO_2 y 10 kPa O_2 + 5 kPa CO_2 disminuyó a lo largo del tiempo de almacenamiento a pesar de las restricciones de oxígeno en los envasases. Altas concentraciones de CO_2 (7,5 kPa CO_2) podrían incrementar la síntesis de carotenos durante el almacenamiento de productos frescos cortados (Wright y Kader 1997a). Kader y Ben-Yehoshua (2000) observaron que la maduración de los tomates se veía acelerada cuando éstos se conservaban en 40-50 kPa O_2. La presencia de O_2 en los envases induce rápidamente la actividad de enzimas asociadas con la formación de etileno (Abeles et al., 1992). Este aumento en etileno provoca cambios fisiológicos, como la maduración de los frutos climatéricos como el tomate (Li et al., 1992).

El licopeno es el caroteno que predomina en tomates frescos cortados, su contenido varió entre 31,4 y 33,7 mg/kg. Otros carotenos precursores del licopeno tales como el fitoflueno y neurosporeno se hallaron en menor cantidad. En general, la concentración de licopeno en las rodajas de tomate permaneció constante durante los primeros 4 días de almacenamiento a 4 ºC y a partir de ese día aumentó considerablemente en los tomates frescos cortados envasados con altas concentraciones de oxígeno ($\geq$ 21 kPa O_2), alcanzando valores de 47-50 mg/kg a los 21 días. Por el contrario, el contenido en neurosporeno disminuyó sustancialmente durante este periodo, alcanzando contenidos por debajo del límite de detección a partir de los 14-21 días de almacenamiento en las muestras envasadas en $\leq$ 10 kPa O_2. En el caso del fitoflueno, el contenido se redujo durante los primeros 11 días de conservación independientemente de la

atmósfera aplicada; no obstante, a partir de este día los tomates frescos cortados envasados en atmósferas ricas en oxígeno aumentaron los niveles hasta 4,1-4,2 mg/kg. Por tanto, atmósferas altas en oxígeno podrían inducir la formación de licopeno a partir de otros carotenos. A través de una serie de reacciones de desaturación el fitoeno se puede transformar en licopeno, dando lugar a productos intermedios como el fitoflueno, ξ-caroteno y neurosporeno (Britton y Hornero-Mendez, 1997). Sin embargo, no existe mucha información sobre los mecanismos de biosíntesis de carotenos en plantas. Las enzimas implicadas en la síntesis de carotenos están codificadas por genes nucleares, y las proteínas precursoras son importadas a los plástidos donde se producirá la biosíntesis de carotenos. Condiciones de estrés, seguidas por exposiciones a distintas atmósferas de envasado, podrían promover la transcripción de los genes y el transporte del mRNA (Bartley y Scolnik, 1995).

Tal como sucedió en el caso del licopeno, el contenido en β-caroteno aumentó a lo largo del almacenamiento en los tomates frescos cortados envasados en ≥ 10 kPa O_2. Por el contrario, el contenido en γ-caroteno se redujo durante la conservación en todas las muestras estudias, debido quizás a su conversión en β-caroteno, ya que la ciclación del γ-caroteno da lugar a una molécula de β-caroteno (Britton y Hornero-Mendez, 1997).

Compuestos fenólicos

El contenido inicial en compuestos fenólicos de las rodajas de tomate, calculado como suma de compuestos fenólicos individuales analizados por HPLC, fue de 45,0-45,7 mg/kg. Si comparamos estos resultados con los niveles de fenoles determinados a través del método del reactivo de Folin-Ciocalteu para la variedad de tomate Bola (302,3 mg/kg) se observa que el contenido en compuestos fenólicos en las rodajas de tomate es mayor cuando se usa el método colorimétrico. El método de Folin-Ciocalteu normalmente sobre estima el contenido en compuestos fenólicos ya que otros compuestos reductores como el ácido ascórbico pueden interferir reaccionando con el reactivo (Martínez-Valverde et al., 2002).

El principal compuesto fenólico encontrado en rodajas de tomate fue el ácido clorogénico (21-22 mg/kg). Se observó un aumento importante de este ácido en tomate fresco cortado envasado en concentraciones altas de O_2. Por otra parte, el contenido en flavonoles se incrementó a lo largo del tiempo independientemente de la atmósfera inicial aplicada. Este incremento del contenido en flavonoles como la quercitina y el kaempferol durante el almacenamiento de tomate fresco cortado podría explicar la disminución del contenido de ácido *p*-cumárico durante este periodo. El metabolismo general fenilpropanoide consiste en una serie de reacciones que llevan a la síntesis de ácido *p*-cumárico a partir de fenilalanina, catalizado por la PAL. A partir de la adición de tres moléculas de malonil-CoA al ácido *p*-cumárico, se pueden obtener chalconas, que a través de reacciones de isomerización, hidroxilación y desaturación se convierten en flavonoles (Heller, 1986). Cabe destacar que el aumento del contenido en flavonoles durante los 21 días de almacenamiento fue menor en las rodajas de tomate envasadas en atmósferas pobres en O_2 (≤ 10 kPa). Zheng et al. (2003) observaron que atmósferas modificadas con altos contenidos en O_2 incrementan el contenido en quercitina y kaempferol en arándanos en mayor proporción que cuando se conservan en aire. Otros compuestos fenólicos minoritarios determinados en tomate fresco cortado fueron el ácido cafeico (2,5-2,7 mg/100ml) y ferúlico (3,9-4,2mg/kg).

Vitamina C

El contenido inicial de vitamina C en los tomates frescos cortados osciló entre 108 a 120 mg/kg. La oxidación de la vitamina C se incrementó por la presencia de oxígeno. A mayor concentración de oxígeno en el interior del envase más importante fue la pérdida de vitamina C a lo largo del tiempo de almacenamiento. El contenido en vitamina C disminuyó substancialmente en tomate frescos cortados envasados en atmósferas ricas en oxígeno (≥ 10 kPa), siendo las rodajas conservadas en 80 kPa O_2 las que presentaron menor contenido en vitamina C a los 21 días (50 mg/kg). Sin embargo, la conservación del tomate fresco cortado envasado en atmósferas pobres en oxígeno (2,5 kPa O_2 + 5 kPa CO_2) previno eficazmente las pérdidas de vitamina C, manteniendo los niveles iniciales durante todo el almacenamiento (108-113 mg/kg). Otros estudios realizados en fruta fresca cortada (Agar et al., 1999; Oms-Oliu et al., 2008a, 2008b) demostraron que la degradación de vitamina C es mayor en atmósferas modificadas ricas en O_2. Además, concentraciones altas de CO_2 (> 5 kPa) en la atmósfera de envasado, como consecuencia de la excesiva respiración de los frutos, podrían ser perjudiciales en la conservación del contenido en vitamina C (Wright y Kader, 1997b). Por tanto, la pérdida de vitamina C de los tomates frescos cortados envasados en atmósferas con alta concentración de oxígeno no solo es debida a la abundancia de oxígeno sino también a los altos niveles de CO_2 presentes en los envases.

Capacidad Antioxidante

La capacidad antioxidante de la fracción hidrofílica y lipofílica de los tomates frescos cortados se evaluó a través de los radicales libres DPPH (1,1-difenil-2-picrilhidrazil) y ABTS (catión 2,2'-azino bis 3-etilbenzotiazoline-6-sulfonato). La capacidad antioxidante de los extractos hidrofílicos disminuyó significativamente a partir del 2º día de almacenamiento, sobretodo en las rodajas de tomate envasados en atmósferas con alta concentración de oxígeno. Según los valores de capacidad antioxidante de la fracción hidrofílica medida a través del radical ABTS, los tomates frescos cortados presentaron valores parecidos independientemente de la atmósfera utilizada. Por otra parte, la capacidad antioxidante de los extractos lipofílicos de los tomates frescos cortados fue mayor en aquellas muestras envasadas en concentraciones altas de oxígeno (≥ 60 kPa O_2).

Además, se observó una clara relación entre la capacidad antioxidante de los extractos hidrofílicos de los tomates frescos cortados y el contenido en vitamina C de los mismos. El método de DPPH no es específico para ningún compuesto antioxidante, sino que da una idea de la capacidad antioxidante global del alimento (Gil et al., 2000). A pesar de que normalmente la capacidad antioxidante determinada mediante el radical ABTS está relacionada con el contenido en flavonoides y fenoles (Millar et al., 2000), no se observó una clara asociación entre estos compuestos y la capacidad antioxidante de los tomates frescos cortados. Las variaciones del contenido en carotenos se relacionaron directamente con los cambios de la capacidad antioxidante lipofílica durante el tiempo de almacenamiento.

Los resultados del presente estudio para cada una de las propiedades antioxidantes sugieren que la conservación de tomate fresco cortado en atmósferas modificadas pobres en O_2 es más adecuada que las atmósferas con alta concentración de O_2 para prevenir la pérdida de los principales compuestos bioactivos presentes en estos productos.

FRESA

Efecto del tiempo y temperatura de almacenamiento sobre las propiedades antioxidantes de fresa fresca cortada

Se estudió el efecto de distintas temperaturas de almacenamiento (5, 10, 15 y 20 ºC) en las propiedades antioxidantes de las fresas frescas cortadas. Además se propusieron diferentes modelos cinéticos para poder predecir los cambios en el potencial antioxidante de las fresas durante los 21 días de envasado. Para ello, los trozos de fresa se almacenaron en atmósferas modificadas altas en O_2, ya que según algunos autores (Van der Steen, et al., 2002) estás condiciones de envasado mantienen la vida útil desde un punto de vista microbiológico y sensorial del producto durante 14 días a 5 ºC. Los modelos cinéticos estudiados fueron el modelo de orden cero, de primer orden y la función de distribución de Weibull.

Antocianinas

El contenido en antocianinas totales en las fresas frescas cortadas dependió del tiempo y la temperatura de almacenamiento. En general, a menor tiempo y temperatura de conservación mayor contenido en antocianinas en los trozos de fresa. Algunos autores (Kırca y Cemeroğlu, 2003; Wang y Xu, 2007) han propuesto un modelo de primer orden para describir la degradación de antocianinas en zumos o concentrados de frutas a lo largo de su conservación a diferentes temperaturas. Estos autores obtuvieron constantes de primer orden que variaron entre $2{,}0\times10^{-3}$-$3{,}3\times10^{-1}$ días^{-1}. Si comparamos éstas constantes cinéticas, con las obtenidas en el presente estudio mediante el modelo de primer orden ($8{,}7\times10^{-3}$-$3{,}9\times10^{-2}$ días^{-1}) se puede concluir que la estabilidad de las antocianinas en los trozos de fresa durante el tiempo de conservación es similar que en zumos o concentrados.

