DEPARTAMENTO DE MEDICINA PREVENTIVA Y SALUD PÚBLICA

ESTUDIO DE PARÁMETROS NUTRITIVOS Y DE CALIDAD EN ALIMENTOS LÍQUIDOS DE ORIGEN VEGETAL PROCESADOS POR TECNOLOGÍAS NO TÉRMICAS

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Estudio de parámetros nutritivos y de calidad en alimentos líquidos de origen vegetal procesados por tecnologías no térmicas.

TESIS DOCTORAL

Presentada por:

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Clara Cortés Fenollar ha disfrutado de una beca para la formación de personal investigador de carácter predoctoral (modalidad A) otorgada por la Oficina de Ciencia y Tecnología de la Conselleria D'innovació i Competitivitat de la Generalitat Valenciana (2001-2005).

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Ana Mª Frígola Cánoves y Mª José Esteve Más, profesor titular de Universidad y profesor ayudante doctor, respectivamente, del Área de Nutrición y Bromatología, del departamento de Medicina Preventiva y Salud Pública, Ciencias de la Alimentación, Toxicología y Medicina Legal, de la Universitat de València.

CERTIFICAN QUE: la licenciada en farmacia Dª Clara Cortés Fenollar ha realizado, bajo su dirección y en los laboratorios del área, el trabajo que lleva por título: "Estudio de parámetros nutritivos y de calidad en alimentos líquidos de origen vegetal procesados por tecnologías no térmicas", y autorizan su presentación para optar al Título de Doctor en Farmacia.

Y para que así conste, expiden y firman el presente certificado en Burjassot, a 12 de septiembre de 2006.

Fdo.: Ana Mª Frígola Cánoves Fdo.: MªJosé Esteve Más

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INTRODUCCIÓN

1. INTRODUCCIÓN

1.1. ALIMENTOS VEGETALES

Dentro del amplio abanico de alimentos que nos ofrece la naturaleza, pocos son tan agradables y directamente comestibles como las frutas y las verduras.

Incluir frutas y verduras diariamente en la dieta es de vital importancia para lograr una alimentación variada y equilibrada. Healthy People 2010 (United States Department of Health and Human Services, 2000) recomiendan tomar cada día, al menos 5 raciones de fruta y verduras. En España, este consejo está siendo fuertemente impulsado por el club "5 al dia" (Cullum, 2003). Aunque solamente una minoría de la población mundial alcanza estos objetivos (Neumark-Sztainer et al., 2002, Field et al., 2003), es importante saber que su ingesta diaria en nuestra dieta comporta muchos beneficios.

Las frutas y verduras presentan en su composición, además de nutrientes indispensables para la vida (hidratos de carbono, proteínas, grasas, etc.), otras sustancias, con un posible efecto protector frente a determinadas enfermedades degenerativas, conocidas como compuestos fitoquímicos o bioactivos (fibra, compuestos fenólicos, carotenoides, vitaminas A, C y E, glucosinolatos, compuestos organosulfurados, etc.), cuya actividad biológica ha sido estudiada en numerosos ensayos *in vitro*, *ex vivo* y mediante ensayos de intervención en humanos (Duthie, 1999; Lampe, 1999; Slaterry et al., 2000; Simon et al., 2001; Sánchez-Moreno et al., 2003; Brandt et al., 2004). Estudios epidemiológicos demuestran un gran efecto protector del consumo de frutas y verduras frente al riesgo de determinadas enfermedades como cáncer, enfermedades cardiovasculares, cataratas, degeneración macular (Van Poppel et al., 1995; Kritchevsky, 1999; Olmedilla et al., 2001; Grassman et al., 2002; Olmedilla et al., 2003; Granado et al., 2004). Lampe (1999) describe varios mecanismos por los que las frutas y sus constituyentes pueden ejercer un efecto protector. Entre

estos mecanismos se incluye la capacidad de modificar el metabolismo del colesterol, regular la detoxificación de enzimas, estimular el sistema inmune, disminuir la agregación plaquetaria, reducir la presión arterial, actuar sobre la concentración y metabolismo de las hormonas esteroideas, su papel como agente antibacteriano y antiviral y por su actividad antioxidante. Fawzi et al. (2004) observan en su estudio que los suplementos multivitamínicos retrasan la progresión de la enfermedad del HIV y propocionan un efectivo, y de bajo coste, instrumento para retrasar la iniciación de la terapia antiretroviral en las mujeres infectadas por HIV.

Si bien las frutas y hortalizas se consumen generalmente frescas, un gran número de ellas son procesadas y/o conservadas por razones económicas, logísticas, para mejorar su digestibilidad, por necesidades culinarias o para facilitar su consumo a determinados grupos de población (niños, ancianos, enfermos, etc.). La creciente demanda por parte del consumidor de alimentos seguros, procesados, y cuya preparación ocupe el menor tiempo posible, ha llevado a la industria alimentaria a aumentar su producción de este tipo de alimentos y a investigadores y tecnólogos de alimentos a realizar un gran esfuerzo para asegurar que los nutrientes y compuestos fitoquímicos presentes en los alimentos de origen vegetal se mantengan o se modifiquen mínimamente durante el procesado y el almacenamiento, hasta llegar al consumidor.

1.1.1. Horchata de chufa

La chufa, *Cyperus Sculentus*, es una planta vivaz, de hojas en roseta y de 40 a 50 centímetros. Posee un sistema radicular rizomático, del que parten raicillas en cuyos extremos se forman las chufas. Estas adquieren dos formas: "llargueta" (alargada) y "armela" (redondeada). La chufa se cultiva en dieciséis pueblos de la comarca valenciana de L´Horta Nord, que reúnen unas exigencias climáticas y

edafológicas y la convierten en la única zona de España donde se cultiva tan singular tubérculo. Se ha otorgado la denominación de origen "Chufa de València" a la producción de las chufas de la variedad-población *Cyperus esculentus L.* obtenidas o multiplicadas, y cultivadas en terrenos ubicados en los siguientes términos municipales: Albalat dels Soreis, Alboraya, Albuixec, Alfara del Patriarca, Almàssera, Bonrepòs y Mirambell, Burjassot, Foios, Godella, Meliana, Moncada, Paterna, Rocafort, Tavernes Blanques, València y Vinalesa.

En 2003, se cultivaron 434 hectáreas de chufas en la comunidad valenciana, con un rendimiento de 18000 chufas por hectárea, lo que supuso una producción de 7812 tm de chufas (MAPA, 2004).

El cultivo de la chufa de Valencia posee un conjunto de valores agrícolas, económicos y sociales que asimilados por el pueblo forman parte de su cultura y hábitos de vida, a la vez que representan el reconocimiento de un producto por su calidad y prestigio. El Reglamento de la Denominación de Origen Chufa de Valencia, desde 1995, vela por la calidad del tubérculo, en todas las fases: plantación, lavado, secado y selección (DOGV, 1995). Asimismo, garantiza al consumidor que en los establecimientos y en los envases de horchata que lleven el logotipo del Consejo Regulador, la horchata ha sido elaborada con autentica Chufa de Valencia y que cumple los parámetros de calidad exigidos. Existe además una Reglamentación Técnico Sanitaria a nivel nacional (BOE, 1988), por la que se clasifica la horchata desde el punto de vista del procesado para su conservación y se establecen las características nutricionales que debe cumplir cada uno de los tipos de horchata descritos.

Entre sus propiedades benefactoras sobre la salud, la chufa ya ocupa su lugar como tal en el siglo XVI, donde Andrés Laguna, médico de Carlos I, atribuía a estos tubérculos propiedades antiinflamatorias de las vías respiratorias y preventivas de problemas estomacales. Hoy en día se considera la chufa como un antidiarreico, debido a su poder astringente.

Fue introducida en España por los árabes en el siglo VII, identificándose desde entonces como un producto típicamente valenciano. Las primeras noticias sobre este fruto y la bebida refrescante que se preparaba de él datan del siglo XII y XIII, entonces en la Comunidad Valenciana se hacía una bebida de gran aceptación llamada "llet de xufes" (Cantalejo, 1996; Virseda, 1997; Torregrosa, 2001).

La entidad de Clasificación Nacional de Actividades Económicas (CNAE-93), clasifica a la horchata como una bebida no alcohólica, sin grasas lácteas. Las bebidas no alcohólicas ocupan un lugar destacado en los hábitos alimentarios de nuestra sociedad, y su consumo sigue un ritmo de crecimiento paralelo con la mejora del nivel de vida. La horchata de chufa es un producto típico de la Comunidad Valenciana, con una gran importancia económica.

Consecuentemente con esta importancia, la fabricación, distribución y venta de la horchata está regulada por la legislación vigente. Sin embargo, el Código Alimentario Español (C.A.E) y las disposiciones que lo desarrollan están lejos de cubrir las necesidades del consumidor en lo que a calidad de la horchata de chufa se refiere.

La horchata de chufa desde el punto de vista nutricional tiene un elevado contenido energético, contiene muy pocas proteínas y son de bajo valor biológico, es pobre en vitaminas, y la relación Ca/P difiere bastante de la recomendada para dietas equilibradas (Morell et al., 1983; Cantalejo, 1996). Sin embargo, la horchata de chufa tiene un gran potencial en el mercado alimentario, con el consiguiente beneficio económico para la comunidad valenciana, el cual está muy limitado por su cortísima vida útil (Selma et al., 2003).

La elaboración de la horchata, de origen familiar, pasó a las horchaterías industriales artesanas, también de carácter familiar, donde se realizaba, no sólo la producción, sino también la venta del producto. Posteriormente surgieron las pequeñas industrias actuales, que fabrican y distribuyen diariamente la horchata

refrigerada a los centros de consumo. La horchata se elabora mediante un proceso tecnológico muy simple, que consta de las siguientes etapas: limpieza, remojado, trituración, maceración, tamizado, incorporación de azúcar y enfriamiento (Morell et al., 1983; Varo et al., 1998). Se necesita un kilo de chufa para producir 6 litros de horchata (Cantalejo, 1996). El esquema de elaboración de la horchata se muestra en la figura 1.

La horchata natural es un alimento altamente perecedero y su alteración se debe principalmente, además de a la gran carga bacteriana que soporta (procedente de los tubérculos de la chufa), a un alto contenido en azúcares y almidón, que la convierten en un medio idóneo para el desarrollo de microorganismos (Arranz et al., 1996; Selma et al., 2003). Se han hecho diversos intentos para reducir la carga microbiana y aumentar la estabilidad de la horchata. Muchos de ellos no han dado buenos resultados, debido a las características intrínsecas de este producto. El uso de conservantes químicos introduce sabores extraños y no inactiva las enzimas presentes en la horchata, los tratamientos térmicos por encima de 72ºC no son convenientes, puesto que bajo estas condiciones, y al poseer un pH elevado (6.3-6.8), el almidón puede gelatinizarse y las proteínas coagular, de modo que se modificarían las características organolépticas del producto (Cantalejo, 1996).

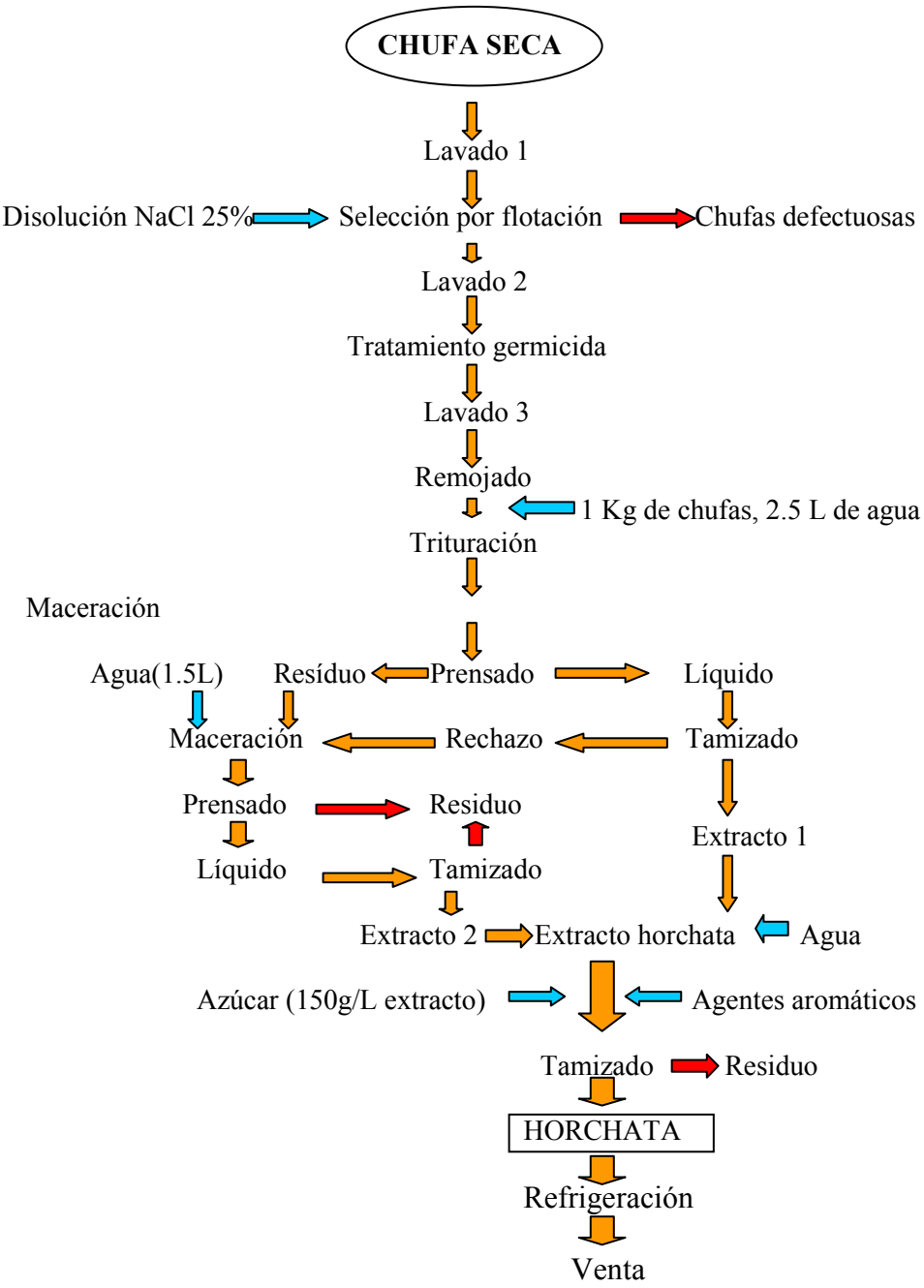

Figura 1. Esquema de elaboración de la horchata de chufa natural.

Desde el punto de vista de su conservación, la horchata de chufa se clasifica en (BOE, 1988):

- **Horchata de chufa natural.** Se preparará con la proporción adecuada de chufa, agua y azúcar para que el producto tenga un mínimo de 12% de sólidos solubles expresados como °Brix a 20°C. Su contenido mínimo de almidón será del 2,2% y el de grasas del 2,5%. Tendrá un pH mínimo de 6,3. Los azúcares totales expresados en sacarosa superarán el 10%. Tanto el almidón como las grasas procederán exclusivamente de los tubérculos utilizados en la preparación del la horchata.

- **Horchata de chufa natural pasterizada.** Es la horchata de chufa natural que ha sido sometida a un tratamiento de pasteurización por debajo de 72°C, sin adición de aditivos ni coadyuvantes tecnológicos. Su composición y características organolépticas y fisicoquímicas serán las mismas que las de la horchata natural.

- **Horchata de chufa pasterizada.** Es la horchata de chufas sometida a un proceso tecnológico que suprima o transforme, total o parcialmente, su contenido de almidón y procesada mediante un tratamiento térmico que asegure la destrucción de los gérmenes patógenos y la mayoría de la flora banal.

- **Horchata de chufa esterilizada.** Es la horchata de chufa sometida a un proceso tecnológico que transforme o suprima total o parcialmente su contenido en almidón y procesada después de su envasado mediante un tratamiento térmico que asegure la destrucción de los microorganismos y la inactividad de sus formas de resistencia.

- **Horchata UHT.** Es la Horchata de chufa sometida a un proceso tecnológico que suprima o transforme, total o parcialmente, su contenido en almidón y procesada mediante un tratamiento térmico UHT que

asegure después de un envasado aséptico la destrucción de los microorganismos y la inactividad de sus formas de resistencia.

- **Horchata de chufa concentrada: refrigerada o congelada.** Es la preparada con las porciones de chufa, agua y azúcar o azúcares adecuados para obtener un producto con una concentración mínima de sólidos disueltos del 42% expresados como °Brix y un pH mínimo de 6, y que por disolución con agua según el modo de empleo permita obtener un producto de características organolépticas y microbiológicas correspondientes a la de la horchata de chufa natural.

- **Horchata de chufa condensada.**

  • Pasteurizada. Es la preparada con las proporciones adecuadas de chufas, agua y azúcares para que el producto resultante tenga un mínimo de 60% de sólidos disueltos, expresados como °Brix a 20°C, un 4,2% de almidón y un 5,4% de grasa, procedentes exclusivamente de las chufas.

  • Congelada. Es la horchata que por sus características de conservación no precisa de una alta concentración de azúcares. Tendrá un mínimo del 50% de sólidos disueltos expresados en °Brix a 20°C, un 5,4% de almidón y un 7,2% de grasa, procedentes exclusivamente de las chufas. Tendrá un pH mínimo de 6. Por disolución, según el modo de empleo, tendrá como mínimo un contenido en almidón y grasas procedentes exclusivamente de las chufas, del 1,3 y del 1,8% respectivamente y un pH mínimo de 6,3. Los azúcares totales expresados en sacarosa serán como mínimo del 40%.

- **Horchata de chufa en polvo.** Es la horchata de chufas sometida a un proceso tecnológico que pueda suprimir o transformar, total o parcialmente, su contenido en almidón en forma de partículas o gránulos

sólidos y obtenida mediante procesos de secado, con un contenido en agua inferior al 5%.

Los tratamientos para mejorar la estabilidad de la horchata son esenciales para la calidad de la misma, pero afectan a su aroma y sabor. Aunque existen muchos productos aceptables en el mercado, ya sea por sus características sensoriales o por su precio, son susceptibles de mejora, y en este sentido se sigue investigando en nuevas tecnologías que permitan conservar la calidad del producto a la vez que lo estabilicen, buscando un alimento lo más parecido al natural.

1.1.2. Zumo de naranja y zumo de naranja-zanahoria

Brasil y USA dominan la producción de cítricos en el ámbito mundial con algo más de 28 millones de toneladas de naranjas al año, lo cual equivale al 45 % de la producción mundial anual de naranjas, que en 2003, supuso 60 millones de toneladas. En Europa, el principal productor es España, seguida de Italia y Grecia, con 3.11, 1.96 y 0.97 millones de toneladas respectivamente (MAPA, 2004).

España es el mayor exportador mundial de frutos cítricos en fresco (3.4 millones de toneladas) (MAPA, 2004) y este mercado puede expandirse mediante una mejor explotación de la imagen de productos frescos, aumentando la vida útil con una calidad óptima y ofreciendo al consumidor opciones novedosas además de los productos tradicionalmente conocidos.

La cantidad de fruta que se destina en España a la industrialización, depende fundamentalmente de la demanda del mercado fresco (exportación y consumo nacional). Brasil y USA suministran el 85% del zumo de naranja que se consume en el mundo, destacando Florida, que industrializa el 95% de su

producción de naranjas como zumo. En Europa, los tres principales productores, España, Italia y Grecia, producen una cantidad de zumo que no cubre la demanda europea, por lo que se recurre a importaciones de zumos de naranja de Brasil y USA (Worsley, 2001). Se estima que la media de transformación en España, en los últimos 5 años es de 600 miles de toneladas de naranja al año, por lo que la producción española de zumo de naranja es de unos 240 millones de litros (Generalitat Valenciana, 2005).

Los cítricos poseen una serie de nutrientes muy importantes para la dieta humana. La naranja (*Citrus sinensis*) es rica en vitamina C, carotenoides y bioflavonoides, y contiene pequeñas cantidades de vitaminas B_1, B_2, B_3, B_5, B_6 y E. El componente mayoritario de los cítricos es la vitamina C, cuya cantidad es muy superior a la de otras frutas. Además, el zumo de naranja tiene también un importante contenido en carotenoides antioxidantes (β-caroteno, β-criptoxantina, zeaxantina y luteína). Los compuestos antioxidantes han adquirido una relevancia notoria puesto que se ha demostrado su participación en la prevención de enfermedades degenerativas, tales como diferentes tipos de cánceres, enfermedades cardiovasculares y neurológicas y otras disfunciones relacionadas con situaciones de estrés oxidativo (fumadores, atmósferas contaminadas, deportistas, dietas inadecuadas, etc) (Gardner et al., 2000; Block et al., 2001; John et al., 2002; Burns et al., 2003; Sánchez-Moreno et al., 2003).

El zumo de naranja es por tanto una fuente muy importante de ácido ascórbico, un nutriente que además de por su acción vitamínica, es importante por su efecto antioxidante, la estimulación del sistema inmune y otros beneficios que están siendo actualmente investigados, como la inhibición de la formación de diversos productos en el estómago, que podrían ser causantes de algunos tipos de cáncer (Kim et al., 2006; Valko et al., 2006).

Las zanahorias (*Daucus carota*) se consumen mucho en fresco, no siendo importante su producción comercial como zumo de zanahoria. Son una excelente

fuente de β-caroteno, con contenidos moderados de ácido ascórbico y diversos minerales. El zumo de zanahoria es una bebida nutritiva si las condiciones de procesado y almacenamiento se eligen cuidadosamente, siendo los problemas más comunes los debidos a la baja acidez, cuajado, cambios en el color y aroma. La acidificación con ácido cítrico o con zumos ácidos mejora algunas de las dificultades del procesado, como la retención del color y la prevención de la coagulación (Ayranci et al., 1993).

En cuanto a la producción de zanahorias, en el año 2003 en España se produjeron 448.3 miles de toneladas (18.7 en la Comunidad Valenciana), de ellas se destinaron 381.1 para el consumo en fresco y 39.1 para su transformación (MAPA, 2004).

El color naranja de la zanahoria se debe a la presencia de carotenos, entre los que destaca el β-caroteno, el cual, tras absorberse en nuestro organismo se transforma en vitamina A o retinol, esencial para la visión, el buen estado de la piel, los tejidos, y para el buen estado del sistema inmunológico. Además, como antioxidante neutraliza los radicales libres, por lo que su consumo frecuente ayuda a prevenir enfermedades degenerativas, cardiovasculares y cáncer. Asimismo contiene vitamina E, que interviene en la estabilidad de las células sanguíneas y en la fertilidad, y tiene acción antioxidante (al igual que las vitaminas A y C), y de vitaminas del grupo B, tales como los folatos y la vitamina B_3 o niacina. El ácido fólico que aportan las zanahorias contribuye a tratar o prevenir anemias y a reducir el riesgo de alteraciones cardiovasculares y de espina bífida en el feto. La niacina interviene en el funcionamiento del sistema digestivo, el buen estado de la piel, el sistema nervioso y en el metabolismo energético. (Consumer.es, 2003).

Stern (1998) abordó el desarrollo de los zumos mezcla de frutas y vegetales enriquecidos con vitaminas, y entre los zumos seleccionados, se dió relevancia al zumo mezcla de naranja-zanahoria por su gran aceptación por parte de los

consumidores. Además de las excelentes y particulares características sensoriales y nutritivas del zumo de naranja, la incorporación de un porcentaje de zanahoria, proporciona una importante contribución para la salud del consumidor (Marx et al., 2003).

1.1.3. Tomate

El tomate (Lycopersicon lycopersicum) es un fruto de la familia de las Solanáceas. Son pocas las Solanáceas comestibles, entre ellas el tomate, el pimiento, la berenjena y la patata, de gran relevancia en la alimentación humana.

Su valor nutritivo y aroma son mayores cuando el tomate madura al sol en pleno campo, es decir, de Agosto a Octubre, aunque tenemos la posibilidad de consumir tomate todo el año cultivado en invernaderos.

Es un alimento poco energético, dos tomates medianos tan sólo aportan 22 calorías. Se le considera una fruta-hortaliza ya que contiene mayor cantidad de azúcares simples que otras verduras, lo que le confiere un ligero sabor dulce. También es fuente importante de ciertas sales minerales (potasio y magnesio, principalmente), y de su contenido en vitaminas destacan la B_1, B_2, B_5, vitamina C y carotenoides como el licopeno (pigmento que da el color rojo característico al tomate). Estas dos últimas sustancias tienen carácter antioxidante con función protectora de nuestro organismo.

En cuanto a la producción de tomates, en el año 2003 en España se produjeron 3.9 millones de toneladas (147431 toneladas en la Comunidad Valenciana), y de ellas se destinaron 2.1millones de toneladas para el consumo en fresco y 1.7 para su transformación (MAPA, 2004).

De este modo, se observa que los tomates se utilizan frecuentemente como un ingrediente para la preparación de las comidas, y por ello, además de su

seguridad microbiológica, la consistencia de los productos obtenidos tras su procesado es un importante aspecto de calidad a tener en cuenta. La consistencia o textura de los productos obtenidos tras el procesado del tomate, es resultado de diferentes transformaciones mediadas por enzimas pectolíticas presentes en el mismo (Krebbers et al., 2003). Polygalacturonasa (PG) y Pectinmetilesterasa (PME) son las enzimas pectolíticas involucradas en la degradación de la pectina del tomate. La acción combinada de estas enzimas conlleva importantes pérdidas de textura y viscosidad durante el procesado industrial de los tomates, y consecuentemente, disminuyen la calidad de sus productos derivados (Gould, 1992; Bartley et al., 1994; Lopez et al., 1997).

Por este motivo, la industria alimentaria intenta encontrar un tipo de tratamiento que permita inactivar de manera selectiva y controlada la enzima PG, mientras que la enzima PME permanezca activa.

1.2. TRATAMIENTOS DE CONSERVACIÓN TRADICIONALES

La estacionalidad y el carácter perecedero de las frutas y vegetales explican la necesidad de aplicar las tecnologías de conservación. El objetivo es combinar el aumento de la vida media con el mantenimiento de las características nutritivas y sensoriales del alimento (Giannakourou et al., 2003).

El procesado térmico es uno de los métodos por los que los alimentos son conservados y se hacen accesibles al consumidor. Durante el tratamiento térmico, además de la inactivación de microorganismos, constituyentes deseables, como nutrientes, color, aroma y textura se destruyen en distintos porcentajes (Rodrigo et al., 1997; Esteve et al., 1998; Prochaska et al., 2000; Alwaazer et al., 2002; Lee et al., 2003; Blasco et al., 2004; Polydera et al., 2003; Polydera et al., 2004; Rodrigo et al., 2006; Sánchez-Moreno et al., 2006).

1.2.1. Esterilización

La esterilización es un tratamiento térmico severo, cuya finalidad es eliminar todos los microorganismos presentes en el alimento, resultando un alimento comercialmente estéril. Cabe citar como ventaja que proporciona una mayor vida útil al alimento, pudiéndose incluso almacenar a temperatura ambiente, sin embargo, 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, en la actualidad, se aplican temperaturas muy elevadas (entre 135 y 150ºC) que permiten tiempos de calentamiento muy cortos (4-15 segundos).

La mayoría de los alimentos estériles comercializados tienen una vida útil de dos años o más. Cualquier deterioro que ocurra transcurrido ese tiempo es debido a cambios en la textura o aromas, no a los crecimientos microbianos (Vaclavik, 1998; Bello, 2000).

1.2.2. Pasteurización

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 tratamientos también tiene inconvenientes, ya que el producto resultante tiene una corta vida media y requiere de otro medio de conservación adicional, como la refrigeración o la congelación. Además es normal que la pasteurización se acompañe de diferentes procedimientos que garanticen la buena conservación del alimento, como el

envasado a vacío, la utilización de envases herméticos, adición de agentes acidulantes, adición de azúcares concentrados, etc. (Bello, 2000).

Hay dos grandes grupos de tecnologías de pasteurización: las que emplean bajas temperaturas (60-65ºC) durante tiempos bastante largos y las que emplean temperaturas más elevadas (75-90ºC) durante tiempos cortos.

Actualmente, los zumos de frutas u hortalizas se someten a temperaturas entre los 90-95ºC durante 15-20 segundos y se envasan asépticamente en caliente. Posteriormente se enfrían y se almacenan para su posterior comercialización (Braddock, 1999; Cano et al., 2003).

En determinados alimentos, como el zumo de naranja, la aplicación de un tratamiento de pasteurización térmica, aunque sea suave, produce una pérdida de calidad (aroma y sabor, independientemente de la pérdida de valor nutritivo) e imposibilita el uso del término "fresco", utilizado por los fabricantes para satisfacer las tendencias del consumidor. Ante esta situación se ha generado un fuerte enfrentamiento entre la FDA (Food and Drug Administration) y los fabricantes de zumos, que ven reducidas sus producciones.

1.3. NUEVAS TECNOLOGÍAS DE CONSERVACIÓN DE ALIMENTOS. TRATAMIENTOS NO TÉRMICOS

Los productos mínimamente procesados han aumentado su demanda en los últimos años debido a la retención de sabor, color, aroma y valor nutritivo, ya que los consumidores demandan productos seguros pero con elevada calidad (con el mínimo de conservantes químicos o sin ellos). Aunque el procesado térmico convencional asegura inocuidad y alarga la vida útil de los alimentos, normalmente conlleva cambios indeseables en la calidad sensorial y nutritiva del producto (Bull et al., 2004). En consecuencia, las tecnologías de procesado no

térmico están en pleno desarrollo como una alternativa a los tratamientos térmicos tradicionales (Selma et al., 2003; Spilimbergo et al., 2003; Sánchez-Moreno et al., 2005).

Adaptarse a las nuevas corrientes y exigencias del consumidor ha sido uno de los objetivos prioritarios del sector alimentario, por lo que desde hace algunos años se están desarrollando nuevos productos, mezclas de zumos, que aumenten la calidad (valor nutritivo, color, etc.) que es el factor que más contribuye a la aceptación por parte del consumidor y a un aumento del valor añadido del producto.

1.3.1. Pulsos Eléctricos de Alta Intensidad (PEAI)

Fruto de la innovación tecnológica en la industria alimentaria es la aparición de esta nueva tecnología: los pulsos eléctricos de alta intensidad (PEAI) con la que se espera tratar alimentos líquidos con determinadas propiedades (ligeramente viscosos, conductividad baja y con pocas partículas y de pequeño tamaño), a los que se les aporte una mayor calidad nutritiva, sensorial y una mayor vida útil, comparándolos con los mismos obtenidos mediante técnicas tradicionales.

El origen de su uso comienza a principios del siglo XX, cuando en 1924, Beattie y Lewis demostraron el efecto letal de las descargas eléctricas sobre los microorganismos al aplicar sobre el alimento un voltaje de 3.000-4.000 V. Posteriormente, otros científicos, como Fetterman (1928) y Getchell (1935), combinaron la corriente eléctrica con la temperatura para pasteurizar leche e inactivar bacterias. Entre 1928 y 1938 la corriente eléctrica se utilizó como medio de generar calor para la pasteurización de unos 200 millones de litros de leche para el consumo (Moses, 1938). Pero no es hasta finales de 1967 cuando Sale y Hamilton (1967, 1968) realizan los primeros estudios para la inactivación de microorganismos aplicando campos eléctricos homogéneos de alto voltaje.

Estos científicos realizaron numerosas observaciones sometiendo suspensiones de microorganismos a campos eléctricos de hasta 25 kV/cm en pulsos de 2 a 20 μs. Observaron que la estructura de la membrana celular presentaba poros irreversibles cuando se les aplicaba un determinado potencial a través de la membrana.

La tecnología de PEAI consiste esencialmente en aplicar una corriente eléctrica en forma de pulsos de corta duración (de 1 a 100μs) a un alimento de conductividad eléctrica adecuada, situado entre dos electrodos, dando lugar a la formación de un campo eléctrico de magnitud comprendida entre 10-70 kV/cm, manteniendo la temperatura de tratamiento por debajo de la empleada en tratamientos térmicos.

Esta técnica se basa en la propiedad que tienen los alimentos fluidos, que están compuestos principalmente por agua y nutrientes como vitaminas, triglicéridos y minerales, de ser muy buenos conductores eléctricos debido a las altas concentraciones de iones que contienen y a su capacidad de transportar cargas eléctricas.

El campo eléctrico afecta a las membranas celulares (Barsotti et al., 1999), pudiendo producir la rotura irreversible de la membrana (Zimmerann, 1986; Pothakamury et al., 1997; Calderón-Miranda et al., 1999), alterando el transporte de iones (Kim et al., 2001) e induciendo cambios en la estructura de las enzimas (Fernández-Díaz et al., 2000). Los mecanismos por los que se inactivan los microorganismos pueden explicarse de diferentes modos:

- Ruptura dieléctrica. La fuerte polarización de las células producida por un campo eléctrico externo, aumenta la permeabilidad y la conductividad de la membrana. El grado de permeabilidad depende de la intensidad y duración del campo aplicado. La ruptura dieléctrica supone una inestabilidad local en la membrana debida a la compresión electromecánica y la tensión del campo eléctrico inducida, dando lugar a la formación de poros.

- Electroporación. Es el fenómeno por el que una célula expuesta a un campo eléctrico sufre una desestabilización temporal de la bicapa lipídica y de las proteínas de su membrana. La consecuencia es la formación de poros en la membrana, por lo que ésta queda parcial o totalmente dañada (Chang, 1992).

En cuanto a la inactivación de enzimas no está muy claro su efecto ni el mecanismo de actuación, ya que en determinadas condiciones de tratamiento algunas enzimas se han conseguido inactivar, mientras que en otras se ha llegado, incluso, a aumentar la actividad de la enzima estudiada (Vega-Mercado et al., 1995; Ho et al., 1997; Giner et al., 2000). En general, el grado de inactivación alcanzada varía mucho de una experiencia a otra, dependiendo del tipo de enzima, de las características del sustrato en el que se encuentra y de las condiciones de tratamiento.

Los elementos básicos de estos sistemas de pulsos eléctricos son:

a) Redes formadoras de pulsos (PFN): la creación de pulsos requiere la descarga rápida de energía eléctrica, en un periodo corto de tiempo. Ello se consigue mediante redes formadoras de pulsos (pulse-forming networks, PFN), que son un circuito eléctrico formado por uno o más generadores de energía, interruptores, resistencias, condensadores y cámaras de tratamiento (Góngora-Nieto et al., 2002). El interruptor debe mantener la energía almacenada en la PFN y controlar su flujo cuando se descargue la misma. La distinta disposición y el número de componentes de cada elemento de la red dan lugar a las distintas formas de los pulsos.

b) Cámaras de tratamiento: la cámara de tratamiento contiene los electrodos, que están situados entre el material aislante y el alimento contenido en el espacio entre los electrodos.

c) *Bomba de alimentación de líquidos*: sistema que permite el suministro del alimento a las cámaras de tratamiento.

d) *Mecanismo de enfriamiento:* Sistema consistente en la recirculación de agua fría a través de los electrodos para controlar la temperatura de los mismos en la cámara de tratamiento.

d) *Sistemas de seguimiento y control:* para conseguir un buen funcionamiento de la PFN, ésta debe estar controlada por un ordenador que rija los periodos de carga y descarga de los condensadores, así como la duración de los mismos (Kreuger, 1989).

Cuando se aplica un campo eléctrico a la cámara de tratamiento que contiene el alimento, la polarización de las moléculas bipolares y el movimiento de los transportadores de cargas, como los iones, en el interior del producto, inducen diferentes tipos de corrientes eléctricas.

El éxito de esta técnica depende de la fuerza del campo eléctrico, de la longitud del pulso, del número de pulsos, de la forma del pulso y de la temperatura de arranque. Un aumento en la intensidad del campo eléctrico y en el tiempo de tratamiento, definido como el producto del número de pulsos y la longitud del pulso, aumenta la inactivación microbiana. La aplicación de los pulsos, cuantificados como intensidad de energía, resultan en una dispersión de la energía térmica y por consiguiente en un aumento de la temperatura del producto (Abram et al., 2003). Los parámetros del tratamiento óptimos dependen de la matriz específica del alimento, de la temperatura, del pH, de la presencia de agentes antimicrobianos y de la conductividad del medio (Ulmer et al., 2002).

La resistencia, la conductividad y las propiedades dieléctricas del alimento a tratar, afectan en la forma en que los alimentos interaccionan con el campo eléctrico (Lewis, 1993).

Los PEAI se han utilizado con gran éxito en productos líquidos, sin embargo también han sido tratados algunos alimentos semisólidos y pulverulentos (Góngora-Nieto et al., 2002).

El tratamiento por PEAI ha ganado un interés creciente al ofrecer algunas ventajas sobre los métodos térmicos que actualmente se utilizan en la elaboración de alimentos y materias frescas (Abram et al., 2003). Mediante el uso de esta tecnología se pretende inactivar los microorganismos presentes en el alimento, así como algunas enzimas, sin destruir los componentes nutritivos y sensoriales. Los alimentos retienen más aromas y vitaminas cuando son elaborados mediante Pulsos Elétricos de Alta Intensidad que con métodos térmicos, manteniendo sus propiedades organolépticas y sus vitaminas casi intactas e inactivando los microorganismos y algunas enzimas. La técnica implica menos cambios en las propiedades físicas y sensoriales del alimento que cuando el mismo se trata por calor (Barbosa-Cánovas et al., 1999; Bendicho et al., 2001; Góngora-Nieto et al., 2002).

Las instituciones que lideran la tecnología, Pure Pulse Technologies, Ohio State University y Diversified Technologies Inc, en USA y Thomson CSF y Centralp, en Francia, son en su mayoría los fabricantes de los equipos de tratamiento.

La investigación se lleva a cabo fundamentalmente a nivel de laboratorio y de planta piloto. El número de equipos de procesado es todavía limitado, sin embargo, existe un equipo a nivel comercial desarrollado por Ohio State University, capaz de procesar desde 200 a 4000 litros por hora, varios equipos a nivel de planta piloto, que procesan desde 80 hasta 200 litros por hora y varios prototipos a nivel de laboratorio (Figura 2).

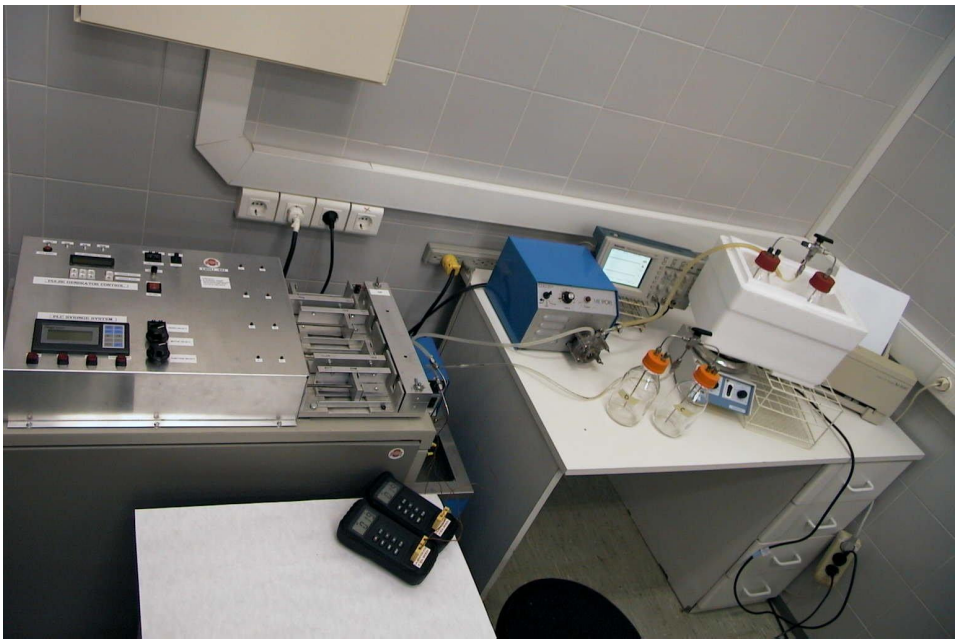

Figura 2. Prototipo a nivel de laboratorio OSU-4D. Equipo de tratamiento por PEAI del Instituto de Agroquímica y Tecnología de Alimentos (IATA, CSIC).

La investigación a nivel de laboratorio se centra en estudios básicos (estudios de fisiología celular, cinéticas de inactivación, mecanismos de inactivación, etc.) aplicados a determinados alimentos (leche, zumos, sopas, etc.), los estudios de planta piloto se encargan de realizar un adecuado escalado de la tecnología (Góngora-Nieto et al., 2002) y los estudios con el equipo comercial existente verifican el potencial de los PEAI, además de deducir el coste económico tanto de implantación del equipo como del procesado (Jin et al., 2002).

1.3.2. Altas Presiones

Esta tecnología, conocida por las siglas de HPP (High Pressure Processing), UHP (Ultra High Pressure) o HHP (High Hydrostatic Pressure) ha sido estudiada como técnica para la conservación de alimentos durante más de un siglo. Esta tecnología se utiliza para conservar alimentos sólidos y líquidos, envasados o no, mediante presiones elevadas (hasta 870 MPa, o aproximadamente 6000 atmósferas), con o sin combinación con calor. Las temperaturas que se alcanzan durante el tratamiento pueden ir desde los 0ºC a los 100ºC y los tiempos de exposición desde pocos segundos hasta 20 minutos.

Los cambios químicos y microbiológicos que se producen en los alimentos dependen de la temperatura y del tiempo de tratamiento. Los efectos de las altas presiones hidrostáticas sobre los microorganismos pueden ser divididos en: efectos relativos a la cubierta celular, cambios celulares inducidos por la presión, aspectos bioquímicos y efectos en los mecanismos genéticos.

La compresión aumenta uniformemente la temperatura de los alimentos aproximadamente 3ºC por cada 100 MPa. La compresión de los alimentos puede cambiar el pH de éstos como efecto de la presión impuesta y debe determinarse para cada proceso de tratamiento de alimentos. La actividad del agua y el pH están entre los factores críticos del proceso en la inactivación de microorganismos por altas presiones. Un aumento de la temperatura del alimento por encima de la temperatura ambiente, y en menor grado, una disminución por debajo de la temperatura ambiente aumentan la tasa de inactivación durante este tratamiento.

En un típico tratamiento por altas presiones, el producto se coloca en un recipiente hermético, flexible y resistente al agua, y se introduce en una cámara de tratamiento que contiene un fluido encargado de transmitirle la presión al

alimento (figura 3). Este fluido (normalmente agua) se presuriza en la cámara mediante una bomba y la presión se transmite al alimento uniformemente. Al transmitirse la presión de manera uniforme y no requerir tratamiento por calor, las características sensoriales del alimento se mantienen sin comprometer la seguridad microbiológica.

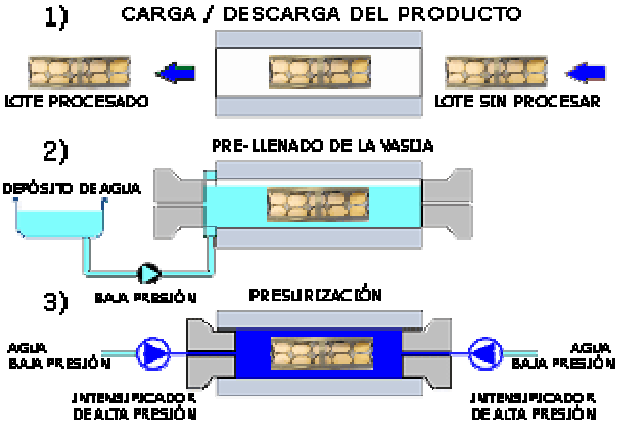

Figura 3. Esquema de funcionamiento de sistema de tratamiento de alimentos por altas presiones.

Actualmente, las altas presiones están siendo aplicadas en USA, Europa y Japón a una selección de alimentos para aumentar su vida útil y su seguridad. Algunos productos tratados por altas presiones que han sido comercializados son: productos cárnicos, productos lácteos, frutas y verduras, pescados y mariscos y zumos y bebidas (figura 4).

Figura 4. Diversos productos comercializados tratados por altas presiones hidrostáticas.

En los cuadros 1 y 2 se muestran los diferentes alimentos vegetales, frutas y zumos tratados por altas presiones, comercializados hasta el momento en todo el mundo.

En USA se comercializan equipos de tratamiento por altas presiones a nivel industrial, así, Avure Technologies (Kent, WA) fabrica equipos con capacidad de hasta 215 litros y con un coste de entre 500000 dólares y 2.5 millones de dólares.

País	Producto	Proceso	Vida útil	Interés y comentarios
Japón (1990)	Mermeladas, salsas y gelatinas	400 MPa, 10-20 min.	2-3 meses, a 4ºC	Mejora la gelificación y penetración de azúcar. Mantiene color, textura y vitamina C
Japón (2000)	Arroz precocinado hipoalergénico	400 MPa	Temperatura ambiente	Desnaturalización de proteínas hipoalergénicas. Uso hospitalario
Italia (2001)	Postres de manzana, pera y fresa	600 MPa 3-5 min.	1-2 meses	Inactivación enzimática e higienización. Mantiene propiedades sensoriales.
USA (2002)	Productos del aguacate	-	-	Inactivación enzimática e higienización. Mantiene propiedades sensoriales.
USA (2002)	Esaladas vegetales	-	-	Higienización e incremento de vida útil sin conservantes
USA (2003)	Cebollas loncheadas	-	45 días	Eliminación del sabor amargo e incremento de vida útil
Canadá (2003)	Salsas y purés de manzana	-	-	Higienización e incremento de vida útil
USA (2004)	Productos de soja, tofú	-	-	Higienización e incremento de vida útil.
España (2005)	Platos preparados de verduras	500 MPa		Higienización e incremento de vida útil.