De los modelos cinéticos estudiados, el modelo de Weibull es el que presentó mayores coeficientes de determinación (R^2_{adj} = 0,800-0,973) y menores valores de RMSE, X^2 y MBE. Las constantes cinéticas obtenidas a través de la distribución de Weibull aumentaron con la temperatura de almacenamiento. Esta tendencia coincide con la obtenida por Shin et al. (2008) quienes observaron un incremento de la degradación de las antocianinas a medida que se incrementaba la temperatura de conservación. La biosíntesis de antocianinas en fresas continúa durante su almacenamiento; no obstante, si estos productos se conservan con elevadas concentraciones de CO_2 la síntesis queda interrumpida (Gil et al., 1997). Niveles altos de CO_2 pueden modificar el pH del medio, diminuyendo la estabilidad de las antocianinas (Brouillard, 1982). De este modo, esta gran disminución de antocianinas durante el tiempo, a medida que aumenta la temperatura, se relacionó directamente con altas presencias de CO_2 en los envases. Por otra parte, se calculó el tiempo medio para la destrucción total de antocianinas y se obtuvieron valores entre 41 y 532 días. La vida útil de las fresas frescas cortadas envasadas en atmósferas ricas en O_2 es de aproximadamente 14 días (Van der Steen, et al., 2002); por tanto, la vida útil comercial de los trozos de fresa almacenados con 80 kPa O_2 finaliza antes de que se produzca una pérdida importante de antocianinas.

Las constantes cinéticas obtenidas mediante la distribución de Weibull se relacionaron con la temperatura de almacenamiento a través de la ecuación de Arrhenius (R^2_{adj} = 0,88) obteniéndose una energía de activación de 101,8 kJ/mol. Markakis (1982) obtuvo una energía de activación de 113,0 kJ/mol en fresas. De aquí se deduce que la susceptibilidad de las

antocianinas al incremento de la temperatura de almacenamiento es similar para fresas cortadas y enteras.

Vitamina C

La concentración inicial de vitamina C disminuyó significativamente durante el tiempo en todas las muestras de fresa analizadas, independientemente de la temperatura de almacenamiento. Ajustando los resultados a los modelos cinéticos, se comprobó que el modelo de orden cero no relaciona adecuadamente las variaciones de vitamina C a lo largo del tiempo en fresas frescas cortadas. En cambio, tanto el modelo de primer orden como el de Weibull predijeron adecuadamente (R^2_{adj} = 0,982-0,994) los cambios en el contenido en vitamina C durante el almacenamiento. Las constantes cinéticas obtenidas mediante el modelo de primer orden y a través de la distribución de Weibull aumentaron con la temperatura. Se ha comprobado que altas temperaturas de conservación favorecen la degradación de la vitamina C (Adisa, 1986). Además, las pérdidas de vitamina C en los trozos de fresa se relacionaron con la acumulación de CO_2 en los envases como consecuencia de las altas temperaturas de conservación. Altas concentraciones de O_2 y de CO_2 aumentan la actividad enzimática y aceleran la pérdida de vitamina C en los productos. Por otra parte, el tiempo medio para la degradación total de la vitamina C en los trozos de fresa fue de 16-30 días, dependiendo de la temperatura de almacenamiento. Considerando que los productos frescos cortados reducen su vida útil comercial a 4-7 días cuando éstos se almacenan a temperaturas ≥ 10 ºC (Artés-Hernández et al., 2007), los resultados sugieren que las fresas frescas cortadas mantendrán niveles altos de vitamina C durante la vida útil comercial del producto.

El efecto de la temperatura de almacenamiento sobre las constantes cinéticas de primer orden y de Weibull se relacionó mediante la ecuación de Arrhenius, obteniéndose valores de energía de activación de 33,3 kJ/mol y 33,8 kJ/mol, respectivamente.

Capacidad antioxidante

La capacidad antioxidante de las fresas frescas cortadas dependió de la temperatura y tiempo de tratamiento. Así, a menor tiempo y temperatura, mayor fue la capacidad antioxidante de los trozos de fresa. En el presente estudio, la disminución de la capacidad antioxidante durante el almacenamiento se asoció a las pérdidas de vitamina C y antocianinas durante el periodo de conservación.

El modelo de orden cero relacionó adecuadamente los cambios de la capacidad antioxidante durante el periodo de conservación a 10 y 15 º C. En cambio, se obtuvieron bajos coeficientes de determinación en fresas frescas cortadas envasadas a 5 y 20 ºC. El modelo de primer orden predijo bien las variaciones de la capacidad antioxidante durante el almacenamiento, excepto para fresas cortadas almacenadas a 5 ºC. Sin embargo, al ajustar el modelo de distribución de Weibull a los resultados se obtuvieron elevados coeficientes de determinación y bajos valores de RMSE, X^2 y MBE. Las constantes cinéticas de degradación de la capacidad antioxidante predichas mediante el modelo de Weibull, se incrementaron con la temperatura de conservación, tomando valores entre $4{,}1\times10^{-3}$ y $6{,}9\times10^{-2}$ días^{-1}. Esta tendencia coincide con la observada por otros autores (Gil et al., 1999) quienes sugirieron que las temperaturas bajas de conservación en frutas y verduras mantienen la capacidad antioxidante de las mismas. Temperaturas altas de conservación aumentan la acumulación de CO_2 en el envase; este hecho acelera el estrés oxidativo y altera la composición en sustancias antioxidantes, dando lugar a variaciones en la capacidad antioxidante (Lana y Tijskens, 2006). La dependencia de las

constantes cinéticas con la temperatura de almacenamiento se relacionó a través de la ecuación de Arrhenius, obteniendo valores de 119,7 kJ/mol. Comparando este valor con el obtenido por Suh et al. (2004) para la capacidad antioxidante de extractos de mora (41,1 a 82 kJ/mol), se observó que la capacidad antioxidante de los trozos de fresa es mas susceptible a un aumento de temperatura de conservación que la de los extractos de mora.

Efecto de la atmósfera de envasado sobre las propiedades antioxidantes de fresa fresca cortada

Tal como se realizó para el tomate fresco cortado, los trozos de fresa se almacenaron en distintas atmósferas de envasado (2,5 kPa O_2 + 7 kPa CO_2, 10 kPa O_2 + 7 kPa CO_2, 21 kPa O_2, 60 kPa O_2, 80 kPa O_2) para poder establecer la influencia de estas atmósferas en las principales propiedades antioxidantes.

Compuestos fenólicos

El ácido elágico (43,3-49,4 mg/kg) fue el ácido fenólico que se halló en mayor cantidad en los trozos de fresa, seguido por el ácido *p*-cumárico (12,9-13,2 mg/kg) y por el ácido *p*-hidroxibenzoico (4,1-7,9 mg/kg). El contenido inicial de ácido elágico se mantuvo durante los primeros cuatro días de almacenamiento, pero a partir de ese momento se observó un aumento de la concentración, alcanzándose valores máximos (69,1-78,3 mg/kg) a los 9-11 días. Tanto las fresas cortadas envasadas con concentraciones ricas en O_2 como en aire, disminuyeron su concentración de elágico alcanzando niveles entre 42,3-52,7 mg/kg al final del periodo de conservación. Zheng et al. (2007) observaron un comportamiento similar del ácido elágico durante el almacenamiento de fresa cortada en atmósferas $\geq$ 21 kPa O_2. Por otra parte, la reducción del ácido *p*-cumárico durante la conservación de fresa fresca cortada podría explicar el incremento del contenido en flavonoides durante este periodo, ya que a partir de una serie de reacciones el ácido *p*-cumárico puede ser transformado en flavonoides. La presencia de altas concentraciones de O_2 en los envases favoreció la oxidación de los ácidos fenólicos. Se observó una mayor pérdida de estos compuestos fenólicos en trozos de fresa conservados con concentraciones altas de O_2 que sin modificación de la atmósfera inicial. Zheng et al. (2007) sugirieron que niveles altos de O_2 pueden inducir la formación de compuestos fenólicos en fresas durante el inicio de la conservación, pero si el almacenamiento es prolongado, estas concentraciones altas de O_2 podrían acelerar la oxidación de los compuestos fenólicos.

La principal antocianina determinada en los trozos de fresa fue pelargonidina-3-glucósido, que representó el 85-86% del contenido inicial en antocianinas. La cianidina-3-glucósido (17,7-18,7 mg/kg) y la pelargonidina-3-rutinósido (56,5-61,1 mg/kg) se encontraron en menor proporción en las muestras analizadas. En general, la síntesis de antocianinas se vio estimulada en los trozos de fresa almacenados en aire o en concentraciones pobres de O_2. La síntesis de antocianinas en fresas almacenadas con altas concentraciones de CO_2 se redujo comparada con las muestras conservadas en aire (Gil et al., 1997). Una investigación más profunda de este fenómeno indicó que las dos enzimas que participan en la biosíntesis de antocianinas se ven influenciadas negativamente por estos altos contenidos en CO_2 (Holcroft y Kader, 1999). Además, la estabilidad de las antocianinas también depende del pH del medio. Altos niveles de CO_2 podrían aumentar el pH del tejido como consecuencia de la disolución del CO_2, dando lugar a la formación de compuestos incoloros a partir de las antocianinas (Brouillard, 1982).

Por otra parte, se determinó el contenido en quercitina (34,1-38,5 mg/kg) kaempferol (6,5-6,8 mg/kg) y miricetina (3,0-3,2 mg/kg) de los trozos de fresa. El contenido en quercitina aumentó considerablemente después de 11-14 días de almacenamiento en muestras envasadas en atmósferas ricas en O_2. Tal como se ha discutido en el caso de tomate fresco cortado, esta acumulación de quercitina podría ser una respuesta al daño fisiológico, favorecido por altas concentraciones de CO_2. A pesar de que los contenidos de kaempferol y miricetina se redujeron durante el almacenamiento en todas las muestras analizadas, las pérdidas fueron menores en fresas frescas cortadas envasadas en niveles altos de O_2.