Cuadro 1. Frutas y verduras del mercado tratadas por altas presiones.

País	Producto	Proceso	Vida útil	Interés y comentarios
Japón (1993)	Licor de arroz (sake)	400 MPa, 30 min.	6-12 meses, 4ºC	Inactiva levaduras y mantiene propiedades sensoriales.
Francia (1994)	Zumos cítricos	400 MPa 1 min.	18 días, 4ºC	Higienización. Mantiene propiedades sensoriales.
México (2000)	Zumos cítricos	500 MPa	-	Higienización. Mantiene propiedades sensoriales.
Líbano (2001)	Zumos de frutas	500 MPa	1 mes	Higienización. Mantiene propiedades sensoriales.
USA (2001)	Zumo de manzana	-	2-3 veces más	Higienización. Mantiene propiedades sensoriales.
Portugal (2001)	Zumo de manzana y zumos cítricos	450 MPa 20-90 s	28 días	Higienización. Mantiene propiedades sensoriales.
Italia (2001)	Zumos de frutas (pera, manzana, fresa, zanahoria)	600 MPa 3-5 min	1-2 meses	Higienización. Mantiene propiedades sensoriales.
USA (2002)	Zumo de naranja, limonada	-	21 días	Higienización. Mantiene propiedades sensoriales.
Republica Checa (2004)	Zumo de brócoli y manzana	500 MPa 1 min	21 días	Higienización. Mantiene propiedades sensoriales.

Cuadro 2. Zumos y bebidas del mercado tratados por altas presiones.

Esta tecnología provoca cambios mínimos en las características nutricionales de los alimentos frescos al eliminar la degradación térmica. Comparado con el procesado térmico, las altas presiones proporcionan alimentos con sabor, apariencia, aroma, textura y nutrientes más similares al producto fresco. La tecnología es especialmente beneficiosa para alimentos sensibles al tratamiento por calor.

Las nuevas tecnologías no térmicas son equivalentes a los procesos de pasteurización pero, no a los de esterilización. Se consiguen reducciones significativas de la población de *Escherichia coli* mediante estas tecnologías, comparables a la pasteurización a 60-70ºC (Lado et al., 2002), sin embargo sólo el tratamiento por altas presiones combinado con calor es capaz de destruir también las esporas. El tratamiento por PEAI es bueno para inactivar, en poco tiempo y contínuo las células vegetativas, pero no consigue eliminar las esporas (Grahl et al., 1996).

BIBLIOGRAFÍA

2. BIBLIOGRAFÍA

Abram, F., Smelt, J.P.P.M., Bos, R., Wouters, P.C., 2003. Modelling and optimization of inactivation of Lactobacillus plantarum by pulsed electric field treatment. Journal of Applied Microbiology, 94, 571-579.

Alwazeer, D., Delbeau, C., Divies, C., Cachon, R., 2003. Use of redox potential modification by gas improves microbial quality, color retention, and ascorbic acid stability of pasteurized orange juice. International Journal of Food Microbiology, 89, 21–29.

Arranz, A., Gascó, R., Périz, R., Coma, J.M., 1996. La educación sanitaria y la calidad microbiológica de la horchata. Alimentaria, 275, 127-132.

Ayranci, G., Tütüncüler, A., 1993. Preparation and storage stability of juices from different carrot species. Die Nahrung, 37 (5), 433-439.

Barbosa-Cánovas, G. V., Góngora-Nieto, M. M., Pothakamury, U. R., & Swanson, B. G., 1999. Academic Press Ltd. London.

Barsotti, L., Cheftel, J. C., 1999. Food processing by pulsed electric fields. II. Biological aspects. Food Reviews International, 5, 181–213.

Bartley, D.A., Nielsen, S.S., Nelson, P.E., 1994. Comparison of invertase and pectinesterase inactivation in processed tomato pulp. Journal of Food Quality, 17, 311–320.

Beattie, J.M., Lewis, F.C., 1924. The electric current (apart from the heat generated). A bacteriological agent in sterilization of milk and other fluids. Journal of Hygiene, 24, 123–137.

Bello, J., 2000. Ciencia Bromatológica. Principios Generales de los Alimentos. Editorial Díaz de Santos, S. A.

Bendicho, S., Arántegui, J., Martín, O., 2001. Procesado de alimentos mediante pulsos eléctricos de alta intensidad de campo. II. Efectos sobre microorganismos y componentes de los alimentos. Alimentaria, 37-44.

Blasco, R., Esteve, M. J., Frígola, A., Rodrigo, M., 2004. Ascorbic acid degradation kinetics in mushrooms in a high-temperature short-time process controlled by a thermoresistometer. Lebensmittel-Wissenschaft + Technologie, 37, 171–175.

Block, G., Norkus, E., Hudes, M., Mandel, S., Helzlsouer, K., 2001. Which plasma antioxidants are most related to fruit and vegetable consumption? American Journal of Epidemiology, 154, 1113–1118.

BOE, 1988. Reglamentación Técnico Sanitaria para la elaboración y venta de horchata de chufa. Real decreto 1338/1988 de 28 de octubre de 1988. Spain.

Braddock, R. J., 1999. Single strength orange juices and concentrate. In Handbook of Citrus by-products and Processing Technology; Wiley, J. New York, 53–83.

Brandt, K., Christensen, L.P., Hansen-Moller, J., Hansen, S.L., Haraldsdottir, J., Jespersen, L., Purup, S., Kharazmi, A., Barkholt, V., Frokiaer, H., Kobaek-Larse, M., 2004. Health promoting compounds in vegetables and fruits: a systematic approach for identifying plant components with impact on human health. Trends in Food Science and Emerging Technologies, 5, 135-149.

Bull, M.K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E.A., Johnson, R.L., Stewart, 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 & Emerging Technologies, 5, 135-149.

Burns, J., Frase, P. D., & Bramley, P. M., 2003. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. Phytochemistry, 62, 939–947.

Calderón-Miranda, M.L., Barbosa-Cánovas, G.V., Swanson, B.G., 1999. Transmission electron microscopy of Listeria innocua treated by pulsed electric fields and nisin in skimmed milk. International Journal of Food Microbiology, 51, 31–39.

Cano, M.P., Plaza, L., Sánchez-Moreno, C., de Ancos, B., 2003. Elaboración y conservación de zumos de naranja. Efecto de nuevas tecnologías sobre su calidad sensorial y nutricional. Alimentación, Nutrición y Salud, 10 (4), 108-119.

Cantalejo, M.J., 1996. Development of new products for earth-almond. Fruit processing, 6 (3), 87-91.

Chang, D.C., 1992. An efficient method of microinjecting attached cells using electroporation. Molecular Biologyof the Cell 3, A90-A90 Suppl. S.

Consumer.es. EROSKI. Alimentación, Hortalizas y Verduras. La zanahoria. Mayo,2003.*www.consumer.es/web/es/alimentacion/aprender_a_comer_bien /guia_alimentos/hortalizas_y_verduras/2003/05/06/60681.php*

Cullum, A., 2003. Increasing fruit and vegetable consumption: the 5 a day programme. Nutrition Bulletin, 28, 159-163.

DOGV (Diari Oficial de la Generalitat Valenciana), 1995. Orden de 25 de septiembre de 1995, de la Conselleria de Agricultura y Medio Ambiente, por la que se aprueba el Reglamento de la Denominación de Origen Chufa de Valencia y su Consejo Regulador. Valencia.

Duthie, G.G., 1999. Determination of activity of antioxidants in human subjects. Proceedings of the Nutrition Society, 58 (4), 1015-1024.

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

Fawzi, W., Spiegelman, D., Villamor, E., Mugusi, F., Essex, M., 2004. A randomized trial of multivitamin supplements and HIV disease progression and mortality. New England Journal of Medicine, 351, 23-32.

Fernandez-Díaz, M.D., Barsotti, L., Dumacy, E., Cheftel, J.C., 2000. Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. Journal of Agricultural and Food Chemistry, 48, 2332–2339.

Fetterman, J.C., 1928. Electrical conductivity method of processing milk. Agricultural Engineering, 9, 107.

Field, A.E., Gillman, M.W., Rosner, B., Rockett, H.R., Colditz, G.A., 2003. Association between fruit and vegetable intake and change in body mass index among a large sample of children and adolescents in the United States, International Journal of Obesity and Related Metabolic Disorders, 27, (7), 821–826.

Gardner, P. T., White, T. A. C., McPhail, D. B., & Duthie, G. G., 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chemistry, 68, 471–474.

Generalitat Valenciana, 2005. Producción de cítricos. Consellería de Agricultura Pesca y Alimentación.

Getchell, B.E., 1935. Electric Pasteurization of Milk. Agricultural engineering, 10, 408-410.

Giannakourou, M.C., Taoukis, P.S., 2003. Kinetics modelling of vitamin C loss in frozen green vegetables under variable storage conditions. Food Chemistry, 83, 33-41.

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

Góngora-Nieto, M.M., Sepúlveda, D.R., Pedrow, P., Barbosa-Cánovas, G.V, Swanson, B.G., 2002. Food processing by pulsed electric fields: treatment delivery, inactivation level, and regulatory aspects. Lebensmittel-Wissenschaft + Technologie, 35, 375-388.

Gould, W.A., 1992. Tomato production, processing and technology, CTI Publications Inc. Maryland, USA, p. 536.

Grahl, T., Märkl, H., 1996. Killing of microorganisms by pulsed electric fields. Applied Microbiology and Biotechnology, 45, 148-157

Granado, F., Olmedilla, B., Blanco, I., 2004. Carotenoid depletion in serum of young type-1 diabetics fed low-carotenoid diets. Annals of nutrition and metabolism, 48, 251-258.

Grassmann, J., Hippeli, S., Elstre, E. F., 2002. Plant's defence mechanism and its benefits for animal and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant physiology and Biochemistry, 40, 471-478.

Healthy People, 2000. U. S. Department of Health and Human Services, Healthy People 2010: Understanding and Improving Health, US Government Printing Office, Washington, DC.

Ho, S.Y., Mittal, G.S., Cross, J.D., 1997. Effect of high field electric pulses on the activity of selected enzymes. Journal of Food Engineering, 31, 69–84

Jin, Z.T., Zhang, Q.H., 2002. Comercial scale integrated PEF processing system and its operation cost. IFT technical paper presentation 91E-21. International Meeting, IFT.

John, J. H., Ziebland, S., Yudkin, P., Roe, L. S., & Neil, H. A. W., 2002. Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomised controlled trial. The Lancet, 359, 1969–1974.

Kim, S. J., Kim, S., 2001. On the effects of the heat generated during an electric field-induced ferroelectric domain switching. International Journal of Solids and Structures, 38 (8), 1311-1325.

Kim, K. N., Pie, J. E., Park, J. H., Park, Y. H., Kim H. W., Kim, M. K., 2006. Retinoic acid and ascorbic acid act synergistically in inhibiting human breast cancer cell proliferation. The Journal of Nutritional Biochemistry, 17, 7, 454-462.

Krebbers, B., Matser, A.M., Hoogerwerf, S.W., Moezelaar, R., Tomassen, M.M.M., van den Berg, R.W., 2003. Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial

inactivation and quality parameters. Innovative Food Science and Emerging Technologies, 4, 377-385.

Kreuger, F. H., 1989. Partial discharge detection in high voltage equipment. London: Butterworths.

Kritchevsky, S. B., 1999. β-carotene, carotenoids and the prevention of coronary heart disease. Journal of Nutrition, 129, 5-8.

Lado, B. H., Yousef, A. E., 2002. Alternative food-presservation technologies: efficacy and mechanisms. Microbes and Infection, 4, 433-440.

Lampe, J.W., 1999. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. American Journal of Clinical Nutrition, 70, 475-490.

Lee, H. S., Coates, G. A., 2003. Effect of thermal pasteurization on Valencia orange juice color and pigments Lebensmittel-Wissenschaft + Technologie, 36, 153-156.

Lewis, M.J., 1993. Propiedades físicas de los alimentos y de los sistemas de procesado. Editorial Acribia, Zaragoza.

Lopez, P., Sanchez, A.C., Vercet, A., Burgos, J., 1997. Thermal resistance of tomato polygalacturonase and pectinmethylesterase at physiological pH. Zeitschrift Für Lebensmittel-Untersuchung und Forschung 204, pp. 146–150.

MAPA, 2004. Ministerio de Agricultura Pesca y Alimentación, Anuario de estadística.

Marx, M., Stuparic, M., Schieber, A., Carle, R., 2003. Effects of thermal processing on trans-cis-isomerization of β-carotene in carrot juices and carotene-containing preparations. Food Chemistry, 83, 609-617.

Morell, J., Barber, S., 1983. Chufa y Horchata: características físicas, químicas y nutritivas. Caja de Ahorros de Valencia, Departamento de investigación y desarrollo agroindustrial, IATA (CSIC).

Moses, D., 1938. Electric pasteurization of milk. Agricultural Engineering, 19, 525-526.

Neumark-Sztainer, D., Wall, M., Perry, C., Story, M., 2003. Correlates of fruit and vegetable intake among adolescents. Findings from Project EAT, Preventive Medicine, 37 (3), 198–208.

Olmedilla, B., Granado, F., Blanco, I., Vaquero, M., Cajigal, C., 2001. Lutein in patients with catarcts anda ge-related macular degeneration: a long-term supplementation study. Journal of the science of food and agricultura, 81 (9), 904-909.

Olmedilla, B., Granado, F., Blanco, I., Vaquero, M., 2003. Lutein, but not alpha-tocopherol, supplentation improves visual function in patients with age-related catarcts: A 2-y double-blind, placebo-controlled pilot study. Nutrition, 19, 21-24.

Polydera, A. C., Stoforos, N. G., Taoukis, P. S., 2003. Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. Journal of Food Engineering, 60, 21–29.

Polydera, A.C., Galanou, E., Stoforos, N.G., Taoukis, P.S., 2004, Inactivation Kinetics of pectin methylsterase of greek Navel orange juice as a function of high hydrostatic pressure abd temperature process conditions. Journal of Food Engineering, 62, 291-298.

Pothakamury, U.R., Barbosa-Cánovas, G.V., Swanson, B.G., Spence, K.D., 1997. Ultrastructural changes in Staphylococcus aureus treated with pulsed electric fields. Food Science and Technology International, 3, 113–121.

Prochaska, L.J., Nguyen, X.T., Donat, N., Piekutowski, W.V., 2000. Effects of food processing on the thermodynamic and nutritive value of foods: literature and data base survey. Medical Hypotheses, 54 (2), 254-262.

Rodrigo, D., Cortés, C., Clynen, E., Schoofs, L., Van Loey, A., Hendrickx, M., 2006. Thermal and high pressure stability of purified Polygalacturonase and

Pectinmethylesterase from four different tomato processing varieties. Food Research Internacional, 39 (4), 440-448.

Rodrigo, C., Rodrigo, M., & Fiszman, S.M. 1997. The impact of high temperature, short time thermal treatment on texture and weight loss of green asparagus. Zeitschrift fuer Lebensmittel -Untersuchung und - Forschung A, 205 (1), 53–58.

Sale, A.J.H., Hamilton, W.A., 1967. Effects of High Electric Fields on Microorganisms I. Killing of Bacteria and Yeast. Biochemical Biophysical Acta, 148, 781-788.

Sale, A.J.H., Hamilton, W.A., 1968. Effects of High Electric Fields on Microorganisms III. Lysis of Erythocytes and Protoplasts; Biochemical Biophysical Acta, 163, 37-43.

Sánchez-Moreno, C., Plaza, L., de Ancos, B., Cano, P., 2003. Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juice. Journal of the Science of Food and Agriculture, 83, 430–439.

Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., Cano, M.P., 2005. Impact of High Pressure and Pulsed Electric Fields on Bioactive compounds and Antioxidant Activity of Orange Juice in comparison with traditional thermal processing. Journal of Agricultural and Food Chemistry, 53, 4403-4409.

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

Selma, M.V., Fernández, P.S., Valero, M., Salmerón, M.C., 2003. Control of Enterobacter aerogenes by high-intensity, pulsed electric fields in horchata, a Spanish low-acid vegetable beverage. Food Microbiology, 20, 105-110.

Simon, J.A., Hudes, E.S., Tice, J.A., 2001. Relation of serum ascorbic acid to mortality among U.S adults. Journal of the American College of Nutrition, 20, 255-263.

Slaterry, M.L., Benson, J., Curtin, K., Ma, K.-N., Schaeffer, D., Potter, J.D., 2000. Carotenoids and colon cancer. American Journal of Clinical Nutrition., 71, 575-582.

Spilimbergo, S., Dehghani, F., Bertucco, A., Foster, N.R., 2003. Inactivation of bacteria and spores by pulsed electric field and high pressure CO_2 at low temperature. Biotechnology and Bioengineering, 82, 118–125.

Stern, P., 1998. In nature's image. Fruit and vegetable beverages with added ingredients. Fluessiges Obst., 65, 126-130

Torregrosa, F. 2001. Características fisico-químicas y de calidad de la horchata natural. Tesina de licenciatura, Universidad de Valencia, España.

Ulmer, H.M., Heinz, V., Gänzle, M.G. Knorr, D., Vogel, R.F., 2002. Effects of pulsed electric fields on inactivation and metabolic activity of Lactobacillus plantarum in model beer. Journal of Applied Microbiology, 93, 326–335.

Vaclavik, V. A.., 1998. Essentials of Food Science. Editorial Chapman & Hall.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions, 160 (1), 1-40.

Van Poppel, G., Goldbohm, R. A., 1995. Epidemiological evidence for β-carotene and cancer prevention. American Journal of Clinical Nutrition, 62, 1493-1503.

Varo, P., Company, R., Guillem, C., 1998. Contribución a la caracterización fisicoquímica de la horchata de chufa natural. Alimentaria, 35 (298), 69-71.

Vega-Mercado, H., Powers, J.R., Barbosa-Cánovas, G.V., Swanson, B.G., 1995. Plasmin inactivation with pulsed electric fields. Journal of Food Science, 60, 1132–1136.

Virseda, P., 1997. Estudio microbiológico de la horchata elaborada en el municipio de Alboraya (Valencia). Alimentación, Equipos y Tecnología, 16, 83-86.

Worsley, S., 2001. USA is challenging for top spot. Foodnews® Juices and Fruit. July 11.

Zimmerann, U., 1986. Electric breakdown, electropermeabilization and electrofusion. Reviews on Physiological Biochemical Pharmacology, 105, 175-256.

OBJETIVOS

3. OBJETIVOS

Las frutas y hortalizas se consumen generalmente frescas, pero un gran número de ellas han de ser procesadas y/o conservadas por razones económicas, logísticas, para mejorar su digestibilidad, por necesidades culinarias o para facilitar su consumo a determinados grupos de consumidores. El aumento de la demanda por parte del consumidor de alimentos seguros, procesados, y cuya preparación ocupe el menor tiempo posible, ha llevado a la industria alimentaria a aumentar su producción de este tipo de alimentos. Para ello, se requiere la valoración y seguimiento de aquellos factores microbiológicos y nutricionales que directa o indirectamente pueden afectar a la salud de los consumidores y de aquellos otros que pueden afectar a la calidad de los alimentos elaborados.

El **OBJETIVO GENERAL** del presente trabajo es estudiar la posible variación de diferentes parámetros nutritivos y de calidad en alimentos líquidos de origen vegetal procesados por tecnologías no térmicas, su comparación cuando se tratan por procesos térmicos convencionales y su almacenamiento en refrigeración.

Para alcanzar este objetivo general se plantean los siguientes **OBJETIVOS ESPECÍFICOS**:

- Puesta a punto y validación de los diversos métodos de análisis.
- Tratamiento por PEAI (distintos campos y tiempos) de horchata de chufa, zumo de naranja-zanahoria y zumo de naranja. Determinación de parámetros nutritivos (vitamina C, carotenoides, vitamina A, fenoles solubles totales y poder antioxidante total) y de calidad (pH, índice de formol, actividad peroxidásica, sustancias reactivas al ácido tiobarbitúrico, ºbrix, color, hidroximetilfurfural, índice de pardeamiento).
- Evolución de las características de calidad de la horchata de chufa tratada por PEAI durante su almacenamiento en refrigeración.

- Estudio de las variaciones en el perfil carotenoide, vitamina A, vitamina C, color y otros parámetros de calidad del zumo de naranja-zanahoria y zumo de naranja tratados por PEAI y durante su almacenamiento en refrigeración.
- Impacto del tratamiento por altas presiones y por calor sobre enzimas de diferentes variedades de tomates utilizados en la industria.

JUSTIFICACIÓN DE LA UNIDAD TEMÁTICA Y PRESENTACIÓN DE LOS TRABAJOS

4. JUSTIFICACIÓN DE LA UNIDAD TEMÁTICA Y PRESENTACIÓN DE LOS TRABAJOS

La innovación tecnológica en la industria alimentaria es uno de los pilares en los que se sustenta el incremento de competitividad y el aporte de alimentos seguros desde un punto de vista microbiológico y con una calidad nutritiva y disponibilidad acordes con las exigencias del consumidor actual. La tendencia en este momento es hacia el consumo de "productos semejantes a fresco", es decir elaborados por procesos "mínimos".

La nueva tecnología "pulsos eléctricos de alta intensidad (PEAI)" de conservación de alimentos líquidos, se basa en el efecto letal de pulsos de muy corto voltaje y muy corta duración (microsegundos) sobre los microorganismos, en principio sin pérdidas significativas para los factores de calidad. La calidad es el efecto que más contribuye a la aceptación por parte del consumidor y a un aumento del valor añadido. Sin embargo cualquier tecnología nueva que se desee aplicar a un alimento en particular, debe demostrar que el producto elaborado tiene unas cualidades superiores al mismo producto elaborado de forma tradicional.

El gran interés de la aplicación de estos estudios a la horchata, zumo de naranja-zanahoria y zumo de naranja, se basa en la creciente demanda de bebidas o zumos naturales, dado su mayor calidad nutritiva y sensorial.

La horchata de chufa natural tiene un gran potencial en el mercado alimentario, el cual está muy limitado por su corta vida útil. Ante la falta de una legislación clara respecto a la composición de la horchata de chufa y sobretodo respecto a la calidad de la misma, se requiere una caracterización de la horchata de chufa comercializada previa al tratamiento por PEAI, para poder obtener conclusiones fiables. En el capítulo 1 se describe la caracterización de este producto y los

resultados obtenidos al estudiar las variaciones de diferentes parámetros de calidad tras el procesado por PEAI del mismo.

Actualmente se está haciendo un gran esfuerzo para asegurar que los compuestos nutritivos y bioactivos presentes en los alimentos de origen vegetal se mantengan o se modifiquen mínimamente durante el tratamiento de conservación de los mismos y su almacenamiento. El zumo mezcla de naranja-zanahoria y el zumo de naranja son ricos en vitamina C y carotenoides. En el capítulo 2 se pone a punto y se valida un método cromatográfico para la determinación de carotenoides en ambos tipos de zumos, y posteriormente, en el capítulo 3, se estudian los cambios que se producen en los carotenoides, vitamina A y vitamina C del zumo de naranja-zanahoria tras su tratamiento mediante PEAI y durante su almacenamiento en refrigeración, comparándose con el zumo de naranja-zanahoria pasteurizado. Además, se estudian las variaciones que se producen en los carotenoides del zumo de naranja-zanahoria sin tratar durante su almacenamiento en congelación a -40ºC.

A lo largo del capítulo 4 se estudia el zumo de naranja, el más consumido a nivel mundial, y se describen los cambios observados en cuanto a color, carotenoides, vitamina C y otros parámetros físico-químicos y de calidad, tras procesar el zumo de naranja por PEAI y pasteurización, así como al almacenarlo en refrigeración.

En el capítulo 5 (trabajo realizado durante una estancia en la Universidad Católica de Leuven, Bélgica), se estudia también la influencia del tratamiento térmico y del tratamiento por Altas Presiones sobre la textura de diferentes variedades de tomate utilizadas en la industria alimentaria para su procesado. Se estudia la inactivación de las enzimas involucradas en la degradación de la pectina del tomate (Polygalacturonasa y Pectinmetilesterasa), responsables de la textura de los productos derivados del mismo.

El trabajo que se presenta proporciona información científica sobre la variación de parámetros nutritivos y de calidad de todos estos alimentos, necesaria para aplicar unas condiciones de tratamiento con PEAI o con Altas Presiones, con el fin de obtener alimentos procesados de alta calidad y de larga vida útil conservados en refrigeración.

Los resultados obtenidos en este trabajo de Tesis Doctoral han dado lugar a las siguientes publicaciones científicas:

Capítulo 1. Horchata de chufa.

1. **Physical and chemical properties of different commercially available types of "horchata de chufa".** Italian Journal of Food Science, 16, 113-121 (2004).

2. **Quality characteristics of horchata (a Spanish vegetable beverage) treated by pulsed electric fields during shelf-life.** Food Chemistry, 91, 319-325 (2005).

Capítulo 2. Puesta a punto de un método de determinación de carotenoides en zumos de frutas y vegetales.

1. **Identification and Quantification of Carotenoids Including Geometrical Isomers in Fruit and Vegetable Juices by Liquid Chromatography with Ultraviolet-Diode Array Detection.** Journal of Agricultural and Food Chemistry, 52, 2203-2212 (2004).

Capítulo 3. Zumo de naranja-zanahoria.

1. **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 (2005).

2. **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 (2006).

3. **Changes in carotenoids including geometrical isomers and ascorbic acid content in orange-carrot juice during frozen storage.** European Food Research and Technology, 221, 125-131 (2005).

Capítulo 4. Zumo de naranja.

1. **Changes of colour and carotenoids contents during high intensity pulsed electric field treatment in orange juices.** Food and Chemical Toxicology, 44, 1932-1939 (2006).

2. **Carotenoid profile modification during refrigerated storage in untreated and pasteurized orange juice and orange juice treated with High Intensity Pulsed Electric Fields.** Journal of Agricultural and Food Chemistry, 54, 6247-6254 (2006).

3. **Ascorbic acid content in refrigerated orange juice after HIPEF and thermal treatment.** Enviado para su publicación.

4. **Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice.** Enviado para su publicación.

5. **Determination of Total Antioxidant Capacity and Phenolic compounds of orange juice refrigerated treated by High Intensity Pulsed Electric Fields.** Enviado para su publicación.

Capítulo 5. Tomate.

1. **Thermal and high pressure stability of purified Polygalacturonase and Pectinmethylesterase from four different tomato processing varieties.** Food Research International, 39, 440-448 (2006).

Así como también a distintas contribuciones a congresos:

1. **Estudio de la calidad y seguridad de las horchatas de chufa del mercado.** Cortés C., Esteve MJ., Frígola A., Torregrosa F. II Congreso Nacional de Ciencia y Tecnología de los Alimentos, Orihuela (Spain), 2003 (ISBN Vol.1 84-95893-75-4).

2. **Pulsed Electric Field Processing Effects on Quality Characteristics of Horchata, a Spanish Vegetable Beverage.** Cortés C., Esteve MJ., Frígola A., Torregrosa F. Workshop on Nonthermal food Preservation, Wageningen (The Netherlands), 2003.

3. **Carotenoid Profile Determination by Liquid Chromatography for Differentiation Orange Juices.** Cortés C., Esteve MJ., Frígola A., Torregrosa F. 3rd Scientific Meeting of the Spanish Society of Chromatography and Related Techniques, Almería (Spain), 2003.

4. **Carotenoid profile determination by liquid chromatography to evaluate the effect of pulsed electric field processing on orange-carrot juice.** Torregrosa F., Cortés C., Esteve MJ., Frígola A. 4th Scientific Meeting of the Spanish Society of Chromatography and Related Techniques, Madrid (Spain), 2004.

5. **Changes of cis/trans carotenoid isomers contents during non-thermal treatments in fruit juices by liquid chromatography with**

diode-array detection. Cortés C., Esteve MJ., Frígola A., Rodrigo D., Torregrosa F. 29th Internacional Symposium on High Performance Liquid Phase Separations and Related Techniques, Stockholm (Sweden), 2005.

6. **Changes of Vitamin C contents during refrigerated storage in orange juice treated by high pulsed electric field and comparison with pasteurized juice.** Cortés C., Esteve MJ., Torregrosa F., Frígola A. Innovations in Traditional Foods (INTRADFOOD2005), Valencia (Spain), 2005.

7. **Thermal and high pressure stability of purified Polygalacturonase and Pectinmethylesterase from four different tomato processing varieties.** Cortés C., Rodrigo D., Clynen E., Schoofs L., Van Loey A., Hendrickx M. Innovations in Traditional Foods (INTRADFOOD2005), Valencia (Spain), 2005.

8. **Antioxidant properties of different commercially available pasteurized orange juices refrigerated.** Cortés C., Esteve MJ., Torregrosa F., Frígola A. VI Congreso Internacional de la Dieta Mediterránea, Barcelona (Spain), 2006.

9. **Effect of the pasteurization on the nutritive value of a squeezed orange juice**. Cortés C., Esteve MJ., Torregrosa F., Frígola A. I World Congress of Public Health Nutrition, Barcelona (Spain), 2006.

RESULTADOS

Capítulo 1

Horchata de chufa

1. **Physical and chemical properties of different commercially available types of "horchata de chufa".** Italian Journal of Food Science, 16, 113-121 (2004).
2. **Quality characteristics of horchata (a Spanish vegetable beverage) treated by pulsed electric fields during shelf-life.** Food Chemistry, 91, 319-325 (2005).

PHYSICAL AND CHEMICAL PROPERTIES OF DIFFERENT COMMERCIALLY AVAILABLE TYPES OF "HORCHATA DE CHUFA"

PROPIETÀ FISICHE E CHIMICHE DEI DIFFERENTI TIPI DI "HORCHATA DE CHUFA" DISPONIBILI IN COMMERCIO

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ABSTRACT

A physicochemical analysis was made of the different types of "horchata de chufa" sold in Spain. The analytical methods for the determination of total fat, total solids, ash, reducing sugars, starch and protein were adapted for these low-acid vegetable beverages. The contents found were: fat, 0.91–3.40 g per 100 g; total solids, 13.61–22.06 g per 100 g; ash, 0.079–0.298 g per 100 g; protein, 0.294–0.742 g per 100 g; starch, not detected–7.45 g per 100 mL; reducing sugars, 11.13–17.86 g per 100 mL; pH, 6.37–7.56; and density, 1.039–1.067 g mL^{-1}. The fat content of all the sterilised horchatas was below the minimum value specified in the regulations.

- Keywords: chemical composition, horchata de chufa, physical properties -

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RIASSUNTO

È stata condotta un'analisi chimicofisica sui differenti tipi di "horchata de chufa" prodotti in Spagna. I metodi analitici per la determinazione dei grassi totali, dei residui totali, delle ceneri, degli zuccheri riducenti, dell'amido e delle proteine, sono stati adattati a questo alimento. La composizione riscontrata è la seguente: grassi, 0.91-3.40 g per 100 g; residui totali, 13.61-22.06 g per 100 g; ceneri, 0.079-0.298 g per 100 g; proteine, 0.294-0.742 g per 100 g; amido, non riscontrato-7.45 g per 100 mL; zuccheri riducenti, 11.13-17.86 g per 100 mL; pH, 6.37-7.56; densità, 1.039-1.067. I contenuto dei grassi de tutte le horchata sterilizzate è sotto il minimo istituito nella regolazione.

Parole chiave: composizione chimica, horchata de chufa, proprietà fisiche

INTRODUCTION

"Horchata de chufa" is a milky-looking Spanish dairy beverage, which is extracted from tiger-nut tubers (*Cyperus esculentus* L.) with the addition of sugar (sucrose). Horchata is a Mediterranean low-acid vegetable beverage that has a high nutritional quality and therefore an important potential in the present food market, limited by a very short shelf-life (Selma et al., 2003). This high nutritional quality is due to its composition. Fat is rich in oleic acid (75% of total fat) and linoleic acid (9–10% of total fat), and in its protein composition arginine is the major amino acid, followed by glutamic acid and aspartic acid. With the exception of His, essential amino acid contents in natural horchata de chufa are higher than the contents in the model protein proposed for adults by the FAO/OMS (Morell and Barber, 1983; Navarro et al., 1984).

The nutritional and sensory characteristics of horchata depend on many factors, including the chemical composition of the tuber and the manufacturing process (Varo et al., 1998a,b). Their lipid and protein content can change without changes in their lipid and amino acid profiles.

Non-alcoholic beverages play an important role in the nutritional habits of European society; their increased consumption in the last few years has paralleled an improvement in the standard of living.

Because of the importance of this product, its manufacture, distribution and sale are regulated by current legislation (BOE, 1988; BOE, 1991). Completion of this legislation is held back by lack of information about the characteristics of the various types of horchata available on the market (Cantalejo, 1996).

The appearance of sterilised horchata de chufa in Valencia in the eighties made it possible for this type of food to reach all parts of Spain, and in recent years sales have started to expand to other countries, such as France, Mexico, Panama, Venezuela, the Dominican Republic, the USA and some Mediterranean countries. Horchata consumption in Spain is about 50 million litres per year. Annual sales of this product amount to over 60 million euros and it has a very well established market (Consejo Regulador Denominación Origen "Chufa de Valencia", 2003).

The various food preservation techniques have given rise to the manufacture of horchata on an industrial scale, but heat treatment at temperatures above 75ºC may gelatinise the starch and coagulate the proteins and so alter the organoleptic characteristics typical of horchata (Cantalejo, 1996; Mosquera et al., 1996).

The aim of the present study was to characterise the various types of horchata commercially available and establish the influence of the preservation treatment on its nutritional characteristics.

MATERIALS AND METHODS

Samples

Commercial products of the various types of horchata de chufa considered representative of the variability of this kind of product on the Spanish market

were selected. Three batches of each type of horchata were purchased, with different manufacture dates. The analyses were performed in duplicate on two subsamples from each batch and type of horchata.

The different types of horchata defined in Spanish legislation (BOE, 1988; BOE, 1991) are the following:

- Untreated horchata: this must be prepared with the appropriate proportion of chufas, water and sugar. The starch and fat obtained must come exclusively from the tubers used for preparation of the horchata. The product may be presented as: refrigerated (A) or frozen (B).
- Pasteurised horchata: this is horchata de chufa subjected to a technological heat process that wholly or partially suppresses or transforms its starch content, and then exposed to a heat treatment that ensures the destruction of pathogenic germs and most of the common flora. It may be presented as: refrigerated (C) or frozen (D).
- Sterilised horchata (E): this is similar to pasteurised horchata, but subjected to heat treatment that ensures destruction of microorganisms and inactivation of their forms of resistance.
- UHT horchata (F): this is similar to sterilised horchata, but with the application of UHT heat treatment and aseptic canning.

All the horchatas mentioned above must have a minimum of 12 g of soluble solids per 100 g, expressed as ºBrix at 20 ºC. Their minimum fat content, obtained exclusively from the tubers used, must be 2 g per 100 g. The minimum pH is 6.3, and total sugar expressed as sucrose must be greater than 10 g per 100 mL.

- Condensed pasteurised horchata (G): this must be prepared with suitable proportions of chufa, water and sugars so that the resulting condensed product has a minimum of 60 g of soluble solids per 100 g, expressed as ºBrix at 20 ºC, 3.5 g of starch per 100 mL, and 4.5 g of fat per 100 g, obtained exclusively from

the chufas. It must have a minimum pH of 6. Total sugar must be at least 50 g per 100 mL, expressed as sucrose.

- Condensed pasteurised horchata without sugar (H): this must be prepared in the same way as condensed pasteurised horchata, but with the addition of fructose instead of sucrose.

Determination Methods

Total fat

The fat content of the horchata was determined by extraction with organic solvents in a Soxhlet-type apparatus (Carpenter et al., 1993). As the sample was liquid, it was decided to use the method used by Folch et al. (1958) and Manglano (1999), with some modifications. A mixture of chloroform and methanol (2:1, v/v) was chosen for the extraction of total fat. Fifty mL of horchata was placed in a decanting funnel, 50 mL of the extractant mixture was added, and the result was agitated for 2 min. The contents were separated into two tubes and centrifuged (3000 rpm, 15 min, 10ºC). The chloroform phase was separated and the entire extraction process was repeated with the aqueous phase. The solvent was eliminated and the residue was heated in an oven at 80ºC for 1 hour and then weighed. The intraday precision (RSD %) obtained was 2.2±0.7% and the interday precision was 3.3±1.1%, using four samples.

Total solids

It was seen that when the samples of horchata were dried to a constant weight at 100±4ºC, they caramelised because of the high percentage of sugar. In order to overcome this difficulty, different tests were made with 5, 10, 15 and 20 mL of horchata, which was dried in a sand bath (60ºC) until a pasty mixture was obtained. This was then placed in a forced air oven at different temperatures, ranging from 80 to 100ºC, for a period varying between 2 and 48 hours. In all cases there was caramelisation of the carbohydrate present in the horchata. The tests were continued without drying the sample in the sand bath. The final

conditions selected were 15 mL maintained at 95ºC in a forced air oven for 2 hours.

Ash

The ash content of the horchata was determined in accordance with the official method (BOE, 1988), but varying the final temperature of the oven to 450ºC. Different volumes of horchata (2, 5, 10 and 25 mL) were tested, and it was found that the percentage of ash was 0.238±0.033, 0.232±0.027, 0.273±0.008 and 0.281±0.005 (g per 100 g ± standard deviation) and the coefficient of variation was 13.7, 11.7, 2.5 and 1.9 for 2, 5, 10 and 25 mL of horchata, respectively. The method used was as follows: The organic matter of 25 mL of sample was destroyed by ashing in a temperature programmed furnace. The temperature was increased slowly, at a rate of 50ºC/30 min, to obtain a final temperature of 450ºC. This temperature was maintained for 24 – 48 h. In order to complete the digestion, the residue was soaked with 1 mL concentrated nitric acid (d = 1.4 g mL–1). Between 48 and 72 h were needed to destroy the organic matter of the sample completely.

Reducing sugars

The volumetric method of Lane-Eynon was used. Four mL of horchata was centrifuged at 7800 rpm for 10 min at 2ºC (see determination of starch), and the supernatant was separated and collected in a 100-mL standard flask. To extract the sugars remaining in the sediment, different volumes of lukewarm water were added (10, 15 and 25 mL), the mixture was centrifuged at 4300 rpm for 10 min at 20ºC, and the supernatant was collected in the standard flask. After 4 extractions with 15 mL of lukewarm water, it was confirmed that the extraction of sugars was complete by means of the Molisch test (the solution to be tested was poured over sulphuric acid with some drops of α-naphthol in a proportion of 15 g per 100 mL. The appearance of a red-violet ring in the contact area indicated the presence of sugar). Four mL of Carrez solution was then added, and the mixture was made up to 100 mL with deionised water and filtered.

Fifty mL of the filtrate was taken and placed in another 100-mL standard flask, 25 mL of deionised water and 5 mL of hydrochloric acid (d = 1.18 g mL^{-1}) were added, and the resulting mixture was placed in a water bath at 70ºC. Various analysis times (3, 5, 7, 10, 12 and 15 min) were studied, and it was found that hydrolysis was complete after 7 min. The flask was cooled, neutralised with 2.5 N NaOH and made up to 100 mL with deionised water. The mixture was tested with 10 mL of Fehling's solution, which had previously been tested with a solution of inverted sucrose at 0.5 g per 100 mL.

In order to calculate the precision of the method, the sugar content was determined in 6 aliquots of a particular horchata, giving a coefficient of 1.4% (17.76±0.24 g per 100 mL of reducing sugars). To estimate the accuracy of the method, 6 recovery assays were prepared, giving a value of 98.1%.

Starch

The starch content of horchata varies in accordance with the preservation treatment to which it is subjected. A method for the determination of starch in horchata was therefore prepared, based on the method proposed by the Ministerio de Sanidad y Consumo (1985). Four mL of horchata was taken and centrifuged (7800 rpm, 10 min, 2ºC), and one drop of iodine solution was added to the supernatant in order to ascertain whether starch was present or not. If starch was present in the supernatant, the sediment alone could not be used to determine the starch content because it would give an underestimate, and so in this case a lyophilised sample of the horchata was analysed. If there was no starch in the supernatant, the sediment obtained by ultracentrifugation or a quantity of lyophilised sample equivalent to 4 mL of horchata was taken, 8 mL of ethanol:petroleum ether (1:3 v/v) was added, the mixture was centrifuged at 3500 rpm for 5 min at 20 ºC, and the supernatant was discarded. In this way the fat was eliminated. The simple sugars were separated from the sediment by washing with lukewarm water as indicated in the section on the determination of

reducing sugars. The starch in the sediment was then hydrolysed. A study was made with perchloric acid at 52 g per 100 mL using various volumes (1, 2, 4, 6 and 8 mL), times (15, 30 and 45 min) and temperatures (60, 75 and 85ºC) for the hydrolysis. The conditions finally selected were as follows: the sediment was dissolved in 5 mL of deionised water and then transferred to a 100-mL standard flask, 6 mL of perchloric acid (52 g per 100 mL) was added, the mixture was shaken and placed in a water bath at 75ºC for 15 min with periodic agitation. In these conditions the hydrolysis of the starch was complete.

Protein

The official method (BOE, 1988) was followed, although for horchata adjustments were made to the sample quantity, the various reagent quantities, the distillation time and the indicator most suitable for testing the protein content.

Different volumes (1, 2, 3 and 5 mL) of horchata were studied. The sample quantity selected was 2 mL, because it gave the smallest coefficient of variation. It was placed in a digestion tube, and 10 mL of concentrated sulphuric acid (d = 1.84 g L^{-1}), 6 drops of (2N) $CuSO_4$, 3 g of K_2SO_4 and some glass beads were added. The mixture was placed on a heating blanket until mineralisation was complete. It was then distilled in a Kjeltec apparatus, with automatic addition of 100 mL of water and 80 mL of NaOH (40 g per 100 mL). Different distillation times (5, 6.8 and 9.9 min) were tested, and the time finally chosen was 6.8 min. When distillation was complete, the NH_3 was tested with (0.1 N) HCl until there was a change in the indicator (methyl red). A parallel test was performed with a blank.

pH

The determination of pH was based on potentiometric measurement at 20ºC (BOE, 1988). The pH was determined in a Crison GLP 21 pH meter equipped with a temperature compensation sensor at 20ºC. The results were expressed to two decimal places.

Density

This was determined at 20ºC with a pycnometer.

Statistical evaluation

The contents were compared using one-way analysis of variance (ANOVA). To determine differences between products and between samples of the same product the least significant difference (LSD) test ($p < 0.05$) was applied. The computer program employed was Statgraphics® Plus for Windows 3.0.

RESULTS AND DISCUSSION

The results obtained in the present study for the various properties are described below. Tables 1 and 2 show the mean values of six determinations (two determinations for each subsample) for each batch and each type of horchata de chufa.

It was observed that the **fat** content of all the sterilised horchatas and horchata F_1 (UHT) was below the minimum value specified in the regulations (2 g per 100 g) (BOE, 1988). The fat content was particularly high in condensed pasteurised horchata without sugar (H), with a value three times greater than that of condensed pasteurised horchata (G), which differs in its sucrose content.

Sample	Fat	Solids	Ash	Protein	Starch	Sugar
	(g per 100 g)				(g per 100 mL)	
A	2.95±0.09	20.03±0.28	0.265±0.021	0.730±0.198	4.31±0.16	17.86±0.13
B	2.84±0.09	19.24±0.34	0.246±0.001	0.438±0.001	2.88±0.06	16.47±0.20
C_1	2.79±0.24	22.06±0.95	0.266±0.027	0.631±0.018	6.04±0.09	17.05±0.08
C_2	3.40±0.18	21.56±0.58	0.240±0.006	0.691±0.010	5.57±0.34	17.24±0.73
C_3	2.75±0.14	21.17±0.42	0.298±0.006	0.742±0.026	0.85±0.05	16.95±0.62
D	2.76±0.19	21.05±0.18	0.242±0.001	0.693±0.023	3.66±0.19	16.35±0.34
E_1	1.09±0.05	15.10±0.09	0.227±0.006	0.378±0.025	6.07±0.52	11.49±0.06
E_2	1.50±0.05	15.55±0.19	0.159±0.004	0.418±0.021	N.D.	13.18±0.16
E_3	0.91±0.05	14.57±0.05	0.230±0.001	0.395±0.023	N.D.	12.89±0.10
E_4	0.92±0.05	15.09±0.03	0.200±0.021	0.366±0.042	1.55±0.03	11.95±0.20
E_5	1.27±0.06	14.62±0.01	0.195±0.001	0.434±0.018	2.83±0.11	11.21±0.03
F_1	1.74±0.04	13.61±0.02	0.217±0.001	0.437±0.004	0.15±0.06	11.13±0.10
F_2	2.24±0.08	13.63±0.39	0.265±0.002	0.446±0.005	N.D.	11.68±0.17
G	0.92±0.03	15.48±0.04	0.079±0.004	0.294±0.025	1.06±0.08	15.06±0.18
H	3.10±0.11	16.54±0.25	0.101±0.001	0.384±0.022	7.45±0.63	12.90±0.21

N.D.: not detected. Untreated horchata: refrigerated, A or frozen, B; Pasteurised horchata: refrigerated, C or frozen, D; Sterilised horchata: E; UHT horchata: F; condensed pasteurised horchata: G or without sugar, H.

All the values for the condensed horchatas (G and H) are expressed in terms of diluted horchata, so that the final result is of the same order as those of the other horchatas.