Vitamina C

La concentración de vitamina C en las fresas frescas cortadas disminuyó sustancialmente durante los 21 de almacenamiento independientemente de la atmósfera inicial, siendo los trozos conservados en atmósferas de 80 kPa O_2 las que presentaron menor contenido en vitamina C a los 21 días (137,8 mg/kg). Al igual que en tomate fresco cortado, la presencia de oxígeno favoreció la oxidación de la vitamina C. Como ya se ha discutido previamente, no sólo las concentraciones altas de O_2 degradan la vitamina C en productos frescos cortado, sino que altos niveles de CO_2 en la atmósfera de envasado también podrían ser perjudiciales en la conservación del contenido en vitamina C. Por tanto, la pérdida de vitamina C de los trozos de fresa envasados en atmósferas con alta concentración de oxígeno es debida no sólo a la alta disponibilidad de oxígeno en el envase sino también a los altos niveles de CO_2 presentes en los mismos.

Capacidad antioxidante

Las fresas frescas cortadas envasadas con atmósferas $\leq$ 21 kPa O_2 mantuvieron mayores capacidades antioxidantes que las envasadas en concentraciones iniciales de 60 ó 80 kPa O_2. A los 21 días de almacenamiento, la capacidad antioxidante fue mayor en el producto envasado con 2,5 kPa O_2 + 7 kPa CO_2, mientras que muestras almacenadas en 80 kPa O_2 mostraron la menor capacidad antioxidante. Según Oms-Oliu et al. (2008a), las atmósferas pobres en O_2 también mantienen mejor la capacidad antioxidante de los trozos de pera que aquéllas con altos contenidos en O_2. Se observó una pérdida importante de la capacidad antioxidante durante los primeros 21 días de almacenamiento, que se relacionó con el contenido en vitamina C de los trozos de fresa. Aunque la capacidad antioxidante de fresa se ha correlacionado bien con el contenido en antocianinas (Zheng et al., 2003), en el presente estudio, los cambios producidos en la capacidad antioxidante de las fresas cortadas durante el almacenamiento estuvieron poco relacionados con las variaciones en el contenido en antocianinas.

BIBLIOGRAFIA

Abeles, F.B., Morgan, P.W., Saltveit, M.E. 1992. Ethylene in plant biology. Academic Press, New York.

Adisa, V.A. (1986). The influence of molds and storage factors on the ascorbic content of orange and pineapple fruits. *Food Chemistry,* 22,139-146.

Agar, I.T., Massantini, R., Hess-Pierce, B., Kader, A.A. (1999). Postharvest CO_2 and ethylene production and quality maintenance of fresh-cut kiwifruit slices. *Journal of Food Science*, 64, 433-440.

Aguayo, E., Escalona, V., Artés, F. (2004). Quality of fresh-cut tomato as affected by type of cut, packaging, temperature and storage time. *European Food Research and Technology*, 219, 492-499.

Aguiló-Aguayo, I., Soliva-Fortuny, R., Martín-Belloso, O. (2007). Effects of pulsed electric field or heat treatments on pectin methylesterase and polygalacturonase activities and viscosity during storage of tomato juice. IFT Annual Meeting, Chicago, IL. Institute of Food Technologists.

Aguiló-Aguayo, I., Sobrino-López, A., Soliva-Fortuny, R., Martín-Belloso, O. (2008). Influence of high-intensity pulsed electric fields processing on lipoxygenase and β-glucosidase activities in strawberry juice. *Innovative Food Science and Emerging Technologies*, 9, 455-462.

Artés, F., Conesa, M.A., Hernández, S., Gil, M.I. (1999). Keeping quality of fresh-cut tomato. *Postharvest Biology and Technology*, 17, 153-162.

Artés-Hernández, F., Rivera-Cabrera, F., Kader, A.A. (2007). Quality retention and potential shelf-life of fresh-cut lemon as affected by cut type and temperature. *Postharvest Biology and Technology*, 43, 245-254.

Bartley, G.E., Scolnik, P.A., 1995. Plant carotenoids: pigments for photoprotection, visual attraction, and human health. *Plant Cell*, 7, 1027-1038.

BOE. (2000). Normas de higiene para la elaboración, distribución y comercio de comidas preparadas. Real Decreto 3484/2000. pp. 1435-1441.

Britton, G., Hornero-Mendez, D. (1997). Carotenoids and colour in fruit and vegetables. En: Tomas-Barberan, F. A., Robins R. J. Phytochemistry of fruit and vegetables. Science Publications, Oxford. pp. 11–27.

Brouillard, R. (1982). Chemical structure of anthocyanins. En: Markakis, P. Anthocyanins as food colors. Food Science and Technology. Academic Press, London. pp. 1-40.

Cano, M.P., Sánchez-Moreno, C., Pascual-Teresa, S., de Ancos, B. (2005). Procesado mínimo y valor nutricional. En: González-Aguilar, G.A.; Gardea, A.A.; Cuamea-Navarro, F. Nuevas tecnologías de conservación. Productos vegetales frescos cortados. Centro de Investigación en Alimentación y Desarrollo, Mexico. Capítulo 7, pp.119-152.

Cortés, C., Esteve, M.J., Rodrigo, D., Torregrosa, F., Frígola, A. (2006) Changes of colour and carotenoids contents during high-intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology,* 44, 1932-1939.

Davey, M.W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science of Food and Agriculture*, 80, 825-860.

Dixon, R.A., Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell*, 7, 1085-1097.

Eberhardt, M.V., Lee, C.Y., Liu, R.H. (2000). Antioxidant activity of fresh apples. *Nature*, 405(6789), 903-904.

Elez-Martínez, P., Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102, 201-209.

FDA (1999). Nutritional labelling manual: a guide for developing and using databases. Center for Food Safety and Applied Nutrition, Washington D.C.

Gahler S., Otto K., Böhm V. (2003). Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products *Journal of Agricultural and Food Chemistry*, 51, 7962-7968.

George, B., Kaur, C., Khurdiya, D.S., Kapoor, H.C. (2004). Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. *Food Chemistry*, 84, 45-51.

Gil, M.I., Holcroft, D.M., Kader, A.A. (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *Journal of Agricultural and Food Chemistry*, 45, 1662-1667.

Gil, M.I., Ferreres, F., Tomás-Barberán F.A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal Agriculture and Food Chemistry*, 47, 2213-2217.

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, M., Kader, A. (2000). Antioxidant activity of pomegrate juice and its relationship with phenolic composition and processing. *Journal of Agriculture and Food Chemistry*, 48, 4581-4589.

Gross, J. (1991). Pigments in Vegetables. Chlorophylls and carotenoids. Van Nostrand Reinold, New York.

Heinonen, M.I., Ollilainen, V., Linkola, E.K., Varo, P.T., Koivistoinen, P.E. (1989). Carotenoids in Finnish food: vegetables, fruits and berries. *Journal Agricultural and Food Chemistry,* 37, 655-659.

Heller, W. (1986). Flavonoid biosynthesis: an overview. In: Cody, V., Middleton, E, Harbone, J.B. Plant flavonoids in biology and medicine: biochemical, pharmacological, and structure-activity relationships. Alan R. Liss, New York, pp. 25-42.

Holcroft, D.M., Kader, A.A. (1999). Controlled atmospheres-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit. *Postharvest Biology and Technology*, 17, 19-32.

Hong, J.H., Gross, K.C. (2001). Maintaining quality of fresh-cut tomato slices through modified atmosphere packaging and low temperature storage. *Journal of Food Science*, 66(7), 960-965.

Jackman, R.L., Smith, J.L. (1997). Anthocyanins and betalains. Natural Food colorants. En: Hendry, G.A.F., Houghton, J.D. Natural Food Colorants. Blackie Academic & Professional, Chapman & Hall, London. pp.244-309.

Jung, M.Y., Kim, S.K., Kim, S.Y. (1995). Riboflavin-sensitized photodynamic UV spectrophotometry for ascorbic acid assay in beverages. *Journal of Food Science*, 60, 360-363.

Kader, A.A. (1987). Respiration and gas exchange of vegetables. En: Weichmann, J. Postharvest Physiology of Vegetables. Marcel Dekker, New York. pp. 25-43.

Kader, A.A., Ben-Yehoshua, S. (2000). Effect of superatmospheric oxygen levels on postharvest physiology and quality on postharvest physiology and quality of fresh-cut fruits and vegetables. *Postharvest Biology and Technology*, 20, 1-13.

Kang, H.M., Salveit, M.E. (2002). Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agricultural and Food Chemistry*, 50, 7536-7541.

Khachik, F., Carvalho, L., Bernstein, P.S., Muir, G.J., Zhao, D.Y., Katz, N.B. (2002). Chemistry, distribution, and metabolism of total carotenoids and their impact on human health. *Experimental Biology and Medicine,* 227, 845-851.

Kidmose, U., Edelenbos, M., Nøbæk, R., Christensen, L. P. (2002).Colour stability in vegetables. En: MacDougall, D.B. Colour in Food: Improving quality. CRC Press, Boca Raton, Florida. pp. 179- 232.

Kırca, A., Cemeroğlu, B. (2003). Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chemistry*, 81, 583-587.

Klim, M., Nagy, S. (1988). An improved method to determine nonenzymic browning in citrus juices. *Journal of Agricultural and Food Chemistry*, 36, 1271–1274.

Klopotek, Y., Otto, K., Böhn, V. (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 53, 5640-5646.

Kosar, M., Kafkas, E., Paydas, S, Baser, K.H. (2004). Phenolic composition of strawberry genotypes at different mutation stages. *Journal of Agriculture and Food Chemistry* 52**,** 1586-1589.

Lana, M.M. (2005). Modelling quality of fresh-cut tomato based on stage of maturity and storage conditions. Wageningen Universiteit, Netherland. Tesis Doctoral.

Lana, M.M., Tijskens, L.M.M. (2006). Effect of cutting and maturity on antioxidant activity of fresh-cut tomatoes. *Food Chemistry*, 97, 203-211.

Lee H.S., Coates G.A. (1999). Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: a storage study. *Food Chemistry*, 65, 165-168.

Lee, S.K., Kader, A.A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology,* 20, 207-220.

Li, N., Parsons, B.L., Liu, D. and Mattoo, A.K. (1992). Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. *Plant Molecular Biology*, 18, 477-487.

Macheix, J.J., Fleuriet, A., Billot, J. (1990). Fruit phenolics. CRC Press, Boca Raton. Florida.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M.C., Lerici, C.R. (2001). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science and Technology*, 11(9-10), 340-346.

Markakis, P. (1982). Anthocyanins as food colors. Food Science and Technology. Academic Press, London.

Martínez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant capacity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture*, 82, 323-330.

Mencarelli, F., Salveit, M.E. (1988). Ripening of mature-green tomato fruit slices. *Journal of the American Society for Horticultural Science,* 113, 742-745.

Miller, H.E., Rigelhof, F., Marquart, L., Prakash A., Kanter, M. (2000). Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of the American College of Nutrition* 19, 312S-319S.

Min, S., Jin, Z.T., Zhang, Q.H. (2003). Commercial scale pulsed electric field processing of tomato juice. *Journal of Agricultural and Food Chemistry*, 51(11), 3338-3344.

Mosqueda-Melgar, J. (2007). Aplicación de pulsos eléctricos de alta intensidad de campo en combinación con sustancias antimicrobianas para garantizar la calidad e inocuidad microbiológica de zumos de frutas. Tesis Doctoral, Universidad de Lleida.

Nguyen, C., Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 34,371-401.

Nguyen, M.L., Schwartz, S.J. (1999). Lycopene: chemical and biological properties. *Food Technology*, 53(2), 38-45.

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008a). Antioxidant content of fresh-cut pears stored in high-O_2 active packages compared with conventional low-O_2 atmospheres and passive modified atmosphere packaging. *Journal of Agricultural and Food Chemistry*, 56, 932-940.

Oms-Oliu, G., Odriozola-Serrano, I., Soliva-Fortuny, R., Martín-Belloso, O. (2008b). The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres. *Food Chemistry*, 106, 1085-1092.

Pinto, A., Lentheric, I., Vendrell, M., Larrigaudiere, C. (2001). Role of fermentative and antioxidant metabolism in the induction of core browning in controlled-atmosphere stored pears. *Journal of the Science and Food Agriculture,* 81, 364-370.

Podsędek, A., Sosnowska, D., Anders, B. (2003). Antioxidative capacity of tomato products. *European Food Research and Technology*, 217(4), 296-300.

Porter L.J., Hrstich, L.N., Chan, B.G. (1986). The conversion of proanthocyanidins and prodelphinidins to cyanidin and delpinidin. *Phytochemistry*, 25, 223-230.

Ramakrishnan, T.V., Francis, F.J. (1980). Autooxidation of carotenoids and their relative polarity. *Journal of Food Quality*, 3, 25-34.

Rein M.J., Heinonen M. (2004). Stability and enhancement of berry juice color *Journal of Agricultural and Food Chemistry*, 52, 3106-3114.

Reyes, L.F., Villarreal, J.E., Cisneros-Zevallos, L.C. (2007). The increase in antioxidant capacity alter wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 101, 1254-1262.

Rodríguez-Amaya D.B. (1993). Stability of carotenoids during the storage of foods. En: Charalambous G. Shelf-life studies of foods and beverages. Elsevier, New-York. pp. 591-628.

Rodríguez-Amaya, D.B. (1997). Carotenoids and food preservation: the retention of provitamin A carotenoids in prepared, processed and stored food. USAID, OMNI Project.

Saint-Cricq de Gaulejac, N.,Glories, Y., Vivas, N. (1999). Free radical scavenging effect of anthocynins in red wines. *Food Research International*, 32, 327-333.

Salomos, T. (1994). Some biological and physical principles underlying modified atmosphere packaging. En: Wiley, R.C. Minimally processed refrigerated fruits and vegetables.Chapman & Hall, New York. Capítulo 5, pp. 183-225.

Salveit, M.E., (1997). Physical and physiological canges in minimally processed fruits and vegetables. En: Tomás-Barberán, F.A., Robin, R.D. Phytochemistry of Fruits and Vegetables. Oxford University Press, Oxford. pp. 205-220.

Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., Cano, P. (2005). Impact o high pressure and pulsed electric fields on bioactive compounds and antioxidant capacity of orange juice in comparison with traditional thermal processing. *Journal of Agricultural and Food Chemistry*, 53(11), 4403-4409.

Sánchez-Moreno, C., Plaza, L., de Ancos, B., Cano, P.M. (2006). Nutritional characterization of commercial traditional pasteurized tomato juices: carotenoids, vitamin C and radical-scavenging capacity. *Food Chemistry*, 98(4), 749-756.

Shi, J., Le Maguer, M. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Technology,* 40, 1-42.

Shin, Y., Ryu, J.A., Liu, R.H., Nock, J.F., Watkins, C.B. (2008). Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. *Postharvest Biology and Technology*, 49, 201-209.

Shukolyukov, S.A., Saakov V.S. (2001). American Cockroach (Periplaneta americana) Synthesizes Carotenoids from the Precursor [14C] Mevalonic Acid Pyrophosphate. *Biochemistry (Moscow)*, 66, 535-540.

Simonetti, P., Pietta, P., Testolin, G. (1997). Polyphenol content and total antioxidant potential of selected Italian wines. *Journal of Agricultural and Food Chemistry***,** 45, 1152-1155.

Soliva-Fortuny, R.C., Oms-Oliu, G., Martín-Belloso, O. (2002). Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67(5), 312-317.

Spagna, G., Barbagallo, R.N., Chisari, M., Branca, F. (2005). Characterization of a tomato polyphenol oxidase and its role in browning and lycopene content. *Journal of Agricultural and Food Chemistry*, 53, 2032-2038.

Stewart, A.J., Bozonnet, S., Mullen, W., Jenkins, G.I., Lean, M.E.J., Crozier, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *Journal of Agricultural and Food Chemistry*, 48, 2663-2669.

Suh, H.J., Kim, J.M., Lee, H., Lee, S.W., Choi, Y.M. (2004). Thermal kinetics on antiradical capacity of mulberry fruit extract. *European Food Research and Technology*, 219, 80-83.

Takeoka, G.R., Dao, L., Flessa, S., Gillespie, D.M., Jewell, W.T., Huebner, B., Bertow, D., Ebeler, S.E. (2001). Processing effects on lycopene content and antioxidant activity of tomatoes. *Journal of Agricultural and Food Chemistry*, 49(8), 3713-3717.

Tannenbaum, S.R., Archer, M.C., Young, V.R. (1985). Vitamins and minerals. En: Fennema O.R. Food Chemistry. Marcel Dekker Inc., New York. pp. 488-493.

Torregrosa, F., Cortés, C., Esteve, M.J., Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *Journal of Agricultural and Food Chemistry*, 53, 9519-9525.

Torregrosa, F., Esteve, M.J., Frígola, A., Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*, 73, 339-345.

Tudela, J.A., Espín, J.C., Gil, M.I. (2002). Vitamin C retention in fresh-cut potatoes. *Postharvest Biology and Technology*, 26, 75-84.

Van der Steen, C., Jacxsens, L., Devlieghere, F., Debevere, J. (2002). Combining high oxygen atmosphere with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberry. *Postharvest Biology and Technology*, 26, 49-58.

Vieira, M.C., Teixeira, A.A., Silva, C.L.M. (2000). Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuaçu (theobroma grandiflorum) nectar. *Journal of Food Engineering*, *43*, 1-7.

Wang, W.D., Xu, S.Y. (2007). Degradation kinetics of anthocyanins in blackberry juice and concentrate, 82, 271-275. *Journal of Food Engineering*, 82, 271-275.

Wright, K.P., Kader, A.A. (1997a). Effect of controlled-atmosphere storage on the quality and carotenoid content of sliced persimmons and peaches. *Postharvest Biology and Technology*, 10, 89-97

Wright, K.P., Kader, A.A. (1997b). Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. *Postharvest Biology and Technology*, 10, 39-48.

Wrolstad, R.E. (2000). Anthocyanins. En: Lauro, G.J., Francis, F.J. Natural Food Colorants. Science and Technology. Marcel Dekker, New York. pp. 237-252.

Zhang, Y., Liao, X. J., Ni, Y.Y., Wu, J.H., Hu, X.S., Wang, Z.F., Chen, F. (2007). Kinetic analysis of the degradation and its color change of cyanidin-3-glucoside exposed to pulsed electric field. *European Food Research and Technology*, 224, 597-603.

Zheng, Y., Wang, C.Y., Wang, S.Y., Zheng, W. (2003). Effect of high-oxygen atmospheres on blueberry phenolics anthocynins, and antioxidant capacity. *Journal of Agriculture and Food Chemistry*, 51, 7162-7169.

Zheng, Y., Wang, S.Y., Wang, C.Y., Zheng, W. (2007). Changes in strawberry phenolics, anthocynins, an antioxidant capacity in response to high oxygen treatments. *LWT- Food Science and Technology*, 40, 49-57.

Conclusiones

CONCLUSIONES

A partir de la información obtenida en este trabajo se puede concluir que los pulsos eléctricos de alta intensidad de campo y el cortado/envasado en atmósfera modificada pueden ser buenas alternativas a los tratamientos tradicionales; ya que con estas técnicas se pueden obtener zumos y frutos frescos cortados con alto potencial antioxidante durante un mayor tiempo.

En particular, se pueden deducir las siguientes conclusiones:

A) Zumos de tomate y fresa tratados mediante PEAIC

- Las condiciones de tratamiento mediante PEAIC se optimizaron en términos de frecuencia, anchura de pulso y polaridad para obtener zumos con alto potencial antioxidante. Tratamientos llevados a cabo con pulsos bipolares de 1 μs a 250 Hz (zumo de tomate) y 232 Hz (zumo de fresa) dieron lugar a máximas retenciones de vitamina C (90%-98% retención), y capacidad antioxidante (89%-100% retención) en ambos zumos, así como licopeno en zumo de tomate (132% retención) y antocianinas (102% retención) en zumo de fresa.

- El efecto combinado de frecuencia, anchura de pulso y polaridad sobre la vitamina C y capacidad antioxidante, así como en licopeno (tomate) y antocianinas (fresas) de los zumos se modelizó adecuadamente a partir de ecuaciones de segundo orden (R^2_{adj} = 0,74-0,94).

- El contenido en vitamina C de los zumos disminuyó, mientras que el de licopeno se incrementó, al aumentar la intensidad de campo, tiempo de tratamiento, anchura de pulso y frecuencia de procesado. Tratamientos aplicados en modo bipolar dieron lugar a zumos con mayor potencial antioxidante, a excepción de la vitamina C, que los tratamientos en modo monopolar.