Table 1. Chemical characteristics of each of the types of “horchata de chufa” commercially available in Spain (n=6)

Sample	pH	Density ($g\ mL^{-1}$)
A	6.59±0.02	1.067±0.005
B	6.55±0.02	1.062±0.001
C_1	6.89±0.12	1.065±0.001
C_2	6.70±0.05	1.064±0.001
C_3	6.37±0.01	1.060±0.003
D	6.78±0.07	1.065±0.002
E_1	7.56±0.09	1.044±0.001
E_2	6.55±0.09	1.046±0.001
E_3	6.96±0.12	1.041±0.001
E_4	7.42±0.05	1.041±0.001
E_5	7.50±0.04	1.039±0.001
F_1	7.25±0.10	1.039±0.002
F_2	6.87±0.01	1.042±0.001
G	7.12±0.01	1.048±0.002
H	6.44±0.03	1.052±0.001

Untreated horchata: refrigerated, A or frozen, B; Pasteurised horchata: refrigerated, C or frozen, D; Sterilised horchata: E; UHT horchata: F; condensed pasteurised horchata: G or without sugar, H.

All the values for the condensed horchatas (G and H) are expressed in terms of diluted horchata, so that the final result is of the same order as those of the other horchatas.

Table 2. Determination of pH and density of each of the types of horchata de chufa commercially available in Spain (n=6)

The smallest percentage (g per 100 g) of **total solids** was found in the sterilised horchatas (E_1 – E_5) and the UHT horchatas (F_1 and F_2), followed by the condensed pasteurised horchatas (all the values for the condensed horchata are expressed in terms of diluted horchata) (G and H) and then the remaining horchatas. These differences were statistically significant ($p < 0.05$).

The quantity of mineral salts in a sample was determined from the ash obtained after incineration of the sample. The mineral composition of horchata de chufa can vary because of agronomic factors such as climate, geographical location of the land, kind of soil, kind of fertiliser, etc.

The lowest percentage of **ash** was found in the condensed pasteurised horchatas (G and H), and these differences were statistically significant ($p < 0.05$). Sterilised and UHT horchata (E and F) had higher values than the condensed pasteurised horchatas and lower values than refrigerated untreated horchata (A), frozen untreated horchata (B), refrigerated pasteurised horchata (C) and frozen pasteurised horchata (D), but these differences were not statistically significant.

For each kind of horchata de chufa, the Technical Health Regulations define the minimum **starch** content (BOE, 1988), which is 1.9 g per 100 mL for frozen untreated horchata (B), refrigerated pasteurised horchata (C) and frozen pasteurised horchata (D), and 0.7 g per 100 mL for condensed horchatas (G and H) when diluted for consumption. However, the regulations in the Valencian Community (DOGV, 1995) establish the slightly higher values of 2.2. and 0.85 g per 100 mL, respectively. The starch content is very variable, even within a given type of horchata. Only sample C_3 had values below those set by the regulations.

The sugar content of the condensed horchatas (G and H) shown in Table 1 is expressed in terms of diluted horchata, so that the final result is of the same order as those of the other horchatas. The Technical Health Regulations establish that total sugar, expressed as sucrose, should be at least 10 g per 100 mL, except in the case of condensed horchatas, in which it should be greater than 50 g per

100 mL (BOE, 1988; DOGV, 1995). The sterilised and UHT horchatas had the lowest content of **reducing sugars**, and these differences were statistically significant.

The **total protein** content was obtained by determining the total nitrogen using the method of Kjendahl and multiplying by a correction factor of 6.25. As can be seen, the protein content varied according to the type of horchata, but in no case was it more than 1 g per 100 g.

In all the horchatas analysed, the **pH** satisfied the Technical Health Regulations (minimum pH of 6.3 except for condensed horchata, which must have a minimum pH of 6 after being reconstituted). The highest pH values (7.05 to 7.19) were found in the sterilised and UHT horchatas and condensed pasteurised horchata, and these horchatas were statistically different ($p < 0.05$) from the others, with the exception of UHT horchata, which did not differ statistically ($p < 0.05$) from pasteurised horchata.

The **density** varied from 1.041 g mL^{-1} for UHT horchata (F) to 1.067 for refrigerated untreated horchata (A).

Although many works on chufa tubers have appeared, few studies on the physical and chemical properties of horchata de chufa have been published. The pH values found by Mestres and Pujadas (1980) for pasteurised and sterilised horchata (6.61 and 6.84 respectively) are slightly lower than those found in the present study for these two types of horchata. They obtained a mean pH value of 6.14 for natural horchata, lower than the minimum required in current legislation (BOE, 1988) and lower than the value found in the present study. The sugar contents found in pasteurised and untreated horchata, 8.16 and 9.4 g per 100 mL, respectively, were lower than those found in the present work, whereas the sugar content in sterilised horchata was of the same order. The same can be said for total solids, for which they obtained values of 14.7, 16.75 and 14.7 g per 100 g in pasteurised, untreated and sterilised horchata respectively. The fat content (3.0 g per 100 g) that they found for pasteurised horchata is similar to the value found

in the present work. For untreated and sterilised horchata they reported values of 3.24 and 2.26 g per 100 g, respectively, which are higher than the levels found in the present work, especially for sterilised horchata. The density values found in the present study are higher than those obtained by Mestres and Pujadas (1980) for untreated, pasteurised and sterilised horchata (1.034, 1.036 and 1.035 g mL^{-1}, respectively).

For untreated horchata, the sugar content found in this work is similar to the value obtained by Navarro et al. (1984) (18.3 g per 100 mL) and higher than the values found by Varo et al (1998a), Beneyto et al. (2000) and Gozalbo et al. (2000), who reported ranges of 14.5–16.9 g per 100 mL, 14.5–16.0 and 14.8–15.8 g per 100 g, respectively.

The total fat value for untreated horchata found in the present work is higher than the values reported by Morell and Barber (1983) and Navarro et al. (1984) (2.70 and 2.62 g per 100 mL, respectively) and similar to the values found by Varo et al. (1998a), Beneyto et al. (2000) and Gozalbo et al. (2000).

In untreated horchata, the values found for solids lie within the range obtained by Varo et al. (1998a) (19.7–21.6 g per 100 g) and are slightly below the values found by Morell and Barber (1983) and Navarro et al. (1984) (22.18 and 22.82 g per 100 g, respectively).

Morell and Barber (1983) and Navarro et al. (1984) obtained protein values of 1.23 and 0.93 g per 100 mL, respectively, in untreated horchata, higher than those found in the present study, and starch values of 3.44 and 2.42 g per 100 mL respectively, lower than those found in this work.

The ash content found in untreated horchata in this study is similar to the values found by Morell and Barber (1983) and Navarro et al. (1984).

In untreated horchata the pH value found in this study and in the literature (Morell and Barber, 1983; Navarro et al., 1984; Varo et al., 1998a; Beneyto et al., 2000 and Gozalbo et al., 2000) is higher than the minimum required by legislation (BOE, 1988).

Morell and Barber (1983) and Varo et al. (1998a) obtained a density value between 1.067 and 1.077 g/mL, similar to the value found in the present study.

CONCLUSION

There were few differences between the different types of horchata (as was verified by the statistical analyses performed), except for the condensed horchatas (G and H), which had lower values for total solids, ash and sugar than the other horchatas. Condensed pasteurised horchata also had lower values for fat and starch. A point to note is the higher protein content in untreated horchata. The Technical Health Regulations were satisfied in all cases, except in the fat content of all the sterilised horchatas and horchata F_1 (UHT), which was below the minimum value specified in the regulations.

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REFERENCES

Beneyto H., Varó P., Guillem C. and Murillo A.D. 2000. Caracterización química de la horchata de chufa natural en las comarcas de l'Alcoià y el Comtat (Alicante). Alimentación, equipos y tecnología. 5: 99-101.

BOE. 1988. Real decreto 1338/1988, de 28 de octubre, por el que se aprueba la Reglamentación Técnica Sanitaria para la Elaboración y Venta de Horchata de Chufa. Boletín Oficial del Estado. Madrid.

BOE. 1991. Real decreto 1410/1991, de 27 de septiembre, por el que se modifica la Reglamentación Técnica Sanitaria para la Elaboración y Venta de Horchata de Chufa, aprobada por el Real Decreto 1338/1988 de 28 de octubre. Boletín Oficial del Estado. Madrid.

Cantalejo M.J. 1996. Development of new products from earth-almond. Fruit Process. 6:87–91.

Carpenter D.E., Ngeh-Ngwainbi J. and Lee S. 1993. Lipid Analysis, in Methods of Analysis for Nutrition Labeling, Ed by Sullivan DM and Carpenter DE. AOAC International. Gaithersburg, pp 85–104.

Consejo Regulador Denominación Origen "Chufa de Valencia". 2003. http://www.chufadevalencia.org

DOGV (Diari Oficial de la Generalitat Valenciana). 1995. Orden de 25 de septiembre de 1995, de la Conselleria de Agricultura y Medio Ambiente, por la que se aprueba el Reglamento de la Denominación de Origen Chufa de Valencia y su Consejo Regulador. Valencia.

Folch J., Lees M. and Sloane S.G.H. 1958. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Gozalbo E., Escoin C., Barreda I. and Gargallo C. 2000. Caracterización química de la horchata de chufa natural. Proceeding IX Simposium Nacional de Laboratorios e Institutos Municipales de Salud Pública. 1:32.

Manglano P. 1999. Estudio de la fracción lipídica de fórmulas para lactantes: Composición e indicadores de oxidación. Degree thesis. Universidad de Valencia. Valencia.

Mestres J. and Pujadas C. 1980. Estudio de la calidad de la horchata de chufa comercializada en Barcelona. 1ª parte. *Alimentaria*. 117:65-68.

Ministerio de Sanidad y Consumo, Análisis de Alimentos. 1985. Ministerio de Sanidad y Consumo, Secretaría General para el Consumo, Dirección General de Control y Análisis de la Calidad. Madrid.

Morell J. and Barber S. 1983. "Chufa y horchata: Características físicas, químicas y nutritivas". Caja de Ahorros de Valencia, Departamento de investigación y desarrollo agroindustrial, IATA (CSIC).

Mosquera L.A., Sims C.A., Bates R.P. and O'Keefe S.F. 1996. Stability of "Horchata de Chufas". J. Food Sci. 61:856–861.

Navarro J. L. Schwartz M., Gasque F., Alberola J., Pérez R. and Lafuente B. 1984. Influencia de la época de recolección de la chufa (*Cyperus esculentus L.*) sobre las características analíticas y sensoriales de la horchata. Rev. Agroquim. Tecnol. Aliment. 24: 199-208.

Selma M. V., Fernández P. S., Valero M. and Salmerón M. C. 2003. Control of *Enterobacter aerogenes* by high-intensity, pulsed electric fields in horchata, a Spanish low-acid vegetable beverage. Food Microbiol. 20: 105-110.

Varo P., Company R. and Guillem C. 1998a. Contribución a la caracterización fisicoquímica de la horchata de chufa natural. *Alimentaria* 35:69–71.

Varo P., Company R. and Guillem C. 1998b. Composición nutricional básica de la horchata de chufa natural. *Alimentación, equipos y tecnología*: 107–110.

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Quality characteristics of horchata (a Spanish vegetable beverage) treated by pulsed electric fields during shelf-life

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Abstract

The application of pulsed electric fields (PEF) is one of the new non-thermal technologies being studied to evaluate their potential as alternative or complementary processes to thermal pasteurization. "Horchata de chufa" (tiger nut milk or earth almond milk) is of high nutritional quality and therefore has great potential in the food market, limited by its very short shelf-life. The present work studies whether PEF can be used to obtain a quality horchata and increase its shelf-life while maintaining its organoleptic characteristics. In order to do so we determined pH, total fat, peroxide index, thiobarbituric acid reactive substances index, formol index, and peroxidase activity in natural (untreated) horchata and horchata subjected to various PEF treatments and studied their stability during storage in refrigeration (2–4ºC). After PEF treatment, only peroxidase activity decreased significantly ($p < 0.05$). This parameter and pH varied during the shelf-life of the horchata, and a negative correlation was obtained between pH and peroxidase activity. © 2004 Elsevier Ltd. All rights reserved.

Keywords: quality, horchata, pulsed electric fields

INTRODUCTION

"Horchata de chufa" is a refreshing, non-alcoholic beverage of milky appearance, obtained from the tubers of the "chufa" (Cyperus esculentus L.). It is a typical product of Spain, and of great economic importance. The annual value of chufa production is close to 3 million euros (MAPA, 1997). According to the industrial production survey, 31749 thousand liters of horchata were manufactured in 1998, representing a retail market value of some 20.1 million euros (INE, 1999). Natural horchata has a pH in the range 6.3–6.8 and is rich in starch. Consequently, it cannot be heated above 72ºC as this would cause the starch to gel and would alter the organoleptic characteristics of the product. Horchata de chufa is of high nutritional quality and therefore has great potential in the food market, limited by its very short shelf-life (Selma, Fernández, Valero & Salmerón, 2003). The fat is rich in oleic acid (75% of total fat) and linoleic acid (9-10% of total fat), and arginine is the major amino acid, followed by glutamic acid and aspartic acid. With the exception of histidine, the essential amino acids in natural horchata de chufa are higher than the amount in the model protein proposed for adults by the FAO/OMS (Morell and Barber, 1983; Navarro et al., 1984). Treatments to improve the stability of horchata are essential for its quality, but they have been applied after significant composition changes, consisting mainly in removal of starch. This has resulted in a loss of aroma and flavor with respect to natural horchata. Consequently, there is a need to explore new technologies that are less drastic and that can preserve product quality and stabilize the product.

Undesirable quality changes may take place during some food pasteurization processes that are used to increase the shelf-life of products in order to obtain an acceptable commercial duration. Preservation of these characteristics is the main aim in foods described as natural, as even mild pasteurization may cause considerable losses in some characteristic properties of natural products (Lu &

Whitaker, 1974). Consequently, because of consumer demand for safe but natural foods, manufacturers have begun to take an interest in finding alternatives to thermal pasteurization (Gould, 1996).

The application of Pulsed Electric Fields (PEF) is one of the non-thermal techniques that have demonstrated their capability of increasing the shelf-life of various pumpable liquid foods.

The main aim of any preservation process is the inactivation of pathogenic or sporulated microorganisms or reduction of their growth in controlled conditions. Various studies have been published which analyze the capability of Pulsed Electric Fields to inactivate microorganisms. Sale and Hamilton (1967) indicated that, of the microorganisms studied, Saccharomyces cerevisiae was the most sensitive to pulse treatment. Consequently, research has been carried out with this microorganism, achieving an inactivation range of between 4 and 9 log reductions, depending on the treatment conditions and substrate used (Rodrigo, Martínez, Harte, Barbosa-Cánovas & Rodrigo, 2001). Pothakamury (1995) achieved 5 decimal reductions with Lactobacillus delbrueckii, Bacillus subtilis and Staphylococcus aureus. Inactivation studies with Pulsed Electric Fields confirm the effectiveness of this treatment and its capability as an alternative technology for food processing.

An important consideration in any technology used for the inactivation of microorganisms is the interpretation of survival curves. The basic models used for interpreting survival curves are based on first-order relations. Hülsheger, Potel and Niemann (1981) were the first to propose a model that described survival curves based on the relation between the logarithm of the microbe survival and treatment time at specific electric field intensity. Peleg (1995) proposed a model based on the Fermi equation, which related the percentage of surviving microorganisms with electric field intensity. Reina, Jin, Zhang and

Yousef (1998) found a relation between the logarithm of the percentage of surviving microorganisms and treatment time at a determined field intensity.

Inactivation studies have concentrated both on model substrates and on real foods (orange juice, apple juice, and milk, among others).

For the study of horchata de chufa we selected the following parameters: pH, total fat, peroxide index, thiobarbituric acid reactive substances index (TBARS), formol index, and peroxidase activity. We studied the possible variation of each parameter with storage. As the shelf-life of natural horchata is considered to be about 48 hours (Barber, 1981), we also studied storage for 5 days in order to establish a relation between variation in the parameters and reduction in the quality of the horchata with time.

The aim of this work is to study whether this new, non-thermal technology (PEF) can be used to obtain a quality horchata and increase its shelf-life while maintaining its organoleptic characteristics.

MATERIALS AND METHODS

Samples. Various batches of samples were obtained directly from the manufacturer involved in the project, which supplied containers of recently made natural horchata de chufa. In total, 7 samples of natural horchata were analyzed, and each was subjected to various times and electric field intensities. The analyses were performed in duplicate, and during the period of the study the samples were stored in refrigeration (2–4ºC). In parallel, for each of the PEF treatments applied we analyzed a sample, in duplicate, to which no treatment was applied and which was designated as the blank throughout the study.

Pulsed Electric Field treatment system. The sample treatments were applied in a continuous PEF treatment system designed by the University of Ohio and located in the Instituto de Agroquímica y Tecnología de los Alimentos (CSIC) in

Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage, and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, OR).

The flow was set at 60 ml/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL). The treatment times varied between 100 and 475 μs and the electric field intensity between 20 and 35 kV/cm. The temperatures during the treatments did not exceed 35 ºC.

Analytic methods.

- pH: The determination of pH was based on the potentiometric measurement at 20ºC (BOE, 1988). It was determined in a Crison GLP 21 pH meter equipped with a temperature compensation sensor at 20ºC. The results were expressed to two decimal places.

- Total fat: We used the method established by Cortés, Torregrosa, Esteve and Frígola, 2004 for the extraction of fat in horchata de chufa, consisting in extraction of fat from the sample with a mixture of chloroform (Merck, Darmstadt, Germany) and methanol (J. T. Baker, Deventer, Holland) (2:1, vol/vol), in accordance with the method described by Angulo (1997) with various modifications.

- Peroxide index: This is defined as the milliequivalents of active oxygen contained in one Kg of fatty matter and it was calculated by iodometry. Iodine formed in the oxidation of iodide ion by the peroxides was determined with sodium thiosulphate. We disolved the fat extracted in 10 ml of chloroform, we added 15 ml of glacial acetic acid and 0.7 g of potassium iodide. After 5 minutes we added 75 ml of water and some drops of starch 1% (vol/vol), and determined

the iodine formed in the reaction with tiosulfato sodium thiosulphate 0.01N (AOAC, 1990).

- Thiobarbituric acid reactive substances index (TBARS): Hydroperoxides, formed by oxidation of lipids, start to degrade into various reaction products. One of the products formed during the oxidation process is malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) to give a colored compound. In order to quantify MDA, initially we reproduced the method described by Angulo (1997), which consists in determining the substances that react with thiobarbituric acid by means of fluorescence with an excitation wavelength of 515 nm and an emission wavelength of 553 nm. However, when we applied this method the spectrofluorimeter did not distinguish the different quantities of horchata. As a result, it was not possible to establish the optimum quantity to be taken, we did not obtain reproducibility in the results, and with time the fluorescence decreased rapidly. After several trials we also observed a considerable influence of temperature on the medium.

After many tests and trials, given the impossibility of solving all the problems posed by the spectrofluorimetric method, we used the method for determination of TBA described by Salih, Smith, Price and Dawson (1987). It is a spectrophotometric technique and is based on the fact that the adduct that forms malondialdehyde with TBA absorbs at the same wavelength as the adduct formed by TBA with 1,1,3,3-tetramethoxypropane (TEP). The intensity of the color is a measure of the concentration of MDA and has been correlated organoleptically with rancidity in fatty products.

For the analysis we homogenized 5 ml of horchata in 15 ml of 0.38 M $HClO_4$ (Merck, Darmstadt, Germany) for 3 minutes in an ice bath. We then added 0.5 ml of a solution of 0.19 M butylhydroxytoluene (Sigma, Steinheim, Germany) in ethanol (J. T. Baker, Deventer, Holland). The mixture was homogenized in an ice bath for 1 minute and centrifuged at 3000 g (Jouan GT 422 refrigerated

centrifuge) for 15 minutes at 5 ºC, and the supernatant was filtered through filter paper (Ø=110 mm).

The original method takes 0.7 ml of this filtrate, but we studied the optimum volume to be taken in our determination, taking volumes ranging from 0.1 to 0.7 ml, and arrived at the conclusion that a volume of 0.2 ml should be used because the most reproducible results were obtained with this volume. Therefore we took 0.2 ml of filtrate, added 0.7 ml of a solution of 0.02 M TBA (Sigma, Steinheim, Germany) and made it up to 1.4 ml with deionized water. The mixture was boiled at 100 ºC for 30 minutes. It was allowed to cool and the absorbance was measured at 532 nm, using deionized water as a blank.

All compounds with aldehyde groups can react with TBA, so that it is necessary to know whether the presence of matrix influences the behavior of the standards. To determine whether or not there was matrix interferences a least squares fit was performed and a covariance analysis was applied to confirm whether the curves were parallel or not. Parallel curves indicate that the solutions behave in a similar way and therefore there are no matrix interferences in the determination. In all the cases studied the result obtained was that the curves were not parallel (Figure 1), indicating that the solutions had different behaviors and therefore there were matrix interferences in the determination. Therefore, for all the thiobarbituric acid reactive substances index determinations curves of standards spiked with matrix should be used. In order to determine the goodness of the method selected we studied the various analytic parameters, obtaining a limit of detection of 0.076 nmol and a recovery percentage of 92.96 ± 0.76%. The instrumental precision expressed as relative standard deviations (RSD%, n=6) was determined on two different days. The interday precision was slightly high, but natural horchata de chufa has a shelf-life of only a few days (intraday precision = 4.3%, interday precision = 13.9%). These parameters indicate that

the method is suitable for the determination of thiobarbituric acid reactive substances.

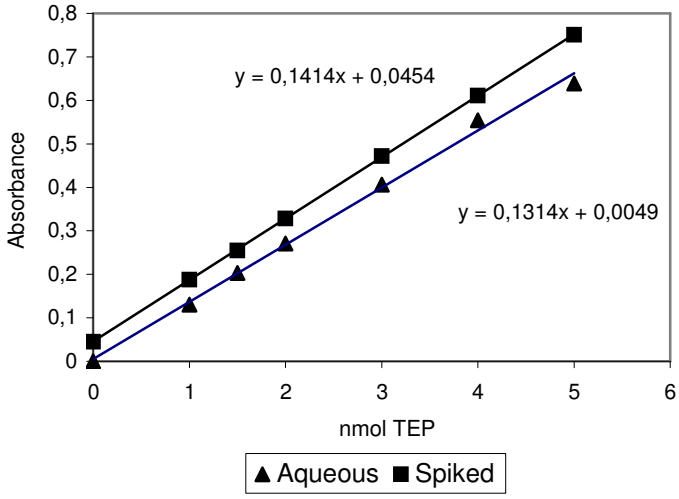

Figure 1: Comparison of slopes in the matrix interference test.

- Formol index: Samples were analysed according to the "Ministerio de Agricultura, Pesca y Alimentación" (MAPA, 1993) methods, in order to determine the formol index.

- Peroxidase activity: We reproduced the method proposed by Moreno, Gasque and Schwartz (1983) for the determination of peroxidase activity in horchata de chufa.

4 ml of horchata were centrifuged at 4300 rpm for 15 minutes at 2ªC, and the supernatant was filtered through filter paper (Ø=110 mm). After filtration of the homogenate, the filtrate was diluted 1:100 with deionized water. POD activity was assayed using 2 ml of the filtrate and adding 2 ml of guaiacol 2% in

methanol (vol/vol) and 1 ml of 0.16% (vol/vol) H2O2. The reaction was carried out for 12 min at 25°C, and the change in absorbance at 480 nm with time was recorded, using a UV-Vis spectrophotometer (Perkin Elmer, USA).

The initial velocity was calculated from the slope of the linear part of the curve obtained. The straight line section of the activity curve was used to express the enzyme activity. Enzymatic activity was defined as an increase in one absorbance unit per minute under the conditions of the assay.

Statistical analysis. The contents were compared using one-way analysis of variance (ANOVA). To determine differences between PEF treatment and the contents during storage of each of the PEF treatments the LSD test ($p < 0.05$) was applied.

RESULTS AND DISCUSSION

The stability of horchata de chufa is related with pH, so that when horchata is altered by the action of microorganisms there is generally a decrease in pH to a greater or lesser degree, according to the degree of alteration. Table 1 shows the results obtained, in which the study at the start time shows that the pH of all the samples is above 6.3, which is the minimum pH established by the Technical Health Regulations (BOE, 1988). During storage there was a clear reduction in these values with time, indicating a decrease in the stability of the horchata. This variation in pH can be fitted to a linear model (Table 2), and the slope of each curve indicates the rate of decrease of this parameter over time. The decrease in the pH of the natural horchata not subjected to any treatment (blank) was faster. Moreover, for a given electric field, when the treatment time increased, the rate of decrease in pH was less. This indicates that the longer the time in which pulses are applied (for a given field), the more effective the treatment is, allowing the stored horchata to have a longer shelf-time. There are almost no studies of the physicochemical characteristics of horchata de chufa subjected to PEF with which to compare our results, but some authors have carried out

studies on other liquid foods (milk, juice, etc.) treated with PEF. Michalac, Alvarez and Zhang (1999) studied the variation in various parameters, including pH, of milk subjected to PEF and concluded that no differences appear in the values obtained before and after treatment. Yeom, Evrendilek, Jin and Zhang (2001) studied a commercial plain low fat yogurt (87.6%) mixed with strawberry jell (8.8%) and strawberry syrup (3.6%) and obtained pH values that did not vary with PEF treatment. In our case there were also no significant differences between the pH values of the blank and those of the samples subjected to PEF at the start time, but we cannot compare the evolution of pH during storage as there are no pH studies comparable to ours.

E	t	Days					
(kV/cm)	(μs)	0	1	2	3	4	5
20	300	6.43 ± 0.01	6.36 ± 0.00	6.38 ± 0.00	5.95[b] ± 0.00	5.56 ± 0.01	5.10 ± 0.00
20	450	6.53 ± 0.02	6.40 ± 0.01	6.35 ± 0.01	6.10[b] ± 0.00	6.01 ± 0.00	5.87 ± 0.01
20	475	6.56 ± 0.01	6.59 ± 0.02	6.41 ± 0.00	6.25[b] ± 0.00	6.19 ± 0.01	6.10 ± 0.02
25	300	6.59 ± 0.01	6.62 ± 0.01	6.35 ± 0.00	6.38 ± 0.00	6.30 ± 0.02	6.25[b] ± 0.01
30	175	6.53 ± 0.00	6.39 ± 0.00	6.30 ± 0.02	6.25[b] ± 0.01	5.91 ± 0.01	5.63 ± 0.01
35	100	6.48 ± 0.00	6.33 ± 0.01	6.27[b] ± 0.01	6.23 ± 0.00	5.84 ± 0.01	5.52 ± 0.00
Blank		6.52 ± 0.01	6.13[b] ± 0.01	5.94 ± 0.00	5.61 ± 0.01	5.25 ± 0.02	4.91 ± 0.00

[a] Mean ± standard deviation for three samples.

[b] Indicates that they are higher than the minimum pH values established by the Technical Health Regulations.

Table 1: Mean pH values during the storage period, according to electric field strength (E) and treatment time (t)[a]

E (kV/cm)	t (μs)	*Equation*	r^a
20	300	- 0.271 x + 6.641	0.938 *
20	450	- 0.135 x + 6.547	0.988 **
20	475	- 0.104 x + 6.611	0.969 **
25	300	- 0.073 x + 6.597	0.913 *
30	175	- 0.171 x + 6.596	0.956 *
35	100	- 0.180 x + 6.562	0.938 *
Blank		- 0.315 x + 6.514	0.997 **

[a] Mean correlation coefficient.

* Fit significant at 95% probability.

** Fit significant at 99% probability.

Table 2: Linear fit of the pH values during storage, according to electric field strength (E) and treatment time (t)

In the horchata samples the fat content exceeded the minimum values established by the legislation for this product (2%) (Cano, Lobo & De Ancos, 1998), and it did not undergo modifications as a result of PEF preservation treatment. Having observed in preliminary tests that the fat percentage did not vary with time, we did not study this parameter during storage in refrigeration. The results appear in Table 3.

E (kV/cm)	t (μs)	Total fat (% wt/wt)
20	300	3.04 ± 0.03
20	450	3.05 ± 0.06
20	475	3.09 ± 0.02
25	300	3.05 ± 0.04
30	175	3.04 ± 0.08
35	100	3.05 ± 0.06
Blank		3.03 ± 0.04

[a] Mean ± standard deviation for three samples.

Table 3: Mean values of total fat (% wt/wt), according to electric field strength (E) and treatment time (t)[a]

Dunn (1995) concentrated on studies of shelf-life and loss of organoleptic and physicochemical characteristics in milk and milk derivatives, obtaining the result, as in our work, that the fat content is not modified as a result of PEF preservation treatments or in the subsequent shelf-life of the product. Similarly, Qin, Pothakamury, Vega-Mercado, Martín-Belloso, Barbosa-Cánovas and Swanson (1995) carried out a study of physicochemical properties and shelf-life of milk and did not observe changes in the percentage (wt/wt) of fat after treatment.

When the peroxide index during storage in refrigeration was determined, none of the PEF-treated samples gave a positive result.

E	t	Days					
(kV/cm)	(μs)	0	1	2	3	4	5
20	300	0.385±0.024	0.358±0.018	0.366±0.020	0.387±0.011	0.374±0.014	0.377±0.010
20	450	0.398±0.014	0.374±0.021	0.401±0.007	0.384±0.023	0.395±0.008	0.391±0.017
20	475	0.401±0.008	0.393±0.014	0.405±0.010	0.416±0.005	0.416±0.010	0.411±0.014
25	300	0.480±0.008	0.463±0.009	0.425±0.012	0.498±0.007	0.455±0.020	0.474±0.021
30	175	0.445±0.007	0.463±0.015	0.453±0.005	0.447±0.017	0.461±0.011	0.457±0.023
35	100	0.464±0.010	0.443±0.018	0.466±0.011	0.457±0.013	0.440±0.012	0.452±0.025
Blank		0.479±0.015	0.450±0.007	0.477±0.013	0.466±0.024	0.462±0.009	0.461±0.017

[a] Mean ± standard deviation for three samples.

Table 4: Mean values of TBARS index (mg MDA/L horchata) during storage period, according to electric field strength (E) and treatment time (t)[a]

Table 4 shows that the TBARS did not undergo modifications as a result of the PEF treatments; in other words, there was no significant increase in this index due to processing with the new technology or subsequently during storage. It can be affirmed, therefore, that this treatment does not cause oxidation of fatty matter, and horchatas treated with this technique can be kept in refrigeration for 5 days without oxidation of fat taking place. Comparison of the thiobarbituric acid reactive substances index of the samples of horchata subjected to PEF with the values obtained by Cortés, Esteve, Frígola and Torregrosa (2003) for various commercially available horchatas subjected to various preservation treatments shows that the PEF-treated samples have the lowest TBARS index, and the index is much higher for all the other horchatas, which were subjected to severe heat treatments, owing to the oxidation of fat caused by the high treatment temperatures.

Similarly, Table 5 shows that the values of the formol index, and therefore the total content of free amino acids, did not undergo modifications as a result of PEF treatment or subsequently during the period of storage in refrigeration.

E	t	Days					
(kV/cm)	(μs)	0	1	2	3	4	5
20	300	11.8±0.0	11.8±0.0	11.6±0.0	11.8±0.0	11.7±0.1	11.8±0.0
20	450	11.9±0.1	12.0±0.0	11.8±0.0	11.8±0.0	11.9±0.1	11.8±0.0
20	475	11.8±0.0	11.6±0.2	11.6±0.0	11.7±0.1	11.8±0.0	11.8±0.0
25	300	12.0±0.0	12.0±0.0	12.0±0.0	12.0±0.0	12.0±0.0	12.0±0.0
30	175	11.9±0.2	11.8±0.0	11.9±0.2	12.0±0.0	12.0±0.0	11.8±0.0
35	100	11.8±0.0	11.8±0.0	11.7±0.1	11.8±0.0	11.8±0.0	11.6±0.0
Blank		11.4±0.0	11.4±0.2	11.6±0.2	11.4±0.0	11.5±0.1	11.4±0.0

[a] Mean ± standard deviation for three samples.

Table 5: Mean values of formol index (% vol/vol) during storage period, according to electric field strength (E) and treatment time (t)[a]

Table 6 shows the peroxidase activity obtained for the horchata not treated by PEF and the horchatas subjected to PEF, and the evolution of this parameter during the period of storage in refrigeration. In all cases, PEF treatment caused partial inactivation of the enzyme. The residual peroxidase activity (0.034–0.097 ΔAbs/min) changed during storage. In those cases in which it increased (which indicates regeneration of the enzyme), it never attained the peroxidase activity of the untreated horchata. Cano et al. (1998) studied peroxidase activity in long-

term frozen stored papaya slices and found that during preservation treatments peroxidase is not totally destroyed but only reduced or inactivated reversibly, so that regeneration of its activity is often observed after long periods of storage, which may lead to deterioration of product quality. These results are similar to those found in our work.

E	t	Days					
(kV/cm)	(μs)	0	1	2	3	4	5
20	300	0.063±0.002^{a}	0.110±0.001^{b}	0.090±0.002^{c}	0.112±0.002^{b}	0.112±0.003^{b}	0.084±0.001^{d}
20	450	0.067±0.001^{a}	0.073±0.002^{b}	0.086±0.004^{c}	0.078±0.002^{d}	0.087±0.005^{e}	0.080±0.001^{d}
20	475	0.075±0.002^{a}	0.072±0.002^{a}	0.085±0.004^{bc}	0.081±0.004^{b}	0.081±0.004^{b}	0.089±0.001^{c}
25	300	0.034±0.001^{a}	0.038±0.005^{a}	0.037±0.002^{a}	0.030±0.001^{b}	0.057±0.002^{c}	0.044±0.004^{d}
30	175	0.039±0.003^{a}	0.063±0.005^{b}	0.065±0.000^{b}	0.066±0.001^{b}	0.073±0.000^{c}	0.092±0.006^{d}
35	100	0.097±0.002^{a}	0.089±0.003^{b}	0.110±0.002^{c}	0.110±0.001^{c}	0.116±0.003^{d}	0.108±0.001^{c}
Blank		0.123±0.002^{a}	0.130±0.006^{b}	0.135±0.002^{c}	0.138±0.003^{c}	0.187±0.000^{d}	0.153±0.002^{e}

[a] Mean ± standard deviation for three samples. Differences in letters within a row indicate significant ($p < 0.05$) differences.

Table 6: Mean values of peroxidase activity (ΔAbs/min) during storage period, according to electric field strength (E) and treatment time (t)a

The p-value, which tests the statistical significance of the correlation (–0.6279) is 0.0000 and it indicates statistically significant non-zero correlations at the 95% confidence level, so that there is a negative correlation between pH and peroxidase activity in the horchata de chufa samples subjected to PEF treatment (Figure 2). As storage time increases, pH decreases, so that it can serve as an indicator of deterioration of horchata in terms of quality, in relation with an

increase in peroxidase activity. Lu and Whitaker (1974) state that the oxidative activity of peroxidase is affected by pH, depending on the kind of food.

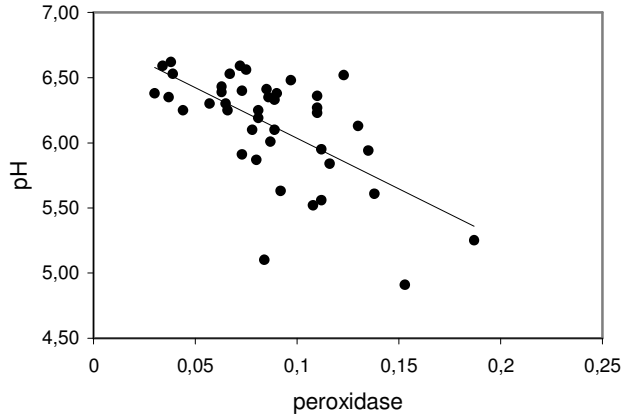

Figure 2: Negative correlation between pH and peroxidase activity.

Selma et al. (2003) studied inactivation of *E. aerogenes* in horchata de chufa subjected to PEF treatments and its possible reactivation or growth after an incubation period, concluding that to prevent growth of this microorganism in horchata treated with PEF it is necessary to monitor contamination of the product in the production line and guarantee its refrigeration during distribution and storage, as recontamination has been detected in the process of extraction of this beverage and in samples incubated at temperatures above 8ºC. These two factors are critical (low contamination and refrigeration) for the obtaining of a new product, PEF-treated horchata de chufa, which would potentially increase the market for this product, as it fulfils the requirements of existing consumers: a natural, healthy, additive-free vegetable beverage.

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REFERENCES

Angulo, A. J. (1997). Indicadores de peroxidación lipídica en productos para nutrición infantil. Ph.D. University of Granada.

AOAC. (1990). Official methods of Analysis. Association of Official Analytical Chemists. Washington, DC.

Barber, S. (1981). Algunas aportaciones recientes de la investigación valenciana sobre productos autóctonos a la Ciencia y a la Tecnología de Alimentos. Revista de Agroquímica y Tecnología de los Alimentos, 21, 175-184.

BOE (1988). Reglamentación Técnico Sanitaria para la elaboración y venta de horchata de chufa. Real decreto 1338/1988 de 28 de octubre de 1988. Spain.

Cano, M. P., Lobo, M. G., & De Ancos, B. (1998). Peroxidase and polyphenol oxidase in long-term frozen stored papaya slices. Differences among hermaphrodite and female papaya fruits. Journal of the Science of Food and Agriculture, 76, 135-141.

Cortés, C., Esteve, M. J., Frígola, A., & Torregrosa, F. (2003). Estudio de la calidad y seguridad de las horchatas de chufa del mercado. Proceedings of the II Congreso de Ciencia y Tecnología de los alimentos, 1, 19-22.

Cortés, C., Esteve, M. J., Frígola, A., & Torregrosa, F. (2004). Physical and chemical properties of different commercially available types of “horchata de chufa”. Italian Journal of Food Science, 1, 113-121.

Dunn, J. E. (1995). Pulsed light and pulsed electric field for foods and eggs. Poultry Science, 75, 1133-1136.

Gould, G. W. (1996). New Methods of Food Preservation (pp. 15-19). In G.W. Gould (ed.), Chapman & Hall, London.

Hülsheger, H., Potel, J., & Niemann, E. G. (1981). Killing of bacteria with electric pulses of high electric field strength. Radiation and Environmental Biophysics, 20, 53-65.

INE. Instituto Nacional de Estadística. (1999). Encuesta Industrial de productos. Madrid.

Jayaram, S., Castle G. S. P., & Margaritis, A. (1992). Kinetics of sterilization of Lactobacillus brevis by the application of high electric voltage pulses. Biotechnology and Bioengineering, 40, 1412-1420.

Lu, A. T., & Whitaker, J. R. (1974). Some factors affecting rates of heat inactivation and reactivation of horseradish peroxidase. Journal of Food Science, 39, 1173-1178.

MAPA. (1993). Ministerio de Agricultura Pesca y Alimentación. Secretaría General Técnica. Métodos Oficiales de Análisis. Madrid.

MAPA. (1997). Ministerio de Agricultura, Pesca y Alimentación. Secretaría General Técnica. Anuario de estadística agraria. Madrid.

Michalac, S. L., Alvarez, V. B., & Zhang, Q. H. (1999). Microbial reduction in skim milk using PEF technology. IFT meeting.

Moreno, J. A., Gasque, F., & Schwartz, M.. (1983). Determinación de la actividad peroxidásica en horchata de chufas (Cyperus sculentus L.). Revista de Agroquímica y Tecnología de Alimentos, 23, 578-584.

Peleg, M. (1995). A model of microbial survival after exposure to pulsed electric fields. Journal of the Science of Food Agriculture, 67, 93-99.

Pothakamury, U. R. (1995). Preservation of foods by non-thermal processes, Ph.D. Dissertation. Washington State University, Pullman, USA.

Pothakamury, U. R., Monsalve-González, A., Barbosa-Cánovas, G. V., & Swanson, B. G. (1995). Inactivation of Escherichia coli and Staphylococcus aureus in model food systems by pulsed electric field technology. Food Research International, 28, 167-171.

Qin, B. L., Pothakamury, U. R., Vega-Mercado, H., Martín-Belloso, O. M., Barbosa-Cánovas, G. V., & Swanson, B. G. (1995). Food pasteurization using high-intensity pulsed electric fields. Food Technology, 12, 55-60.

Reina, L., Jin, Z. T., Zhang, Q. H., & Yousef, A. E. (1998). Inactivation of Listeria monocytogenes in milk by pulsed electric fields. Innovative Food Science and Emerging Technologies, 1, 21-29.

Rodrigo D., Martínez, A., Harte, F., Barbosa-Cánovas, G. V., & Rodrigo, M. (2001). Study of inactivation of Lactobacillus plantarum in orange-carrot juice by means of Pulsed Electric Fields: Comparison of inactivation kinetics models. Journal of Food Protection, 64, 259-263.

Sale, A. J. H., & Hamilton, W. A. (1967). Effects of high electric fields on microorganisms. I. Killing of bacteria and yeast. Biochimica et Biophysica Acta, 143, 781-788.

Salih, A. M., Smith, D. M., Price, J. F., & Dawson, L. E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poultry Science, 66, 1483-1488.

Selma M. V., Fernández, P. S., Valero, M., & Salmerón, M. C. (2003). Control of Enterobacter aerogenes by high-intensity pulsed electric fields in horchata, a Spanish low acid beverage. Food microbiology, 20, 105-110.

Torregrosa Verdú, F. (2001). Características fisico-químicas y de calidad de la horchata natural. Degree thesis. University of Valencia, Spain.

Yeom H. W., Evrendilek, G. A., Jin, Z. T. & Zhang, Q.H. (2001). Processing of yoghurt-based product with pulsed electric fields. IFT meeting.

Capítulo 2

Puesta a punto de un método de determinación de carotenoides en zumos de frutas y vegetales.

1. **Identification and Quantification of Carotenoids Including Geometrical Isomers in Fruit and Vegetable Juices by Liquid Chromatography with Ultraviolet-Diode Array Detection.** Journal of Agricultural and Food Chemistry, 52, 2203-2212 (2004).

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Identification and Quantification of Carotenoids Including Geometrical Isomers in Fruit and Vegetable Juices by Liquid Chromatography with Ultraviolet-Diode Array Detection

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A method was established for the identification and quantification of carotenoids including geometrical isomers in fruit and vegetable juices by liquid chromatography with an ultraviolet-diode array detector, using a C_{18} Vydac 201TP54 column. The mobile phase used was the ternary methanol mixture (0.1 M ammonium acetate), *tert*-butyl methyl ether and water, in a concentration gradient, and a temperature gradient was applied. Retinol palmitate was added as an internal standard. An extraction process (ethanol/hexane, 4:3, v/v) was performed, followed by saponification with diethyl ether/methanolic KOH (0.1%, w/v, BHT) (1:1, v/v) for 0.5 h at room temperature. Seventeen different (*cis* and *trans*) carotenoids were identified by UV-vis spectra and retention times in HPLC in the juices analyzed. The analytic parameters show that the method proposed is sensitive, reliable, accurate, and reproducible.

KEYWORDS: Carrot juice; *cis-trans* carotenoids; citric juice; *Citrus sinensis*; food analysis; liquid chromatography

INTRODUCTION

Adapting to new trends and consumer demands has been one of the primary objectives of orange juice producers, so that for some years these manufacturers have been producing juices with mild pasteurization, marketed in refrigerated conditions and with limited shelf life. New products are being produced in this line with juice mixtures that provide increased quality (nutritive value, color, etc.), this being the factor that contributes most to consumer acceptance and an increase in the value added to the product.

Citrus is a complex source of carotenoids, with the largest number of carotenoids found in any fruit (*1*). About 700 kinds of carotenoids have been isolated in nature (*2*). The carotenoids present in citrus are a complex mixture of >115 natural substances (*3*). Various carotenoids, including α-carotene, β-carotene, and β-cryptoxanthin (Figure 1), have provitamin A activity, being transformed into retinal by mammals. The xanthophylls (oxocarotenoids) lutein and zeaxanthin (Figure 1) are also known to provide protection against macular degeneration connected with age, through their ability to capture free oxygen and blue light in the retina (*4*). Carotenoids have a range of important and well-documented biological activities. They are potent antioxidants and free radical scavengers (*5*), and they modulate the pathogenesis of cancers (*6*, *7*) and coronary heart disease (*8*). The application of various industrial treatments can lead to the formation of cis isomers, which do not have the same vitamin activity as the all-trans isomers. This is vital for the accurate determination of the dietary intake of these micronutrients and in the development of comprehensive food tables (*9*, *10*).

Health professionals consider the consumption of fruit and vegetables to be of great importance as a means of protection against illnesses such as cancer and coronary heart disease, partially because of their high content of antioxidant vitamins, such as vitamins C and E, phenolic compounds, and carotenoids (*10*). Vitamin A is an essential nutrient for man and all mammalian species because it

cannot be synthesized within the body. Citric fruits such as oranges contain a certain carotenoid number, but not all of them are precursors of vitamin A. However, carotenoids are important in the prevention of certain degenerative diseases including coronary heart disease and cancers.

Various procedures have been described for the determination of carotenoids. High-performance liquid chromatography (HPLC) is considered to be the method of choice for the separation, identification, and quantification of carotenoids found in biological tissues (*11-14*). On the other hand, determination of the complete carotenoid composition is complicated, costly, and time-consuming (*15*). The best separation of carotenoids is achieved with C_{18} columns (*11*, *14*, *16-20*). The reasons for choosing the C_{18} package include compatibility with many solvents, usability for the complete polarity range of carotenoids, and wide commercial availability. The good separation of geometrical isomers of carotenoids by the polymeric phases of C_{18} is evident (*21*, *22*). Many authors have used a 5-μm polymeric C_{18} column, a Vydac 201TP54 (*23-29*). Historically, the efficiency of the separation has been improved by using smaller particle sizes, changing the shape of the particles from irregular to spherical, ensuring uniform particle size, and making the columns more reproducible. The advantages of a small particle size include high efficiency and low consumption of solvents because shorter columns are used. The disadvantage is the decrease in column life as a result of obstruction and the use of high pressure. This disadvantage can be reduced by filtering the samples and by using precolumns (*21*, *25*, *29*).