- La función de distribución de Weibull (R^2_{adj} =0,73-0,99) fue una herramienta útil para describir las variaciones de vitamina C, capacidad antioxidante y antocianinas (fresa) de los zumos en función del tiempo de tratamiento, mientras que el modelo propuestos por Peleg (R^2_{adj} = 0,83-0,99) fue el más adecuado para predecir los cambios cinéticos del licopeno en zumo de tomate. Además, se obtuvieron ecuaciones para asociar adecuadamente la variación de vitamina C, licopeno (tomate), antocianinas (fresa) y capacidad antioxidante (fresa) con el efecto combinado de la intensidad de campo y tiempo de tratamiento.

- Los zumos de tomate pasteurizados por PEAIC (35 kV/cm durante 1500 μs; bipolar 4 μs anchura de pulso a 100 Hz) mostraron mayores concentración de licopeno (7,84 mg/100 ml), β-caroteno (0,40 mg/100 ml) y fitoflueno (1,37 mg/100 ml) que los tratados por calor y los frescos. Del mismo modo, los zumos de tomate tratados por PEAIC mantuvieron mayores cantidades de carotenos (licopeno, neurosporeno y γ-caroteno), quercitina, vitamina A y C que los tratados térmicamente durante el almacenamiento.

- En zumo de fresa, el tratamiento de pasteurización mediante PEAIC (35 kV/cm durante 1700 μs; bipolar 4 μs anchura de pulso a 100 Hz) afectó en menor medida al contenido en vitamina C (98% retención) que el tratamiento térmico (94-95% retención). Además, los zumos de fresa tratados por PEAIC mostraron mayores niveles de antocianinas, ácido elágico y ácido *p*-cumárico que los procesados térmicamente durante el tiempo de almacenamiento.

- La vida útil de los zumos, estimada como la reducción del 50% del compuesto limitante (vitamina C), fue de 21 y 28 días para zumo de tomate pasteurizado por PEAIC o por calor (90 ºC-60 s), respectivamente; mientras que en zumo de fresa la vida útil fue de 21 días independientemente del tratamiento aplicado.

- Las variaciones de la capacidad antioxidante en zumo de tomate se asociaron con cambios en el contenido en licopeno y vitamina C, mientras que en los zumos de fresa se atribuyó principalmente al contenido en antocianinas y, en menor medida, al contenido en vitamina C.

B) Tomate y fresa frescos cortados

- La variedad determinó significativamente el contenido en compuestos bioactivos de los tomates. Los tomates de la variedad Bodar presentaron el máximo contenido en licopeno (80,5 mg/kg); sin embargo la variedad Durinta mostró la mayor concentración de vitamina C (212,3 mg/kg) y compuestos fenólicos (335,9 mg acido gálico/kg). Sin embargo, la variedad no influyó en la evolución del potencial antioxidante a lo largo del tiempo que, en general, se mantuvo constante o presentó ligeras variaciones respecto al contenido inicial.

- El almacenamiento de tomate fresco cortado a 5 ºC, no sólo alargó la vida útil del producto desde un punto de vista microbiológico (14 días), sino que también conservó el contenido en compuestos bioactivos del mismo. Del mismo modo, la concentración de vitamina C y antocianinas, así como la capacidad antioxidante de fresa fresca cortada se mantuvo mejor en frutas cortadas almacenadas a 5 ºC, que a temperaturas más altas.

- El modelo de Weibull permitió predecir bien (R^2_{adj} = 0,80-0.99) los cambios en el contenido en vitamina C y antocianinas, así como la variación de la capacidad antioxidante en fresa fresca cortada almacenada a distintas temperaturas con velocidad de degradación comprendidas entre $1,3\times10^{-1}$ y $4,4\times10^{-3}$ días^{-1}. Estas constantes cinéticas obtenidas mediante el modelo de Weibull se relacionaron adecuadamente con la temperatura de conservación a través de la ecuación de Arrhenius (E_a = 33,3-119,7 kJ/mol).

- El uso de atmósferas altas de O_2 provocó un estrés en ambos frutos dando lugar a la formación de quercitina durante el almacenamiento de tomate y fresa frescos cortados. Además, estas condiciones de envasado acarrearon un aumento sustancial en el contenido en carotenos (licopeno y β-caroteno) y ácido clorogénico en tomate fresco cortado.

- Las atmósferas con concentraciones reducidas en O_2 evitaron las pérdidas de vitamina C y mantuvieron mejor la capacidad antioxidante en tomate y fresa frescos cortados. Además, en fresas frescas cortadas, estas condiciones de envasado conservaron mayores contenidos en antocianinas y ácidos fenólicos durante el almacenamiento, en comparación con el envasado en concentraciones altas de O_2 o aire.

Anexo

Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits

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ABSTRACT
Vitamin C is one of the most important antioxidant supplied by fruits and vegetables. Therefore a reliable and easy method is needed for its determination. In this work, two UV-HPLC methods for the determination of ascorbic acid were validated and compared in strawberries, tomatoes and apples. In addition, two different reducing agents [DL-1,4-dithiotreitol (DTT) or 2,3-dimercapto-1-propanol (BAL)] were tried for differentiate dehydroascordic acid and determine vitamin C. Reliability resulted satisfactory for the UV-HPLC methods in each fruit. UV-HPLC methods resulted linear up to 50 mg/L and the least detection and quantification limits were < 0.18 mg/ 100g and < 0.61 mg/ 100g, respectively. Precision, as relative standard deviation, ranged from 0.6% to 3.9% and the recovery between 93.6 and 104.4%. Although, the UV-HPLC methods resulted useful for the routine analysis of AA and vitamin C in fruits, the best reliability was achieved when using a C18 column and DTT as reducing agent. Moreover, it may be the UV-HPLC method of choice because it is the easiest and cheapest to perform.

Key words: ascorbic acid, vitamin C, fruits, reliability, validation, comparison.

INTRODUCTION

The term vitamin C is used as the generic descriptor for all compounds exhibiting the biological activity of ascorbic acid. It includes esters of ascorbic acid, synthetic forms such as 6-deoxy-L-ascorbic acid and oxidized compounds (Davey et al., 2000). However, vitamin C in fruits is assumed to be the sum of the content of ascorbic acid (AA) plus dehydroascorbic acid (DHAA) (Combs, 1998). These two substances are readily oxidized, especially when exposed to elevated temperatures, some divalent cations (e.g. copper and iron), oxygen, alkaline pH, light, or degradative enzymes (Gregory, 1996). While the oxidation of AA to DHAA is reversible, DHAA can undergo irreversible hydrolysis to diketogulonic acid, which is not biologically active as vitamin C (Russell, 2004). Vitamin oxidation and loss during processing and cooking is of great concern for nutritionists, processors and consumers. Vitamin C is used as an index of the health-related quality of fruits, since, as compared to other beneficial compounds, it is more sensitive to degradation by processing and storage.

Due to the labile nature of vitamin C, preparation procedures are designed to avoid loss of vitamin. Metaphosphoric acid is the most common solvent used which inhibits L-ascorbic oxidase and metal catalysis, and, in addition, precipitates proteins (Eitenmiller and Lande, 1999). On the other hand, in order to determine vitamin C content, DHAA should be reduce to AA in samples. Usually, the DHAA content in samples can be calculated by the difference between the vitamin C (after DHAA reduction) and the ascorbic acid concentrations (previous to reduction) (Fernández-Muiño et al., 2002). Various thiol-containing compounds such as dithiotreitol (DTT) and dimercaptopropanol (BAL) can be used as a reducing agent. However, Diop et al. (1988) observed an incomplete reduction of DHAA to AA depending on the reducing agent used.

Various methods have been employed for the analysis of vitamin C in food, including electrochemical (Calokerinos and Hadjiioannou, 1983), spectrophotometric (Liu et al., 1982), spectrofluorimetric (Sánchez-Mata et al., 2000) and chromatographic methods. However, high-performance liquid chromatographical (HPLC) methods have some advantages regarding specificity, sensitivity or easy operation (Gökmen and Acar, 1996). Reversed-phased (Furusawa, 2001), bonded-phase NH_2 (Arakawa et al., 1981), ion-exchange (Nelis et al., 1997) or ion-pair reversed columns (Madigan et al., 1996) have been the most commonly employed columns for vitamin C analysis. Regarding the way of detection, AA can be easily detected by UV at wavelengths between 245 nm and 254 nm. Although, UV detectors are usually included in HPLC systems and are simpler and faster than others, few UV-HPLC methods have been validated to be used for vitamin C determination in foods. Most of these methods have been validated in beer, wine and fruit beverages. However, fruits are different from those fermented products and more complex matrices than beverages and so naturally occurring compounds could affect the detection or interfere in the identification and quantification of AA. Davey et al. (2000) reported that considerable caution should be taken when using methods that have been developed for the analysis of specific plant tissue in the assay of other different matrixes. On the other hand, according to the available literature, vitamin C concentration varied greatly among the type of fruit and cultivars. The content of vitamin C in fruits ranged from 200-210 mg/100g for blackcurrant to 2-10 mg/100g for apple (Davey et al., 2000; Russell, 2000).

In general, fruits tend to be a good source of vitamin C; however, fruits such as pears, plums and apples, contain only a very modest concentration of this vitamin. Consequently,

obtaining an adequate method is needed for measuring the concentration of vitamin C in specific fruits.

The aim of this work was to evaluate the feasibility of using different UV-HPLC methods for determining vitamin C in fruits with different concentration of vitamin C. Strawberry (high vitamin C concentration), tomato (medium concentration) and apples (low concentration) were chosen. Two reducing agents and two UV-HPLC methods were tried. Moreover, AA was determined before and after reduction to calculate the DHAA in the sample. The reliability of the methods was evaluated in terms of linearity, sensitivity, precision and recovery. A comparative study was carried out between reducing agents as well as UV-HPLC methods.

MATERIALS AND METHODS

Reagents

Metafosforic acid, DL-1,4-dithiotreitol (DTT) and 2,3-dimercapto-1-propanol (BAL) were purchased from Acros Organics (NJ, USA); ascorbic acid, acetonitrile, potassium dihydrogen phosphate and sulphuric acid were obtained from Scharlau Chemie, SA (Barcelona, Spain).