Diode array detectors (DAD) were used commonly for the identification of carotenoids in citrus and vegetables due to the specific spectral data of many of these pigments (*1*, *15*, *18*, *30*).

In the selection of the mobile phase gradient, it is necessary to take into account the fact that the main solvent should be slightly organic and have low viscosity; it should also allow suitable solubility of carotenoids and permit the work to be

performed in low-pressure conditions. These criteria limit the choice to acetonitrile and methanol. There have been reports that in most columns solvents based on methanol provide greater recovery of carotenoids than those based on acetonitrile, and they also have a lower cost and toxicity (*31*, *32*).

Carotenoids are fat soluble but because of the high moisture content of plant tissues, a preliminary extraction solvent miscible with water is generally necessary to allow for penetration of the solvent. Methanol is often used as an initial extractant (*2*). Water-immiscible solvents can be used after the sample is dehydrated; however, it has been found that more efficient extractants are composed of a slightly polar solvent in addition to the nonpolar solvent (*33*).

Saponification is used to evaluate the presence of carotenoid esters, because the saponification of esterified carotenoids with fatty acids gives rise to their hydroxycarotenoid derivatives, considerably simplifying the chromatogram profile (*20*). Saponification causes a clear transformation of the carotenoid profile, with considerable loss of carotenoids, especially xanthophylls (*19*). Saponification of carotenoid extracts may be necessary to remove neutral fats, chlorophylls, and chlorophyll derivatives that interfere in the spectrophotometric assay of carotenoids and in the release of esterified xanthophylls (*2*, *34*).

Various compounds are normally used as internal standard: β-apo-8'-carotenal (trans) (*35*, *36*), Sudan I (*20*, *29*), canthaxanthin (*37*, *38*), echinenone (*39-41*), nonapreno-β-carotene (*42*), or *all-trans*-retinol palmitate (*43-45*).

In this work we established and validated a method for the determination of the various (trans and cis) carotenoids in a fresh orange-carrot juice mixture and in fresh orange juice in order to evaluate their nutritive qualities.

MATERIALS AND METHODS

Reagents. β-Carotene, *all-trans*-retinol palmitate, and *tert*-butyl hydroxytoluene (BHT) (special grade) were purchased from Sigma (Steinheim, Germany). Lutein and zeaxanthin were provided free as standard substances by Roche

(Basel, Switzerland). Ammonium acetate (HPLC grade), petroleum ether, hexane (HPLC grade), potassium hydroxide (85%), and *tert*-butyl methyl ether (TBME) (HPLC grade) were purchased from Scharlau (Barcelona, Spain); acetonitrile (special grade) and magnesium hydroxide carbonate (40-45%) from Panreac (Barcelona, Spain); and ethanol, diethyl ether, methanol, and sodium chloride (special grade) from Baker (Deventer, The Netherlands). Chloroform was obtained from Merck (Darmstadt, Germany).

Samples. Natural orange juice (produced from *Citrus sinensis*) and an orange-carrot juice mixture (80:20, v/v) were provided by the manufacturer involved in the project. The juices were packaged aseptically and frozen at -40 °C until the time for their analysis. We verified that their vitamin profiles did not change during the storage period.

Preparation of Stock Standard Solutions. To prepare the stock standard solution, 12.5 mg of β-carotene, 4 mg of lutein, 5.2 mg of zeaxanthin, and 35 mg of retinol palmitate were weighed and dissolved in 25 mL of chloroform with 0.1% BHT (w/v). They were stored in sealed amber vials under N_2, at the lowest temperature possible (< -20ºC), for use over an extended period.

The precise concentration of each of the standards was checked periodically by its extinction coefficient. To do this, 100 μL of the stock standard solution of β-carotene, lutein, zeaxanthin, and retinol palmitate was taken and evaporated with N_2, and in each case the result was redissolved with 10 mL of the appropriate solvent (see Table 1) for subsequent spectrophotometric measurement after filtration of the solution (*40*). The concentration of each standard was calculated by applying eq 1: $C\ (\mu g/ml) = A \cdot 106 / E^{1\%}$, where C is concentration, A absorbance and $E^{1\%}$ is the extinction coefficient.

The working solution was prepared each day from the stock solution of each of the standards. To do so, we took 25, 150, 90, and 100 μL of β-carotene, lutein, zeaxanthin, and retinol palmitate, respectively, evaporated them with N_2, and diluted them with 1 mL of methanol/TBME (70:30, v/v). The concentration of each solution was checked by its extinction coefficient.

Instrumentation. The LC system consisted of a series 1050 chromatograph with a quaternary pump system, a diode array detector (Hewlett-Packard, 1100 series), a column thermostat (Agilent 1100 series), an on-line degassing system, and a ChemStation (series A.06.03) data system (Hewlett-Packard, Waldbronn, Germany).

A Vydac 201TP54 column with a particle size of 5 μm, 250 × 4.6 mm, reverse phase C18 and a precolumn (guard column) Vydac 201TP (4.6 mm i.d. cartridge with 5-μm particles) (Hesperia, CA) were used.

Table 1. Extraction coefficients of various standards

Compound	Solvent	λ (nm)	$E^{1\%}$
Lutein	Ethanol	445	2550
Zeaxanthin	Ethanol	452	2480
Canthaxanthin	Petroleum Ether	466	2200
β-Carotene	Ethanol	453	2620
Retinol Palmitate	Ethanol	328	975

RESULTS AND DISCUSSION

Method Development and Evaluation. A number of factors that affect the chromatographic responses of carotenoids and contribute to analytical variations and inaccuracies in their quantitative determination are discussed and solutions provided. The following gives the result of our method development and assesses the "robustness" of the method.

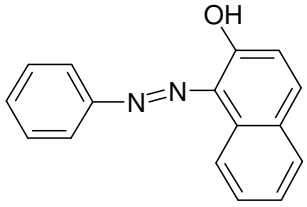
Selection of the Internal Standard. The incorporation of an internal standard is highly recommended because for the quantitative measurement of carotenoids it is essential to take into account losses due to incomplete recovery, evaporation, and variability in the injection. A suitable internal standard should not be present in the sample to be determined, but it should have a similar structure and, therefore, behave similarly to the compound that it is desired to extract. Also, it should not appear at a retention time that could interfere with other components present in the sample.

Retinol palmitate was selected as the internal standard (Figure 1), and it also served to indicate whether saponification was complete, because it is transformed into retinol (Figure 1), which appears at much lower retention times (*43*). Figure 2 shows the chromatogram before and after saponification of the internal standard, retinol palmitate (λ = 350.8 nm).

Fig. 1. Structures of Sudan I & carotenoids.

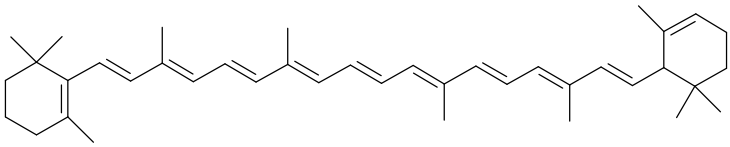
INTERNAL STANDARD

Sudan I: (Mr=248.29)

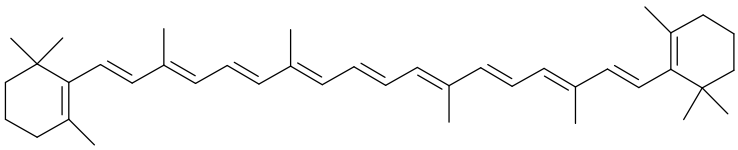


CAROTENES

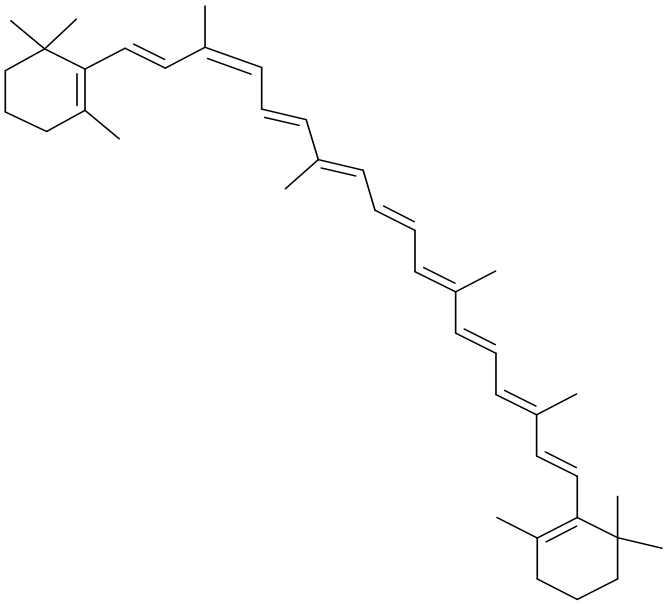
α-carotene: (Mr=536.89)



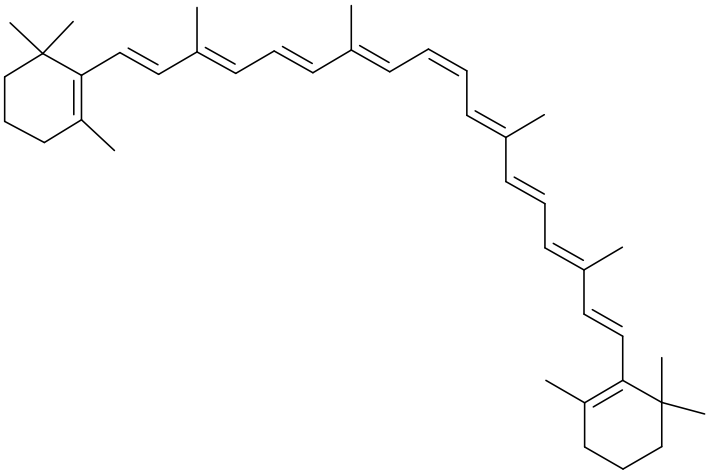
β-carotene: (Mr=536.89)



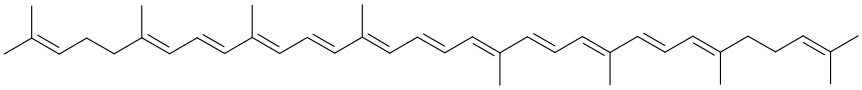
9-cis-β-Carotene: (Mr=536.89)



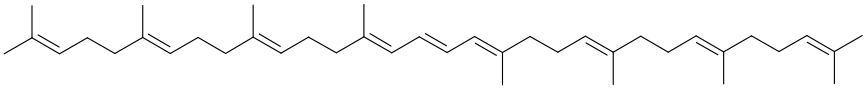
15-cis-β-Carotene: (Mr=536.89)



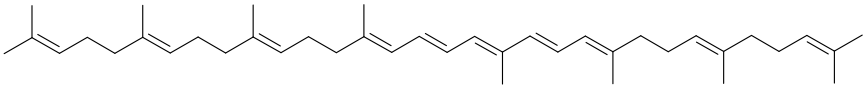
Lycopene: (Mr=536.89)



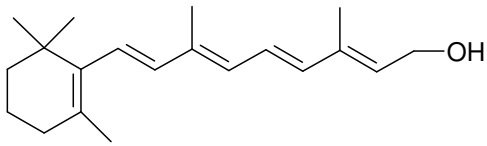
Phytoene: (Mr=544.96)



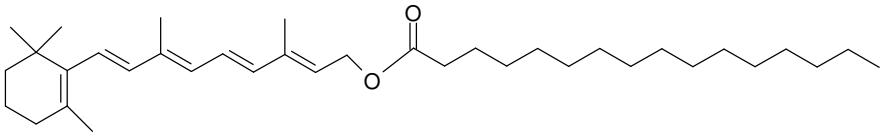
Phytofluene: (Mr=542.94)



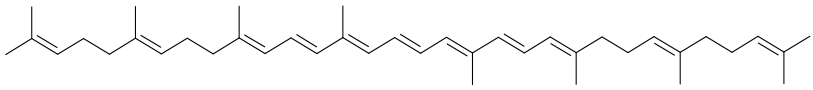
Retinil palmitate: (Mr=524.88)



Retinol: (Mr=286.46)

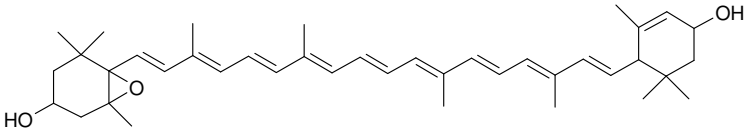


ζ-carotene: (Mr=540.92)

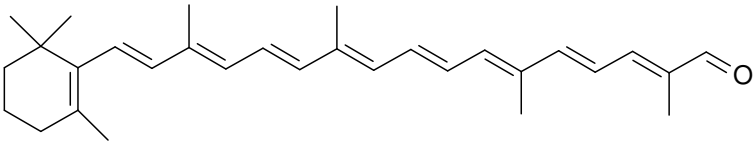


XANTHOPHYLLS

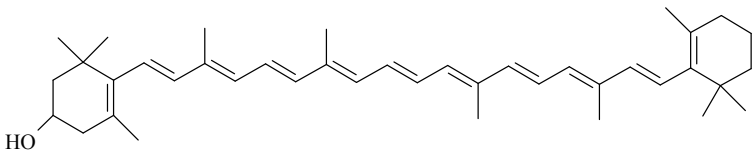
Antheraxanthin: (Mr=584.89)



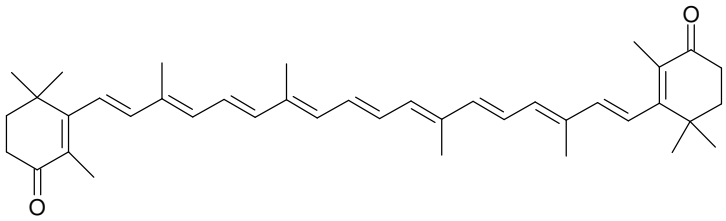
β-apo-8'-carotenal: (Mr=416.65)



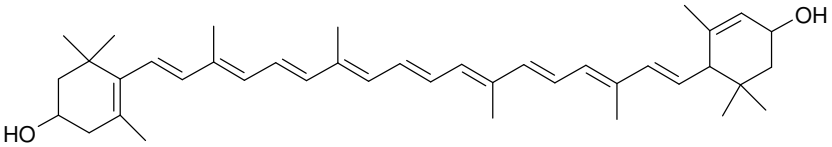
β-cryptoxanthin: (Mr=552.89)



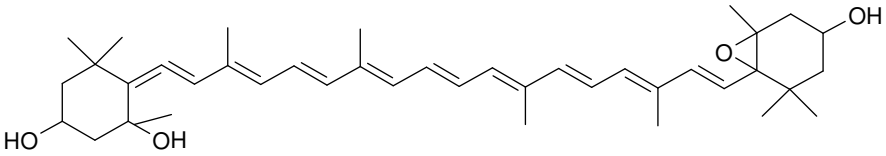
Canthaxanthin: (Mr=564.86)



Lutein: (Mr=568.89)



Neoxanthin: (Mr=618.95)



Zeaxanthin: (Mr=568.89)

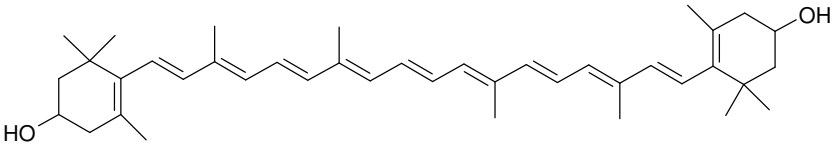
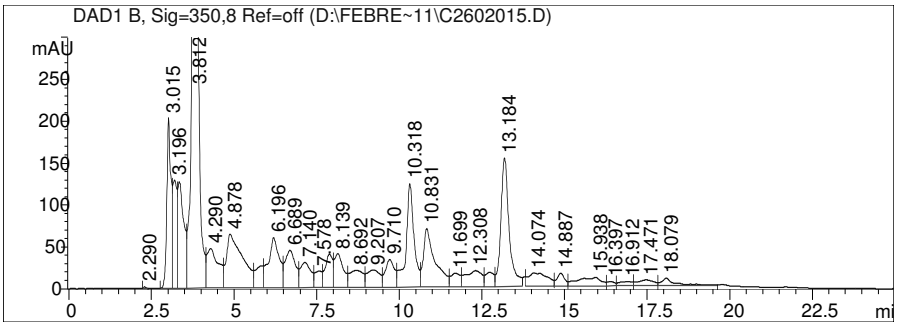


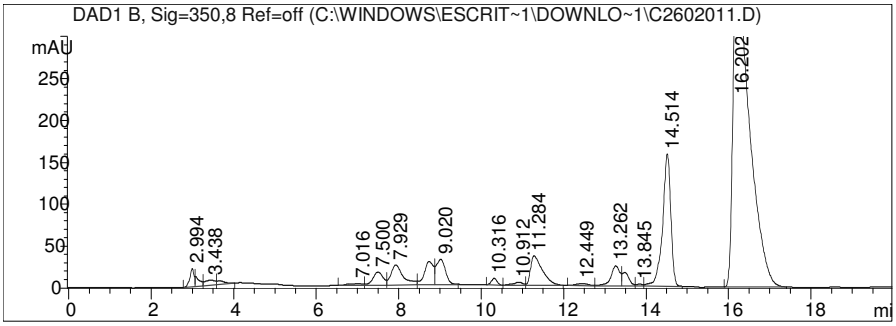
Fig. 2. Identification of internal standard (Retinol Palmitate) in saponified orange juice (A) and in an unsaponified mixture of standards (B) (λ=350.8 nm).

(A)



t_R=3.812 min is the saponified IS.

(B)



t_R =16.202 min is the unsaponified IS.

Extraction and Saponification of Carotenoids. When we applied the extraction and subsequent saponification proposed by Rouseff et al. (*1*), we observed the separation of distinct chromatographic peaks, with a clear identification of β-carotene, although there was baseline drift. To avoid precipitation with Carrez reagents and the use of solvents (petroleum ether and acetone) with oxidant power, we applied and modified the extraction method proposed by Taungbodhitham et al. (*46*). We increased the sample size to 25 g of juice and added BHT (0.1%, w/v), 100 μL of internal standard, 0.05 g of magnesium hydroxycarbonate, and 35 mL of ethanol/hexane (4:3, v/v). We studied several agitation times (5, 15 and 30 min), obtaining the best results with a time of 15 min (under N_2 and protected from the light), and filtered in a low-pressure. The residue was washed with 35 mL of ethanol/hexane (4:3, v/v) and filtered. The residue was washed twice with 12.5 mL of ethanol and finally with 12.5 mL of hexane (until it was colorless). All of the liquid filtrates were combined and washed twice with 50 mL of 10% NaCl in an amber decanting funnel and then with 50 mL of water (three times). The organic phase was evaporated at 40ºC in a rotary evaporator (Eyela NE-1). We saponified the residue obtained and varied the volumes of diethyl ether (5, 10, and 15 mL) and methanolic KOH (5, 10, and 15 mL) added for the saponification, because completion of saponification varied with the kind of juice (orange-carrot, plain orange, etc.), and finally we added 10 mL of diethyl ether and 10 mL of (0.5 M) methanolic KOH with 0.1% BHT (w/v) and left it overnight at room temperature protected from the light. To avoid possible alterations (oxidations) and reduce the time for saponification, we studied various saponification times: 0.5, 1, 3, and 24 h. We verified that saponification was complete after 30 min, and so this was the time selected. We performed the saponification in an inert atmosphere (N_2).

At the end of this time we added 20 mL of diethyl ether and extracted twice with 50 mL of 10% NaCl (w/v). The ether phase was washed three times with 50 mL

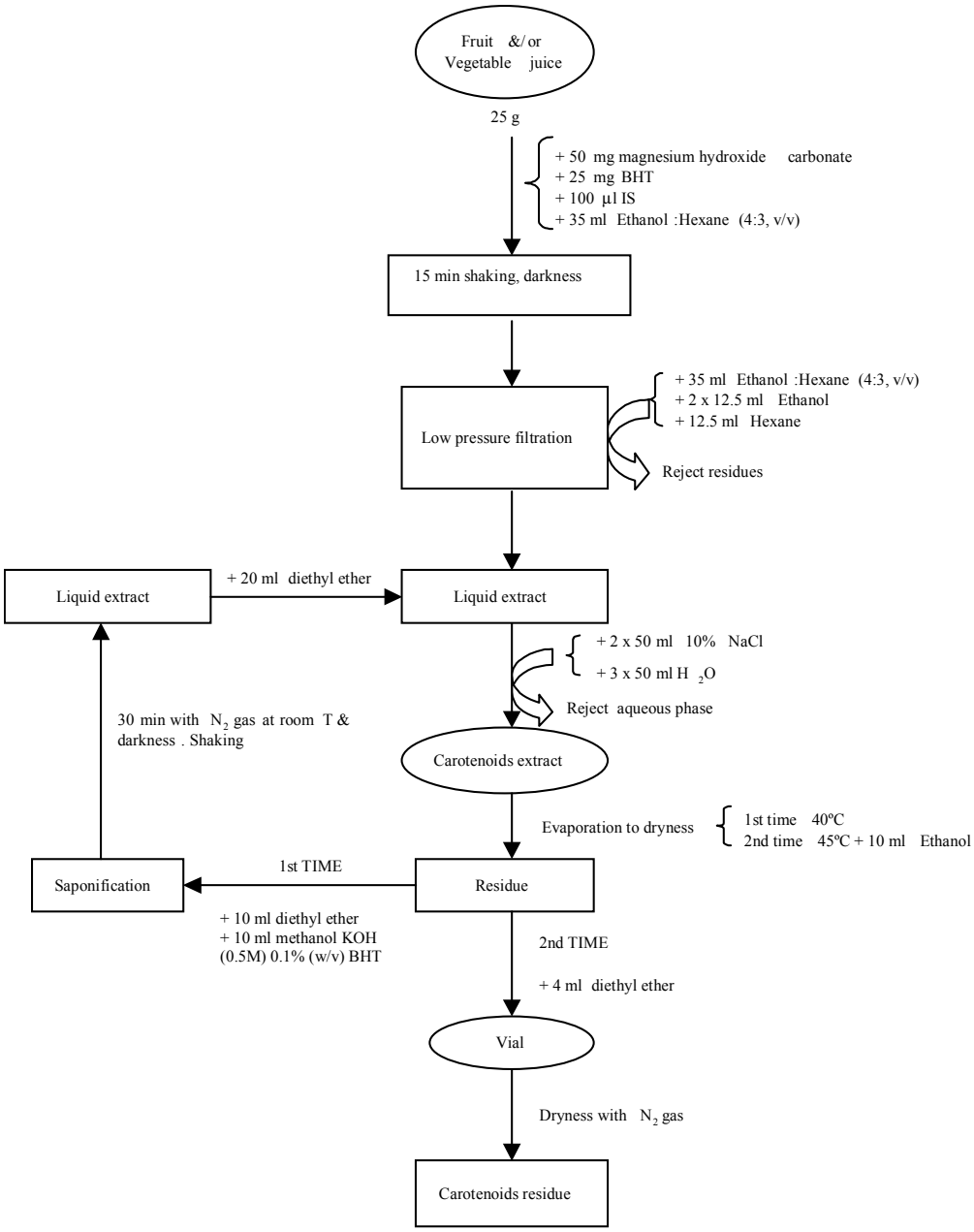
of H_2O until a neutral pH was obtained. It was filtered in the presence of anhydrous Na_2SO_4, but we observed that in some of the samples there were remains of water in the ether phase. Therefore, to ensure that the water was totally eliminated, we added 10 mL of absolute ethanol (after testing various volumes: 2, 5, and 10 mL), and in some samples it was necessary to add a further 5 mL to achieve dryness, evaporating at 45ºC. The residue was dissolved with 4 mL of diethyl ether and placed in an amber glass flask, the solvent was evaporated under N_2, and the residue was stored at -20ºC until time for chromatographic determination (we verified that the results remained invariable up to 4 days after extraction). Figure 3 shows a diagram of the final extraction process.

The carotenoid extract was reconstituted with 1 mL of MeOH/TBME (70:30, v/v) at the moment of injection.

Some authors employ 2, 3, 5, and 6 h (refs *27*, *42*, *47*, and *30* respectively) to carry out the saponification process in the dark at room temperature. Other authors employ >12 h (*1*, *22*, *24*, *37*, *38*, *49-51*). We have checked that 30 min was adequate to carry out the saponification, the same time used by Wingerath et al. (*52*).

Mobile Phase. Carotenoids may undergo losses or degradation on the column. Studies have indicated that compounds which act as modifiers improve the recovery of carotenoids from the column and reduce or eliminate on-column degradation. Ammonium acetate has also been reported to improve column recovery. Addition of ammonium acetate to MeOH used in the mobile phase increased the recovery from all columns (*14*, *32*). First, methanol was chosen, and ammonium acetate was added to it in various concentrations of 0.05, 0.1, and 0.2 M. The best results were obtained with a concentration of 0.1 M, as there was an increasing improvement in the separation of the different chromatographic peaks and at the same time a better recovery of them.

Fig. 3. Diagram of extraction of carotenoid pigments.



TBME encourages elution of the more apolar compounds, and therefore it was introduced in a small proportion in the first minutes and gradually increased to 25% in the final minutes of the chromatogram. We observed that in the first minutes the chromatographic peaks did not resolve correctly, and therefore we decided to add a small percentage of H_2O during the first 10 min, taking care not to have water and TBME at the same time (to avoid formation of small bubbles). In the end we selected the introduction of 5% water with 95% methanol during the first 3 min, after which the water was eliminated and we eluted with methanol. TBME was then introduced and gradually increased to 25% at 15 min, after which it was decreased so as to have 100% methanol at the end of the chromatogram. Table 2 shows the mobile phase gradient selected.

The influence of ambient temperature on the elution of carotenoids was standardized by selecting a temperature gradient (0 min, 20ºC; 6 min, 30ºC; 22 min, 20ºC). Some authors use column temperatures from 20 to 40ºC (*10*, *29*, *38*, *39*, *41*, *50*, *51*), but above 40ºC, the separation of α-carotene and β-carotene is poor, where a curvature around 40ºC is caused by a phase change of polymeric ODS from solid-like state to liquid-like state (*22*).

Figures 4 and 5 show the chromatograms obtained by applying the method established for the separation of carotenoids in orange-carrot juice mixtures and orange juices. The most common retention times and wavelengths are shown in Table 3.

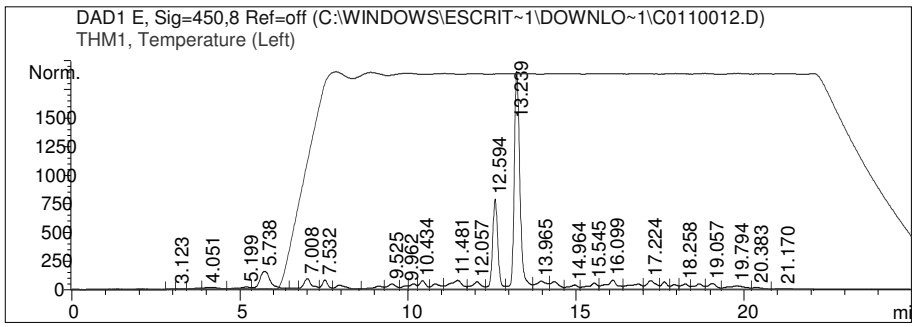
Identification and Quantification of Carotenoids. Basically because of the lack of commercial standards of carotenoids, especially of cis isomers, we used various identification techniques employed by many authors, including the following:

The UV-vis spectra and the maximum λvalues of the carotenoids were compared, which indirectly indicates the greater or lesser conjugation between their bonds, because the greater the conjugation, the higher the maximum λ values are.

The polarity of the carotenoids was examined because in reverse phase liquid chromatography the retention times follow the order from greater to lesser polarity.

Fig. 4. Chromatogram of orange-carrot juice mixture at λ=450 nm (A) and λ=350 nm (B).

(A)



(B)

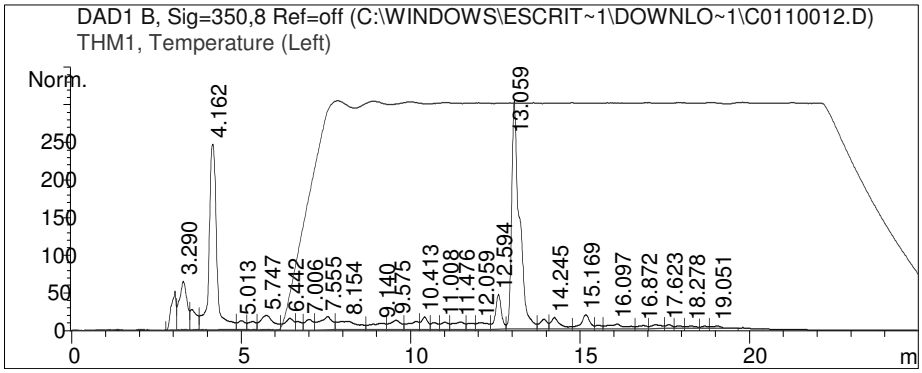


Table 2. Mobile phase gradient for determination of carotenoids by HPLC

Time (min)	MeOH + AA (%)	H_2O (%)	MTBE (%)
0	95	5	0
3	100	0	0
5	95	0	5
10	86	0	14
15	75	0	25
22	95	0	5
23	100	0	0

AA: 0.1 M ammonium acetate.

Various spectral fine structural values were determined, including the following:
%III/II is the percentage of the quotient between band III and band II (normally λ_{max}), taking the trough between the two bands as the baseline. It is used to identify carotenoids (*49-51*, *53*, *54*).
$\%D_B/D_{II}$ is the percentage of the quotient between the cis peak band and band II (normally λ_{max}). It is used to identify the different cis isomers of carotenoids (*1*, *31*, *32*, *40*, *43*, *46*).
The Q ratio = D_{IImax}/D_B is the quotient between band II (normally λ_{max}) and the cis peak band. This is also used to identify the different cis isomers of carotenoids (*23-25*, *47*, *55*).
With all of these techniques, it was possible to identify the most characteristic carotenoids in orange and orange-carrot juices.
Lutein and zeaxanthin (peaks 6 and 7, respectively) are usually very difficult to resolve as they differ only in the position of a single bond in one of the terminal

rings (Figure 1), and some authors cannot resolve them (*35*, *38*), but they are completely resolved with this chromatographic system. However, 9-*cis*-violaxanthin is not completely resolved from neoxanthin (peak 2). The coelution of these peaks is due to the similar structures (Figure 1) and therefore similar spectral characteristics (Table 3). The chromatographic purity of this incompletely resolved carotenoid pair is shown in Figure 6.

Table 4 shows the chromatographic and spectral characteristics of carotenoids obtained in orange juices. Comparative chromatographic and spectral characteristics of carotenoids in orange juice obtained from the literature are shown in Table 5.

For the quantification of the various carotenoids identified in the samples studied, we quantified the different carotenoids in accordance with their similarity in terms of chemical-structural behavior and polarity with respect to the validated standards for which we had a calibration curve: lutein, zeaxanthin, and β-carotene. Accordingly, for the quantification we proceeded as follows:

The chromatogram was separated into three parts; all of the carotenoids up to and including lutein were quantified as such, and the remaining xanthophylls were quantified as zeaxanthin. The carotenes were quantified as β-carotene.

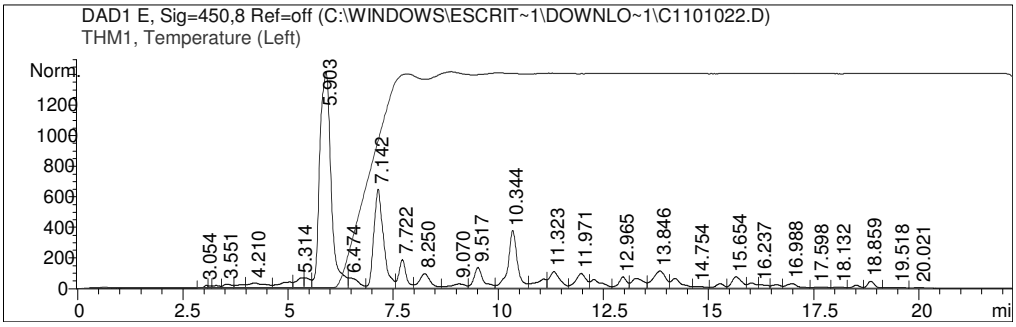
Each carotenoid was quantified in accordance with whether its maximum λwas close to 290, 350, 430, or 450 nm. The content of each carotenoid in micrograms per 100 g of sample was determined by applying eq 2:

$$(A_{sample} / A_{IS}) \times \mu g \text{ Standard} \times 100 / (A_{standard} / A_{IS}) \times g \text{ sample},$$

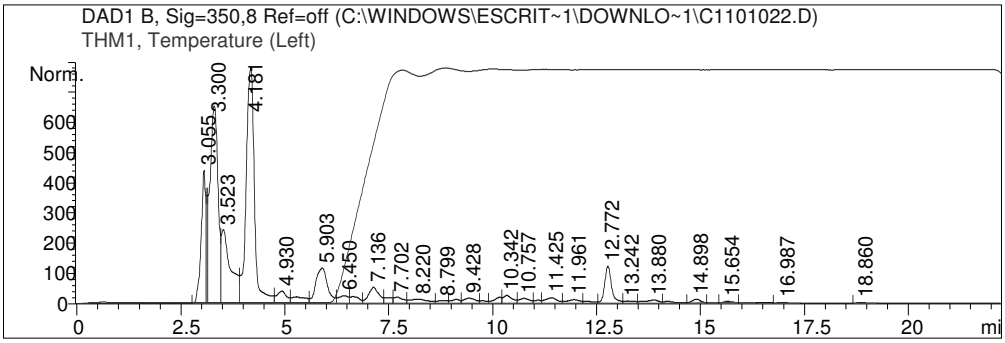
(IS = internal standard).

Fig. 5. Chromatogram of orange juice at λ=450 nm (A) and λ=350 nm (B).

(A)



(B)



Analytical parameters. To check the reliability and usefulness of the proposed method the analytical parameters were determined for the carotenoids for which commercial standards were available: lutein, zeaxanthin and β-carotene.

The responses were linear in the following ranges and equations: 2.02-56.55 μg/ml (n=7) with an equation y=0.022x + 0.0425 (r=0.991) for Lutein, 2.56-51.29 μg/ml (n=7) with an equation $y=0.026x - 3\cdot10^{-5}$ (r=0.991) for Zeaxanthin and 5.54-1776.15 μg/ml (n=7) with an equation y=0.0034x + 0.7654 (r=0.989) for β-Carotene. The concentration of carotenoids in all samples is included in the studied concentration interval.

The limit of detection was calculated by preparing five reagent standard solutions and applying the quotient between 3 times the standard deviation and the slope of the calibration curve ($LOD = 3 \times S_{n\text{-}1}/m$). The detection limits were 0.0196, 0.0166, and 0.1404 μg/mL for lutein, zeaxanthin, and β-carotene, respectively. The limit of quantitation corresponds to the minimum quantity with which it is possible to quantify without uncertainty ($LOQ = 10 \times S_{n\text{-}1}/m$). The limits of quantitation were 0.0652, 0.0552, and 0.4680 μg/mL for lutein, zeaxanthin, and β-carotene, respectively. To calculate the LOD and LOQ parameters, we used the slope of the external calibration curve and the standard deviation based on the maximum peaks of the reagents standard solutions at 450 nm.

Table 3. Instrumental relative standard deviation (RSD%, n=6) and relative standard deviation of the method for an orange-carrot juice mixture.

Name	Instrumental precision (RSD%, n=6)			Precision of the method (RSD%, n=6)		
	Intraday			Intraday		
	Day 1	Day 2	Interday	Day 1	Day 2	Interday
Valenciaxanthin	2.92	2.29	2.60	11.24	5.43	8.34
Neoxanthin	1.72	0.17	0.94	6.42	1.48	3.95
Antheraxanthin	5.26	8.27	6.76	5.63	7.46	6.55
Lutein (all trans)	5.38	8.04	6.71	7.66	6.04	6.85
Zeaxanthin	1.69	8.09	4.89	5.49	6.85	6.17
α-Cryptoxanthin	1.73	7.94	4.83	5.39	5.69	5.54
β-Cryptoxanthin	0.67	9.89	5.28	6.87	4.43	5.65
α-Carotene	1.72	6.64	4.18	8.53	3.65	6.09
Phytone + Phytofluene mixture	2.63	7.98	5.31	5.23	1.50	3.36
β-Carotene	1.99	7.81	4.90	8.49	3.28	5.88

Table 4. Wavelengths and retention times of orange-carrot juice mixture and orange juice

		Orange-carrot	Orange
		t_R	t_R
Carotenoids	λ	(min)	(min)
Valenciaxanthin	430	3.226	3.856
Neoxanthin	430	3.784	5.902
cis-Antheraxanthin	430		6.676
Antheraxanthin	450	4.380	7.142
Lutein (all trans)	450	4.774	7.722
Zeaxanthin	450	5.280	8.250
α-Cryptoxanthin	450	9.472	9.652
cis-β-Cryptoxanthin	450		9.934
β-Cryptoxanthin	450	8.677	10.344
9-cis-α-Carotene	430		12.216
α-Carotene	450	11.639	12.276
Phytoene + Phytofluene	290	12.026	12.729
β-Carotene	450	12.536	12.965
13-cis-β-Carotene	450	13.281	
ζ-Carotene	430	13.662	12.827
9-cis-β-Carotene	450	14.919	13.380
15-cis-β-Carotene	450	15.012	

Table 5. Quantification of carotenoids in orange-carrot juice mixture and in orange juice

	Orange-carrot		Orange	
Carotenoids	µg/100 g	SD	µg/100 g	SD
Valenciaxanthin	39.99	14.61	14.44	1.27
Neoxanthin	404.17	140.77	698.89	71.87
cis-Antheraxanthin			26.88	3.40
Antheraxanthin	176.18	32.65	220.75	29.44
Lutein (all trans)	72.74	7.60	48.86	8.78
Zeaxanthin	55.47	5.63	35.09	3.39
α-Cryptoxanthin	51.83	3.55	42.29	2.34
cis-β-Cryptoxanthin			16.58	2.36
β-Cryptoxanthin	67.62	8.10	118.31	3.61
9-cis-α-Carotene			50.38	15.53
α-Carotene	132.29	11.84	22.41	3.25
Phytoene + Phytofluene	185.60	1.75	36.54	2.61
β-Carotene	238.72	18.77	20.63	3.12
13-cis-β-Carotene	30.68	13.77		
ζ-Carotene	40.66	5.49	34.76	3.50
9-cis-β-Carotene	14.30	1.64	26.92	7.38
15-cis-β-Carotene	8.68	1.79		

The detection and quantification limits described above show that the method is very sensitive for the carotenoids studied.

Accuracy was estimated by means of recovery assays. A sample (16.69, 14.14, and 17.02 μg for lutein, zeaxanthin, and β-carotene, respectively) to which known amounts of standard lutein, zeaxanthin, and β-carotene (38.30, 30.32, and 12.37 μg, respectively) had been added was subjected to the entire extraction and determination process. The recovery percentages obtained were 94.0, 89.2, and 92.8 for lutein, zeaxanthin, and β-carotene, respectively.

Instrumental precision was checked from six consecutive injections of a standard solution, and the relative standard deviations (RSDs) obtained were 0.55, 0.34, and 5.33% for lutein, zeaxanthin, and β-carotene, respectively. When the standard solution was prepared and measured on alternate days, the RSD values were 0.57, 0.51, 5.67 % for lutein, zeaxanthin, and β-carotene, respectively. Instrumental precision was also checked from six consecutive injections of a sample extract (Table 6). The precision of the method was determined by preparing six aliquots of the same sample of orange juice and six aliquots of the same sample of orange-carrot juice. In both cases, precision was expressed as a coefficient of variation. The results obtained are shown in Table 6.

It can be seen that the instrumental and method precision levels obtained are acceptable both in standards and in sample (orange-carrot juice) for the carotenoids studied.

Analytical parameters are similar to those found by Chen et al. (*24*), Hart et al. (*40*), and Konings et al. (*27*), highlighting that the linearity interval studied is wider in this work.

Figures 4 and 5 show the chromatograms of the two types of samples that we analyzed: an orange-carrot juice mixture and orange juice, respectively. The differences between the two samples can be seen clearly: the mixed juice has a greater quantity of carotenoids, especially of α- and β-carotene, but the most representative carotenoids (9-*cis*-violaxanthin, neoxanthin, antheraxanthin,

lutein, zeaxanthin, α- and β-cryptoxanthin, 9-*cis*-α-carotene, phytoene, and phytofluene) are present in both juices. The most common retention times and wavelengths are shown in Table 3. A slight variation can be seen in the retention times obtained for the sample of orange-carrot juice mixture and the sample of orange juice, because they are different matrices. It must also be emphasized that not all of the cis isomers were found in all of the samples analyzed, and the particular isomers obtained depended on each matrix and on their different conservation treatments.

The contents of the various carotenoids identified in accordance with the proposed method in orange juice and in an orange-carrot juice mixture are shown in Table 7.

Conclusions. The utilization of photodiode array detection is a valuable tool for characterization of *cis*- and *trans*-carotenoids in vegetable and citric juices (orange juice and an orange-carrot juice mixture). Using the rapid procedure described, the major carotenoids have been characterized from spectral and retention time data obtained with authentic standards or literature values. Major carotenoids were separated within 22 min with ternary gradient elution. The low toxicity and danger of most of the reagents and products used during the extraction and separation of carotenoids do not call for special safety measures in the laboratory. As the analytical parameters show, the proposed method permits determination of *cis*- and *trans*-carotenoids in vegetable juices, because the method is sensitive, reliable, accurate, and reproducible.

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LITERATURE CITED

1. Rouseff, R.; Raley, L.; Hofsommer, H. J. Application of diode-array detection with a C30 reversed-phase column for the separation and identification of saponified orange juice carotenoids. *J. Agric. Food Chem.* **1996**, *44*, 2176-2181.

2. O'Neal, A.; Schwartz, S. J. Chromatographic analysis of cis/trans carotenoid isomers. Review. *J. Chromatogr.* **1992**, *624*, 235-252.

3. Klaui, H.; Bauernfeind, J. C. *Carotenoids as Colorants and Vitamin A Precursors*; Bauernfeind, J. C., Ed.; Academic Press: New York, 1981; Chapter 2, p 47.

4. Landrum, J. T.; Bone, R. A. Lutein, Zeaxanthin and the Macular Pigment. *Arch. Biochem. Biophys.* **2001**, *385*, 28-40.

5. Grassmann, J.; Hippeli, S.; Elstre, E. F. Plant's defence mechanism and its benefits for animal and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant Physiol. Biochem.* **2002**, *40*, 471-478.

6. Van Poppel, G.; Goldbohm, R. A. Epidemiological evidence for β-carotene and cancer prevention. *Am. J. Clin. Nutr.* **1995**, *62*, 1493-1503.

7. Giovannucci, E. Tomatoes, tomato-based products, lycopene and cancer: a review of the epidemiological literature. *J. Natl. Cancer Inst.* **1999**, *91*, 317-331.

8. Kritchevsky, S. B. β-carotene, carotenoids and the prevention of coronary heart disease. *J. Nutr.* **1999**, *129*, 5-8.

9. Souci, S. W.; Fachmann, W.; Krant, H. *Food Composition and Nutrition Tables*; Wissenschaftliche Verlagsgessellschaft: Stuttgart, Germany, 1987.

10. Burns, J.; Fraser, P. D.; Bramley, P. M. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry* **2003**, *62*, 939-947.

11. Bushway, R. J. Determination of α- and β-carotene in some raw fruits and vegetables by high-performance liquid chromatography. *J. Agric. Food Chem.* **1986**, *34*, 409-412.

12. Barua, A. B.; Kostic, D.; Olson, J. A. New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum. *J. Chromatogr*. **1993**, *617*, 257-264.

13. van Breemen, R. B.; Schmitz, H. H.; Schwartz, S. J. Continuous-flow fast atom bombardment liquid chromatography/mass spectrometry of carotenoides. *Anal*. *Chem*. **1993**, *65*, 965-969.

14. Emenhiser, C.; Simunovic, N.; Sander, L. C.; Schwartz, S. J. Separation of geometrical carotenoid isomers in biological extracts using polymeric C30 column in reversed-phase liquid chromatography. *J. Agric*. *Food Chem*. **1996**, *44*, 3887-3893.

15. Kimura, M.; Rodriguez-Amaya, D. B. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chem*. **2002**, *78*, 389-398.

16. Cano, M. P. HPLC separation of chlorophyll and carotenoid pigments of four kiwi fruit cultivars. *J. Agric*. *Food Chem*. **1991**, *39*, 1786-1791.

17. Biacs, P. A.; Daood, H. G.; Huszka, T. T.; Biacs, P. K. Carotenoids and carotenoid esters from new cross-cultivars of paprika. *J. Agric*. *Food Chem*. **1993**, *41*, 1864-1867.

18. Chen, B. H.; Chen, Y. Y. Stability of chlorophylls and carotenoids in sweet potato leaves during microwave cooking. *J. Agric*. *Food Chem*. **1993**, *41*, 1315-1320.

19. Cano, M. P.; de Ancos, B. Carotenoid and carotenoid ester composition in mango fruit as influenced by processing method. *J. Agric*. *Food Chem*. **1994**, *42*, 2737-2742.