Sample Preparation

Ascorbic acid

Strawberries, tomatoes and apples were bought from a local supermarket at commercial maturity and storaged at 4 (±1) ºC before analysis. The extraction was based on a procedure proposed by Brubacher, Müller-Mulot & Southgate (1985). A portion of 25 g of fruit was added to 25 ml of 4.5% metaphosphoric solution. The mixture was homogenized and centrifuged at 22100xg for 15 min. at 4 ºC. The supernatant was vacuum-filtered through Whatman No. 1. Then, 10 ml of the vacuum-filtered sample were passed through a Millipore 0.45 µm membrane and thus were ready to be injected in the HPLC system.

Vitamin C

To quantify the total concentration of vitamin C, two different reductors were tried. A solution of DTT (20 mg/mL) was prepared and an aliquot of 0.2 ml was added to 1 ml of the vacuum-filtered sample, obtained in AA analysis, following the method proposed by Sánchez-Mata et al. (2000). An aliquot of 2 µl of the other reductor (BAL) was combined with each ml of the vacuum-filtered sample based on a method proposed by Soliva-Fortuny and Martín-Belloso (2003). The mixtures were kept in the darkness for 2 h. Then they were passed through a Millipore 0.45 µm membrane and injected into the HPLC system.

The DHAA was calculated as the difference between the vitamin C (after reduction) and AA (without reduction) (Sánchez-Mata et al., 2000).

Chromatographic conditions

The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 245 nm. Samples were introduced onto the column through a manual injector equipped with a sample loop (20 µl). The flow rate was fixed at 1.0 ml/min at room temperature. Two different chromatographic conditions were tried: a) A reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6mm x 250mm) was used as stationary phase. The mobile phase was a 0.01% solution of sulphuric acid adjusted to pH=2.6 (Sanchez-Mata et al., 2000). b) A NH_2-Spherisorb S5 Column (250x4.6mm, 5µm) was employed. The eluent was 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile in a ratio 60:40 under isocratic conditions (Soliva-Fortuny and Martín-Belloso, 2003).

Validation

The reliability of HPLC-methods was validated through their linearity, sensitivity, precision and recovery.

Linearity

Once verified the normal distribution of the results, linearity was evaluated through the relationship between the concentration of acid ascorbic (independent variable) and the absorbance obtained thought the HPLC-UV detector (dependent variable). Then an analysis of variance of the regression and a residual plot were carried out. The experimental Fisher value (F_{cal}) was compared to its tabulated value (F_{tab}) for 1 and n-2 degrees of freedom (Steel and Torrie, 1980). The determination coefficient (r^2) was calculated by means of the least-squares analysis. Three calibration lines were carried out for each chromatographic condition and reducing agent. Moreover, every calibration line was done through three replicates of each concentration of ascorbic acid (0.5, 1, 1.5, 3 and 5 mg/100g) to know the extent of the total variability of the response that could be explained by the linear regression model.

Sensitivity

The detection limit (DL) and quantification limit (QL) were calculated from the calibration lines that defined linearity, using the Long and Winefordner criterion (Long and Winefordner, 1983) as expressed in Equation 1 and 2.

$$DL = \frac{3 \times S}{a} \qquad (1)$$

$$QL = \frac{10 \times S}{a} \qquad (2)$$

where a is the slope of the calibration line and S is the standard error of the intercepted point.

Precision

The precision of the method indicates the degree of dispersion within a series of determinations on the same sample. Six measurements were performed for each tried UV-HPLC method and reducing agent in strawberries, tomatoes and apples giving a total of 144 samples. The relative standard deviations (RSD_{exp}) were calculated dividing the standard deviation by the mean of the concentration, and the adequacy of the (RSD_{exp}) to the Horwitz criterion (Horwitz, 1982) was evaluated.

Recovery

Recovery was tested by the standard addition procedure at two levels for each method on strawberries, tomatoes and apples. The concentrations of AA added to the sample were: 30 and 60 mg/100g in strawberry, 10 and 20 mg/100g for tomatoes and 1.5 and 3 mg/100 for apples. In each addition level, six determinations were carried out for each UV-HPLC method, reducing agent and fruit (216 samples), and the recovery (%) was calculated in every case. The homogeneity of variances between levels of addition was verified by a Cochran test (Steel and Torrie, 1980). The mean recoveries of each level were compared using a Student's t-test, the experimental value (t_{exp}) was compared to the tabulated value (t_{tab}) for (n-1) degrees of freedom (Steel and Torrie, 1980). Therefore, an average value of both levels could be considered when t_{exp} was lower than t_{tab}.

Comparison of the methods

A comparison procedure was carried out to find significant differences among the mean values obtained throughout the UV-HPLC methods, with or without addition of reducing agent. The least significant difference test was employed to determine differences among means at a 5% significance level. Moreover the principle of Bland and Altman (1986) was used to compare UV-HPLC methods and kind of reducing agent. The statistical treatments were performed with Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md).

A comparative study was carried out in terms of linearity from three calibration lines with their respective r-value, sensitivity by DL and QL and precision through the RSD values. To carry out the comparison test on recovery terms, all the values of recovery of each set of analysis were considered in each case.

RESULTS AND DISCUSSION

Validation of the methods

HPLC-methods methods were validated through their linearity, sensitivity, precision and recovery, with and without reducing agent added.

Linearity

Absorbance responses of AA, with and without reducing agent addition, were significantly linear up to 5 mg/100g according to the determination coefficient (R^2) shown in Table 1. In addition, the residuals are randomly distributed around the line with zero mean (Figure 1). Therefore the regression model represents the data correctly for all HPLC-UV methods, with or without reducing agent addition.

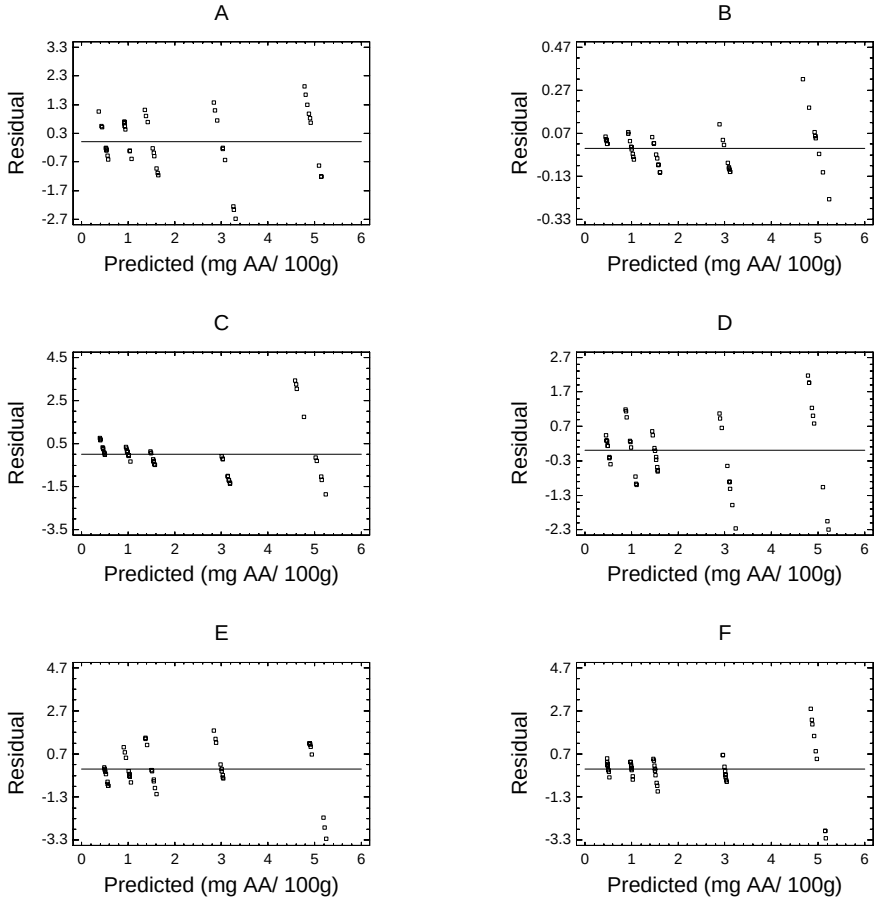

Figure 1.-Residual Plots of the regression model for the evaluated UV-HPLC methods to determine ascorbic acid (A) through C18 column and without reducing agent (B) through NH_2 column without reducing agent (C) through C18 column and DTT as reducing agent (D) through C18 column and BAL as a reducing agent (E) through NH_2 column and DTT as a reducing agent and (F) thought a NH_2 column and BAL as a reducing agent.

There is a good relationship between the concentration of AA and the area obtained throughout both UV-HPLC methods, C18 column with mobile phase of sulphuric acid (0.01%) adjusted to pH=2.6 and NH_2 column with 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile (60:40) as a mobile phase. The coefficients of determination (R^2) were higher than 99.36% in every method and reducing agent used. On the other hand, similar slopes of the calibration lines were observed between UV-HPLC methods without reducing agent addition. However, slopes were lower when using a NH_2 column than a C18 column. The consequence of these different slopes may affect the sensitivity of the methods.

Detection (DL) and Quantification (QL) Limits

DL can be defined as the minimum concentration capable of giving a chromatographic signal three times higher than background noise. The QL is the lowest amount of analyte in the sample which can be quantitatively determined with precision and accuracy. Lower standard errors of the intercepted point were achieved throughout a NH_2 column than a C18 column with or without reducing agent addition (Table 1). In addition, the higher the standard error of the intercepted point the lower the sensitivity was. The DL and QL obtained for AA through a C18 column without reducing agent were 0.17 and 0.57 mg/100g, respectively, while 0.10 and 0.34 mg/100g were the corresponding limits using a NH_2 column (Table 1). On the other hand, DL and QL were lower than 0.18 and 0.61 mg/100g, respectively, when AA was determined with reducing agent irrespective of the UV-HPLC method. The QL values achieved through the tried UV-HPLC methods, with or without reducing agent addition, were lower than the content of vitamin C present in fruits, thus they can be considered sensible enough for general determination of vitamin C in fruits.

Table 1.- Linearity and sensitivity for the evaluated UV-HPLC methods to determine ascorbic acid.