20. Cano, M. P.; de Ancos, B.; Lobo, M. G.; Monreal, M. Carotenoid pigments and colour of hermaphrodite and female papaya fruits (*Carica papaya* L.) cv. Sunrise during post-harvest ripening. *J. Sci*. *Food Agric*. **1996**, *71*, 351-358.

21. Craft, N. E.; Sander, L. C.; Pierson, H. F. Separation and relative distribution of *all-trans*-β-carotene and its *cis* isomers in β-carotene preparations. *J. Micronutr. Anal*. **1990**, *8*, 209-221.

22. Jinno, K.; Lin, Y. Separation of carotenoids by high-performance liquid chromatography with polymeric and monomeric octadecylsilica stationary phases. *Chromatographia* **1995**, *41*, 311-317.

23. Saleh, M. H.; Tan, B. Separation and identification of cis/trans carotenoid isomers. *J. Agric. Food Chem*. **1991**, *39*, 1438-1443.

24. Chen, T. M.; Chen, B. H. Optimization of mobile phases for HPLC of cis-trans carotene isomers. *Chromatographia* **1994**, *39*, 346-354.

25. Chen, B. H.; Peng, H. Y.; Chen, H. E. Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. *J. Agric. Food Chem*. **1995**, *43*, 1912-1918.

26. Chen, H. E.; Peng, H. Y.; Chen, B. H. Stability of carotenoids and vitamin A during storage of carrot juice. *Food Chem*. **1996**, *57*, 497-503.

27. Konings, E. J. M.; Roomans, H. S. Evaluation and validation of an LC method for the analysis of carotenoids in vegetables and fruit. *Food Chem*. **1997**, *59*, 599-603.

28. Ben-Amotz, A.; Fishler, R. Analysis of carotenoids with emphasis on 9-*cis*-β-carotene in vegetables and fruits commonly consumed in Israel. *Food Chem*. **1998**, *62*, 515-520.

29. Pupin, A. M.; Dennis, M. J.; Toledo, M. C. F. HPLC analysis of carotenoids in orange juice. *Food Chem*. **1999**, *64*, 269-275.

30. Khachik, F.; Beecher, G. R.; Lusby, W. R. Separation, identification, and quantification of the major carotenoids in extracts of apricots, peaches, cantaloupe, and pink grapefruit by liquid chromatography. *J. Agric. Food Chem*. **1989**, *37*, 1465-1473.

31. Craft, N. E. Carotenoid reversed-phase high performance liquid chromatography methods: Reference compendium. In *Methods in Enzymology*; Academic Press: New York, 1992; pp 185-205.

32. Epler, K. S.; Craft, N. E.; Ziegler, R. G. Liquid chromatographic method for the determination of carotenoids, retinoids and tocopherols in human serum and in food. *J. Chromatogr*. **1993**, *619*, 37-48.

33. De Ritter, E.; Purcell, A. E. Carotenoid analytical methods. In *Carotenoids as Colorants and Vitamin A Precursors*; Bauernfeind, J. C., Ed.; Academic Press: London, U.K., 1981; Chapter 10, pp 815-923.

34. Lesellier, E.; Tchapla, A. Analysis of carotenoids by high-performance liquid chromatography and supercritical fluid chromatography. *J. Chromatogr*. **1993**, *633*, 9-23.

35. Noga, G.; Lenz, F. Separation of Citrus Carotenoids by Reversed-Phase High-Performance Liquid Chromatography. *Chromatographia* **1983**, *17*, 139-142.

36. Marx, M.; Schieber, A.; Reinhold, C. Quantitative determination of carotene stereoisomers in carrot juices and vitamin supplemented (ATBC) drinks. *Food Chem*. **2000**, *70*, 403-408.

37. Matus, Z.; Deli, J.; Szabolcs, J. Carotenoid composition of yellow pepper during ripening: isolation of β-cryptoxanthin 5,6-epoxide. *J. Agric*. *Food Chem*. **1991**, *39*, 1907-1914.

38. Mouly, P. P.; Gaydou, E. M.; Corsetti, J. Characterization of paprika (*Capsicum annuum*) extract in orange juices by liquid chromatography of carotenoid profiles. *J. Agric*. *Food Chem*. **1999**, *47*, 968-976.

39. Scott, K. J.; Hart, D. J. Further observations on problems associated with the analysis of carotenoids by HPLC-2: Column temperature. *Food Chem*. **1993**, *47*, 403-405.

40. Hart, D. J.; Scott, K. J. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid

content of vegetables and fruits commonly consumed in the UK. *Food Chem*. **1995**, *54*, 101-111.

41. Muller, H. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z. Lebensm. Unters. Forsch. A* **1997**, *204*, 88-94.

42. Khachik, F.; Beecher, G. R. Application of a C-45-β-carotene as an internal standard for the quantification of carotenoids in yellow/orange vegetables by liquid chromatography. *J. Agric. Food Chem*. **1987**, *35*, 732-738.

43. Oliver, J.; Palou, A. Chromatographic determination of carotenoids in foods. *J. Chromatogr. A* **2000**, *881*, 543-555.

44. Olmedilla, B.; Granado, F.; Blanco, I.; Rojas-Hidalgo, E. Determination of nine carotenoids, retinol, retinyl palmitate and α-tocopherol in control human serum using two internal standards. *Food Chem*. **1992**, *45*, 205-213.

45. Granado, F.; Olmedilla, B.; Blanco, I.; Rojas-Hidalgo, E. Carotenoid composition in raw and cooked spanish vegetables. *J. Agric. Food Chem*. **1992**, *40*, 2135-2140.

46. Taungbodhitham, A. K.; Jones, G. P.; Wahlqvist, M. L.; Briggs, D. R. Evaluation of extraction method for the analysis of carotenoids in fruits and vegetables. *Food Chem*. **1998**, *63*, 577-584.

47. O'Neil, C. A.; Schwartz, S. J.; Catignani, G. L. Comparison of liquid chromatographic methods for determination of cis-trans isomers of β-carotene. *J. Assoc. Off. Anal. Chem*. **1991**, *74*, 36-42.

48. Khachik, F.; Beecher, G. R. Separation and identification of carotenoids and carotenol fatty acid esters in some squash products by liquid chromatography. 1. Quantification of carotenoids and related esters by HPLC. *J. Agric. Food Chem*. **1988**, *36*, 929-937.

49. Mercadante, A. Z.; Britton, G.; Rodriguez-Amaya, D. B. Carotenoids from yellow passion fruit (*Passiflora edulis*). *J. Agric. Food Chem*. **1998**, *46*, 4102-4106.

50. Lee, H. S. Characterization of carotenoids in juice of red navel orange (*Cara cara*). *J. Agric. Food Chem.* **2001**, *49*, 2563-2568.

51. Lee, H. S.; Castle, W. S.; Coates, G. A. High-performance liquid chromatography for the characterization of carotenoids in the new sweet orange (Earlygold) grown in Florida, USA. *J. Chromatogr. A* **2001**, *913*, 371-377.

52. Wingerath, T.; Stahl, W.; Kirsch, D.; Kaufmann, R.; Sies, H. Fruit juice carotenol fatty acid esters and carotenoids as identified by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. *J. Agric. Food Chem.* **1996**, *44*, 2006-2013.

53. Britton, G. UV/Visible spectroscopy. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhauser Verlag: Boston, MA, 1995; Vol. 1B, Spectroscopy, pp 13-62.

54. Psomiadou, E.; Tsimidou, M. Simultaneous HPLC determination of tocopherols, carotenoids, and chlorophylls for monitoring their effect on virgin olive oil oxidation. *J. Agric. Food Chem.* **1998**, *46*, 5132-5138.

55. Tsukida, K.; Saiki, K.; Takii, T.; Koyama, Y. Separation and determination of cis/trans-β-carotenes by high-performance liquid chromatography. *J. Chromatogr.* **1982**, *245*, 359-364.

56. Phillip, D.; Molnar, P.; Toth, G.; Young, A. J. Light-induced formation of 13-*cis*-violaxanthin in leaves of *Hordeum vulgare*. *J. Photochem. Photobiol. B: Biol.* **1999**, *49*, 89-95.

57. Chen, B. H.; Yang, S. H. An improved analytical method for the determination of carotenes and xanthophylls in dried plant materials and mixed feeds. *Food Chem.* **1992**, *44*, 61-66.

58. Simpson, K. L.; Tsou, S. C. S.; Chichester, C. O. Carotenes. In *Methods Vitamins Assay*, 4th ed.; Augustin, J., Klein, B. P., Becker, D., Venugopal, P. B., Eds.; Forte Association of Vitamin Chemists; Wiley: New York, 1985.

59. Chandler, L. A.; Schwartz, S. J. HPLC separation of cis-trans carotene isomers in fresh and processed fruits and vegetables. *J. Food Sci*. **1987**, *52*, 669-672.

Capítulo 3

Zumo de naranja-zanahoria

1. **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 (2005).
2. **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 (2006).
3. **Changes in carotenoids including geometrical isomers and ascorbic acid content in orange-carrot juice during frozen storage**. European Food Research and Technology, 221, 125-131 (2005).

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Effect Of High-Intensity Pulsed Electric Fields Processing And Conventional Heat Treatment On Orange–Carrot Juice Carotenoids

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Liquid chromatography (LC) was the method of choice for quantification of carotenoids (including geometrical isomers) to evaluate the effects of high-intensity pulsed electric field (HIPEF), a non-thermal preservation method, with different parameters (electric field intensities and treatment times), on an orange–carrot juice mixture (80:20, v/v). In parallel, a conventional heat treatment (98ºC, 21 s) was applied to the juice. HIPEF processing generally caused a significant increase in the concentrations of the carotenoids identified as treatment time increased. HIPEF treatment at 25 and 30 kV/cm provided a provitamin A concentration higher than that found in the pasteurized juice.

KEYWORDS: Carotenoid; High- intensity pulsed electric field; Heat treatment; Orange–carrot juice

1. Introduction

The demand for minimally processed food has increased in recent years, owing to its greater retention of flavour, colour and nutritive value and consumer demand for safe but high-quality natural foods (with minimal or no chemical preservatives). Although conventional thermal processing ensures safety and extends the shelf life of foods, it often leads to detrimental changes in the sensory qualities of the product (*1*). Consequently, non-thermal processing for the preservation of foods is being developed as an alternative to traditional thermal methods (*2-14*). New products are being produced in this line, with juice mixtures that provide increased quality (nutritive value, colour, etc.), this being the factor that contributes most to consumer acceptance and an increase in the value added to the product. The consuunption of refrigerated juices in the USA is currently of the order 4.4 bilion (*15*).

In addition to the excellent intrinsic sensory and nutritive characteristics of orange juice, the incorporation of a proportion of carrot makes a valuable contribution to the health of the consumer since oranges have a high vitamin C content, and carrots have the highest content of carotenes of fresh foods. Stern (*16*) discussed the development of vitamin-enriched fruit and vegetable juice mixtures, and among the suitable juices he highlighted orange–carrot juice because of its greater consumer acceptance.

Carotenoids are one of the main kinds of natural pigments because their distribution in the plant kingdom is extremely widespread (*17*). About 700 kinds of carotenoids have been isolated in nature (*18*). The carotenoids present in citrus fruits are a complex mixture of >115 natural substances (*19*), but not all of them are precursors of vitamin A. Various carotenoids, including α-carotene, β-carotene, and β-cryptoxanthin, have provitamin A activity, being transformed into retinal by mammals. The xanthophylls (oxocarotenoids) lutein and zeaxanthin are also known to provide protection against macular degeneration

connected with age, through their ability to capture free oxygen and blue light in the retina (*20-23*). Carotenoids have a range of important and well-documented biological activities. They are potent antioxidants and free radical scavengers, and they modulate the pathogenesis of cancers and coronary heart disease (*24-29*).

Vitamin A deficiencies are widespread in developing countries, influencing the growth of young children severely (*30, 31*). UNICEF and WHO consider that improving the vitamin A status of young children with marginal deficiency may reduce the mortality by 23% on average. Dietary approaches are needed to replace supplementation programs, ensuring sustainability and an adequate coverage of children in need (*32*). Although fruits and vegetables containing carotenoids are available in developing countries, deficiencies are often found during the off season (*33*). The application of various industrial treatments can lead to the formation of *cis* isomers, which do not have the same vitamin activity as *all-trans* isomers.

Non-thermal treatment technologies can make a significant contribution to improving the vitamin A intake during the off season, as conventional thermal treatments lead to partial degradation of vitamins, especially carotenoids.

Pulsed electric field (HIPEF) treatment has gained increasing interest because it offers some attractive advantages over thermal methods currently used in processing raw materials and foods (*10*). HIPEF has been most successful with fluid products; however, some semisolids and powders have also been treated (*34*). HIPEF processing inactivates microorganisms and enzymes without significant adverse effects on flavour and nutrients (*3-7, 35-37*). An increase in electric field intensity or in treatment time, defined as the product of the number of pulses and the pulse length, increases microbial inactivation. Application of pulses, quantified as energy input, results in thermal energy dissipation and consequently in an increase of the temperature of the product (*10*). Optimal treatment parameters depend on the specific food matrix, temperature, pH,

presence of antimicrobial compounds, and conductivity of media (*38*). However, no reference about the effects of HIPEF on carotenoid changes in orange-carrot juice have found in the literature.

Liquid chromatography (LC) is considered to be the method of choice for the separation, identification and quantification of carotenoids found in biological tissues (*39-48*). The use of photodiode array detection in the identification of carotenoids by liquid chromatography is a valuable tool for characterization of *cis*- and *trans*- isomers in vegetable and citric juices (*49-53*).

The purpose of this study is reflected the effects of HIPEF, with different electric field intensities (25, 30, 35 and 40 kV/cm) and different treatment times (30–340 μs) of carotenoids on an orange–carrot juice mixture (80:20, v/v) prepared by a manufacturer and packaged aseptically. In parallel, a conventional heat treatment was applied to the orange–carrot juice and the results were compared.

2. Experimental

2.1. Chemicals

α-Carotene, *all-trans*-retinol palmitate and *tert*-butyl hydroxytoluene (BHT) (special grade) were purchased from Sigma (Steinheim, Germany). Lutein and zeaxanthin were provided free as standard substances by Roche (Basel, Switzerland). Ammonium acetate (LC grade), petroleum ether, hexane (LC grade), potassium hydroxide (85%), and *tert*-butyl methyl ether (TBME) (LC grade) were purchased from Scharlau (Barcelona, Spain); acetonitrile (special grade) and magnesium hydroxide carbonate (40–45%) from Panreac (Barcelona, Spain); and ethanol, diethyl ether, methanol and sodium chloride (special grade) from Baker (Deventer, The Netherlands). Chloroform was obtained from Merck (Darmstadt, Germany).

2.2. Samples

The process for obtaining the mixed orange and carrot juice was: after appropriate washing and hygienization of the oranges, the juice was extracted (FMC juice extractors with a 2-mm-diameter perforated plate) and placed in a tank. Carrot juice was obtained after washing the vegetables first with a diluted solution of sodium hydroxide and afterwards with drinking water. The washed vegetables were ground, and the juice was sifted and mixed with the orange juice (80% orange and 20% carrot). The mixed juice was packaged in Elopack packages and frozen (–40ºC).

2.3. Instrumentation

The LC system consisted of a series 1050 chromatograph with a quaternary pump system, a diode array detector (Hewlett-Packard, 1100 series), a column thermostat (Agilent, 1100 series), an on-line degassing system, and a ChemStation (series A.06.03) data system (Hewlett-Packard, Waldbronn, Germany).

A 250 x 4.6 mm Vydac 201TP54 reverse phase C_{18} column with a particle size of 5 μm and a Vydac 201TP precolumn (guard column) (4.6 mm i.d. cartridge with 5-μm particles) (Hesperia, CA) were used.

2.4. HIPEF treatment system

The sample treatments were applied in a continuous HIPEF treatment system designed by the University of Ohio and located in the Instituto de Agroquímica y Tecnología de los Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage, and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, OR U.S.A.).

The flow was set at 60 ml/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL).

Treatment time ranged from 30 to 340 μs and the electric field was set at 25, 30, 35 and 40 kV/cm. Samples were collected after each treatment. The experiments were performed in duplicate.

2.5. Thermal treatment

The thermal treatment intensity given to T samples (98ºC, 21 s) was similar to the one given by manufacturers of refrigerated juice. To treat the samples an ARMFIELD FT74P equipment with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (98ºC, 21 s) are reached. Heating of orange juice at 90–99ºC for 15–30 s is normal in commercial practice (*54*). After treatment, the juice was cooled with cold water from a cooler (ARMFIELD FT61), and it was packed and stored until analysis. Experiment were performed in duplicate.

2.6. Chromatographic determination

The carotenes (including geometric isomers) were identified and quantified by liquid chromatography with an ultraviolet diode array, using a Vydac 201TP54 C_{18} column and a Vydac 201TP precolumn (guard column). The mobile phase used was *tert*-butyl methyl ether, methanol with ammonium acetate (0.1 M) and water (in a concentration gradient) and a temperature gradient was applied with retinol palmitate as an internal standard. An extraction process (ethanol/hexane, 4:3, v/v) was performed, followed by saponification with diethyl ether/methanolic KOH (0.1%, w/v, BHT) (1:1, v/v) for 0.5 h at room temperature. The carotenoids were identified by UV–vis spectra and retention times in LC in the juices analyzed (*53*).

2.7. Determination of vitamin A

Vitamin A was expressed as retinol equivalents (RE), using the following conversion (*55*): RE= (μg of β-carotene)/6 + (μg of β-cryptoxanthin + α-carotene)/12.

3. Results and discussion

3.1. Carotenoid profile and effect of heat treatment

The LC profile of the saponified extract of fresh orange–carrot juice is shown in Figure 1, and the carotenoids identified appear in Table 1.

Table 2 shows the mean concentration of each of the carotenes quantified in the orange–carrot juice and their vitamin activity expressed as retinol equivalents. It also gives the concentration of each of the carotenoids in the heat-treated orange–carrot juice. Of all the carotenoids studied, only 5 decreased significantly ($p<0.05$): 9-*cis*-violaxanthin+neoxanthin (32.9%), antheraxanthin (3.9%), α-cryptoxanthin (15.5%), 9-*cis*-α-carotene (25.9%) and 9-*cis*-β-carotene (27.3%), while there was a significant increase in the others, with the exception of lutein, zeaxanthin, β-carotene and ξ-carotene. The greatest increase in concentration was in 13-*cis*-β-carotene (163.9%), followed by mutatoxanthin (46.5%) and *cis*-β-cryptoxanthin (46.3%). Thermal processing is reported to increase carotenoid concentration, perhaps owing to greater stability enzymatic degradation and unaccounted losses of moisture and soluble solids which concentrate the sample per unit weight (*56*). Heat induces *cis*/*trans* isomerization (13-*cis*-β-carotene) and different carotenoid by-products can be formed (*57*-*59*). Lee and Coates (*60*) studied the effect of heat treatment (90ºC, 30 s) on the colour and pigments of oranges and found that the carotenoids that decreased were violaxanthin (46.4%), *cis*-violaxanthin (19.7%) and antheraxanthin (24.8).

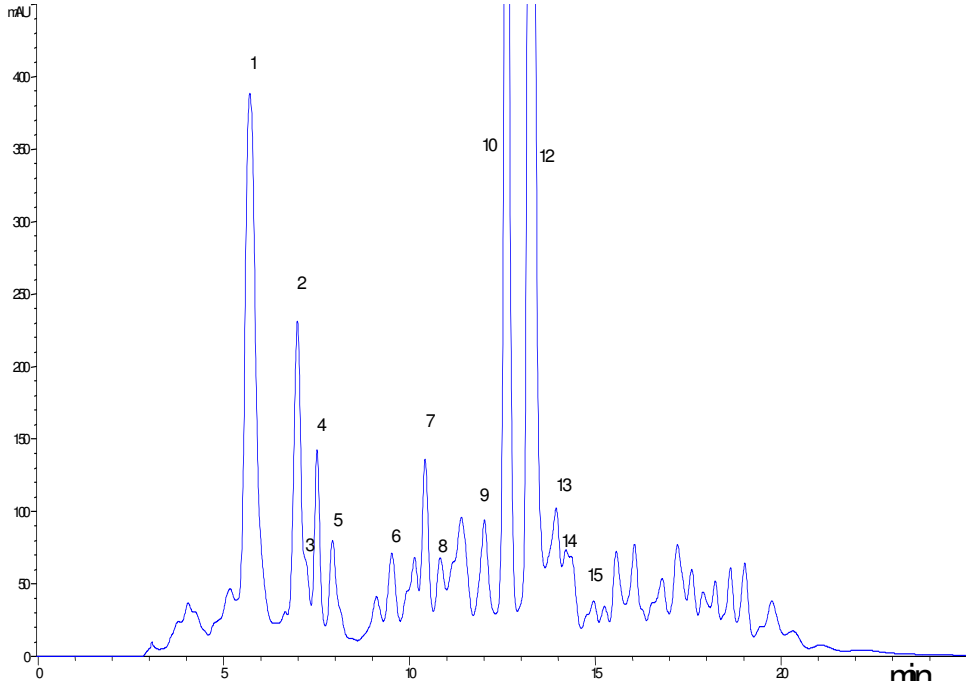
Table 1. Wavelengths and retention times of orange–carrot juice mixture

Peak no.	Carotenoids	λ (nm)	Orange–carrot t_R (min) ± SD
1	9-*cis*-violaxanthin+neoxanthin	430	5.75 ± 0.04
2	antheraxanthin	450	7.01 ± 0.03
3	mutatoxanthin	430	7.20 ± 0.01
4	lutein	450	7.54 ± 0.02
5	zeaxanthin	450	7.96 ± 0.04
6	α-cryptoxanthin	450	9.53 ± 0.03
7	β-cryptoxanthin	450	10.45 ± 0.04
8	*cis*-β-cryptoxanthin	450	10.83 ± 0.05
9	9-*cis*-α-carotene	430	12.06 ± 0.04
10	α-carotene	450	12.63 ± 0.03
11	phytoene+phytofluene	290	13.06 ± 0.02
12	β-carotene	450	13.28 ± 0.03
13	13-*cis*-β-carotene	450	13.95 ± 0.02
14	ζ-carotene	430	14.28 ± 0.04
15	9-*cis*-β-carotene	450	14.96 ± 0.03

Retention time: t_R

Figure 1. Chromatogram of untreated orange–carrot juice mixture at λ=450 nm (A) and λ=290 nm (B).

(A)



(B)

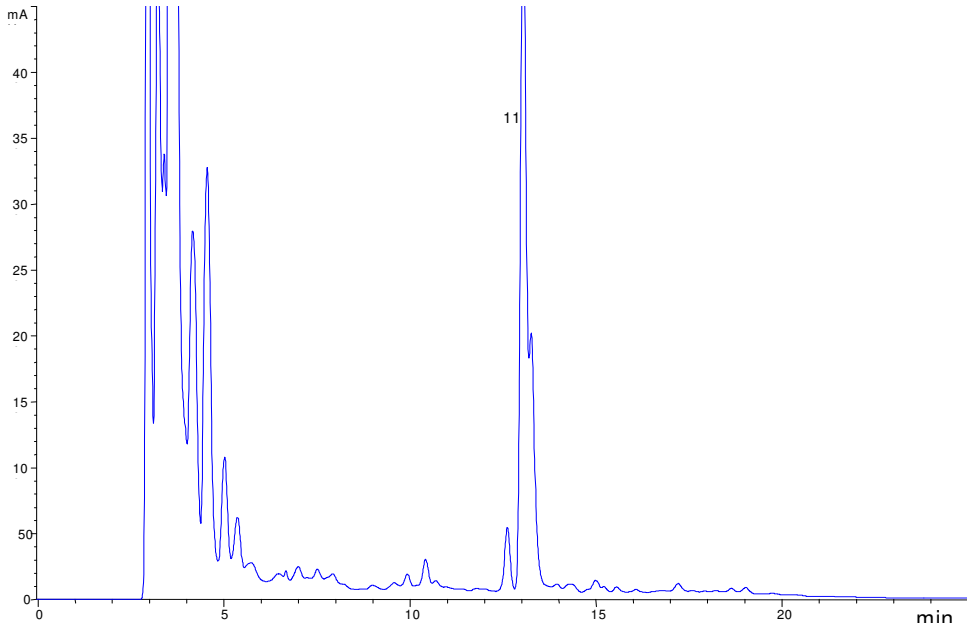


Table 2. Concentration of carotenoids (μg/100g) and vitamin A (RE) in untreated orange–carrot juice and pasteurized orange–carrot juice

		Untreated orange–carrot juice	Pasteurized orange–carrot juice
1	9-*cis*-violaxanthin+neoxanthin	332.29±19.94	223.03±10.95
2	antheraxanthin	132.12±5.28	126.97±6.33
3	mutatoxanthin	13.87±0.83	15.08±0.96
4	lutein	45.17±2.71	47.20±3.01
5	zeaxanthin	37.15±2.19	54.42±1.56
6	α-cryptoxanthin	25.46±0.81	21.51±1.51
7	β-cryptoxanthin	38.81±2.87	50.17±2.03
8	*cis*-β-cryptoxanthin	26.29±0.98	38.46±1.22
9	9-*cis*-α-carotene	106.40±3.23	78.76±1.58
10	α-carotene	691.02±18.56	818.63±16.32
11	phytoene+phytofluene	334.36±11.02	401.90±15.27
12	β-carotene	1789.69±108.36	1893.49±112.23
13	13-*cis*-β-carotene	99.71±7.38	263.13±15.63
14	ξ-carotene	100.14±4.66	99.14±3.97
15	9-*cis*-β-carotene	41.33±3.02	30.05±2.85
	Vitamin A	367.45±22.05	396.24±31.64

They found a clear increase in the concentration of luteoxanthin (30.9%), mutatoxanthin (74.2%), lutein (13.4%) and ξ-carotene (23.1%). Doering et al. (*61*) reported that when β-carotene is exposed to temperatures below 100ºC the carotenoids formed are mainly 13- and 15-*cis*-β-carotene, and 9-*cis*-carotene is formed when the temperature is above 100ºC. Similar results were obtained by

Kuki et al. (*62*), who found that when β-carotene is heated to 80ºC for 30 min 13-*cis*- and 15-*cis*-β-carotene are formed. However, Chen et al. (*63*) and Johnsson (*64*) found that β-carotene in carrot juice was stable to *cis*-isomerization when pasteurized at 100ºC. Marx et al. (*65*) studied the effects of heat treatment on *trans*-*cis*-isomerization of β-carotene in carrot juice and found that pasteurization at 95ºC and sterilization at 121ºC caused a lower percentage of 13-*cis*-β-carotene to be formed than when the juice was sterilized at 130ºC. Lavelli and Giovanelli (66) evaluated the effect of heat during storage of products derived from tomatoes and found that the β-carotene content decreased even at 30ºC. Bull et al. (*1*) report that high pressure processing and thermal treatment of orange juice do not significantly reduce its β-carotene content.

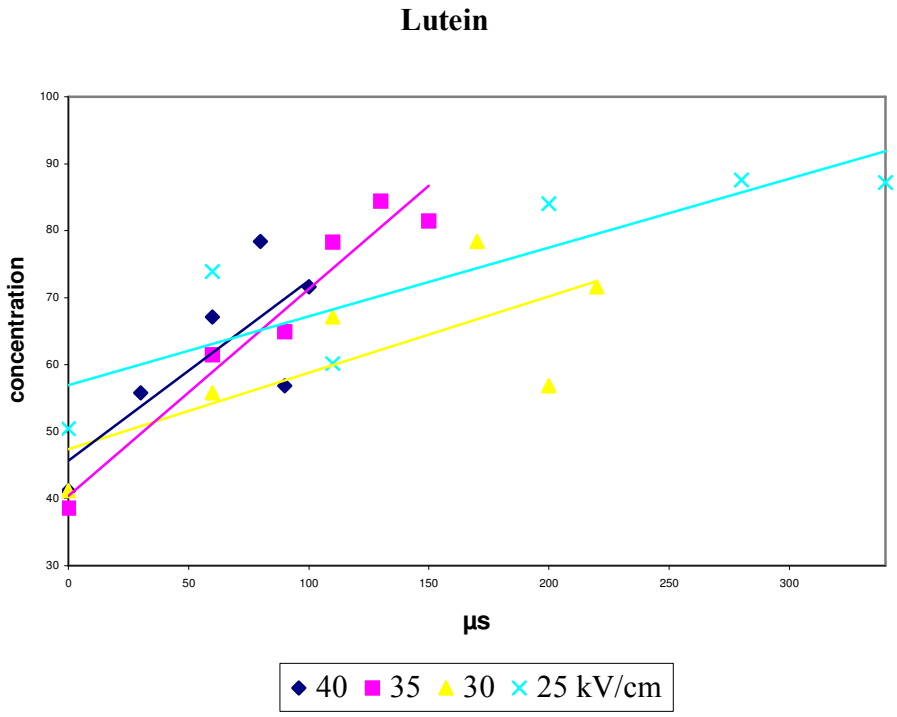
3.2. Effect of HIPEF treatment on carotenoid content of orange–carrot juice

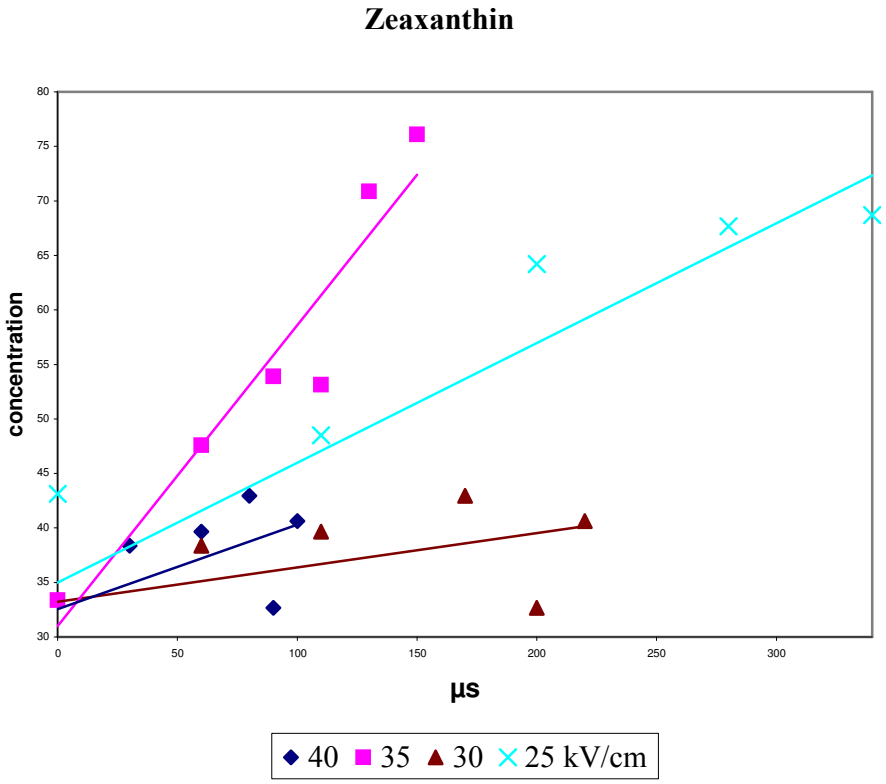
In order to establish the effect of HIPEF treatment, different field intensities (25, 30, 35 and 40 kV/cm) were applied for different times (from 30 to 340 μs), and in all cases the results were compared with the results for untreated orange-carrot juice. Tables 3–6 show the results obtained. The tables give the maximum temperature attained in each treatment, which did not exceed 65ºC in any case.

It can be seen that the concentration of the 9-*cis*-violaxanthin+neoxanthin mixture increases with treatment time, and although the fit is not significant it can be seen that the rate of formation of these carotenoids increases with treatment intensity: 0.728±0.183, 0.965±0.347, 1.628±0.794 and 1.863±0.686 μg/100g of juice per μs, for juice treated at 25, 30, 35 and 40 kV/cm, respectively. Something similar happens with the concentration of antheraxanthin, which increases with treatment time, and its rate of formation increases with treatment intensity: 0.259±0.069, 0.423±0.084, 0.745±0.118 and 0.941±0.272 μg/100g of juice per μs, for juice treated at 25, 30, 35 and 40

kV/cm, respectively. In the concentration of mutatoxanthin there are no significant differences when the HIPEF field is 25 kV/cm, but the concentration increases when the field is 30 kV/cm and decreases when the treatment field is 35 or 40 kV/cm, and the rate of degradation is greater in the 40 kV/cm field. The concentration of lutein and zeaxanthin increases with treatment time and intensity (see Figure 2).

Figure 2. Concentration of Lutein and Zeaxanthin (µg/100g) for different electric fields in orange–carrot juice.





The concentration of α-cryptoxanthin increases in the treatments of 25 and 30 kV/cm, whereas it decreases in treatments of 35 and 40 kV/cm. When the HIPEF treatment applied is 25 kV/cm the concentration of β-cryptoxanthin increases significantly in the first 110 µs and then remains stable until 340 µs. In fields of 30, 35 and 40 kV/cm the concentration of this carotenoid increases with longer treatment times, and the rate of formation increases with treatment intensity: 0.236±0.070, 0.454±0.110 and 0.494±0.066 µg/100g of juice per µs, for treatments of 30, 35 and 40 kV/cm, respectively. Similar behaviour can be seen

in the evolution of the concentration of *cis*-β-cryptoxanthin and 9-*cis*-α-carotene. In the 25 kV/cm field there is a significant increase ($p<0.05$) in the first 110 μs of treatment, decreasing slightly when the treatment time increases. When the field intensity is increased (30, 35 and 40 kV/cm), the concentration of *cis*-β-cryptoxanthin increases as treatment time increases, whereas the concentration of 9-*cis*-α-carotene increases when the field intensity applied is 30 kV/cm but decreases when the intensity is higher. The concentration of α-carotene increases slightly when a 25 kV/cm field is applied, it is maintained in treatments of 30 and 35 kV/cm, and it decreases significantly ($p<0.05$) when a field of 40 kV/cm is applied. It can be seen that this result is not influenced by treatment time. The concentration of β-carotene increases when a 25 kV/cm field is applied, although the increase is not significant ($p>0.05$). When the field is 30, 35 or 40 kV/cm no significant differences ($p<0.05$) are observed when the treatment time increases. The concentration of 13-*cis*-β-carotene, ξ-carotene and 9-*cis*-β-carotene increases when a 25 kV/cm field is applied. With a field of 30 or 35 kV/cm no significant differences are observed in the concentrations of 13-*cis*-β-carotene and ξ-carotene at the various treatment times, but they decrease significantly ($p<0.05$) when the treatment intensity increases (40 kV/cm). The concentration of 9-*cis*-β-carotene decreases in fields of 30 or 40 kV/cm and increases in a field of 35 kV/cm, so that a trend cannot be established.

Table 3. Concentration of carotenoids (μg/100g) and vitamin A (RE) in orange-carrot juice treated by HIPEF with a field of 25 kV/cm

	Treatment time (μs)	0	60	110	200	280	340
	Maximum temperature (ºC)		40	44	51	58	65
1	9-*cis*-violaxanthin+neoxanthin	381.46	522.73	493.74	648.35	602.03	662.53
2	antheraxanthin	135.32	177.04	154.22	226.07	215.17	223.91
3	mutatoxanthin	11.54	28.58	44.02	30.22	19.91	26.19
4	lutein	50.46	73.92	60.14	84.06	87.57	87.18
5	zeaxanthin	43.11	26.56	48.50	64.19	67.67	68.69
6	α-cryptoxanthin	26.16	30.47	37.21	37.24	42.68	36.60
7	β-cryptoxanthin	38.35	66.89	99.16	90.90	87.86	84.47
8	*cis*-β-cryptoxanthin	30.70	50.64	58.37	55.22	46.17	31.28
9	9-*cis*-α-carotene	59.86	75.26	193.74	177.92	165.33	144.85
10	α-carotene	722.17	920.14	848.29	975.48	793.19	812.20
11	phytoene+phytofluene	320.56	629.95	402.90	506.90	497.26	598.53
12	β-carotene	1928.78	2132.48	1950.31	2365.17	1996.79	1935.79
13	13-*cis*-β-carotene	97.21	91.04	220.73	157.88	199.71	83.56
14	ξ-carotene	80.98	146.51	194.77	154.26	141.91	142.65
15	9-*cis*-β-carotene	30.13	66.75	57.49	47.62	62.96	75.52
	Vitamin A (RE)	391.59	449.88	420.24	495.92	418.05	409.24

Table 4. Concentration of carotenoids (μg/100g) and vitamin A (RE) in orange-carrot juice treated by HIPEF with a field of 30 kV/cm

	Treatment time (μs)	0	60	110	170	200	220
	Maximum temperature (ºC)		42	47	56	61	64
1	9-*cis*-violaxanthin+neoxanthin	381.46	486.82	511.07	549.00	494.07	693.25
2	antheraxanthin	135.32	191.40	214.41	227.16	219.28	247.36
3	mutatoxanthin	11.54	20.37	25.06	24.36	24.60	31.26
4	lutein	50.46	65.93	83.46	86.09	85.96	96.96
5	zeaxanthin	43.11	57.54	66.31	67.06	70.68	84.50
6	α-cryptoxanthin	26.16	45.05	43.22	43.71	42.04	50.35
7	β-cryptoxanthin	38.35	60.30	73.71	87.59	67.79	107.22
8	*cis*-β-cryptoxanthin	30.70	56.59	47.77	47.44	67.61	75.68
9	9-*cis*-α-carotene	59.86	143.14	161.89	155.03	169.49	259.14
10	α-carotene	722.17	1002.91	1121.57	1100.34	934.37	1185.56
11	phytoene+phytofluene	320.56	364.02	467.99	504.10	646.99	560.87
12	β-carotene	1928.78	1051.66	2127.89	2435.42	2007.34	2965.81
13	13-*cis*-β-carotene	97.21	126.94	165.09	179.01	188.93	202.03
14	ξ-carotene	80.98	176.66	168.19	162.15	189.62	211.77
15	9-*cis*-β-carotene	30.13	69.34	70.25	63.68	83.93	89.64
	Vitamin A (RE)	391.59	278.60	468.27	518.42	433.87	619.68

Table 5. Concentration of carotenoids (μg/100g) and vitamin A (RE) in orange-carrot juice treated by HIPEF with a field of 35 kV/cm

	Treatment time (μs)	0	60	110	170	200	220
	Maximum temperature (ºC)		42	47	56	61	64
1	9-*cis*-violaxanthin+neoxanthin	261.00	501.00	313.75	383.64	537.22	552.20
2	antheraxanthin	93.73	148.99	157.14	204.03	194.79	198.43
3	mutatoxanthin	16.38	18.00	14.89	15.52	13.43	15.31
4	lutein	38.56	61.48	64.98	78.33	84.48	81.43
5	zeaxanthin	33.39	47.6	53.89	53.13	70.88	76.09
6	α-cryptoxanthin	29.11	33.92	29.04	28.28	27.03	29.21
7	β-cryptoxanthin	35.71	45.08	53.47	71.49	105.24	92.70
8	*cis*-β-cryptoxanthin	25.20	36.13	30.79	27.83	31.39	36.21
9	9-*cis*-α-carotene	222.69	137.15	161.81	143.87	153.04	133.3
10	α-carotene	867.62	472.61	577.91	571.76	721.70	489.43
11	phytoene+phytofluene	423.15	447.64	348.67	368.46	387.53	375.32
12	β-carotene	2253.27	1086.74	1564.31	644.78	2102.18	1000.86
13	13-*cis*-β-carotene	157.95	118.04	115.41	112.51	103.72	41.51
14	ξ-carotene	205.50	132.83	122.48	116.38	145.25	126.47
15	9-*cis*-β-carotene	70.66	40.44	45.11	48.89	46.68	50.74
	Vitamin A (RE)	467.95	235.33	323.54	337.43	431.38	225.86

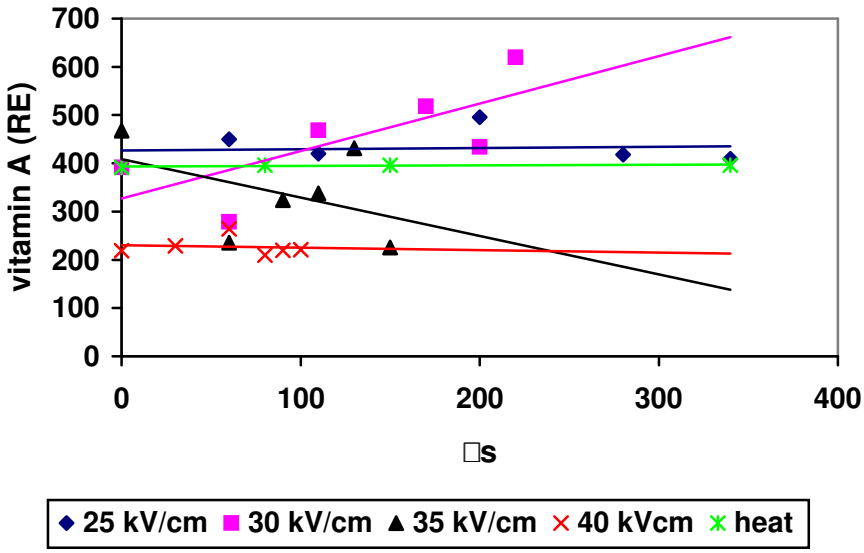
Table 6. Concentration of carotenoids (μg/100g) and vitamin A (RE) in orange-carrot juice treated by HIPEF with a field of 40 kV/cm

	Treatment time (μs)	0	30	60	80	90	100
	Maximum temperature (ºC)		43	53	61	60	65
1	9-*cis*-violaxanthin+neoxanthin	305.23	484.92	519.44	507.22	466.93	562.35
2	antheraxanthin	164.09	189.46	220.31	258.31	276.77	225.64
3	mutatoxanthin	16.03	10.67	8.76	12.63	10.97	8.97
4	lutein	41.19	55.79	67.13	78.40	56.84	71.63
5	zeaxanthin	29.00	38.36	39.67	42.95	32.66	40.62
6	α-cryptoxanthin	20.39	18.03	14.36	13.79	13.93	15.19
7	β-cryptoxanthin	42.79	57.68	63.86	83.86	93.16	87.09
8	*cis*-β-cryptoxanthin	18.57	42.82	31.19	39.13	46.18	36.83
9	9-*cis*-α-carotene	83.18	78.92	59.56	70.47	77.78	66.57
10	α-carotene	452.13	490.87	541.20	443.47	452.12	462.32
11	phytoene+phytofluene	273.15	201.90	203.13	208.70	226.27	230.53
12	β-carotene	1047.93	1073.35	1264.06	975.06	1025.15	1032.81
13	13-*cis*-β-carotene	46.46	67.25	92.68	48.54	53.52	45.43
14	ξ-carotene	33.10	49.10	39.01	36.79	42.02	33.74
15	9-*cis*-β-carotene	34.41	6.60	9.21	6.98	13.24	4.84
	Vitamin A (RE)	218.66	228.70	264.35	209.77	219.8	220.73

Comparison of these results with pasteurized orange–carrot juice shows that it is only in the HIPEF treatments with an intensity of 25 or 30 kV/cm that the vitamin A content is higher than in the pasteurized juice (Figure 3).

The results obtained when HIPEF is applied to the juice cannot be compared with other authors because no similar works have been found in the literature.

Figure 3. Variation of vitamin A (RE) with HIPEF treatment and heat.



3. Conclusions

HIPEF processing is an alternative to conventional thermal treatment of orange-carrot juice. HIPEF processing generally causes a significant increase in the concentration of the various carotenoids identified as treatment time increases, whereas when the juice is processed with conventional heat treatment the concentrations of most of the carotenoids decrease. To select the best HIPEF treatment conditions it is necessary to bear in mind not only microbiological and enzymatic inactivation but also organoleptic characteristics and nutritive value. HIPEF treatment at 25 and 30 kV/cm provides a higher provitamin A content than that found in heat treatment juice.

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REFERENCES

1. Bull, M. K.; Zerdin, K.; Howe, E.; Goicoechea, D.; Paramanandhan, P.; Stockman, R.; Sellahewa, J.; Szabo, E. A.; Johnson, R. L.; Stewart, C. M. The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innov. Food Sci. Emerg. Technol.* **2004**, 5, 135-149.

2. Qin, B. L.; Pothakamury, U. R.; Barbosa-Cánovas, G. V.; Swanson, B. G. Nonthermal pasteurization of liquid foods using high-intensity pulsed electric fields. *Crit. Rev. Food Sci. Nutr.*. **1996**, 36, 603-627.

3. Grahl, T.; Märkl, H. Killing of microorganisms by pulsed electric fields. *Appl. Microbiol. Biot*. **1996**, 45, 148-157.

4. Jia, M.; Zhang, Q. H.; Min, D. B. Pulsed electric field processing effects on flavor compounds and microorganisms of orange juice. *Food Chem.* **1996**, 65, 445-451.

5. Qiu, X.; Sharma, S.; Tuhela, L.; Jia, M.; Zhang, Q. An integrated peiac pilot plant for continuous nonthermal pasteurization of fresh orange juice. *Agric. Eng.*. **1998**, 41, 1069-1074.

6. Bendicho, S.; Arántegui, J.; Martín, O.. Procesado de alimentos mediante pulsos eléctricos de alta intensidad de campo. II. Efectos sobre microorganismos y componentes de los alimentos. *Alimentaria*, **2001**, junio, 37-44.

7. Rodrigo, D.; Martínez, A.; Harte, F.; Barbosa-Cánovas, G. V.; Rodrigo, M. Study of inactivation of Lactobacillus plantarum in orange-carrot juice by means of pulsed electric fields: comparison of inactivation kinetics models. *J. Food Protect*. **2001**, 64, 259-263.