Column	Reducing agent	Calibration line[a]	R^2(%)	Standard Error [(b)]	DL* (mg/100g)	QL* (mg/100g)
-C18	-	y =651431x -15425	99.44	36983	0.17	0.57
	DTT	y =619745x + 48537	99.36	37702	0.18	0.61
	BAL	y =649402x - 414	99.60	31373	0.14	0.48
-NH_2	-	y =616385x + 44301	99.80	20882	0.10	0.34
	DTT	y =524769x + 13532	99.71	21455	0.12	0.41
	BAL	y =544883x ± 25944	99.87	14838	0.08	0.27

[a] y = slope · x ± intercepted point (n=9) DL = Detection limit; QL = Quantification limit
[b]Standard error of the intercept point of the calibration line.
*****No significant differences were found among UV-HPLC methods and reducing agent.
BAL=2,3-dimercapto-1-propanol; DTT =1,4-dithiotreitol

Table 2.- Precision of the assayed UV-HPLC methods for the determination of ascorbic acid (AA), dehydroascorbic acid (DHAA) and vitamin C (VitC) in strawberry (STR), tomatoes (TOM) and apples (APP).

Column	RA	Subst.	Fruit	Concentration[a]	RSD_{exp} (%)*	RSD Horwitz[b]
-C18	DT	AA	STR	57.2 ± 0.4	0.7[a]	6.153
			TOM	21.7 ± 0.2	0.9[a]	7.119
			APP	1.78 ± 0.02	1.1[a]	10.373
		DHAA	STR	2.2 ± 0.6	27.3	10.048
			TOM	1.8 ± 0.3	16.7	10.356
			APP	0.07 ± 0.06	85.7	16.882
		VitC	STR	59.1 ± 0.9	1.5[a]	6.122
			TOM	23.6 ± 0.2	0.9[a]	7.030
			APP	1.78 ± 0.06	3.4[a]	10.373
	BA	AA	STR	52.7 ± 0.3	0.6[a]	6.229
			TOM	18.0 ± 0.4	2.2[a]	7.323
			APP	3.6 ± 0.1	2.8[a]	9.330
		DHAA	STR	7.8 ± 0.9	11.5	8.305
			TOM	2.8 ± 0.5	17.9	9.689
			APP	0.48± 0.08	16.7	12.635
		VitC	STR	60.6 ± 0.5	0.8[a]	6.100
			TOM	20.9 ± 0.4	1.9[a]	7.160
			APP	4.11 ± 0.05	1.2[a]	9.146
$-NH_2$	DT	AA	STR	53.0 ± 1.9	3.6[b]	6.224
			TOM	19.0 ± 0.4	2.1[b]	7.263
			APP	2.7 ± 0.1	3.7[b]	9.743
		DHAA	STR	10.3 ± 1.8	17.5	7.964
			TOM	9.6 ± 1.0	10.4	8.049
			APP	2.4 ± 0.3	12.5	9.917
		VitC	STR	63.4 ± 0.8	1.3[b]	6.058
			TOM	28.6 ± 0.4	1.4[b]	6.830
			APP	5.1 ± 0.2	3.9[b]	8.853
	BA	AA	STR	54.5 ± 2.1	3.8[b]	6.198
			TOM	22.7 ± 0.7	3.1[b]	7.071
			APP	3.1 ± 0.1	3.2[b]	9.542
		DHAA	STR	7.8 ± 2.4	30.8	8.305
			TOM	5.1 ± 1.3	25.5	8.853
			APP	0.7 ± 0.2	28.6	11.938
		VitC	STR	62.3 ± 1.0	1.6[b]	6.074
			TOM	27.8 ± 1.0	3.6[b]	6.859
			APP	3.8 ± 0.1	2.6[b]	9.254

[a]Mean ± standard deviation (n=6) (mg/100g); **Subst**: substance; **RA**: reducing agent
[b]Acceptable RSD value based on the Horwitz criterion.
DTT =1,4-dithiotreitol BAL=2,3-dimercapto-1-propanol.
*Values in the same column with different letters are significant different ($p < 0.05$).
Values of RSD of DHAA were not analyzed because of the high RSD shown.

Precision

The relative standard deviations (RSD) achieved for each UV-HPLC method and reducing agent was less than 5% for AA and vitamin C determination (Table 2). According to the Horwitz criterion (Horwitz, 1982), all RSD obtained were satisfactory, thus all UV-HPLC methods irrespective of the reducing agent tried may be considered precise for AA and vitamin C determination. The RSD values range from 0.6% to 3.9% in AA analysis. The lowest value of RSD for vitamin C determination was obtained in strawberries (0.8%) using BAL as a reducing agent and the UV-HPLC system with a C18 column, whereas the results obtained throughout a NH_2 column and DTT led the maximal value of RSD (3.9%) in apples. The precision values obtained in the present work were also in the range recommended by the Association of Official Analytical Chemists for substances around 10 mg/L (AOAC, 1998). Sanchez-Mata et al. (2000) proposed the calculation of DHAA by difference between the content of vitamin C and AA. These authors calculated, with good results, the content of DHAA in green beans. However, neither the methods with a C18 column nor those using a NH_2 column gave precise results for DHAA in the studied fruits because of their high RSD values (Table 2). On the other hand, as can be observed in Table 2, the concentration of AA and vitamin C in strawberries, tomatoes and apples obtained in this work are in the range of those published in the literature which varied from 40-90 mg/100g in strawberries, 20-30 mg/100g for tomatoes and 2-10 mg/100g for apple (Davey et al., 2000; Rusell, 2000) and confirm the different content of vitamin C depending on the type of fruit. Breene et al. (1994) reported that variability of vitamin C within the type of fruit might be attributed to environmental and cultural practices. Vitamin C content varied considerably among cultivars, ripeness and growing condition. Harvest maturity, soil fertilization, irrigation, light intensity and day/night temperatures could also affect vitamin C content in fruits (Davey et al., 2000).

Recovery

Mean recovery percentages ranged from 93.6% to 104.4% (Table 3). All the variances of the recovery obtained for UV-HPLC methods, with or without reducing agent addition, were homogeneous through the Cochran test. The Student test showed that the recovery of AA did not depend on the addition of this compound in each fruit, and thus, the final recovery was the average of the results obtained in both levels of addition for each fruit. Moreover, recovery was similar to the theoretical 100% for each assay, so all recovery values were satisfactory according to Student's t-test.

Table 3.- Recovery of the assayed UV-HPLC methods to determine ascorbic acid (AA) and vitamin (Vit C) in strawberry (STR), tomatoes (TOM) and apples (APP).

Column	RA	Subst.	Fruit	Recovery (%)[a]		Mean* Recovery	100-Student test[b]
				Level I	Level II		
-C18	-	AA	STR	100.7 ± 1.9	101.9 ± 3.1	$101.3^{aA\alpha}$	1.802
			TOM	95.7 ± 2.3	96.3 ± 4.6	$96.0^{aA\alpha}$	3.925
			APP	107.9 ± 2.5	100.5± 5.7	$104.2^{aB\alpha}$	2.561
	DTT	VitC	STR	104.5 ± 0.9	101.3 ± 2.1	$102.9^{aA\alpha}$	4.235
			TOM	100.1 ± 2.9	97.6 ± 3.4	$98.8^{aA\alpha}$	1.202
			APP	106.4 ± 1.6	101.2 ± 2.8	$103.8^{aB\alpha}$	3.795
	BAL	VitC	STR	107.8 ± 1.9	100.9 ± 4.7	$104.3^{aA\beta}$	3.174
			TOM	96.0 ± 7.2	95.4 ± 5.9	$95.7^{aA\beta}$	3.506
			APP	107.0 ± 3.7	101.8 ± 2.8	$104.4^{aB\beta}$	3.681
$-NH_2$	-	AA	STR	91.0 ± 4.4	96.1 ± 8.9	$93.6^{bA\alpha}$	3.098
			TOM	96.0 ± 3.6	95.4 ± 5.1	$95.7^{bA\alpha}$	3.492
			APP	91.2 ± 5.1	100.8 ± 11.3	$96.0^{bB\alpha}$	1.427
	DTT	VitC	STR	91.6 ± 5.6	98. 9 ± 5.5	$95.2^{bA\alpha}$	2.511
			TOM	104.0 ± 3.5	100.3 ± 7.5	$102.1^{bA\alpha}$	0.411
			APP	97.1 ± 9.9	99.5 ± 8.6	$98.3^{bB\alpha}$	0.662
	BAL	VitC	STR	105.7 ± 1.8	96.8 ± 1.5	$101.2^{bA\beta}$	1.298
			TOM	104.0 ± 3.5	98.7 ± 2.3	$101.3^{bA\beta}$	1.179
			APP	105.3 ± 1.0	96.1 ± 1.8	$100.7^{bB\beta}$	0.495

[a] Recovery mean ± standard deviation (n=6 in each level); **Subst**: substance; **RA**: reducing agent
[b] Test to determinate differences among the mean recovery obtained and the theoretical 100% $t_{tab(11,0.001)}$= 4.437
DTT =1,4-dithiotreitol **BAL**=2,3-dimercapto-1-propanol
* Different values of lower case letter stand for significant differences between columns.
Different values of capital letter in the same column correspond to significant differences among fruits.
Different values of symbol in the same column correspond to significant differences between reducing agents

Comparison of the methods

UV-HPLC methods were significantly linear up to 5 mg/100g and sensitive enough to determine AA using or not reducing agent (Table 1 and Figure 1). However, lower slopes and standard errors of the intercepted point were observed using a NH_2 column than a C18 column. As a result, the sensitivity of the methods was different. DL and QL were lower when a NH_2 column and the mobile phase was 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile than those methods using a C18 column with the mobile phase of 0.01% solution of sulphuric acid adjusted to pH=2.6. Through this UV-HPLC method, minimal values of DL and QL of 0.08 and 0.27 mg/100g were found when the determinations of AA

were performed with BAL as a reducing agent. On the contrary, DL and QL were < 0.18 and < 0.61 mg/100g, respectively, when the analysis was done throughout a C18 column irrespective of the reducing agent used. However, the differences were not significant for linearity and sensitivity among UV-HPLC methods with or without addition of reducing agent. On the contrary, precision results depended significantly on the UV-HPLC method. As can be seen in Figure 2a, there was lack of agreement between UV-HPLC methods in strawberry samples determination. In the same way and complementary, the ANOVA test indicated that the obtained RSD throughout a C18 column was significantly better than that achieved by a NH_2 column with or without reducing agent addition (Table 2). However, Bland and Altman (1986) and ANOVA test indicated that both reducing agents might be used to determine vitamin C in fruits irrespective of the content of this vitamin in the fruits (Figure 2b, Table 2). In contrast, neither method with NH_2 nor with C18 column is precise for DHAA irrespective of the reducing agent added (Table 2). Ball (1997) reported that errors in DHAA content can be observed if the concentration of this compound in the sample is very low in comparison to the content of AA. Some authors (Wills, et al., 1984; Fernández-Muiño, et al., 2002) have reported that DHAA is present at low levels in fresh fruit; consequently, the high RSD values found in this study for DHAA may be due to its low concentration in the sample. Furthermore high RSD values might be a consequence of an incomplete reduction of DHAA to AA. Diop et al. (1988) observed a DHAA reduction of 55% using homocysteine as a reducing agent. In contrast, Deutsch and Santhosh-Kumar (1996) observed that using sulfhydryl compounds in the reduction of DHAA to AA, other substances different to DHAA can be transformed to AA.