8. Bendicho, S.; Espachs, A.; Arántegui, J.; Martín, O. Effect of high intensity pulsed electric fields and heat treatments on vitamins of milk.*J. Dairy Res.* **2002**, 69, 113-123.

9. Hodgins, A. M.; Mittal, G. S.; Griffiths, M. W. Pasteurization of fresh orange juice using low-energy pulsed electrical fiend. *J. Food Sci*. **2002**, 67, 2294-2299.

10. Abram, F.; Smelt, J. P. P. M.; Bos, R.; Wouters, P. C. Modelling and optimization of inactivation of Lactobacillus plantarum by pulsed electric field treatment. J. *Applied Microbiol.* **2003**, 94, 571-579.

11. Espachs-Barroso, A.; Barbosa-Cánovas, G. V.; Martín-Belloso, O. Microbial and enzymatic changes in fruit juice induced by high intensity pulsed electric fields. *Food Rev. Int*. **2003**, 19, 253-273.

12. Selma, M. V.; Fernández, P. S.; Valero, M.; Salmerón, M. C. Control of Enterobacter aerogenes by high-intensity, pulsed electric fields in horchata, a Spanish low-acid vegetable beverage. *Food Microbiol.* **2003**, 20, 105-110.

13. Spilimbergo, S.; Dehghani, F.; Bertucco, A.; Foster, N. R. Inactivation of bacteria and spores by pulse electric field and high pressure CO_2 at low temperature. *Biotechnol. Bioeng.* **2003**, 82**,** 118-125.

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

15. Jago, D. New food products around the world. *Food Beverage Int.* **2004**, 3 (2), 11-15.

16. Stern, P. In nature's image. Fruit and vegetable beverages with added ingredients. *Fluessiges Obst.* **1998**, 65, 126-130.

17. Meléndez-Martínez, A. J.; Vicario, I. M.; Heredia, F. J. A routine high-performance liquid chromatography meted for carotenoid determination in ultrafrozen orange juices. *J. Agric.* Food Chem. **2003**, 51, 4219-4224.

18. O'Neal, A.; Schwartz, S.J. Chromatography analysis of cis/trans carotenoid isomers. *Review. J. Chromatogr*. **1992,** 624, 235-252.

19. Klaui, H.; Bauernfeind, J. C. In *Carotenoids as colorants and vitamin A precursors*. Bauernfeind, J. C. Ed.; Academic Press: New York, NY, 1981; Chapter 2, p 47.

20. Granado, F.; Olmedilla, B.; Blanco, I. Carotenoid depletion in serum of young type-1 diabetics fed low-carotenoid diets. *Annals of nutrition and metabolism*. **2004**, 48, 251-258.

21. Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M. Lutein, but not alpha-tocopherol, supplentation improves visual function in patients with age-related catarcts: A 2-y double-blind, placebo-controlled pilot study. *Nutrition.* **2003**, 19, 21-24.

22. Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M.; Cajigal, C. Lutein in patients with catarcts anda ge-related macular degeneration: a long-term supplementation study. *J. sci. food agric.* **2001**, 81 (9), 904-909.

23. Landrum, J. T.; Bone, R. A. Lutein, Zeaxantin and the Macular Pigment. *Arch. Biochem. Biophys.* **2001**, 385, 28-40.

24. Sanchez-Moreno, C.; Cano, M. P.; de Ancos, B.; Plaza, L.; Olmedilla, B.; Granado, F.; Elez-Martínez, P.; Martín-Belloso, O.; Martín, A. Pulsed electric fields-processed orange juice consumption increase plasma vitamin C and decreases F2-isoprostanes in healthy humans. *J. nutr. Biochem.* **2004**, 15, 601-607.

25. Sanchez-Moreno, C.; Cano, M.P.; de Ancos, B.; Plaza, L.; Olmedilla, B.; Granado, F.; Martín, A. Consumption of high-pressurized vegetable soup

increases plasma vitamin C and decreases oxidative stress and inflammatory biomarkers in healthy humans. *J. nutr.* **2004**, 134 (11), 3021-3025.

26. Grassmann, J.; Hippeli, S.; Elstre, E. F. Plant's defence mechanism and its benefits for animal and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant physiol. Biochem*., **2002**, 40, 471-478.

27. Van Poppel, G.; Goldbohm, R. A. Epidemiological evidence for β-carotene and cancer prevention. *Am. J. Clin. Nutr*. **1995**, 62, 1493-1503.

28. Giovannucci, E. Tomatoes, tomato-based products, lycopene and cancer: a review of the epidemiological literature. *J. Natl. Cancer Inst*. **1999**, 91, 317-331.

29. Kritchevsky, S. B. β-carotene, carotenoids and the prevention of coronary heart disease. *J. Nutr.* **1999**, 129**,** 5-8.

30. Fawzi, W.; Herrera, M. G.; Willett, W. C.; Nestel, P.; El Amin, A.; Lipsitz, S.; Mohamed, K. A. Dietary vitamin A intake and the risk of mortality among children. *Am. J. Clin. Nutr*. **1994,** 59, 401-408.

31. Chytil, F. Vitamin A: not for vision only. *Br. J. Nutr.* **1999**, 82, 161-162.

32. Scrimshaw, N. S.; Murray, E. B. Special issue on dietary approaches to vitamin A deficiency. In *Food Nutrition Bulletin*; United Nation University Press: Tokio, Japan, 2000; 2 (2); pp 115-116.

33. Zeitlin, M. F.; Megawangi, R.; Kramer, E. M.; Amstrong, H. C. Mothers' and children's intakes of vitamin A in rural Bangladesh. *Am. J. Clin. Nutr.* **1992**, 56, 136-147.

34. Góngora-Nieto, M. M.; Sepúlveda, D. R.; Pedrow, P.; Barbosa-Cánovas, G. V; Swanson, B. G. Food processing by pulsed electric fields: treatment delivery, inactivation level, and regulatory aspects. *Lebensm. –Wiss. u.-Technol*. **2002**, 35, 375-388.

35. Qin, B. L.; Pothakamury, U. R.; Vega, H.; Martin, O.; Barbosa-Cánovas, G. V.; Swanson, B. G. Food pasteurization using high-intensity pulsed electric fields. *Food Technol.* **1995**, 49**,** 55-60.

36. Bendicho, S.; Espachs, A.; Stevens, D.; Arántegui, J.; Martín, O. Effect of high intensity pulsed electric fields on vitamins of milk. *European Conference of Emerging Food Sci. and Technol.* **1999***,* Tampere, Finland.

37. Cortes, C; Esteve, M. J.; Frígola, A.; Torregrosa, F. Quality characteristics of horchata (a Spanish vegetable beverage) treated with pulsed electric fields during shelf-life. *Food Chem.* **2005**, 91, 319-325.

38. Ulmer, H. M.; Heinz V.; Gänzle M. G.; Knorr D; Vogel R. F. Effects of pulsed electric fields on inactivation and metabolic activity of Lactobacillus plantarum in model beer. *J. Appl. Microbiol.* **2002***,* 93, 326-335.

39. Bushway, R. J. Determination of α- and β-carotene in some raw fruits and vegetables by high-performance liquid chromatography. *J. Agric. Food Chem*. **1986**, 34, 409-412.

40. Barua, A. B.; Kostic, D.; Olson, J. A. New simplified procedures for the extraction and simultaneoushigh-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum. *J. Chromatogr.* **1993**, 617, 257-264.

41. Van Bremen, R. B.; Schmitz, H. H.; Schwartz, S. J. Continuous-flow fast atom bombardment liquid chromatography/mass spectrometry of carotenoids. *Anal. Chem.* **1993**, 65, 965-969.

42. Chen, H. E.; Peng, H. Y.; Chen, B. H. Stability of carotenoids and vitamin A during storage of carrot juice. *Food Chem.* **1996**, 57 (4), 497-503.

43. Emenhiser, C.; Simunovic, N.; Sander, L. C.; Schwartz, S. J. Separation of geometrical isomers in biological extracts using polymeric C30 column in reversed-phase liquid chromatography. *J. Agric. Food Chem*. **1996**, 44, 3887-3893.

44. Kidmose, U.; Knuthsen, P.; Edelenbos, M.; Justesen, U.; Hegelung, E. Carotenoids and flavonoids in organically grown spinash (Spinacia oleracea L.) genotypes after deep frozen storage. *J. Sci. Food Agric.* **2001**, 81, 918-923.

45. De Sio, F.; Servillo, L.; Loiuduce, R.; Laratta, B.; Castaldo, D. A chromatographic procedure for the determination of carotenois and chlorophylls in vegetable products. *Act. Aliment.* **2001**, 30 (4), 395-405.

46. De Ancos, B.; Sgroppo, S.; Plaza, L., Cano, M. P. Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *J. Sci. Food Agric.* **2002**, 82, 790-796.

47. Humphries, J.M.; Khachik, F. Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat and pasta products. *J. Agric. Food Chem*. **2003**, 51, 1322-1327.

48. Mendes-Pinto, M.M.; Ferreira, A.C; Oliveira, M.B.; Guedes de Pinho, P. Evaluation of some carotenoids in grapes by reversed- and normal-phase liquid chromatography: a qualitative analysis. *J. Agric. Food Chem.* **2004**, 52 (10), 3182-3188.

49. Khachik, F.; Beecher, G. R.; Lusby, W. R. Separation, identification and quantification of the major carotenoids in extracts apricots, peaches, cantaloupe, and pink grapefruit by liquid chromatography. *J. Agric. Food Chem.* **1989**, 37, 1465-1473.

50. Chen, B. H.; Chen, Y. Y. Stability of chlorophylls and carotenoids in sweet potato leaves during microwavw cooking. *J. Agric. Food Chem.* **1993,** 41, 1315-1320.

51. Rouseff, R.; Raley, L.; Hofsommer, H. J. Application of diode-array detection with a C30 reversed-phase column for the separation and identification of saponified orange juice carotenoids. J. Agric. Food Chem. 1996, 44, 2176-2181.

52. Kimura, M.; Rodriguez-Amaya, D. B. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chem*. **1996**, 44, 3887-3893.

53. Cortés C.; Esteve M. J.; Frígola A; Torregrosa F. Identification and quantification of carotenoids including geometrical isomers in fruit and

vegetable juices by liquid chromatography with ultraviolet-diode array detection. *J. Agric. Food Chem*. **2004**, 52, 2203-2212.

54. Braddock, R. J. Single strength orange juices and concentrate. In *Handbook of Citrus by-products and Processing Technology*; Wiley, J., Ed.; New York, NY, 1999; pp 53–83.

55. FAO/WHO Joint Expert Consultation. Requirements of vitamin A, iron, folate, and vitamin B12. In FAO Food Nutrition Series, 23; Rome, Italy, 1988.

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

57. Khachick, F.; Beecher, G.R.; Goli, M.B.; Lusby, W.R. Separation, identification and quantification of carotenoids in fruits, vegetables and human plasma by high-performance liquid chromatography. *Pure Appl.Chem.* **1991**, 63, 71-80.

58. Khachick, F.; Goli, G.R.; Beecher, G.R.; Holden, J.; Lusby, W.K.; Tenorio, M.D.; Barrera, M.R. Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *J. Agric. Food Chem*. **1992**, 40, 390-398.

59. Quackembush, F.W. Reverse phase HPLC separation of cis and trans-carotenoids and its application to β-carotenes in food materials. *J. Liq. Chromatogr*. **1987**, 10, 643-653.

60. Lee, H.S; Coates, G.A. Effect of thermal pasteurization on Valencia orange juice color and pigments. *Lebensm. Wiss U. Technol*. **2003**, 36, 153-156.

61. Von Doering, W.E.; Sotiriou-Leventis, C.; Roth, W.R. Thermal interconversions among 15-cis-, 13-cis-, and all-trans-β-carotene: kinetics, Arrhenius parameters, thermochemistry, and potential relevance to anticarcinogenity of all-trans-β-carotene. *J. Am. Chem. Soc.* **1995**, 117, 2747-2757.

62. Kuki, M.; Koyama, Y.; Nagae, H. Triplet-sensitized and thermal isomerization of all-trans, 7-cis, 9-cis, 13-cis, and 15-cis-isomers of β-carotene: configurational dependence of the quantum yield of isomerization via T_1 state. *J. Phys. Chem*. **1991**, 95, 7171-7180.

63. Chen, B.H.; Peng, H.Y.; Chen, H.E. Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. *J Agric. Food Chem.* **1995**, 43, 1912-1818.

64. Johnsson, L. Thermal degradation of carotenes and influence on their physiological functions. In Nutritional and toxicological consequences of food processing, Friedman, M., Ed.; Plenum Press, New York, NY, 1991; 75-82.

65. Marx M.; Stuparic M.; Schieber A.; Carle R. Effects of thermal processing on trans-cis-isomerization of β-carotene in carrot juices and carotene-containing preparations. Food Chem. 2003, 83, 609-617.

66. Lavelli, V.; Giovanelli, G. Evaluation of heat and oxidative damage during storage of processed tomato products. II. Study of oxidative damage indices. J. Sci. Food Agric. 2003, 83, 966-971.

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Ascorbic Acid stability during refrigerated storage of orange–carrot juice treated by High Pulsed Electric Field and comparison with pasteurized juice

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Abstract

The degradation kinetics of ascorbic acid was determined in orange–carrot juice treated by PEF in order to establish its shelf life. Different electric field intensities (25, 30, 35, and 40 kV/cm) and different treatment times (from 30 to 340 μs) were studied. The ascorbic acid degradation rate (k) obtained was -0.009 ± 0.0008 μs^{-1}, -0.0140 ± 0.0009 μs^{-1}, -0.0220 ± 0.0023 μs^{-1} and -0.0187 ± 0.0049 μs^{-1} for fields of 25, 30, 35, and 40 kV/cm respectively. The treatment selected was 25 kV/cm. The shelf life of the orange–carrot juice treated by pulses at 25 kV/cm for two times (280 μs and 330 μs) was compared with a heat-treated juice (98 ºC, 21 s) kept in refrigerated storage at 2 and 10 ºC. The remaining concentration of ascorbic acid in the pasteurized orange-carrot juice was 83%, whereas in the PEF-treated juice it was 90%. The ascorbic acid degradation rate in the juice stored at 2 ºC was less than in the juice stored at 10 ºC, and in the pasteurized juice it was greater. PEF treatment at 25 kV/cm for 280–330 μs extended the half-life of the juice stored at 2 ºC to 50 days.

keywords: Ascorbic Acid; storage; shelf life; kinetics; PEF

1. Introduction

Consumption of fruit and vegetables can prevent certain diseases such as cancer and cardiovascular diseases, as these foods are rich in antioxidant vitamins, such as vitamins C and E, phenolic compounds, and carotenes (McCall & Frei, 1999; Williamson, 1999; Gardner, White, McPhail & Duthie, 2000; Block, Norkus, Hudes, Mandel & Helzlsouer, 2001; John, Ziebland, Yudkin, Roe & Neil, 2002; Burns, Frase & Bramley, 2003; Sánchez-Moreno, Plaza, de Ancos & Cano, 2003).

It is clear that, in addition to the particular excellent sensory and nutritive characteristics of orange juice, the incorporation of a proportion of carrot provides a considerable contribution to the health of the consumer. Oranges have a high vitamin C content, and the carrot is the food product with the highest level of carotene. Citric juices contain a high level of carotene but not all are precursors of vitamin A. The mixture of orange juice and carrot juice is rich in antioxidants and therefore is a rich dietetic source of them.

Thermal processing is one of the methods by which appropriate foods are preserved and made available to the consumer. During thermal treatment, in addition to the inactivation of microorganisms, varying percentages of desirable constituents such as nutrients, color, flavor, and texture are destroyed (Chen, Shaw & Paris, 1993; Lessin, Catigani & Schwartz, 1997**;** Rodrigo, Rodrigo & Fiszman, 1997; Parish, 1998; Bezman, Rousseff & Naim, 2001; Manso, Oliveira, Oliveira & Frias, 2001; Alwazeer, Cachon & Divies, 2002; Lee & Coates, 2003; Polydera, Galanou, Stoforos & Taoukis, 2004). Retention studies of vitamins to asses the effects of food processing on the nutritive value of foods are of great importance to food technologists and consumers. Vitamin C is thermolabile and therefore in fruit and vegetables it provides an indication of the loss of other vitamins and acts as a valid criterion for other organoleptic or

nutritional components, such as natural pigments and aromatic substances. Its concentration decreases during storage, depending on storage conditions, such as temperature, oxygen content, and light (Esteve, Farré, & Frígola, 1995; Murata, Shinoda & Homma, 2002; Alwazeer, Delbeau, Divies & Cachon, 2003; Polydera, Stoforos & Taoukis, 2003; Zerdin, Rooney & Vermuë, 2003; Blasco, Esteve, Frígola & Rodrigo, 2004).

Pulsed electric field electro-technology (PEF) is an emerging technology in the field of food preservation. PEF has the potential to pasteurize several foods nonthermally via exposure to high-voltage short pulses while the material is between the electrodes of a treatment chamber. The electric field affects the cell membranes (Barsotti & Cheftel, 1999), and may cause irreversible membrane breakage (Zimmermann, 1986; Pothakamury, Barbosa-Cánovas, Swanson & Spence, 1997**;** Calderón-Miranda, Barbosa-Cánovas & Swanson, 1999), alteration in transport of ions (Kim, Ye & Li, 2001), and changes in enzyme structure (Vega-Mercado, Powers, Barbosa-Cánovas & Swanson, 1995; Fernandez-Díaz, Barsotti, Dumay & Cheftel, 2000). There are many studies on the effect that this new technology has on microorganisms and their half-life.

Various authors (Rodrigo, Martínez, Harte, Barbosa-Cánovas & Rodrigo, 2001; Ulmer, Heinz, Gänzle, Knorr & Vogel, 2002; Abram, Smelt, Bos & Wouters, 2003) have studied the inactivation of *Lactobacillus plantarum*, and the inactivation of *E. coli*, *Enterobacter aerogenes*, mesophile flora, molds, and yeast flora has also been studied (McDonald, Lloyd, Vitale, Petersson & Innings, 2000; Rodrigo, Barbosa-Cánovas, Martínez & Rodrigo, 2003[a]; Rodrigo, Barbosa-Cánovas, Martínez & Rodrigo, 2003[b]; Selma, Fernández, Valero & Salmerón, 2003; Spilimbergo, Dehghani, Bertucco & Foster, 2003).

A number of authors have studied the evolution of quality factors in orange juice after PEF treatment, in some cases making a comparison with the evolution after heat treatment (Ho, Mittal & Cross, 1997; Jia, Zhang & Min, 1999**;** Giner, Gimeno, Espachs, Elez, Barbosa-Cánovas & Martin, 2000; Yeom, Streaker,

Zhang & Min, 2000[a]; Yeom, Streaker, Zhang & Min, 2000[b]; Ayhan, Yeom, Zhang & Min, 2001; Van Loey, Verachtert & Hendrick, 2001; Min & Zhang, 2002[a]; Min, Jin, Yeom, Min & Zhang, 2002[b]). We have not found any papers which study the half-life of orange–carrot juice treated by PEF and compare it with heat treatment.

The aim of the present study was to establish the shelf life of orange–carrot juice treated by PEF. To do this, we studied the degradation kinetics of ascorbic acid in the juice according to the PEF conditions applied. We selected the best treatment and studied the evolution of ascorbic acid in the PEF-treated juice stored at 2 and 10 ºC. In parallel we applied a conventional thermal treatment to orange–carrot juice and compared the results.

2. Materials and methods

2.1. Samples.

The process for obtaining the mixed orange and carrot juice was: after appropriate washing and hygienization of the oranges, the juice was extracted (FMC juice extractors with a 2-mm-diameter perforated plate) and placed in a tank. Carrot juice was obtained after washing the vegetables first with a diluted solution of sodium hydroxide and afterwards with drinking water. The washed vegetables were ground, and the juice was sifted and mixed with the orange juice (80% orange and 20% carrot). The mixed juice was packaged in Elopack packages and frozen (–40 ºC).

2.2. PEF treatment system

An OSU-4D bench-scale continuous PEF system, designed at Ohio State University, was used to treat the samples. Six co-field treatment chambers with a diameter of 0.23 cm and gap distance of 0.293 cm were connected in series. Two

cooling coils were connected before and after each pair of chambers, and submerged in a circulating refrigerated bath to maintain the treatment temperature within the designed range. The temperature was monitored with a thermocouple reader. Pulse waveform, voltage, and intensity in the treatment chambers were recorded with a digital oscilloscope (Tektronix TDS 210, Tektronix Inc., OR U.S.A.). The flow rate was set at 60 mL/min with a peristaltic pump. A square-wave bipolar pulse duration of 2.5 μs was selected. In order to decide on the most appropriate PEF conditions, a number of alternative processing conditions were tested. Treatment time ranged from 30 to 340 μs and the electric field was set at 25, 30, 35, and 40 kV/cm. Samples were collected after each treatment. The experiments were performed in duplicate.

2.3. Thermal treatment

The thermal treatment intensity given to T samples (98 ºC, 21 s) was similar to the treatment given by manufacturers of refrigerated juice. To treat the samples an ARMFIELD FT74P unit with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (98 ºC, 21 s) were reached. Heating of orange juice at 90–99 ºC for 15–30 s is normal in commercial practice (Braddock, 1999). After treatment, the juice was cooled with cold water from a cooler (ARMFIELD FT61), and it was packaged and stored until the analysis.

2.4. Packaging and storage

The treated juice was packaged inside a laminar flux chamber in clean, sterile twist-off jars. The closed jars were stored in two chambers (2 ºC and 10 ºC, in darkness, and with relative humidity control). Samples stored at 2 ºC were analyzed after 7, 17, 35, 52, and 70 days, and those stored at 10 ºC were analyzed after 7, 17, 24, 42, and 59 days.

2.5. Polarographic determination of ascorbic acid

5 mL of juice was diluted to 25 mL with the extraction solution: oxalic acid (Panreac, Barcelona, Spain) 1%, w/v, trichloroacetic acid (Baker, Deventer, Holland) 2%, w/v, sodium sulfate (Baker, Deventer, Holland) 1%, w/v. After vigorous shaking, the solution was filtered through a folded filter (Whatman no. 1). 9.5 mL of 1% oxalic acid (w/v) and 2 mL of 2M acetic acid (Panreac, Barcelona, Spain) / sodium acetate (Panreac, Barcelona, Spain) buffer (pH = 4.8) were added to an aliquot of 0.5 mL of filtrate, and the solution was transferred to the polarographic cell. The following instrumental conditions were applied: DP_{50}, mode DME, drop size 2, drop time 1 s, scan rate 10 mV/s, initial potential –0.10 V. Determinations were carried out by using the peak heights and standard additions method (Aparicio, Farré & Frígola, 1992).

3. Results and discussion

3.1. Selection of processing conditions

We studied different fields (25, 30, 35, and 40 kV/cm), and five treatment times (from 30 to 340 μs) in each field. Table 1 shows the results obtained. The concentration of ascorbic acid in the fresh orange–carrot juice not subjected to any treatment was 27.11 ± 0.44 mg/100 mL. When the remaining concentration of ascorbic acid versus treatment time in each of the fields was adjusted by least squares, we observed that it followed a zero-order kinetics (see Figure 1): $C = C_0 - kt$, where C is the concentration of ascorbic acid after treatment (mg/100 mL), C_0 is the concentration in the untreated juice, k is the ascorbic acid loss rate (μs^{-1}), and t is the treatment time (μs). The ascorbic acid degradation rate (k) obtained was –0.009 ± 0.0008 μs^{-1} (correlation coefficient 0.984, standard error 0.250); –0.014 ± 0.0009 μs^{-1} (correlation coefficient 0.992, standard error 0.177); –0.022 ± 0.0023 μs^{-1} (correlation coefficient 0.981, standard error

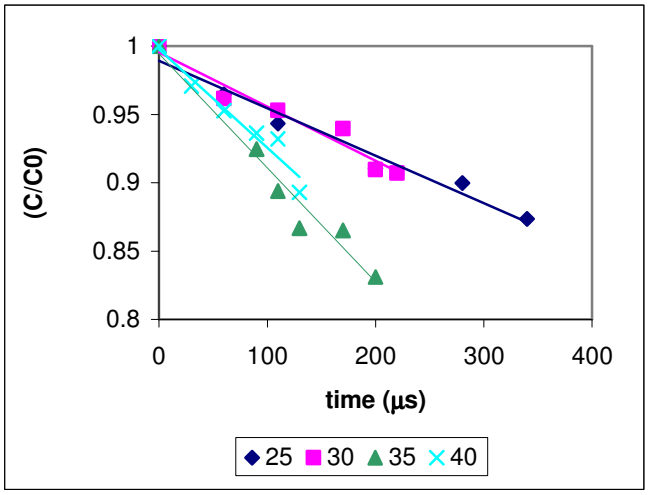
0.0352); and –0.0187 ± 0.0049 μs^{-1} (correlation coefficient 0.884, standard error 0.545) for a field of 25, 30, 35, and 40 kV/cm, respectively.

Table 1. Concentration of ascorbic acid (mg/100 mL) in orange–carrot juice for different fields and treatment times.

Field (kV/cm)	Time (μs)	Ascorbic acid ± s[a] (mg/100 mL)
25	60	26.16 ± 0.635
25	110	25.57 ± 0.317
25	200	24.69 ± 0.864
25	280	24.40 ± 0.690
25	340	23.68 ± 1.499
30	60	26.07 ± 0.466
30	110	25.83 ± 1.043
30	170	25.47 ± 1.025
30	200	24.66 ± 0.868
30	220	24.60 ± 1.03
35	90	25.07 ± 0.680
35	110	24.24 ± 0.267
35	130	23.50 ± 0.260
35	170	23.46 ± 0.439
35	200	22.53 ± 1.192
40	30	26.32 ± 0.255
40	60	24.83 ± 0.388
40	90	25.38 ± 0.147
40	110	25.27 ± 0.307
40	130	24.21 ± 0.292

s[a]: standard deviation.

Figure 1. Degradation (k) of ascorbic in orange–carrot juice in each of the PEF treatments studied.



The fit was significant at 99% ($p < 0.01$) in all cases except the field of 40 kV/cm, where the fit was significant at 95% ($p < 0.05$).

The degradation of ascorbic acid studied by other authors fits a first-order kinetics. This can be explained by taking into account the fact that in PEF the treatment time lasts for µs, whereas in thermal processes the time during which heat is applied is longer, and in this case the degradation curves divide clearly into two linear sections which correspond to two types of degradation, one aerobic and the other anaerobic (Blasco et al., 2004; Nagy, 1980**;** Eison-Perchonok & Downes, 1982; Robertson & Samaniego, 1986; Kennedy, Rivera, Lloyd, Warner & Jumel, 1992; Ariahu, Adekunle & Nkpa, 1997; Esteve, Frígola, Martorell & Rodrigo, 1998; Esteve, Frígola, Martorell & Rodrigo, 1999). When PEF is applied, the treatment times are so short (µs) that anaerobic conditions are not achieved.

The results obtained indicated that the ascorbic acid degradation rate was less in PEF treatment at 25 kV/cm, and therefore this was the treatment chosen for

preservation of the orange–carrot juice and subsequent estimation of its shelf life.

3.2. Study of the degradation kinetics of ascorbic acid during storage

In order to compare the shelf life of pasteurized and PEF-treated orange–carrot juice, first we studied the degradation kinetics of ascorbic acid when the juice was treated by PEF at 25 kV/cm for two times of 280 μs (attaining a temperature of 65 and 68 ºC) and for 330 μs (attaining a temperature of 74 ºC), and thermal treatment (98 ºC for 21 s) was applied to another aliquot, keeping the samples in refrigerated storage at 2 and 10 ºC during the period of the study. The initial concentration of vitamin C in the orange–carrot juice mixture was 23.49 ± 0.53 mg/100 mL. Throughout the study the pH and ºBrix of the juice were monitored. The treatment did not affect these parameters, and no modification was observed during storage. The pH values were 3.87 ± 0.05, 3.88 ± 0.18, 3.83 ± 0.06, and 3.79 ± 0.09 for the pasteurized juice, the juice treated by PEF for 280 μs at 65 ºC, the juice treated by PEF for 280 μs at 68 ºC, and the juice treated by PEF for 330 μs, respectively. The ºBrix were 10.1 ± 1.4, 9.98 ± 1.3, 10.8 ± 0.9, and 10.4 ± 0.7 for the pasteurized juice, the juice treated by PEF for 280 μs at 65 ºC, the juice treated by PEF for 280 μs at 68 ºC, and the juice treated by PEF for 330 μs, respectively.

The remaining concentration of ascorbic in the pasteurized orange–carrot juice was 83%, whereas in the juice treated by PEF it was 90% (see Table 2). This clearly shows that treatment by PEF affects the nutritional value less than pasteurization does. Qiu, Sharma, Tuhela, Jia & Zhang (1998) observed that degradation of vitamin C in orange juice was less when PEF was applied than when it was processed thermally. Grahl & Märkl (1996) found that the ascorbic acid remaining in milk after applying PEF treatment was greater than 90%.

Table 2. Rate constants (k_T) and correlation coefficients (R^2) for degradation of ascorbic acid in PEF and thermally pasteurized orange–carrot juice during storage at 2 and 10 ºC.

Juice treatment	C_0 (mg/100 mL)	k_T (days^{-1}) (SE[a])	R^2
	10 ºC		
Pasteurized	19.52	–0.0963 (0.0097)	0.980
PEF 280µs 65ºC	21.21	–0.0261 (0.0034)	0.967
PEF 280µs 68ºC	20.99	–0.0231 (0.0036)	0.954
PEF 330µs	21.68	–0.0414 (0.0047)	0.975
	2 ºC		
Pasteurized	19.52	–0.0466 (0.0017)	0.997
PEF 280µs 65ºC	21.21	–0.0080 (0.0015)	0.938
PEF 280µs 68ºC	20.99	–0.0101 (0.0013)	0.967
PEF 330µs	21.68	–0.0211 (0.0030)	0.963

SE [a], standard error.

During storage, the concentration of vitamin C in the juice decreases according to the storage conditions. Taking previous studies (Kinkal & Giray, 1987; Esteve et al., 1995) into account, it is considered that ascorbic acid follows a first-order degradation kinetics: $C = C_0\exp(-k_T t)$, where C_0 is the total initial ascorbic acid content (mg/100 mL), k_T is the rate constant (day^{-1}), C is ascorbic acid content at time t (mg/100 mL), and t is the storage time (days).

In order to study the degradation of ascorbic acid, Ln (C/C_0) was plotted against storage time for each treatment applied (Figure 2). Table 2 shows the ascorbic acid degradation rate in the juice studied and the correlation coefficient, during storage at 2 and 10 ºC, for each of the preservation treatments studied. It can be seen that the ascorbic acid degradation rate is less when the juice is stored at 2 ºC than when the storage temperature is 10 ºC (as one would expect).

Figure 2. Degradation kinetics of ascorbic acid in orange–carrot juice treated by PEF (field 25 kV/cm) and pasteurized, during storage at 2 ºC and 10 ºC

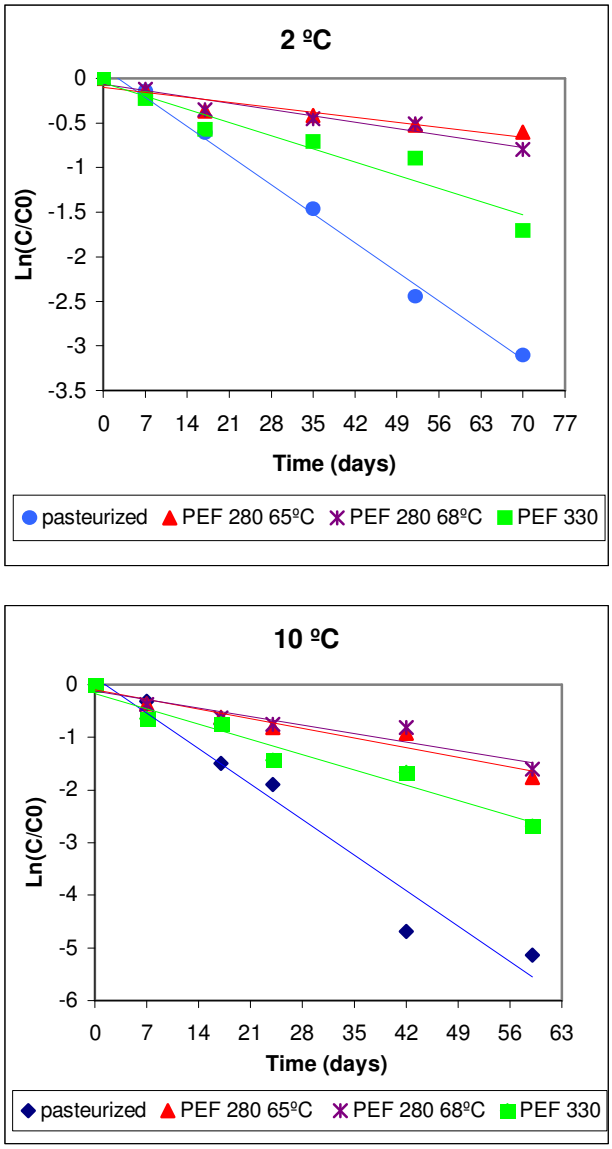


Table 3. Half-life (days) of pasteurized and PEF-treated orange–carrot juice when stored at 2 and 10 ºC.

	Storage temperature	
	10 ºC	**2 ºC**
	Half-life (days)	*Half-life (days)*
Pasteurized	5	11
PEF 280µs 65ºC	19	62
PEF 280µs 68ºC	22	50
PEF 330µs 74ºC	12	24

The degradation rate is greater in the pasteurized juice than in the juice treated by PEF. No significant difference is observed between treatment by PEF for 280 µs at 65 ºC and at 68 ºC, whereas the degradation rate is significantly higher when PEF treatment is applied for 330 µs.

The shelf life of pasteurized juice and juice treated by PEF (in various conditions) stored at 2 and 10 ºC was calculated as the time taken for the ascorbic acid concentration to reduce to 50%, taking the value of the degradation rate (k_T) obtained by applying the Arrhenius equation into account (Table 2).

Table 3 shows the half-life of ascorbic acid in the orange–carrot juice stored at 2 and 10 ºC for each of the treatments studied. The lower degradation rate in the juices treated by PEF causes the shelf life of the juice to be much greater than for pasteurized juice. There is also a great increase in the shelf life of the juice when the preservation temperature is 2 ºC, and it is therefore recommendable to store the juice at this temperature to ensure that it reaches the consumer with a high nutritive value. If we compare the juices treated by PEF for 280 µs and 330 µs, we see that in the first case the shelf life of the juice is greater, so that this would be the treatment of choice. No significant difference is found when the temperature is 65 or 68 ºC. From the results obtained we can deduce that the

half-life of orange–carrot juice treated by PEF is 50 days if stored at 2 ºC, whereas if the juice is pasteurized it is 11 days. Qiu et al. (1998) observed similar results during storage of orange juice, with samples that had been treated by PEF suffering less loss of vitamin C than samples treated by heat.

Esteve, Farré & Frígola (1996) studied the stability of ascorbic acid in fresh orange juice and commercial orange juices maintained at 4 and 10 ºC, finding that at 4 ºC the loss of ascorbic acid was less than 10% after 7 days of storage. Choi, Kim & Lee (2002) found that, for pasteurized juice (90 ºC, 90 s), during refrigerated storage (4.5 ºC) more than 50% of the ascorbic acid was lost within 3 weeks of storage, and it was completely degraded after 5 weeks of storage. Shivashankara, Isobe, Al-Haq, Takenaka & Shiina (2004) studied the ascorbic acid content in Irwin mango fruits stored at 5 ºC after a high electric field pretreatment, observing that the ascorbic acid decreased after 20 days of storage. Yeom et al. (2000[b]) studied effects of PEF (35 kV/cm, 59 μs) and heat (94.6 ºC, 30 s) on the concentration of ascorbic acid in orange juice during storage at 4 ºC and 22 ºC. PEF-treated orange juice retained a significantly higher content of ascorbic acid than heat-pasteurized orange during storage at 4 ºC. They found that in the juice treated by PEF the concentration of ascorbic acid reduced to 50% after 47 days of storage at 4 ºC, whereas in the juice treated by heat this reduction had taken place after 31 days. If the storage temperature is 25 ºC the concentration of ascorbic acid reduces to 50% after 13 and 12 days, for juice treated by PEF and juice treated by heat, respectively.

4. Conclusions

PEF processing is an alternative to conventional thermal pasteurization of orange–carrot juice. To select the best PEF treatment conditions it is necessary to bear in mind not only microbiological and enzymatic inactivation but also organoleptic characteristics and nutritive value. Ascorbic acid is a good

parameter for this. PEF treatment at 25 kV/cm for 280–330 μs allows the half-life of the juice to reach 50 days when stored at 2 ºC, whereas when the preservation temperature is 10 ºC the half-life is 19 days. The ascorbic acid content in juice treated by PEF is greater than in juice pasteurized conventionally, and it is maintained for a longer period when stored in refrigeration, so that its nutritive value is also greater.

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References

Abram, F., Smelt, J.P.P.M., Bos, R., & Wouters, P.C. (2003). Modelling and optimization of inactivation of *Lactobacillus plantarum* by pulsed electric field treatment. *Journal of Applied Microbiology*, 94, 571–579.

Alwazeer, D., Cachon, R., & Divies, C. (2002). Behavior of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in fresh and thermally processed orange juice. *Journal of Food Protection*, 65, 1586–1589.

Alwazeer, D., Delbeau, C., Divies, C., & Cachon, R. (2003). Use of redox potential modification by gas improves microbial quality, color retention, and ascorbic acid stability of pasteurized orange juice. *International Journal of Food Microbiology*, 89, 21–29.

Aparicio, P., Farré, R., & Frígola, A. (1992). Determinación del ácido ascórbico en vegetales por polarografía diferencial de impulsos. *Anales de Bromatología*, XLIV, 257–261.

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

Ayhan, Z., Yeom, H.Y., Zhang, Q.H., & Min, D.B. (2001). Flavour, colour, and vitamin C retention of PEF processed orange juice in different packaging materials. *Journal of Agricultural and Food Chemistry*, 49, 669–674.

Barsotti, L., & Cheftel, J.C. (1999). Food processing by pulsed electric fields. II. Biological aspects. *Food Reviews International*, 5, 181–213.

Bezman, Y., Rousseff, R.L., & Naim, M. (2001). 2-methyl-3-furanthiol and methional are possible off-flavors in stored orange juice: aroma-similarity, NIF/SNIF GC-O, and GC analyses. *Journal of Food Science*, 62 (1), 85–88.

Blasco, R., Esteve, M.J., Frígola, A., & Rodrigo, M. (2004). Ascorbic acid degradation kinetics in mushrooms in a high-temperature short-time process controlled by a thermoresistometer. *Lebensmittel-Wissenschaft + Technologie*, 37, 171–175.

Block, G., Norkus, E., Hudes, M., Mandel, S., & Helzlsouer, K. (2001). Which plasma antioxidants are most related to fruit and vegetable consumption? *American Journal of Epidemiology*, 154, 1113–1118.

Braddock, R.J. (1999). Single strength orange juices and concentrate. In J. Wiley (Ed.), *Handbook of Citrus by-products and Processing Technology* (pp 53–83). New York.

Burns, J., Frase, P.D., & Bramley, P.M. (2003). Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry*, 62, 939–947.

Calderón-Miranda, M.L., Barbosa-Cánovas, G.V., & Swanson, B.G. (1999). Transmission electron microscopy of *Listeria innocua* treated by pulsed

electric fields and nisin in skimmed milk. *International Journal of Food Microbiology*, 51, 31–39.

Chen, C.S., Shaw, P.E., Paris, M.E. (1993). Orange and tangerine juices. In S. Nagy, C.S Chen, P.E. Shaw (Eds.), *Fruit Juice Processing Technology* (pp. 110–165) Agscience: Auburndale, Florida.

Choi, M.H., Kim, G.H., & Lee, H.S. (2002). Effects of ascorbic acid retention on juice color and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. *Food Research International,* 35, 753–759.

Eison-Perchonok, M.H., & Downes, T.W. (1982). Kinetics of ascorbic acid autoxidation as a function of dissolved oxygen concentration and temperature. *Journal of Food Science*, 47, 765–773.

Esteve, M. J., Frígola, A., Martorell, L., & Rodrigo, C. (1999). Kinetics of green asparagus ascorbic acid heated in a high-temperature thermoresistometer. *Zeitschrift fuer Lebensmittel -Untersuchung und -Forschung A*, 208, 144–147.

Esteve, M.J., Farré, R., & Frígola, A. (1995). Changes in ascorbic acid content of green asparagus during the harvesting period and storage. *Journal of Agricultural and Food Chemistry***,** 43, 2058–2061.

Esteve, M.J., Farré, R., & Frígola, A. (1996). Stability of ascorbic acid in orange juices after initial use at home begins. *Journal of Food Quality*, 19, 243-249.

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.

Fernandez-Díaz, M.D., Barsotti, L., Dumay, E., & Cheftel, J.C. (2000). Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. *Journal of Agricultural and Food Chemistry***,** 48, 2332–2339.

Gardner, P.T., White, T.A.C., McPhail, D.B., & Duthie, G.G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*, 68, 471–474.

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

Grahl, T., & Märkl, H. (1996). Killing of microorganisms by pulsed electric fields. *Applied Microbiology and Biotechnology*, 45, 148–157.

Ho, S.Y., Mittal, G.S., & Cross, J.D. (1997). Effect of high field electric pulses on the activity of selected enzymes. *Journal of Food Engineering*, 31, 69–84

Jia, M., Zhang, Q., & Min, D. (1999). Pulsed electric field processing effects on flavor compounds and microorganism of orange juice. *Food Chemistry*, 65, 445–451.

John, J.H., Ziebland, S., Yudkin, P., Roe, L.S., & Neil, H.A.W. (2002). Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomised controlled trial. *The Lancet*, 359, 1969–1974.

Kennedy, J.F., Rivera, Z.S., Lloyd, L.L., Warner, F.P., & Jumel, K. (1992). L-ascorbic acid stability in aseptically processed orange juice in TetraBrik cartons and the effect of oxygen. *Food Chemistry*, 45, 327–331.

Kim, H., Ye, J., Li, Y. (2001). Inactivation of *Listeria monocytogenes* in chilling brine using a flow through electrochemical treatment. *Institute of Food Technologists*, *Annual Meeting*. Paper 59H–22.

Kinkal, N.S., & Giray, C. (1987). Kinetics of ascorbic acid degradation in potato blanching. *International Journal of Food Science and Technology,* 22, 249–254.

Lee, H.S., & Coates, G.A. (2003). Effect of thermal pasteurization on Valencia orange juice color and pigments. *Lebensmittel-Wissenschaft + Technologie*, 36, 153–156.

Lessin, W.J., Catigani, G.L., & Schwartz, S.J. (1997). Quantification of *cis-trans* isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. *Journal of Agricultural and Food Chemistry*, 45, 3728–3732.

Manso, M.C., Oliveira, F.A.R., Oliveira, J.C., & Frias, J.M. (2001). Modelling ascorbic acid thermal degradation and browning in orange juice: kinetics of pectinmethylesterase inactivation. *Journal of Food Science*, 66 (2), 328–331.

McCall, M.R., & Frei, B. (1999). Can antioxidant vitamins materially reduce oxidative damage in humans?. *Free Radical Biology and Medicine*, 26, 1034–1053.

McDonald, 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 field strengths of 30 and 50 kV/cm. *Journal of Food Science*, 65, 984–989.

Min, D.B., Zhang, Q.H. (2002ª). Pulsed electric field processing to improve the freshness of orange and tomato juices. *International Meeting, IFT.*

Min, S., Jin, Z.T., Yeom, H., Min, S.K., & Zhang, Q.H. (2002^{b}). Optimisation of processing conditions and effects of commercial scale PEF processing on the microbiological, chemical, physical, and sensory properties of orange juice. *International Meeting, IFT.*

Murata, M., Shinoda, Y., & Homma, S. (2002). Browning of model orange juice solution and changes in the components. *International Congress Series*, 1245, 459–460.

Nagy, S. (1980). Vitamin C content of citrus fruit and their products: a review. *Journal of Agricultural and Food Chemistry*, 25, 602–604.

Parish, M.E. (1998). Orange juice quality after treatment by thermal pasteurization or isostatic high pressure. *Journal of Food Science and Technology*, 31, 439–442.

Polydera, A.C., Galanou, E., Stoforos, N.G., & Taoukis, P.S. (2004). Inactivation kinetics of pectin methylesterase of Greek Navel orange juice as a function of high hydrostatic pressure and temperature process conditions. *Journal of Food Engineering*, 62, 291–298

Polydera, A.C., Stoforos, N.G., Taoukis, P.S. (2003). Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. *Journal of Food Engineering*, 60, 21–29.

Pothakamury, U.R., Barbosa-Cánovas, G.V., Swanson, B.G., & Spence, K.D. (1997). Ultrastructural changes in *Staphylococcus aureus* treated with pulsed electric fields. *Food Science and Technology International*, 3, 113–121.

Qiu, X., Sharma, S., Tuhela, L., Jia, M., & Zhang, Q. (1998). An integrated PEF pilot plant for continuous nonthermal pasteurization of fresh orange juice. *American Society of Agricultural Engineers*, 41(4), 1069-1074

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, 192.

Rodrigo, C., Rodrigo, M., & Fiszman, S.M. (1997). The impact of high temperature, short time thermal treatment on texture and weight loss of green asparagus. *Zeitschrift fuer Lebensmittel -Untersuchung und -Forschung A*, 205 (1), 53–58.

Rodrigo, D., Barbosa-Cánovas, G. V., Martínez, A., & Rodrigo, M. (2003[b]). Effect of PEF on 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.

Rodrigo, D., Barbosa-Cánovas, G.V., Martínez, A., & Rodrigo, M. (**2003[a]**). Weibull distribution function based on an empirical mathematical model for inactivation of *Escherichia coli* by pulsed electric fields. *Journal of Food Protection*, 66, 1007–1012.

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

Sánchez-Moreno, C., Plaza, L., de Ancos, B., & Cano, P. (2003). Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juice. *Journal of the Science of Food and Agriculture*, 83, 430–439.

Selma, M.V., Fernández, P.S., Valero, M., & Salmerón, M.C. (2003). Control of *Enterobacter aerogenes* by high-intensity, pulsed electric fields in horchata, a Spanish low-acid vegetable beverage. *Food Microbiol*. **2003**, *20*, 105–110.

Shivashankara, K.S., Isobe, S., Al-Haq, M.I., Takenaka, M., & Shiina, T. (2004). Fruit antioxidant activity, ascorbic acid, total phenol, quercitin, and carotene of Irwin mango fruits stored at low temperature after high electric field pretreatment. *Journal of Agricultural and Food Chemistry*, 52, 1281–1286.

Spilimbergo, S., Dehghani, F., Bertucco, A., & Foster, N.R. (2003). Inactivation of bacteria and spores by pulsed electric field and high pressure CO_2 at low temperature. *Biotechnology and Bioengineering*, 82, 118–125.

Ulmer, H.M., Heinz, V., Gänzle, M.G. Knorr, D., & Vogel, R.F. (2002). Effects of pulsed electric fields on inactivation and metabolic activity of *Lactobacillus plantarum* in model beer. *Journal of Applied Microbiology*, 93, 326–335.

Van Loey, A., Verachtert, B., & Hendrick, M. (2001). Effects of high electric field pulses on enzymes. *Trends of Food Science and Technology*, 12, 94–102.

Vega-Mercado, H., Powers, J.R., Barbosa-Cánovas, G.V., & Swanson, B.G. (1995). Plasmin inactivation with pulsed electric fields. *Journal of Food Science*, 60, 1132–1136.

Williamson, G. (1999). Protective effects of fruits and vegetables in the diet. *Nutrition and Food Science*, 1, 6–10.

Yeom, H.W., Streaker, C.B., Zhang, Q.H., & Min, D.B. (2000[a]). 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, Q.H., & Min, D.B. (2000[b]). 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.

Zerdin, K., Rooney, M.L., & Vermuë, J. (2003). The vitamin C content of orange juice packed in an oxygen scavenger material. *Food Chemistry*, 82, 387–395.

Zimmermann, U. (1986). Electrical breakdown, electropermeabilization and electrofusion. *Reviews on Physiological Biochemical Pharmacology*, 105, 175–256.

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ORIGINAL PAPER

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Changes in carotenoids including geometrical isomers and ascorbic acid content in orange-carrot juice during frozen storage

Abstract

Fruits and vegetables are highly perishable foods, which explains the need to apply preservation technologies, such as freezing. The aim is to combine shelf life extension with maintenance of sensory and nutrient characteristics. The stability of orange-carrot juice stored at –40ºC for 132 days was studied. The ascorbic acid and carotene content and influence of storage time were analysed. Carotenes were identified and quantified by LC and ascorbic acid was determined by differential pulse polarography. The initial concentration of ascorbic acid was 27.09 mg/100mL and decreased 4.1% during the storage period. Vitamin A activity in the orange-carrot juice increased during the period studied, fitting a linear model (R^2 = 0.87). Of the 14 carotenes identified, only antheraxanthin and the 9-*cis*-violaxanthin + neoxanthin mixture decreased in concentration during the storage period.

Keywords: ascorbic acid, carotenoids, frozen storage, orange-carrot juice, vitamin A

Introduction

Fruits and vegetables, important components of a balanced human diet, provide us with significant levels of some micronutrients. Epidemiological studies show that the consumption of fruits and vegetables gives considerable protection against the risk of certain diseases connected with aging, such as cancer, cardiovascular diseases, cataract, macular degeneration and diabetes [1-9]. Multivitamin supplements delay the progression of HIV disease and provide an effective, low-cost means of delaying the initiation of antiretroviral therapy in HIV-infected women [10]. Certain carotenoids, such as β-carotene, have been very thoroughly studied. Vitamin C, vitamin E and β-carotene are referred to collectively as the antioxidant vitamins [11]. However, the antioxidant potential of citrus juices has been related mainly to vitamin C content [12, 13].

Orange juice is a very important source of ascorbic acid, a nutrient that, besides its vitamin action, is valuable for its antioxidant effect, stimulation of the immune system and other health benefits which are being actively investigated and reported, such as inhibition of formation of cancer-causing N-nitroso compounds in the stomach [14, 15]. Orange juice undergoes a considerable number of deterioration reactions during storage (degradation of ascorbic acid, cloud loss, microbial spoilage, development of off-flavour, changes in colour, texture and appearance), which result in a loss of product quality [16-20].

In addition to the excellent intrinsic sensory and nutritive characteristics of orange juice, the incorporation of a proportion of carrot makes a valuable contribution to the health of the consumer since oranges have a high vitamin C content, and carrots have the highest content of carotenoids of fresh foods [21].

Carotenoids are one of the main types of natural pigments because their distribution in the plant kingdom is extremely wide [22]. The importance of carotenoids and of the foods that they contain is based on their two main properties, their provitamin A activity and their antioxidant capacity [23], although not all the carotenoids present in fruits and vegetables are precursors of

vitamin A. Some, including α-carotene, β-carotene and β-cryptoxanthin, have provitamin A activity, being transformed into retinal by mammals [24]. Carotenoids also have a considerable antioxidant capacity [25], and they modulate the pathogenesis of cancers [26, 27] and coronary heart disease [28].
The application of some industrial treatments may lead to the formation of *cis*-isomers, which do not possess the same provitamin activity as *trans*-isomers but have greater bioavailability than *trans*-isomers [29]. The most notable positive effect of processing on the overall quality or health capacity of food is the increased antioxidant status. The bioavailability of carotenoids increases fivefold in some processed products as a consequence of moderate heating or enzymatic disruption of vegetable cell structure [30]. In general, the antioxidant activity of various vegetables increases significantly after heat treatment [31, 32].

The seasonality and perishability of fruits and vegetables explain the need for the application of preservation technologies, such as freezing. The aim is to combine shelf life extension with maintenance of sensory and nutrient characteristics [33].
Freezing of fruit and vegetable juices is one of the most common ways of maintaining the quality of these products. It has been specified that the most important nutritional changes in frozen foods are due to storage time [34]. However, freezing does not prevent development of off-flavour and colour and texture deterioration in frozen vegetables, because enzyme systems remain active even at sub-zero temperatures [35]. The level of vitamin C, besides being an indicator of nutrient value, can be used in the case of frozen juices as a reliable and representative index for estimating quality deterioration. The stability of carotenoids during storage is very important if the final product is to be attractive and acceptable. Like ascorbic acid, carotenoids are very susceptible to deterioration during storage of the foods that contain them. The degradation of carotenoids affects not only the colour of foods but also their nutritive value and flavour.

The aim of the present work is to study the stability of an orange-carrot juice stored at –40ºC and to establish the possible nutritional changes. To do this, the ascorbic acid and carotene content (including geometrical isomers) and the influence of storage time were studied.

Materials and methods

Samples

The process for obtaining the mixed orange and carrot juice was: after appropriate washing of the oranges, the juice was extracted (FMC juice extractors with a 2-mm-diameter perforated plate) and placed in a tank where it was mixed with carrot juice. The carrot juice was obtained after washing the vegetables first with a diluted solution of sodium hydroxide and afterwards with drinking water. The washed carrots were ground and the juice was sieved and mixed with the orange juice (80% orange and 20% carrot). The mixed juice was packaged in Elopack packages (lightweight paperboard cartons) and frozen (–40 ºC) for 132 days. Periodically the samples were analysed in triplicate.

Instrumentation

The LC system consisted of a series 1050 chromatograph with a quaternary pump system, a diode array detector (Hewlett-Packard, 1100 series), a column thermostat (Agilent 1100 series), an on-line degassing system, and a ChemStation (series A.06.03) data system (Hewlett-Packard, Waldbronn, Germany). A Vydac 201TP54 reverse phase C18 column (250 x 4.6 mm, with a particle size of 5 μm) and a Vydac 201TP precolumn (guard column) (4.6 mm i.d. cartridge with 5-μm particles) (Hesperia, CA) were used.

A Metrohm (Herisau, Switzerland) 746 analyser equipped with a Metrohm 747 VA stand was used. The working electrode was a Metrohm multi-mode electrode operated in the dropping mercury mode. A platinum wire counter electrode and a saturated calomel reference electrode were used.

Methods

Chromatographic determination of carotenes

The identification and quantification of carotenes (including geometrical isomers) was performed by liquid chromatography with an ultraviolet-diode array detector, using a Vydac 201TP C18 column. The mobile phase used was methanol + 0.1M ammonium acetate (A), tert-butyl methyl ether (B) and water (C) in a concentration gradient: at 0 min 95% (A) and 5% (C), at 3 min 100% (A), at 5 min 95% (A) and 5% (B), at 10 min 86% (A) and 14% (B), at 15 min 75% (A) and 25% (B), at 22 min 95% (A) and 5% (B) and at 23 min 100% (A); and a temperature gradient was applied with retinol palmitate as an internal standard. An extraction process (ethanol/hexane, 4:3, v/v) was performed, followed by saponification with diethyl ether/methanolic KOH (0.1%, w/v, BHT) (1:1, v/v) for 0.5 h at room temperature (figure 1). The carotenoids were identified by UV-vis spectra and retention times in HPLC in the juices analysed [36].

Determination of vitamin A

Vitamin A was expressed as retinol equivalents (RE), using the following conversion [37]:

RE = (μg of β-carotene)/6 + (μg of ζ-carotene + β-cryptoxanthin + α-carotene)/12.

Polarographic determination of ascorbic acid

5 mL of juice was diluted to 25 mL with the extraction solution (oxalic acid 1%, w/v, trichloroacetic acid 2%, w/v, sodium sulphate 1%, w/v). After vigorous shaking the solution was filtered through a folded filter (Whatman no. 1). The 9.5 mL of oxalic acid 1% (w/v) and 2 mL of acetic acid/sodium acetate 2M buffer (pH = 4.8) were added to an aliquot of 0.5 mL of filtrate and the solution was transferred to the polarographic cell. The following instrumental conditions were applied: DP_{50}, mode DME, drop size 2, drop time 1 s, scan rate 10 mV/s, initial potential –0.10 V. Determinations were carried out by using the peak heights and standard additions method [38].

Stadistical analysis

The content wre compared using one-way analysis of variance (ANOVA). To determine differences between products and/or samples of the same product the Tukey tes ($p<0.05$) was applied.

Results and discussion

Ascorbic acid

The initial concentration of ascorbic acid found in the orange-carrot juice was 27.09±0.18 mg/100mL. The contents were compared using one-way analysis of variance (ANOVA). As there were differences a Tukey test was applied, which indicated that the ascorbic acid concentration after 132 days of storage at –40ºC (25.98±0.18 mg/100mL) was significantly lower than the initial concentration. Regression analysis applied to the loss of ascorbic acid with time indicated that the loss followed a linear model to describe the relationship between ascorbic acid and storage time (days). The equation of the fitted model was: ascorbic acid (mg/100mL) = 26.99 – 0.010·time (days). See Figure 2. The slope of the equation indicates the ascorbic acid degradation rate during storage at –40ºC,

0.010 ± 0.004 mg/100mL per day. The R squared statistic indicates that the model as fitted explains 56.4% of the variability in ascorbic. The correlation coefficient is –0.751, indicating a moderately strong relationship between the variables. The standard error of the estimate shows the standard deviation of the residuals to be 0.372. Navarro et al. [39], who studied storage of concentrated juice (60 ºBrix) at 0, –8 and –18 ºC in order to establish appropriate conditions for preservation of orange juice, found that there were no variations in the concentration of ascorbic acid during 6 months' storage. Lee and Coates [40] studied the variation in vitamin C in a frozen, freshly squeezed, unpasteurized orange juice during storage at –23ºC and found that after 24 months there was a 19.2% loss of vitamin C, the rate of vitamin C loss being about 0.011 mg/100mL per day and the ascorbic acid degradation rate being higher than that found in the present study for orange-carrot juice stored at –40ºC. Korus et al. [41] found that vitamin C in grass pea decreased (7–8%) after 6 months of frozen storage (at –30 and –40ºC). Giannakourou and Taoukis [42] established that, for frozen green vegetables (green peas, spinach, green beans and okra), the temperature dependence of vitamin C loss in the –3 to –20 ºC range was adequately modelled by the Arrhenius equation. Martins and Silva [43] studied frozen green beans, performing a quality profile evaluation during home storage at –6, –12 and –18 ºC, and found that the losses of ascorbic acid were significant after 4 days of storage at –6ºC (51.47%), and when the storage temperature was –12ºC the ascorbic acid decreased very rapidly during the first 5 days (45.03%) and after 14 days the residual ascorbic acid was 10.72%. When the green beans were stored at –18ºC, the residual concentration of ascorbic acid after 15 days was 10%. Bahçeci et al. [44] found that ascorbic acid in green beans decreased following a first-order kinetics during storage at –18ºC. Sahari et al. [45] found that the greatest losses of ascorbic acid occurred during the first 15 days of storage, the percentages being 64.5, 10.7 and 8.9 at –12, –18 and –24ºC, respectively, and no statistically significant differences were observed between –

18 and –24ºC.

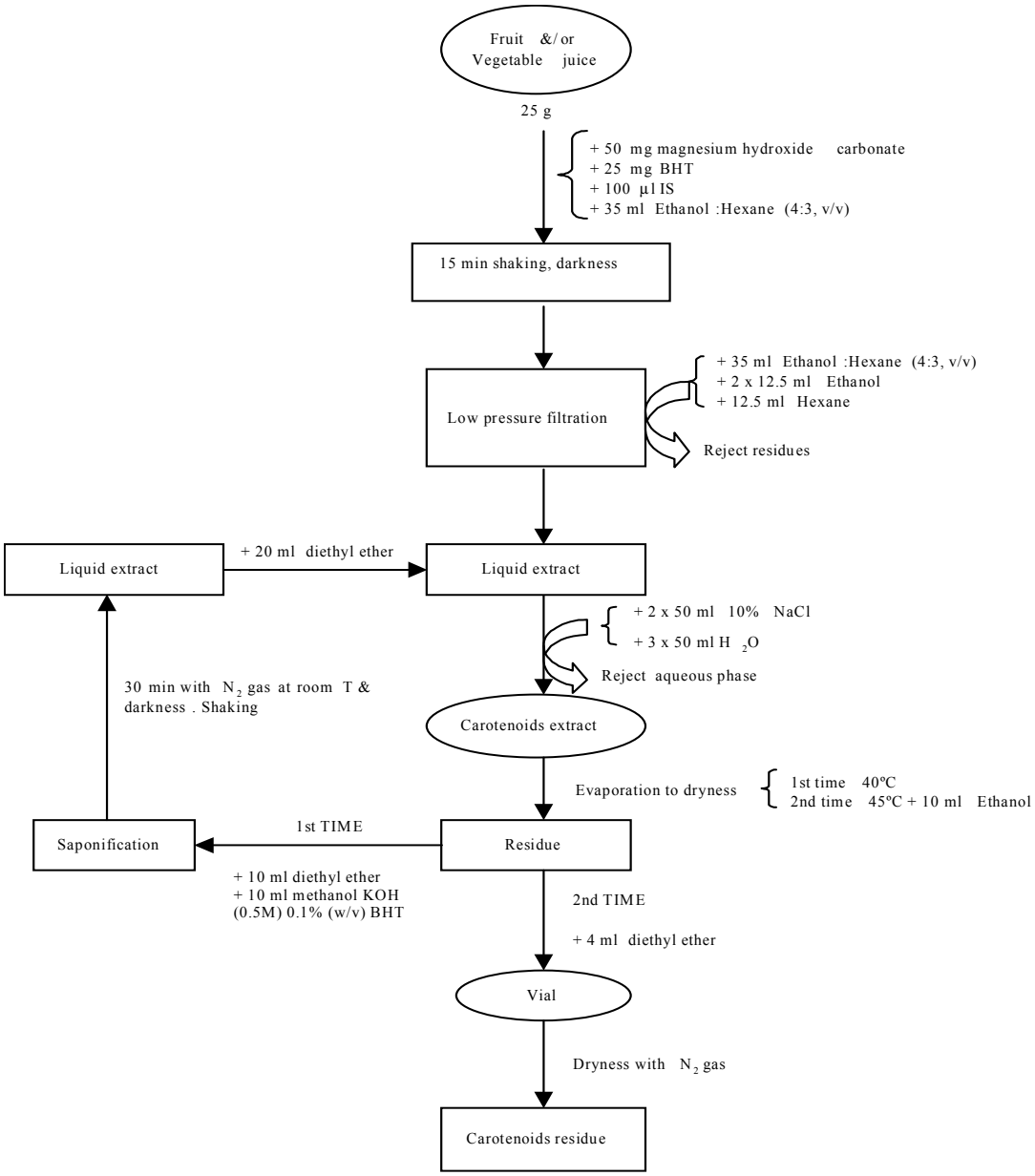


Fig. 1 Extraction of carotenoid pigments

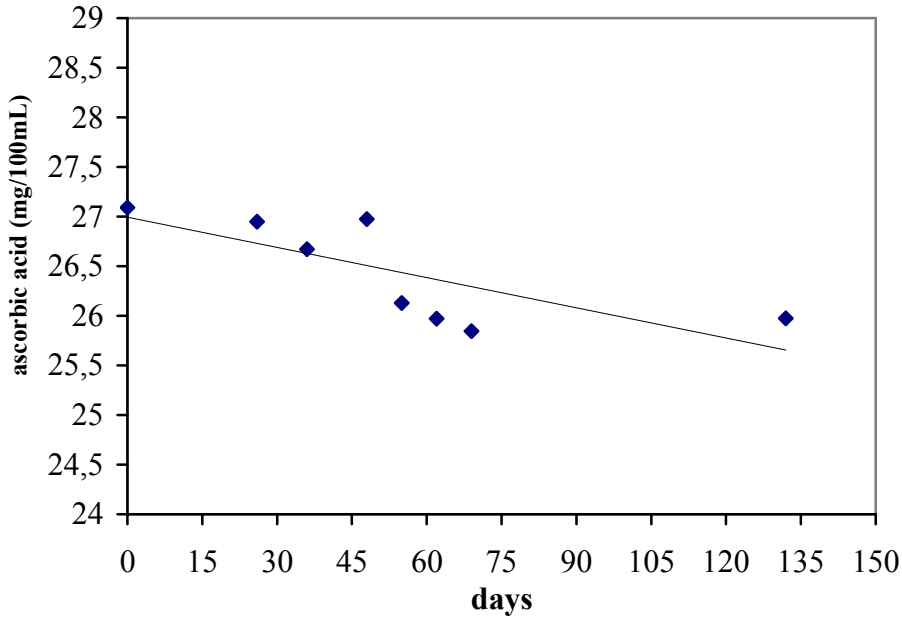
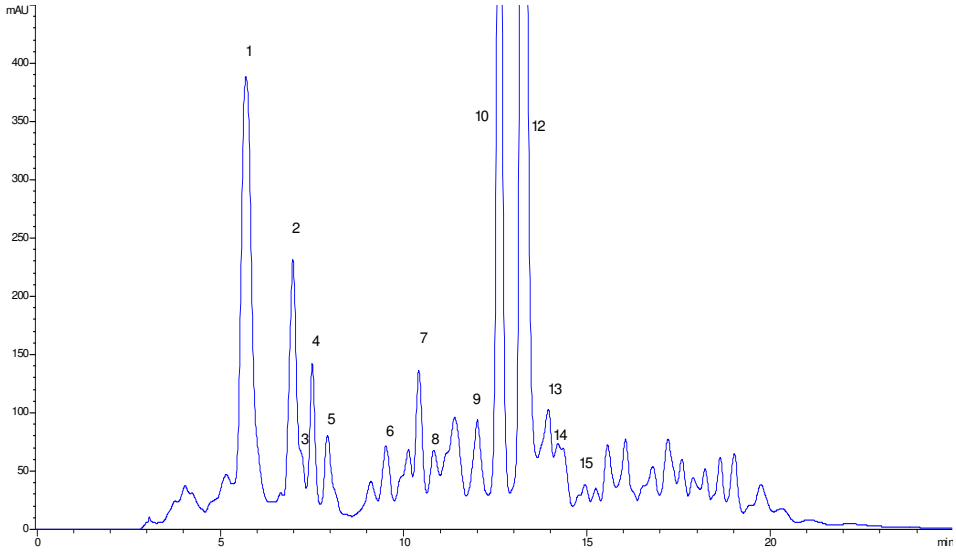


Fig. 2 Variation in ascorbic acid in orange-carrot juice during storage at –40ºC.

Carotenes

Figure 3 shows a chromatogram of the orange-carrot juice, and Table 1 indicates the carotenes identified, their retention time and the wavelength at which they were detected.

(A)



(B)

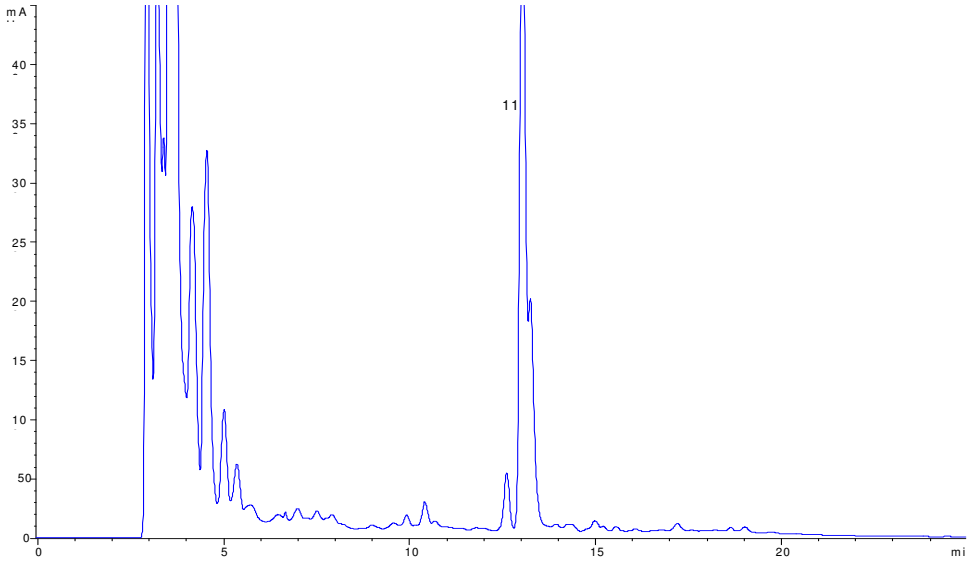


Fig. 3 Chromatogram of untreated orange-carrot juice mixture at λ=450 nm (A) and λ=290 nm (B).

Peak no.	Carotenoids	λ (nm)	Orange–carrot t_R (min) ± SD
1	9-*cis*-violaxanthin+neoxanthin	430	5.75 ± 0.04
2	antheraxanthin	450	7.01 ± 0.03
3	mutatoxanthin	430	7.20 ± 0.01
4	lutein	450	7.54 ± 0.02
5	zeaxanthin	450	7.96 ± 0.04
6	α-cryptoxanthin	450	9.53 ± 0.03
7	β-cryptoxanthin	450	10.45 ± 0.04
8	*cis*-β-cryptoxanthin	450	10.83 ± 0.05
9	9-*cis*-α-carotene	430	12.06 ± 0.04
10	α-carotene	450	12.63 ± 0.03
11	phytoene+phytofluene	290	13.06 ± 0.02
12	β-carotene	450	13.28 ± 0.03
13	13-*cis*-β-carotene	450	13.95 ± 0.02
14	ζ-carotene	430	14.28 ± 0.04
15	9-*cis*-β-carotene	450	14.96 ± 0.03

Retention time: t_R

Table 1 Wavelengths and retention times of orange-carrot juice mixture in the frozen orange-carrot juice (–40ºC)

If we study the variation in vitamin A (RE) in the orange-carrot juice during storage at –40ºC we see that its concentration increases significantly with time ($p<0.05$), from 44.75 to 468.33 RE (Figure 3). Fitting by least squares, we find that the cloud of points provides a significant fit ($p<0.01$) with a linear model:

vitamin A (RE) = 3.89·time (days) + 9.54. The R^2 value indicates that the model as fitted explains 75.0% of the variability in vitamin A content. The correlation coefficient equals 0.866, indicating a moderately strong relationship between the variables. The standard error of the estimate shows the standard deviation of the residual to be 93.66. There is a negative correlation between the evolution of the concentrations of ascorbic acid and vitamin A during storage at –40ºC: ascorbic acid = 26.99 – 0.003·vitamin A (ascorbic acid in mg/100mL, and vitamin A in RE). The correlation coefficient equals –0.833 and the standard error estimate (0.312) indicates a moderately strong relationship between the variables.

We went on to study the evolution of each of the carotenoids in the frozen orange-carrot juice (–40ºC). Figures 5–8 give a graphic representation of the results obtained. Fitting by least squares, we obtained a significant linear fit ($p>0.05$) in 8 of the carotenes studied, with the following slopes: –2.429±0.810 (R^2 = 0.775), –1.037±0.312 (R^2 = 0.799), 7.148±1.796 (R^2 = 0.852), 2.339±0.483 (R^2 = 0.892), 19.084±4.518 (R^2 = 0.865), 1.138±0.296 (R^2 = 0.843), 1.560±0.285 (R^2 = 0.913) and 0.524±0.190 (R^2 = 0.748) μg/100g per day, for 9-*cis*-violaxanthin + neoxanthin, antheraxanthin, α-carotene, phytoene + phytofluene, β-carotene, 13-*cis*-β-carotene, ξ-carotene and 9-*cis*-β-carotene, respectively. Only 9-*cis*-violaxanthin + neoxanthin and antheraxanthin decreased with storage time. Figure 4 shows the remaining percentage of the concentration of each of these 2 carotenes. After 36 days the decrease in the concentration of antheraxanthin was 43.1%, whereas there was no variation in the concentration of 9-*cis*-violaxanthin + neoxanthin, but it decreased sharply (41.5%) after 48 days of storage at –40ºC. At the end of the period studied (132 days), the losses were 50.5 and 64.8% for 9-*cis*-violaxanthin + neoxanthin and antheraxanthin, respectively. The other carotenes (lutein, zeaxanthin, mutatoxanthin, α-cryptoxanthin, β-cryptoxanthin, *cis*-β-cryptoxanthin and 9-*cis*-α-carotene) underwent modifications during storage at –40ºC, although the application of

simple regression analysis did not show any significant relationship between the variables.

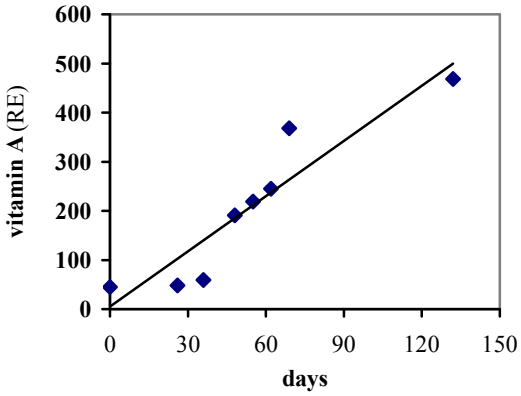


Fig. 4 Evolution of vitamin A content in orange-carrot juice during storage at –40ºC.

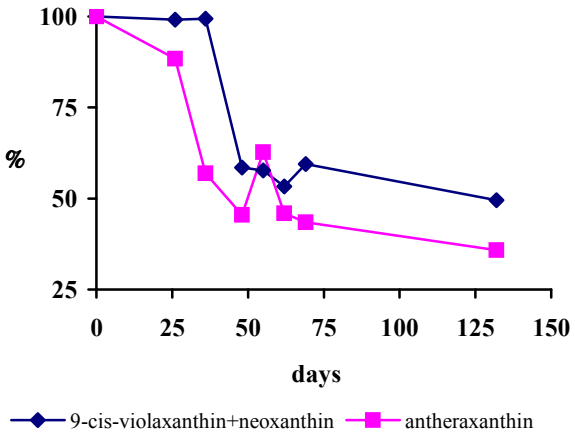


Fig. 5 Evolution of 9-cis-violaxanthin+neoxanthin and antheraxanthin in the frozen orange-carrot juice (–40ºC)

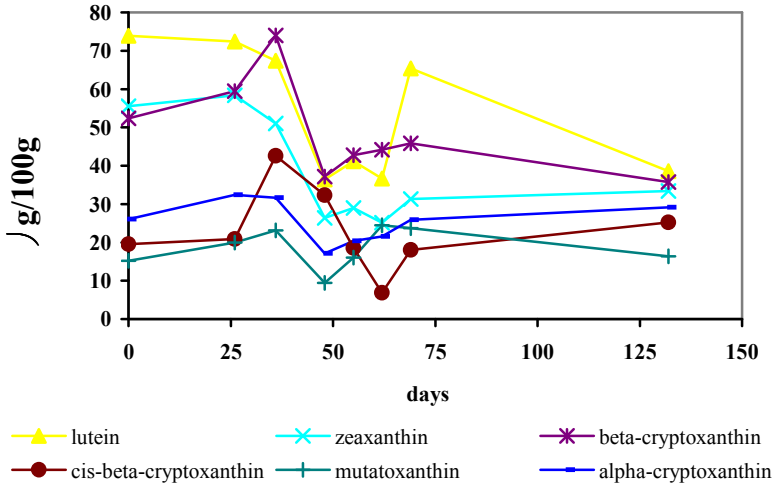


Fig. 6 Evolution of lutein, zeaxanthin, β-cryptoxanthin, cis-β -cryptoxanthin, mutatoxanthin and α-cryptoxanthin in the frozen orange-carrot juice (–40ºC)

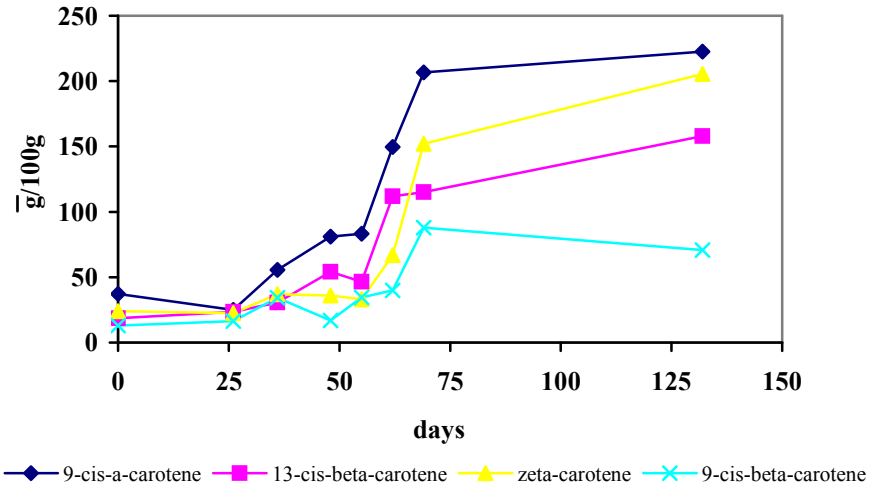


Fig. 7 Evolution of 9-cis-α-carotene, 13-cis-β-carotene, ζ-carotene and 9-cis-β-carotene in the frozen orange-carrot juice (–40ºC)

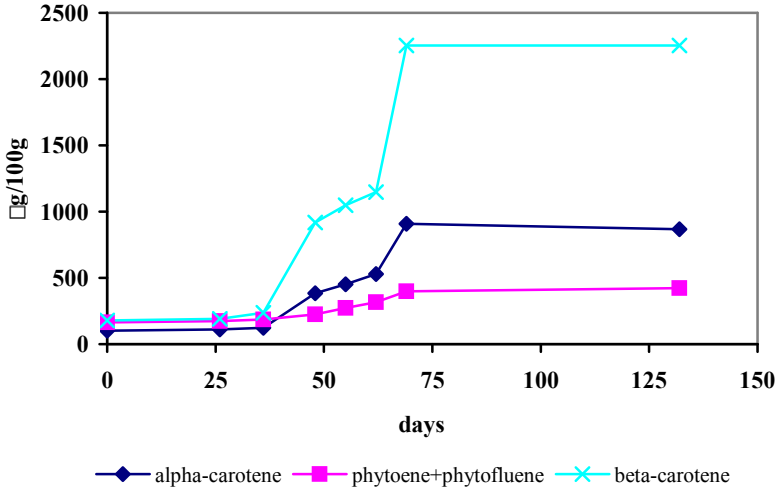


Fig. 8 Evolution of α-carotene, phytoene+phytofluene and β-carotene in the frozen orange-carrot juice (–40ºC)

The p-value, which tests the statistical significance of the correlation, is 0.000 and indicates statistically significant non-zero correlations at the 95% confidence level. It can be seen that when the concentrations of α-carotene, phytoene + phytofluene, β-carotene and ξ-carotene increase there is a significant decrease in the concentrations of 9-*cis*-violaxanthin + neoxanthin and antheraxanthin (Table 2).

	antheraxanthin	lutein	zeaxanthin	mutatoxanthin	α-cryptoxanthin	β-cryptoxanthin	*Cis*-β-cryptoxanthin	9-*cis*-α-carotene	α-carotene	Phytoene+phytofluene	β-carotene	13-*cis*-β-carotene	ζ–carotene	9-*cis*-β-carotene
9-*cis*-violaxanthin +neoxanthin	0.813*	0.864**	0.955**	0.124	0.621	0.853**	0.371	-0.480	-0.839**	-0.815*	-0.821*	-0.560	-0.674	-0.565
Antheraxanthin		0.691	0.810*	-0.060	0.353	0.521	-0.064	-0.470	-0.797*	-0.788*	-0.793*	-0.643	-0.744*	-0.689
Lutein			0.863**	0.363	0.681	0.751*	0.150	-0.121	-0.494	-0.489	-0.480	-0.296	-0.382	-0.158
Zeaxanthin				0.115	0.765*	0.760*	0.304	-0.592	-0.715	-0.672	-0.686	-0.366	-0.486	-0.447
Mutatoxanthin					0.479	0.487	-0.259	0.477	0.144	0.226	0.109	-0.091	0.085	0.443
α-cryptoxanthin						0.673	0.290	-0.407	-0.213	-0.121	-0.181	0.111	0.061	0.131
β-cryptoxanthin							0.460	-0.230	-0.675	-0.624	-0.671	-0.506	-0.556	-0.303
Cis-β-cryptoxanthin								-0.433	-0.319	-0.340	-0.271	0.095	-0.104	-0.163
9-*cis*-α-carotene									0.551	0.491	0.503	0.406	0.252	0.559
α-carotene										0.986**	0.997**	0.835**	0.926**	0.904**
Phytoene+ phytofluene											0.983**	0.826*	0.940**	0.914**
β-carotene												0.873**	0.946**	0.906**
13-*cis*-β-carotene													0.955**	0.810*
ζ-carotene														0.887**

** significant fit at 99%; * significant fit at 95%.

Table 2. Correlation coefficient between carotenoids in the frozen orange-carrot juice (–40ºC).

Lisiewska and Kmiecik [46] studied the effect of storage period and temperature on the chemical composition and organoleptic quality of frozen tomato cubes and found significant differences in the contents of vitamin C, carotenoids and β-carotene. After 6 months of frozen storage, these tomatoes, in comparison with the raw material, contained 38 and 26% less vitamin C, 18 and 7% less carotenoids, and 21 and 7% less β-carotene. Lisiewska et al. [47] found that the level of β-carotene was stable in all samples of dill during freezing and refrigerated storage (–20, –30 and –40 ºC) for up to three months. The prevailing opinion is that freezing does not prevent the degradation of carotenoids. According to Biacs and Wissgott [48], the losses are mainly due to the activity of enzymes, particularly in an oxygen environment.

Conclusions

Storage of orange-carrot juice at –40ºC caused a decrease in the concentration of ascorbic acid (4.1%). Provitamin A activity A (RE) increased during storage of the juice, thus helping to maintain the organoleptic characteristics of the product. Nine of the fourteen carotenes identified increased significantly after 132 days of storage at –40ºC. Only antheraxanthin and the 9-*cis*-violaxanthin + neoxanthin mixture decreased in concentration during the period studied.

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References

1. Law MR, Morris JK (1998) European Journal of. Clinical Nutrition 52 (8): 549-556

2. Kritchevsky SB, Tell GES, Shimakawa T, Dennis B, Li RL, Kohlmeier L, Steere E, Heiss G (1998) American Journal of Clinical Nutrition 68 (3): 726-733

3. Tibble DL (1999) American Journal of Clinical Nutrition 68: 521-522

4. Slaterry ML, Benson J, Curtin K, Khe-Ni M, Schaeffer D, Potter JD (2000) American Journal of Clinical Nutrition 71: 575-582

5. Virtamo J, Edwards B, Virtanen M, Taylor P, Maliha N, Albanes D, Huttunen JK, Hartman AM, Hietanen P, Maenpaa H, Koss L, Nordling S, Heinonen OP (2000) Cancer Causes Control, 11, 933-939

6. Albanes D, Malila N, Taylor PR, Huttunen JK, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Barret MJ, Pietinen P, Hartman TJ, Sipponen P, Lewin K, Teerenhovi L, Hietanen P, Tangrea JA, Virtanen M, Heinonen OP (2000) Cancer Causes Control, 11, 197-205

7. Forastier F, Pistelli R, Sestini P, Fortes C, Renzoni E, Rusconi F, Dell'Orco V, Ciccone G, Bisanti L (2000) SIDRIA Collaborative Group, Italy (Italian Studies on Respiratory Disorders in Children and the Environment). Thorax, 55, 283-288

8. Simopoulos AP (2001) Journal of Nutrition, 131, 3065S-3073S

9. Jacques P, Chylack LJ, Hankinson S, Khu P, Rogers G, Friend J, Tung W, Wolfe JK, Padhye N, Willett WC, Taylor A (2001) Arch. Opthalmol., 119, 1009-1019

10. Fawzi WW, Spiegelman D, Villamor E, Mugusi F, Essex M (2004) New England Journal of Medicine 351: 23-32

11. Kaur C, Kapoor HC (2001) J. Food Sci. Technol., 36, 703-725

12. Rapisarda P, Tomaino A, Lo Cascio R, Bonina F, De Pasquale A, Saija A (1999) Journal of Agricultural and Food Chemistry 47: 4718-4723

13. Gardner PT, White TAC, McPhail DB, Duthie GG (2000) Food Chemistry 68: 471-474

14. Hussein A, Odumeru JA, Ayanbadejo T, Faulkner H, McNab WB, Hager H, Szijarto L (2000) Food Research International 33: 131-136

15. Sanchez-Mata MC, Cámara-Hurtado M, Diez-Marques C, Torija-Isasa M (2000) European Food Research Technology 210: 220-225

16. Ewaidah EH (1992) Food Chemistry 44 (2): 103-111

17. Goyle A, Ojha P (1998) Journal of Food Science and Technology 35 (4): 346-348

18. Roig MG, Bello JF, Rivera ZS, Kennedy JF (1999) Food Research International 32: 609-619

19. Ayhan Z, Yeom HW, Zhang QH, Min DB (2001) Journal of Agricultural and Food Chemistry 49 (2): 669-674

20. Bezman Y, Rouseff RL, Naim M (2001) Journal of Agricultural and Food Chemistry 49 (1): 5425-5432

21. Bureau J, Bushway RJ (1986) Journal of Food Science 51: 128

22. Meléndez-Martínez AJ, Vicario IM, Heredia FJ (2003) Journal of Agricultural and Food Chemistry 51: 4219-4224

23. Granado F, Olmedilla B, Blanco I, Rojas-Hidalgo E (1992) Journal of Agricultural and Food Chemistry 40: 2135-2140

24. Landrum JT, Bone RA (2001) Archives of Biochemistry and Biophysics 385: 28-40

25 Grassman J, Hippeli S, Elstre EF (2002) Plant physiology and Biochemistry

40: 471-478

26. Van Poppel G, Goldbohm RA (1995) American Journal of Clinical Nutrition 62: 1493-1503

27. Giovannucci E (1999) Journal of the National Cancer Institute 91: 317-331

28. Kritchevsky SB (1999) Journal of Nutrition 129: 5-8

29. Shi J, Le Maguer M (2000) Crit. Rev. Food Sci. Nutr., 40, 1

30. Southon S (2000) Food Research International, 33, 211-217

31. Gazzani G, Papetti A, Masssolini G, Daglia M (1998) J Food Chem. 6, 4118-4122

32. West C, Castenmiller J (1998) Food processing and bioavailability of food antioxidants. In: Proceedings. FAIR CT95-0158 Symposium Natul. Antioxidants in Processed Foods-Effects on storage characteristics and nutricional value. Denmark: Agril. University of Federiksberg

33. Giannakourou MC, Taoukis PS (2003) Food Chem. 83: 33-41

34. Sahari MA, Boostani MF, Hamidi ZE (2004) Food Chem. 86: 357-363

35. Rodriguez-Saona LE, Barrett DM, Selivonchick DP (1995) J. Food Sci. 60: 1041-1044

36. Cortés C, Esteve MJ, Frígola A, Torregrosa F (2004) J. Agric. Food Chem. 52: 2203-2212

37. FAO/WHO Joint Expert Consultation, 1988

38. Aparicio P, Farré R, Frígola A (1992) An. Bromatol. XLIV: 257-261

39. Navarro JL, Lafuente B, Gasque F, Izquierdo L, Nadal MI (1981) Rev. Agroquim. Tecnol. Aliment., 21, 505-512

40. Lee HS, Coates GA (1999) Food Chem. 65, 165-168

41. Korus A, Lisiewska Z, Kmiecik W (2002) Nahrung, 46 (4), 233-237

42. Giannakourou MC, Taoukis PS (2003) Food Chem. 83: 33-41

43. Martins RC, Silva CLM (2004) J. Food Eng. 64: 481-488

44. Bahçeci KS, Serpen A, Gökmen V, Acar J (2004) J. Food Eng., in press.

45. Sahari MA, Boostani M, Hamidi Z (2004) Food Chem., 86: 357-363

46. Lisiewska Z, Kmiecik W (2000) Food Chem., 70: 167-173

47. Lisiewska Z, Kmiecik W, Slupski J (2004) Food Chem., 84, 511-518

48. Biacs P, Wissgott U (1997) Nahrung, 41, 306-310

Capítulo 4

Zumo de naranja

1. **Changes of colour and carotenoids contents during high intensity pulsed electric field treatment in orange juices.** Food and Chemical Toxicology, 44, 1932-1939 (2006).
2. **Carotenoid profile modification during refrigerated storage in untreated and pasteurized orange juice and orange juice treated with High Intensity Pulsed Electric Fields.** Journal of Agricultural and Food Chemistry, 54 (17), 6247-6254 (2006).
3. **Ascorbic acid content in refrigerated orange juice after HIPEF and thermal treatment**. Enviado para su publicación.
4. **Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice.** Enviado para su publicación.
5. **Determination of Total Antioxidant Capacity and Phenolic compounds of orange juice refrigerated treated by High Intensity Pulsed Electric Fields.** Enviado para su publicación.

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Changes of colour and carotenoids contents during high intensity pulsed electric field treatment in orange juices

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Abstract

Liquid chromatography (LC) was the method chosen to evaluate the effects of High Intensity Pulsed Electric Fields (HIPEF), with different electric field intensities (25, 30, 35 and 40 kV/cm) and different treatment times (30-340 μs), on orange juice cis/trans carotenoid contents. In parallel, a conventional heat treatment (90 ºC, 20 s) was applied to the orange juice in order to compare the effect on the carotenoid contents.

HIPEF processing of orange juice is an alternative to the thermal treatment of pasteurization, provided that it is kept refrigerated, because, when the most extreme conditions of this kind of treatment are applied, the decrease in the concentration of carotenoids with vitamin A activity is very small, and also most of the carotenoids identified have a slightly increased concentration after application of the most intense treatments, although always less than in untreated fresh juice. In any case, pasteurization treatment causes a greater decrease in the concentration of most of the carotenoids identified and the carotenoids with vitamin A activity. The total carotenoid concentration decreased by 12.6% in pasteurized orange juice with respect to untreated fresh orange juice, as opposed to decreases of 9.6%, 6.3% or 7.8% when fields of 25, 30 or 40 kV/cm were applied. Orange juice treated with HIPEF shows a greater tendency towards the colour yellow and a lesser tendency towards red with respect to untreated orange juice, while the luminance of the juice remains practically invariable. This tendency is less than in pasteurized orange juice.