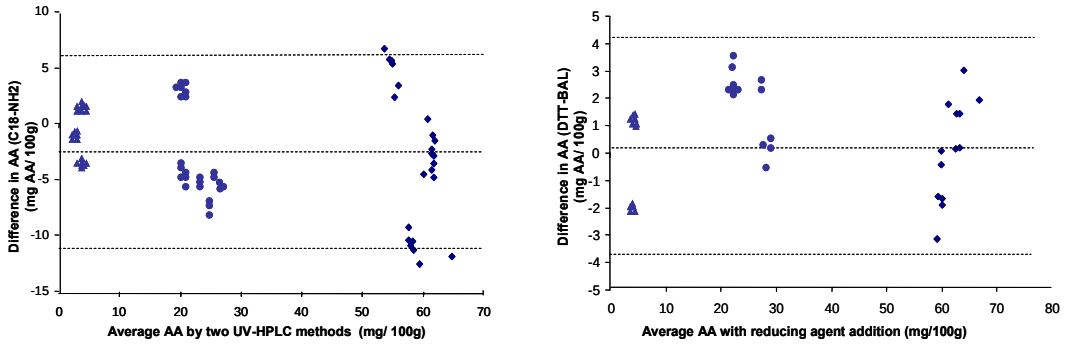

Figure 2.- Comparison of two UV-HPLC methods (a): a C18 column with a mobile phase of sulphuric acid (0.01%) adjusted to pH=2.6 and a NH_2 column with 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile (60:40) as a mobile phase and two different reducing agent (b): DTT (DL-1,4-dithiotreitol and BAL (2,3-dimercapto-1-propanol) to determine ascorbic acid content (AA) in strawberry (♦), tomato (●) and apple (▲) according to the Bland and Altman test. In both cases the detection took place at 245 nm.

On the other hand, differences in recoveries among UV-HPLC methods and reducing agents were observed (Figure 3).The HPLC system constituted by a C18 column with mobile phase of sulphuric acid (0.01%) adjusted to pH=2.6 led to better recoveries of AA and vitamin C, according the LSD test (Table 3). In addition, recoveries closer to 100% were observed using DTT when reducing agent was added (Table 3). Significantly better recoveries were achieved in strawberries and tomatoes than in apples (Table 3). Vitamin C content added to apples was between 10 and 20 times lower than those used in tomatoes and strawberries, consequently, differences in recovery results for apples might be due to the low concentration of vitamin C in this fruits.

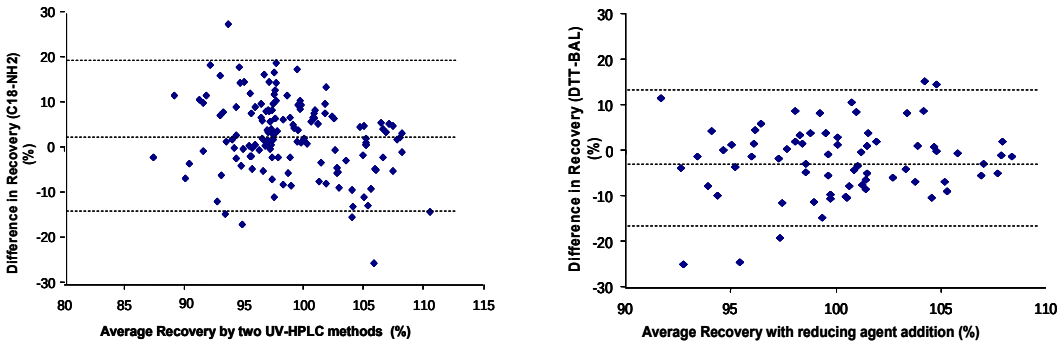

Figure 3.-Comparison of the recovery obtained with two UV-HPLC methods (A): a C18 column with a mobile phase of sulphuric acid (0.01%) adjusted to pH=2.6 and the other with a NH_2 column with 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile (60:40) as a mobile phase and two different reducing agent (B): DTT (DL-1,4-dithiotreitol) and BAL (2,3-dimercapto-1-propanol) in strawberry, tomato and apple according to the Bland and Altman test. In both cases the detection took place at 245 nm.

CONCLUSIONS

Reliability has been satisfactory for all the evaluated UV-HPLC methods, reducing agents and fruits. In every case, suitable linearity, sensitivity, precision and accuracy through recovery for AA and vitamin C analysis in strawberries, tomatoes and apples were obtained. Both UV-HPLC methods and reducing agent studied are useful for determining the content of AA and vitamin C in strawberries, tomatoes and apples. However, the determination of AA through the method where a C18 column is used, results more adequate in terms of precision and recovery. Furthermore, it is easier and cheaper to carry out than the UV-HPLC method with a NH_2 column. So, the UV-HPLC method with C18 column may be chosen for routine analysis. On the other hand, significantly better recoveries values were reached using DTT as reducing agent, thus DTT may be selected for the analysis of vitamin C.

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REFERENCES

AOAC (1998). Peer Verified Methods program. Manual on Policies and Procedures. AOAC, Arlington, Virginia.

Arakawa, N., Otsuka, M., Kurata, T., Inaka, C. (1981). Separative determination of ascorbic acid and erythorbic acid by high-performance liquid chromatography. *Journal of Nutrition Science and Vitaminology,* 27 (1) 9-15.

Ball, G.F.M. (1997). Bioavailability and analysis of vitamins in foods. Chapman & Hall. New York.

Bland, J.M., Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1(8476), 307-310.

Breene W. M. (1994). Healthfulness and nutritional quality of fresh versus processed fruit and vegetables: A review. *Journal of Foodservice Systems*, 8 (1),1-45

Brubacher, G., Müller-Mulot, W., Southgate, D.D.T. (1985). Methods for determination of vitamins in foods. Elsevier, London.

Calokerinos, A.C., Hadjiioannou, T.P. (1983). Direct potentiometric titration of thiosulfate, thiourea and ascorbic acid with iodate using and iodide ion-selective electrode. *Microchemical journal,* 28 (4), 464-469.

Combs, G.F. (1998). The Vitamins. Fundamental Aspects in Nutrition and Health. Academic Press, California.

Davey, M.W., Van Montagu, M., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science of Food and Agriculture,* 80, 825-860.

Deutsch, J.C., Santhosh-Kumar, C.R. (1996). Dehydroascorbic acid undergoes hydrolysis on solubilization which can be reversed with mercaptoethanol. *Journal of Chromatography A,* 724, 271-278.

Diop, P.A., Franck, D., Grimm, P., Hasselmann, C. (1988). High-performance liquid chromatographic determination of vitamin C in fresh fruit from West Africa. J*ournal of Food Composition and Analysis,* 1(3), 265-269.

Eitenmiller R.R., Lande, W.O. (1999). Vitamin Analysis for the health and food Sciences. CRC Press, Florida.

Fernández-Muiño, M.A., Sancho-Ortiz, M. T., Valls-García, F. (2002). Water-Soluble Vitamins. In W.J. Hurst, *Methods of Analysis for Functional Foods and Nutraceuticals*, (pp. 275-294). CRC Press, Florida.

Furusawa, N. (2001). Rapid high-performance liquid chromatographic identification /quantification of total vitamin C in fruit drinks. *Food Control*, 12(1), 27-29.

Gökmen, V., Acar, J. (1996). A simple HPLC method for the determination of Total Vitamin C in fruit juices and drinks. *Fruit Process*, 5, 198-201.

Gregory, J.F. (1996). Vitamins (C). In:. Fennema, O.R. Food Chemistry. Marcel Dekker, New York. (pp. 559-606).

Horwitz, W. (1982). Evaluation of analytical methods used for regulation of foods and drugs. *Analytical Chemistry*, 54(1), 67-76.

Liu, T.Z., Chin, N., Kiser, M.D. Bigler,W.N. (1982). Specific spectrophotometry of ascorbic-acid in serum or plasma by use of ascorbate oxidase. *Clinical Chemistry*, 28 (11), 2225-2228.

Long, G.L., Winefordner, J.D. (1983). Limit of detection. A closer look at the IUPAC definition. *Analytical Chemistry* 55(7), 712-724.

Madigan, D., McMurrough, I., Smyth, M.R. (1996). Improved method for the determination of ascorbic acid in beer by using high-performance liquid chromatography with electrochemical detection. *Analytical Communications,* 33(1), 9-10.

Nelis, H.J., De Leenheer, A.P., Merchie, G., Lavens, P., Sorgeloos, P. (1997). Liquid chromatographic determination of vitamin C in aquatic organisms. *Journal of Chromatographic Science* 35(7), 337-341.

Russell, L.F. (2000). Vitamins in Animal and Human Nutrition. Iowa State University Press, Iowa.

Russell, L.F. (2004). Water-Soluble vitamins. In: Nollet, L.M.L. Handbook of food analysis. Physical characterization and nutrient analysis. Marcel Dekker, New York. Vol1 pp.487-571.

Sánchez-Mata, M.C., Cámara-Hurtado, M., Díez-Marqués, C., Torija-Isasa, M.E. (2000). Comparison of high-performance liquid chromatography and spectrofluorimetry for vitamin C analysis of green beans (*Phaseolus vulgaris* L.). *European Food Research and Technology*, 210(3), 220-225.

Soliva-Fortuny, R.C., Belloso-Martín, O. (2003) Microbiological and biochemical changes in minimally processed fresh-cut Conference pears. *European Food Research and Technology*, 217(1), 4-9.

Steel, R.G.D., Torrie J.H. (1980). Principles and procedures of statistics. A biometrical approach. Mcgraw-Hill Book Company, New York.

Wills, R.B.H., Wimalasiri, P., Greenfield, H. (1984). Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin C activity. *Journal of Agricultural and Food Chemistry*, 32(4), 8.