Keywords: carotenoid, colour, pulsed electric field, liquid chromatography, orange juice

1. Introduction

Over 63 million tons of oranges are produced annually throughout the world (FAOSTAT, 2004). Orange juice is the predominant juice manufactured by the beverage processing industry worldwide. The consumption of refrigerated juices in the USA is currently of the order of $4.4 billion (Jago, 2004). Conventional thermal processing ensures safety and extends the shelf life of orange juice, but it often leads to detrimental changes in the sensory qualities of the product. Consumers desire high quality foods that are nutritious, with freshly prepared flavour, texture and colour, with minimal or no chemical preservatives, and above all safe (Bull et al., 2004). Consequently, newly developed food technologies usually focus on preservation while keeping food quality attributes. High Intensity Pulsed Electric Fields (HIPEF) is one of the non-thermal emerging technologies being developed for the preservation of foods as an alternative to traditional thermal methods (Yeom et al., 2000; Butz and Tauscher, 2002; Selma et al., 2003; Spilimbergo et al., 2003; Shivashankara et al., 2004; Elez-Martínez et al., 2005; Cortés et al., 2005a). HIPEF treatment has gained increasing interest as it offers some attractive advantages over thermal methods currently used in processing raw materials and foods (Qin et al., 1996; Grahl and Märkl, 1996; Jia et al., 1996; Qiu et al., 1998; Bendicho et al., 2001; Bendicho et al., 2002; Abram et al., 2003). On an industrial level, at Ohio State University (United States) there is equipment which processes up to 2000 litres of juice per hour and which in turn is connected to a UHT/HTST unit. Application of HIPEF is restricted to food products that can withstand high electric fields, i.e. that have low electrical conductivity and do not contain or form bubbles. The particle size of liquid food is also a limitation (Butz and Tauscher, 2002; Góngora-Nieto et al., 2002; Ulmer et al., 2002).

HIPEF can be used to avoid the detrimental effects, including vitamin losses, of traditional high temperature pasteurization of many foods. Traditional thermal processing of orange juice causes vitamin losses, including loss of vitamin C

(Farnworth et al., 2001) and changes in carotenoids (important to the colour and nutritional value of the juice) (Bull et al., 2004).

Carotenoids are one of the main classes of natural pigments because their distribution in the plant kingdom is extremely wide (Meléndez-Martínez et al., 2003). They are a diverse group of over 600 structurally related isoprenoids biosynthesized by plants, fungi and bacteria (Ben-Amotz and Fisher, 1997). The carotenoids present in citrus are a complex mixture of more than 115 natural substances, but not all of them are precursors of vitamin A (Klaui and Bauernfeind, 1981).

Orange juice is the most important dietary source of vitamin A carotenoids (β-carotene, α-carotene and β-cryptoxanthin) and antioxidant carotenoids (β-carotene, β-cryptoxanthin, zeaxanthin and lutein), because of its contents and high consumption. These carotenoids have been involved in the reduction of degenerative human diseases, such as heart disease and cancer (Giovannucci, 1999; Kritchevsky, 1999; Tibble, 1999; Slaterry et al., 2000; Temple, 2000), because of their antioxidant and free-radical scavenging properties (Landrum and Bone, 2001; De Ancos et al., 2002).

The objective of this study is to evaluate the effects of HIPEF, with different electric field intensities and different treatment times, on orange juice cis/trans carotenoid contents, characterized by HPLC, and to compare the results with the effect that the thermal treatment of pasteurization has on carotenoid contents in orange juice.

2. Materials and Methods

2.1. Chemicals

Lutein and zeaxanthin were provided free as standard substances by Roche (Basel, Switzerland). β-Carotene, all-trans-retinol palmitate, and tert-butyl hydroxytoluene (BHT) (special grade) were purchased from Sigma (Steinheim,

Germany). Ammonium acetate (HPLC grade), petroleum ether, hexane (HPLC grade), potassium hydroxide (85%), and tert-butyl methyl ether (TBME) (HPLC grade) were purchased from Scharlau (Barcelona, Spain); acetonitrile (special grade) and magnesium hydroxide carbonate (40-45%) from Panreac (Barcelona, Spain); and ethanol, diethyl ether, methanol and sodium chloride (special grade) from Baker (Deventer, The Netherlands). Chloroform was obtained from Merck (Darmstadt, Germany).

2.2. HIPEF and thermal treatment of orange juice

Oranges (*Citrus sinensis L.*) of the Navel variety (Spain) were purchased from a local supermarket. The orange juice was obtained, after suitable washing and hygienization of the fruits, by using a squeezer (FMC juice extractor with a 2-mm-diameter perforated plate), and it was then filtered with a 0.23-mm-pore-diameter filter. The juice was then packaged aseptically and frozen at –40º C until analysis. During storage period a modification in carotenoids concentration is observed. Initial carotenoid concentration in untreated orange juice of each tratment is different, because treatments are applied in different days. The same modification was observed in carotenoid concentration of orange-carrot juice stored at -40ºC during 132 days (Cortés et al., 2005b).

HIPEF treatments were carried out in a continuous flow bench scale system (OSU-4D, Ohio State University, Columbus, OH) located in the Instituto de Agroquímica y Tecnología de los Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, OR USA). The flow was set at 60

ml/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL).

Treatment time ranged from 30 to 340 μs and the electric field was set at 25, 30, 35 and 40 kV/cm. Samples were collected after each treatment. The experiments were performed in triplicate.

The thermal treatment intensity given to the samples was 90 ºC, 20 s. To treat the samples an ARMFIELD FT74P unit with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (90 ºC, 20 s) were reached. Heating of orange juice at 90–99 ºC for 15–30 s is normal in commercial practice (Braddock, 1999).

2.3. Analysis of carotenoids. Chromatographic determination

Carotenoid pigments were extracted, saponified and analysed by the HPLC method, following a procedure described by Cortés et al. (2004).

The LC system consisted of a series 1050 chromatograph with a quaternary pump system, a diode array detector (Hewlett-Packard, 1100 series), a column thermostat (Agilent 1100 series), an on-line degassing system, and a ChemStation (series A.06.03) data system (Hewlett-Packard, Waldbronn, Germany). A Vydac 201TP54 column with a particle size of 5 μm, 250 x 4.6 mm, reverse phase C18 and a Vydac 201TP precolumn (guard column) (4.6 mm i.d. cartridge with 5-μm particles) (Hesperia, CA) were used.

The carotenoids (including geometrical isomers) were identified and quantified by liquid chromatography with an ultraviolet diode array. The mobile phase used was tert-butyl methyl ether and water (in a concentration gradient), and a temperature gradient was applied with retinol palmitate as an internal standard. An extraction process (ethanol/hexane, 4:3, v/v) was performed, followed by saponification with diethyl ether/methanolic KOH (0.1%, w/v, BHT) (1:1, v/v) for 0.5 h at room temperature. For the quantification of the various carotenoids identified in the samples studied we quantified the different carotenoids in

accordance with their similarity in terms of chemical-structural behaviour and polarity with respect to the validated standards for which we had a calibration curve: lutein, zeaxanthin and β-carotene. Accordingly, for the quantification we proceeded as follows: all the samples were spiked with retinol palmitate (internal standard, 350 nm); the chromatogram was separated into 3 parts: all the carotenoids up to and including lutein were quantified as such, and the remaining xanthophylls were quantified as zeaxanthin. The carotenes were quantified as β-carotene. Each carotenoid was quantified in accordance with whether its maximum λ was close to 290, 350, 430 or 450 nm. The content of each carotenoid in μg/100 g of sample was determined after applying equation (2): $(A_{sample} / A_{IS}) \cdot \mu g$ Standard $\cdot$ 100 / $(A_{standard} / A_{IS}) \cdot$ g sample. Carotenoids were identified by UV-vis spectra and retention times (table 1) in HPLC in the juices analysed (Cortés et al., 2004).

2.4. Determination of vitamin A

Vitamin A was expressed as retinol equivalents (RAE), using the following conversion (Trumbo et al, 2003; IOM, 2001):

RAE = (μg of β-carotene)/12 + (μg of β-cryptoxanthin + α-carotene)/24

2.5. Colour analysis

Colour (CIE L*, a*, b*) analysis was performed using a Hunter Labscan II spectrophotometric colorimeter controlled by a computer that calculates colour ordinates from the reflectance spectrum (Calvo and Durán, 1998). The results were expressed in accordance with the CIELAB system with reference to illuminant D65 and with a visual angle of 10º.

The CIE L*, a* and b* values were used to calculate the total colour differences (ΔE*), using the formula:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)2 + (\Delta b^*)^2]^{1/2}$$

where ΔL*, Δa* and Δb* are differences between the orange juice colour of fresh and pulsed juice subjected to the electric field and time in which the greatest treatment temperature is attained (Lee and Coates, 1999).

2.5. Statistical analysis

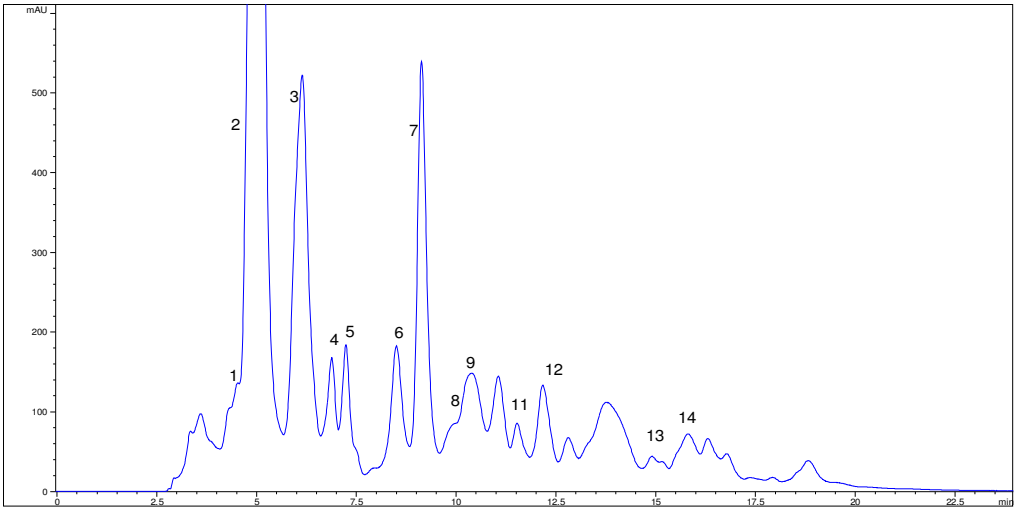
Significant differences between the results were calculated by analyses of the variance (ANOVA). One-way ANOVA was calculated on the three triplicate measurements. Differences at $p<0.05$ were considered to be significant. An LSD test was applied to indicate the samples between which there were differences. All statistical analyses were performed using Statgraphics Plus 5.0 (Statistical Graphics Corporation, Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Effect of HIPEF treatment on carotenoid profile of orange juice

To establish the effect of the HIPEF treatment, different fields (25, 30, 35 and 40 kV/cm) were applied for different times (from 30 to 340 μs), and in all cases the results were compared with untreated orange juice. The juice was also subjected to a pasteurization treatment for 20 seconds at 90 ºC, in order to compare it with untreated fresh orange juice and with the juice treated with HIPEF. Figure 1 shows a chromatogram of the fresh orange juice before any HIPEF or thermal treatment, in which one can see the various separate peaks after applying the extraction procedure described by Cortés et al. (2004). Table 1 shows the carotenoids identified, their retention time and the wavelength at which they were detected. Tables 2-5 show the results obtained and indicate the maximum temperature reached in each of the HIPEF treatments applied. The results are a mean of three performances of each treatment, carried out on different dates and each analysed in duplicate.

(A)



(B)

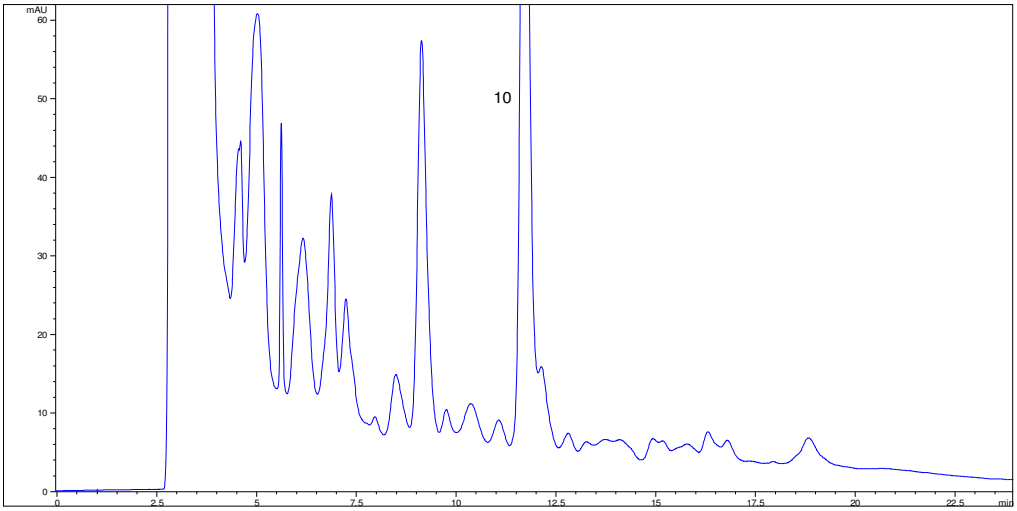


Figure 1: Chromatogram of fresh orange juice at λ=450 nm (A) and λ=290 nm (B).

Peak	Carotenoids	λ (nm)	t_R (min) ± SD
1	13-cis-violaxanthin	430	4.3 ± 0.1
2	neoxanthin + 9-cis-violaxanthin	430	4.9 ± 0.1
3	antheraxanthin	450	6.0 ± 0.1
4	lutein	450	6.7 ± 0.1
5	zeaxanthin	450	7.1 ± 0.1
6	isolutein	450	8.2 ± 0.1
7	β-cryptoxanthin	450	8.8 ± 0.1
8	α-carotene	450	10.1 ± 0.2
9	9-cis-α-carotene	430	10.7 ± 0.2
10	phytoene + phytofluene	290	11.3 ± 0.2
11	7,8,7',8'-tetrahydrolycopene	430	11.4 ± 0.2
12	β-carotene	450	11.8 ± 0.2
13	13-cis-β-carotene	450	14.5 ± 0.2
14	9-cis-β-carotene	450	15.4 ± 0.2

Table 1: Wavelengths and retention times of carotenoids in orange juice.

When the 25 kV/cm field is applied (Table 2), we see that the carotenoid concentrations generally tend to decrease or remain practically constant while the treatment time increases, that is, while the maximum temperature to which the juice is subjected increases. In some cases, when the two most intense treatments (35 and 40 kV/cm) are applied, attaining the highest temperatures (about 70º C), the concentration of some carotenoids increases slightly, perhaps owing to greater stability, enzymatic degradation and losses of moisture and soluble solids that concentrate the sample per unit weight (Rodriguez-Amaya, 1997). This is the case with the neoxanthin + 9-cis-violaxanthin mixture, antheraxanthin, lutein, zeaxanthin, β-cryptoxanthin, phytoene + phytofluene, isolutein and 7,8,7',8'-tetrahydrolycopene. Heat induces cis/trans isomerization (13-cis-β-carotene) and various carotenoid by-products may be formed (Khachik et al., 1991, 1992; Quackembush, 1987). When the 30 kV/cm treatment is applied (Table 3), we see that the concentrations of the various carotenoids remain practically constant, and there is also a clear increase in the concentrations of some of them when the highest treatment times are applied. When the orange juice is treated with the 35 kV/cm and 40 kV/cm electric fields (Tables 4 and 5), we see that there is a clear increase in the concentration of 13-cis-violaxanthin and 7,8,7',8'-tetrahydrolycopene when the most intense treatments are applied with these electric fields. On the other hand, there is a clear decrease in the concentrations of β-cryptoxanthin, α-carotene and β-carotene when higher treatment times are applied. Nevertheless, these decreases are not statistically significant ($p>0.05$). Similarly, Bull et al. (2004) indicate that high pressure processing and thermal treatment of orange juice do not significantly decrease the concentration of β-carotene.

	Treatment time (μs)	**0**	**40**	**100**	**170**	**240**	**300**	**340**
	Temperature (ºC)		**37**	**44**	**52**	**64**	**67**	**72**
1	13-cis-violaxanthin	19.1	22.5	21.5	21.8	20.5	21.7	21.9
2	neoxanthin + 9-cis-violaxanthin	663	524	530	455	477	601	597
3	antheraxanthin	198	155	154	135	140	185	184
4	lutein	42.9	33.9	35.5	29.9	30.3	42.5	41.9
5	zeaxanthin	65.7	56.4	57.4	48.2	49.0	65.1	62.9
6	isolutein	81.9	64.8	65.2	58.3	61.2	71.1	70.5
7	β-cryptoxanthin	185	147	147	136	141	175	170
8	α-carotene	69.9	59.6	66.1	64.9	62.9	65.8	60.7
9	9-cis-α-carotene	42.1	32.4	38.2	37.2	35.3	36.4	35.9
10	phytoene + phytofluene	26.4	19.1	19.4	18.2	18.4	23.7	23.9
11	7,8,7',8'-tetrahydrolycopene	28.0	21.3	24.1	20.5	21.3	26.7	27.8
12	β-carotene	33.6	25.8	29.6	28.2	27.3	30.6	28.2
13	13-cis-β-carotene	8.78	7.27	8.73	7.98	9.81	7.05	6.84
14	9-cis-β-carotene	20.5	18.7	21.2	23.8	19.8	21.2	19.7
	Total Carotenoids	1714	1357	1393	1265	1285	1567	1549
	Vitamin A	12.3	9.59	10.1	9.56	9.59	11.3	10.9

Table 2: Concentration of carotenoids (μg/100g) and vitamin A (RAE) in orange juice processed by HIPEF (25 kV/cm).

	Treatment time (μs)	**0**	**40**	**80**	**100**	**150**	**190**	**240**
	Temperature (ºC)		**38**	**46**	**49**	**58**	**63**	**69**
1	13-cis-violaxanthin	33.2	45.3	22.3	22.6	24.5	27.5	36.6
2	neoxanthin + 9-cis-violaxanthin	642	474	481	456	507	539	540
3	antheraxanthin	200	141	147	137	158	165	168
4	lutein	43.6	30.8	32.0	30.5	39.3	37.2	38.1
5	zeaxanthin	68.1	52.6	54.5	51.5	58.0	64.7	66.8
6	isolutein	69.7	57.2	54.4	50.5	57.1	74.0	80.6
7	β-cryptoxanthin	184	143	143	131	156	172	182
8	α-carotene	65.4	50.5	51.2	41.9	42.6	67.6	76.9
9	9-cis-α-carotene	37.2	28.7	27.2	23.9	26.9	40.0	46.0
10	phytoene + phytofluene	26.6	18.9	18.6	18.0	20.1	22.6	24.5
11	7,8,7',8'-tetrahydrolycopene	25.1	18.9	18.6	17.7	21.8	26.7	27.9
12	β-carotene	32.0	23.8	22.1	19.9	22.3	29.3	33.8
13	13-cis-β-carotene	8.16	6.76	6.31	5.33	5.81	7.52	9.99
14	9-cis-β-carotene	19.6	15.7	15.7	12.8	14.4	17.1	22.7
	Total Carotenoids	1657	1221	1218	1132	1284	1466	1551
	Vitamin A	11.9	9.27	9.19	8.36	9.65	11.5	12.6

Table 3: Concentration of carotenoids (μg/100g) and vitamin A (RAE) in orange juice processed by HIPEF (30 kV/cm).

	Treatment time (μs)	**0**	**40**	**80**	**100**	**150**	**190**	**240**
	Temperature (ºC)		**38**	**46**	**49**	**58**	**63**	**69**
1	13-cis-violaxanthin	17.8	14.4	23.9	28.9	22.4	21.0	21.9
2	neoxanthin + 9-cis-violaxanthin	589	482	515	607	663	615	635
3	antheraxanthin	174	142	151	182	202	190	191
4	lutein	36.8	29.2	31.9	39.5	44.9	41.7	40.5
5	zeaxanthin	63.8	53.1	57.1	47.7	55.6	53.8	54.7
6	isolutein	79.5	71.4	70.8	53.6	65.7	65.9	67.9
7	β-cryptoxanthin	188	159	161	129	149	150	152
8	α-carotene	77.3	71.7	62.1	52.0	60.4	65.3	70.3
9	9-cis-α-carotene	42.6	39.8	38.1	29.8	34.9	39.2	41.4
10	phytoene + Phytofluene	24.1	20.7	20.5	18.9	21.0	21.6	22.3
11	7,8,7',8'-tetrahydrolycopene	22.5	20.6	22.7	22.0	25.9	26.7	27.2
12	β-carotene	31.5	28.5	26.9	23.0	26.4	28.3	29.9
13	13-cis-β-carotene	8.30	9.17	7.25	6.17	6.93	9.45	8.71
14	9-cis-β-carotene	20.0	22.2	16.8	12.2	11.7	16.0	20.5
	Total Carotenoids	1556	1321	1333	1371	1545	1535	1571
	Vitamin A	12.2	10.6	10.5	8.50	9.85	10.2	10.5

Table 4: Concentration of Carotenoids (μg/100g) and vitamin A (RAE) in orange juice processed by HIPEF (35 kV/cm).

	Treatment time (μs)	**0**	**30**	**40**	**60**	**80**	**100**	**130**
	Temperature (C)		**41**	**44**	**49**	**55**	**61**	**69**
1	13-cis-violaxanthin	17.3	16.5	16.1	16.0	24.7	25.2	32.1
2	neoxanthin + 9-cis-violaxanthin	580	539	543	519	561	529	542
3	antheraxanthin	175	163	168	158	171	170	186
4	lutein	38.3	35.2	36.1	36.0	37.7	41.9	42.2
5	zeaxanthin	61.9	58.6	58.9	55.0	59.8	59.3	66.1
6	isolutein	72.3	73.8	74.4	65.1	66.7	61.7	72.6
7	β-cryptoxanthin	173	164	1657	149	158	157	165
8	α-carotene	65.6	76.8	72.9	64.6	54.7	46.5	53.8
9	9-cis-α-carotene	34.7	42.6	39.8	35.8	31.8	29.3	33.4
10	phytoene + phytofluene	23.6	21.7	21.9	19.9	21.0	19.9	21.7
11	7,8,7',8'-tetrahydrolycopene	22.1	24.9	26.5	24.0	24.4	23.4	25.4
12	β-carotene	31.2	30.7	31.5	25.7	24.7	24.0	25.4
13	13-cis-β-carotene	8.64	8.58	8.03	6.52	5.68	5.63	7.25
14	9-cis-β-carotene	16.2	20.3	23.3	17.5	17.4	14.7	14.2
	Total Carotenoids	1495	1502	1479	1350	1413	1375	1378
	Vitamin A	12.7	12.8	12.5	11.0	10.9	10.4	11.5

Table 5: Concentration of carotenoids (μg/100g) and vitamin A (RAE) in orange juice processed by HIPEF (40 kV/cm).

We performed an analysis of variance (ANOVA) to test whether there were significative differences between the untreated juice and the juice treated with HIPEF. The concentrations of zeaxanthin, isolutein, β-cryptoxanthin, phytoene + phytofluene and β-carotene decreased significantly ($p<0.01$) in the juices treated with HIPEF. A multiple regression analysis was performed to see the influence of field intensity, treatment time and temperature on each of the carotenoids analysed. Only the field intensity had a significant ($p<0.01$) effect on the concentration of valenciaxanthin, with an increase in field intensity being accompanied by a lower concentration of valenciaxanthin (valenciaxanthin = 40.39 - 0.85*field, R-squares = 13.72).

	Untreated juice	Pasteurized juice
Neoxanthin + 9-cis-violaxanthin	445±23.2	351±10.7
Antheraxanthin	162±10.1	138±4.26
Lutein	27.3±1.89	24.0±0.90
Zeaxanthin	68.7±0.95	56.4±0.85
isolutein	62.0±2.76	57.8±0.45
β-Cryptoxanthin	206±14.3	176±2.14
α-carotene	49.9±1.82	32.9±14.6
9-cis-α-carotene	31.6±0.21	24.0±4.61
Phytoene + Phytofluene	23.3±0.19	21.5±0.27
7,8,7',8'-tetrahidrolycopene	28.2±0.84	25.4±0.16
β-Carotene	37.2±1.44	33.6±0.02
9-cis-β-carotene	12.6±0.55	10.4±0.12
Total Carotenoids	1367±64.7	1195±31.6
Vitamin A	14.8±0.66	12.3±0.70

Table 6: Concentration of carotenoids (μg/100g) and vitamin A (RAE) in orange juice untreated and orange juice processed by pasteurization.

Comparison of the results shows that pasteurization of orange juice produces a greater decrease in carotenoid concentrations than the application of HIPEF. Results are shown in table 6. Thus, the concentration of total carotenoids decreases 12.6% in pasteurized orange juice with respect to untreated fresh orange juice, compared with decreases of 9.6%, 6.4% or 7.8% when highest times of fields 25, 30 or 40 kV/cm are applied.

3.2. Effect of HIPEF treatment on vitamin A in orange juice

In all the fields applied, vitamin A decreases slightly when the treatment time increases, owing mainly to the very marked decrease in the concentration of β-cryptoxanthin, and also, to a lesser extent, to the decrease in α-carotene and β-carotene. When the highest treatment times for each electric field are applied, the decrease in vitamin A with respect to untreated fresh orange juice is 11.1%, 13.9% and 9.9% in the 25 kV/cm, 35 kV/cm and 40 kV/cm fields, respectively. However, in the 30 kV/cm field, vitamin A increases 6% when the most intense treatment time is applied. In some cases we see that when the orange juice is subjected to the highest treatment times the vitamin A activity increases slightly with respect to the lowest treatment times, owing to the increase in the concentration of β-cryptoxanthin, β-carotene and α-carotene, as commented earlier. In the pasteurized orange juice, the concentration of vitamin A decreases 15.6% with respect to untreated fresh orange juice (table 6). A thermal processing study (Lessin et al., 1997) with various fruits and vegetables, including orange juice, indicated a relatively large loss (36%) of vitamin A carotenoids in orange juice processing.

A multiple linear regression was fitted to describe the relationship between vitamin A and 3 independent variables. The equation of the model fitted was:

$$\text{Vitamin A} = 8.06 + 0.21*\text{field} + 0.02*\text{time} - 0.13*\text{Tmax}$$

Since the P-value in the ANOVA table is less than 0.01, there is a statistically significant relationship between the variables at the 99% confidence level. The

R-Squared statistic indicates that the model as fitted explains 21.8% of the variability in vitamin A.

3.3. Effect of HIPEF treatment on colour parameters of orange juice

With the various HIPEF treatments applied, the CIE b* values (77.14±4.90) gradually changed towards a more positive direction (84.24±2.06) in all samples. When longer treatment times were applied, this variation was statistically significant (p=0.0027). On the other hand, the CIE a* values (6.15±0.95) decreased as the treatment time increased in all the samples studied (4.41±0.62), and this decrease was also significant (p=0.0030). The decrease in CIE a* was greater than the increase in CIE b*, so that when the quotient a*/b* is plotted one can clearly see a decrease in its value as the treatment times increase for a given electric field (Figure 2). These variations indicate that the orange juice treated with HIPEF shows a greater tendency towards the colour yellow and a lesser tendency towards red, with respect to the untreated orange juice. These results are in agreement with those obtained for the pasteurized orange juice, in which the variations were slightly greater, with the CIE a* values decreasing 60% with respect to the untreated orange juice and the CIE b* values increasing 14%. Lee and Coates (2003) obtained similar results when they studied colour in fresh orange juice and pasteurized orange juice. The direction of the colour change was different from the colour changes observed in red grapefruit juice (Lee and Coates, 1999). The value of CIE L* indicates the luminance of the juice. The CIE L* values did not alter significantly (72.61±0.41) when any of the treatments studied were applied. The results obtained for the CIE L* of the orange juice treated with HIPEF and pasteurized differ from those reported by Lee et al. (2001) for orange juice subjected to conventional preservation treatments by pasteurization and those reported by Lee and Coates (1999) when they studied red grapefruit juices also processed by heat treatments. They found a slight decrease in CIE L* when the juice was processed, and they attributed it

to partial precipitation of unstable particles in the juice, as described by Genovese et al. (1997).

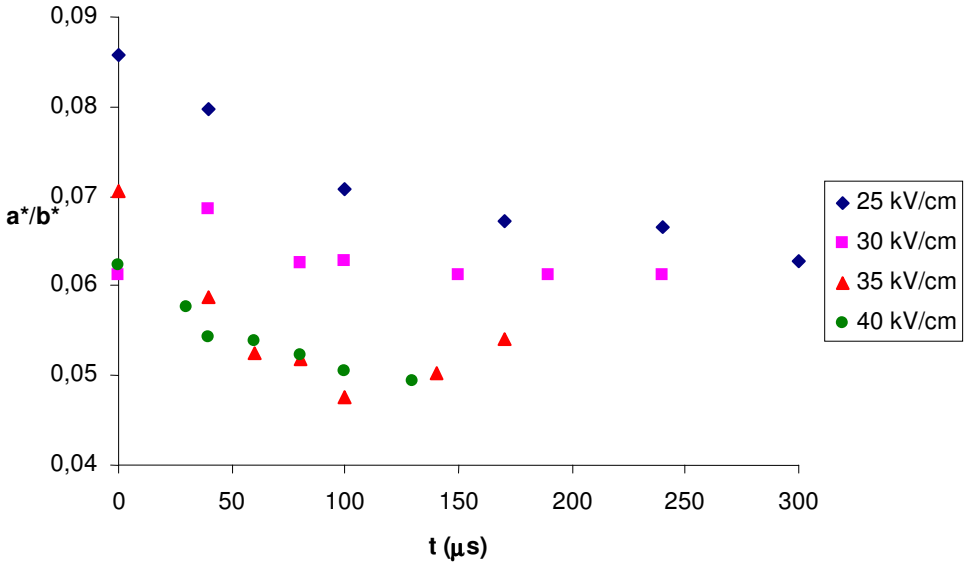


Figure 2: CIELAB (a/b) after HIPEF treatments in orange juice.

The total colour differences (ΔE*), which indicate the magnitude of the colour difference between fresh and pulsed orange juices subjected to the electric field and time in which the highest treatment temperature was reached (25kV/cm, 340 µs), were 7.32±2.86, and this difference is statistically significant ($p<0.05$). It has been considered (Francis & Clydesdale, 1975) that a ΔE* of 2 would be a noticeable visual difference for a number of situations. Lee and Coates (2003) observed similar results when they studied fresh orange juice and pasteurized orange juice, also obtaining ΔE* values greater than 2.

Color parameters are influenced by the presence and quantities of different carotenoids in juices analyzed and correlations between color and carotenoids

has been observed. The 9-cis-β-carotene, 13-cis-β-carotene and 13-cis-violaxanthin had a linear relationship ($p<0.05$) with CIE a* (positive) and CIE L* (negative). Similarly, the neoxanthin + 9-cis-violaxanthin mixture, lutein, antheraxanthin, zeaxanthin, isolutein, β-cryptoxanthin, β-carotene, phytoene + phytofluene and 9-cis-α-carotene had a statistically significant ($p<0.05$) linear relationship with CIE a* (positive), CIE b* (positive) and CIE L* (negative).

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References

Abram, F., Smelt, J. P. P. M., Bos, R., Wouters, P. C., 2003. Modelling and optimization of inactivation of *Lactobacillus plantarum* by pulsed electric field treatment. Journal of Applied Microbiology 94, 571-579.

Ben-Amotz, A., Fisher, R., 1997. Analysis of carotenoids with emphasis on 9-cis-beta-carotene in vegetables and fruits commonly consumed in Israel. Food Chemistry 62 (4), 515-520.

Bendicho, S., Arántegui, J., Martín, O., 2001. Procesado de alimentos mediante pulsos eléctricos de alta intensidad de campo. II. Efectos sobre microorganismos y componentes de los alimentos. Alimentaria, June, 37-44.

Bendicho, S., Espachs, A., Arántegui, J., Martín, O., 2002. Effect of high intensity pulsed electric fields and heat treatments on vitamins of milk. Journal Dairy Research 69, 113-123.

Braddock, R.J., 1999. Single strength orange juices and concentrate. In J. Wiley (Ed.), Handbook of Citrus by-products and Processing Technology, New York, pp. 53–83.

Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E. A., Johnson, R. L., Stewart, 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.

Butz, P., Tauscher, B., 2002. Emerging technologies: chemical aspects. Food Research International 35, 279-284.

Calvo, C., Durán, L., 1998. Lightness and gloss of peach gels: effect of fruit and sugar content. Zeitschrift für Lebensmittel-Untersuchung und -Forschung a - food research and technology 207 (2), 101-104.

Cortés, C., Esteve, M. J., Frígola, A., Torregrosa, F., 2004. Identification and quantification of carotenoids including geometrical isomers in fruit and vegetable juices by liquid chromatography with ultraviolet-diode array detection. Journal of Agricultural and Food Chemistry 52, 2203-2212.

Cortés, C., Esteve, M. J., Frígola, A., Torregrosa, F., 2005a. Quality characteristics of horchata (a Spanish vegetable beverage) treated with pulsed electric fields during shelf-life. Food Chemistry 91, 319–325.

Cortes, C.; Esteve, M.J.; Frigola, A.; Torregrosa, F., 2005b. Changes in carotenoids including geometrical isomers and ascorbic acid content in orange-carrot juice during frozen storage. European Food Research and Technology 221, 125-131.

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 Agriculture 82, 790-796.

Elez-Martínez, P., Escolá-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.

FAOSTAT (FAO Statistical Databases), 2004.

Farnworth, E. R., Lagace, M., Couture, R., 2001. Thermal processing, storage conditions, and the composition and physical properties of orange juice. Food Research International, 34 (1): 25-30.

Francis, F. J., Clydesdale, F. M., 1975. Food colorimetry: Theory and applications. Westport, CT: The AVI publishing Co., Inc.

Genovese, D. B., Elustondo, M. P., Lozano, J. E., 1997. Color and cloud stabilization in cloudy apple juice by steam heating during crushing. Journal of Food Science 62, 1171-1175.

Giovannucci, E., 1999. Tomatoes, tomato-based products, lycopene and cancer: a review of the epidemiological literature. Journal of the National Cancer Institute 91, 317-331.

Góngora-Nieto, M. M., Sepúlveda, D. R., Pedrow, P., Barbosa-Cánovas, G. V., Swanson, B. G., 2002. Food processing by pulsed electric fields: treatment delivery, inactivation level, and regulatory aspects. Lebensmittel-Wissenschaft Technologie 35, 375-388

Grahl, T., Märkl, H., 1996. Killing of microorganisms by pulsed electric fields. Applied Microbiology and Biotechnology 45, 148-157.

IOM, 2001. Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press.

Jago, D., 2004. New food products around the world. Food Beverage International. 3 (2), 11-15.

Jia, M., Zhang, Q. H., Min, D. B., 1996. Pulsed electric field processing effects on flavor compounds and microorganisms of orange juice. Food Chemistry 65, 445-451.

Khachik, F., Beecher, G. R., Goli, M. B., Lusby, W. R., 1991. Separation, identification and quantification of carotenoids in fruits, vegetables and human plasma by high-performance liquid chromatography. Pure Applied Chemistry 63, 71-80.

Khachik, F., Goli, G. R., Beecher, G. R., Holden, J., Lusby, W. K., 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.

Klaui, H., Bauernfeind, J. C., 1981. In: Carotenoids as colorants and vitamin A precursors. Bauernfeind, J. C. (Ed.), Academic Press, New York, Chapter 2, p. 47.

Kritchevsky, S. B., 1999. β-carotene, carotenoids and the prevention of coronary heart disease. Journal of Nutrition 129**,** 5-8.

Landrum, J. T., Bone, R. A., 2001. Lutein, Zeaxantin and the Macular Pigment. Archives of Biochemistry and Biophysics 385, 28-40.

Lee, H. S., Coates, G. A., 1999. Thermal pasteurization effects on color of red grapefruit juices. Journal of Food Science 64, 663-666.

Lee, H. S., Castle, W. S., Coates, G. A., 2001. High-performance liquid chromatography for the characterization of carotenoids in the new sweet orange (Earlygold) grown in Florida, USA. Journal of Chromatography A 913 (1-2), 371-377.

Lee, H. S., Coates, G. A., 2003. Effect of thermal pasteurization on Valencia orange juice color and pigments. Lebensmittel-Wissenschaft + Technologie 36, 153-156.

Lessin, W. J., Catigani, G. L., Schwartz, S. J., 1997. Quantification of cis-trans isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. Journal of Agricultural and Food Chemistry 45, 3728-3732.

Meléndez-Martínez, A. J., Vicario, I. M., Heredia, F. J. A., 2003. Routine high-performance liquid chromatography method for carotenoid determination in ultrafrozen orange juices. Journal of Agricultural and Food Chemistry 51, 4219-4224.

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.

Qiu, X., Sharma, S., Tuhela, L., Jia, M., Zhang, Q., 1998. An integrated PEIAC pilot plant for continuous nonthermal pasteurization of fresh orange juice. ASAE transaction 41, 1069-1074.

Quackembush, F. W., 1987. Reverse phase HPLC separation of cis and trans-carotenoids and its application to β-carotenes in food materials. Journal of Liquid Chromatography 10, 643-653.

Rodríguez-Amaya, D. B., 1997. Carotenoids and Food Preparation: The retention of provitamin A Carotenoids in prepared, processed and stored foods; OMNI Project: Washington DC, pp. 13-51.

Selma, M. V., Fernández, P. S., Valero, M., Salmerón, M. C., 2003. Control of Enterobacter aerogenes by high-intensity, pulsed electric fields in horchata, a Spanish low-acid vegetable beverage. Food Microbiology 20, 105-110.

Shivashankara, K. S., Isobe, S., Al-Haq, M. M., Takenaka, M., Shiina, T., 2004. Fruit antioxidant activity, ascorbic acid, total phenol, quercetin, and carotene of irwin mango fruits stored at low temperature after high electric field pretreatment. Journal of Agricultural and Food Chemistry 52 (5), 1281-1286.

Slaterry, M. L., Benson, J., Curtin, K., Khe-Ni-Ma, Schaeffer, D., Potter, J. D., 2000. Carotenoids and Vitamin A. American Journal of Clinical Nutrition 71, 575-582.

Spilimbergo, S., Dehghani, F., Bertucco, A., Foster, N. R., 2003. Inactivation of bacteria and spores by pulse electric field and high pressure CO_2 at low temperature. *Biotechnology and Bioengineering* 82**,** 118-125.

Temple, N. J., 2000. Antioxidants and disease: more questions than answers. Nutrition Research 20 (3), 449-459.

Tibble, D. L., 1999. Further evidence of the cardiovascular benefits of diet enriched in carotenoids. American Journal of Clinical Nutrition 68, 521-522.

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.

Ulmer, H. M., Heinz, V., Gänzle, M. G., Knorr, D., Vogel, R. F., 2002. Effects of pulsed electric fields on inactivation and metabolic activity of Lactobacillus plantarum in model beer. Journal of Applied Microbiology 93, 326-335.

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 heat pasteurization. Journal of Agricultural and Food Chemistry 48, 4597-4605.

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Carotenoid profile modification during refrigerated storage in untreated and pasteurized orange juice and orange juice treated with High Intensity Pulsed Electric Fields

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A comparative study was made of the evolution and modification of various carotenoids and vitamin A in untreated orange juice, pasteurized orange juice (90ºC, 20 s), and orange juice processed with High Intensity Pulsed Electric Fields (HIPEF) (30 kV/cm, 100 μs), during 7 weeks of storage at 2ºC and 10ºC.

The concentration of total carotenoids in the untreated juice decreased by 12.6% when the juice was pasteurized, whereas the decrease was only 6.7% when the juice was treated with HIPEF. Vitamin A was greatest in the untreated orange juice, followed by orange juice treated with HIPEF (decrease of 7.52%), and, lastly, pasteurized orange juice (decrease of 15.62%). The decrease in the concentrations of total carotenoids and vitamin A during storage in refrigeration was greater in the untreated orange juice and the pasteurized juice than in the juice treated with HIPEF.

During storage at 10ºC, auroxanthin formed in the untreated juice and the juice treated with HIPEF. This carotenoid is a degradation product of violaxanthin. The concentration of antheraxanthin decreased during storage and it was converted into mutatoxanthin, except in the untreated and pasteurized orange juices stored at 2ºC.

KEYWORDS: carotenoids; orange juice; pulsed electric fields; vitamin A; pasteurized; storage

INTRODUCTION

Orange juice is an excellent source of vitamin A and is a product desired by many consumers who are interested in maintaining a healthy diet. Carotenoids are amongst the most abundant bioactive compounds in vegetables and fruits, and on a worldwide basis about 60% of vitamin A is estimated to come from provitamin A carotenoids, while in developing countries they provide up to 82% (*1*). The importance of carotenoids and the foods that contain them is based on their two most important properties, their antioxidant capacity and their vitamin A activity, with β-carotene having the highest activity of them all, and they are associated with anti-cancer, anti-aging, and anti-ulcer properties (*2*, *3*). Not all carotenoids present in fruits and vegetables are vitamin A precursors. Several of them, including α-carotene, β-carotene and β-cryptoxanthin, have provitamin A activity, being transformed into retinal by mammals (*4*). The main problems associated with carotenoids come from the instability of these pigments, because they are highly unsaturated molecules and are subject to isomerization. The stability of carotenoids during storage is very important for consumer acceptance of the end product. For many years orange juice has been produced in numerous forms, such as frozen concentrate, orange juice from concentrate, and pasteurized juice. Thermal processing is one of the methods by which appropriate foods are preserved and made available to the consumer. During thermal treatment, in addition to the inactivation of microorganisms, varying percentages of desirable constituents such as nutrients, color, flavor, and texture are destroyed. Although these products must conform to strict guidelines which prevent unnatural changes in the juice, concern about diet and nutrition has led consumers to seek a more natural product (*5*-*8*).

The citrus industry has been exploring innovative methods with minimal heat treatment to increase markets by improving nutritional and flavor qualities. High Intensity Pulsed Electric Field (HIPEF) processing, a non-thermal method,

inactivates microorganisms without significant adverse effects on flavor and nutrients (*9-14*).

HIPEF has the potential to pasteurize various foods non-thermally by exposure to short high-voltage pulses while the material passes between electrodes in a treatment chamber. The electric field affects the cell membranes (*15*), and may cause irreversible membrane breakage (*16*), alteration in transport of ions, and changes in enzyme structure (*17*). There are many studies on the effect that this new technology has on microorganisms and their half-life (*18-22*).

A number of authors have studied the evolution of quality factors in orange juice after HIPEF treatment, in some cases making a comparison with the evolution after heat treatment (*12, 23-27*).

Liquid chromatography (LC) is considered to be the method of choice for the separation, identification, and quantification of carotenoids found in biological tissues (*28-31*) and the use of photodiode array detection in the identification of carotenoids by liquid chromatography is a valuable tool for characterization of *cis*- and *trans*- isomers in vegetable and citric juices *(32-34*).

The aim of this work is to study carotenoid profile modifications when natural orange juice is treated by means of pasteurization or HIPEF, and also its evolution during seven weeks of storage in refrigeration at two different temperatures (2ºC and 10ºC), and the transformations of some carotenoids as a result of that storage. A study is also made of the evolution of vitamin A in the different orange juices during the storage period.

MATERIALS AND METHODS

Reagents

α-Carotene, *all-trans*-retinol palmitate, and butylated hydroxytoluene (BHT) (special grade) were purchased from Sigma (Steinheim, Germany). Lutein and zeaxanthin were provided free as standard substances by DSM (Basle, Switzerland). Ammonium acetate (special grade), petroleum ether, hexane (LC

grade), potassium hydroxide (85%), and *tert*-butyl methyl ether (TBME) (LC grade) were purchased from Scharlau (Barcelona, Spain); acetonitrile (LC grade) and magnesium carbonate hydroxide pentahydrate (40–45%) from Panreac (Barcelona, Spain); and ethanol, diethyl ether, methanol, and sodium chloride (LC grade) from Baker (Deventer, The Netherlands). Chloroform was obtained from Merck (Darmstadt, Germany).

Sampling of orange juice

After appropriate washing and hygienisation of the fruits, they were subjected to an extraction process (FMC juice extractors with a 2-mm-diameter sieve) and the juice was introduced into a tank.

HIPEF treatment system

Sample treatments were carried out in a continuous HIPEF treatment system designed by the University of Ohio and located in the Instituto de Agroquímica y Tecnología de Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage, and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, OR U.S.A.). Flow was set at 60 ml/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL). Treatment time was 100 μs and the electric field was set at 30 kV/cm. These treatment conditions were selected on the basis of the results on carotenoid concentration, color, vitamin C, enzymes and microorganisms, obtained when the orange juice was treated using different fields (25, 30, 35, and 40 kV/cm) and different times (30–340 μs) (*5, 18, 19*). Samples were collected after treatment. The experiments were performed in duplicate.

Thermal treatment

To treat the samples, Armfield FT74P unit with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (90ºC, 20 s) were reached. Heating of orange juice at 90–99ºC for 15–30 s is normal in commercial practice (*35*). After treatment, the juice was cooled with cold water from a cooler (ARMFIELD FT61), and it was packed and stored until analysis. The experiments were performed in duplicate.

Storage conditions

Juices were packaged in Elopack packages (pure-pack®), and they were stored in refrigeration and darkness at 2ºC and 10ºC (±1ºC) with controlled humidity. Samples were analyzed in duplicate immediately after processing, then after 1, 2, 3, 4, 6, and 7 weeks of storage.

Determination of Carotenoids

The carotenoids (including geometrical isomers) were identified and quantified by liquid chromatography with UV/Vis-diode array detector (Hewlett-Packard, 1100 series) and a column thermostat (Agilent 1100 series).

An extraction process (ethanol/hexane, 4:3, v/v) was performed, followed by saponification with diethyl ether/methanolic KOH (0.1%, w/v, BHT) (1:1, v/v) for 0.5 h at room temperature. The injection volume used was 20 µl.

A 250 x 4.6 mm Vydac 201TP54 reverse phase C_{18} column with a particle size of 5 µm and a Vydac 201TP precolumn (guard column) (4.6 mm i.d. cartridge with 5-µm particles) (Hesperia, CA, USA) were used. The mobile phase used was methanol (0.1 M ammonium acetate), *tert*-butyl methyl ether, and water (in a concentration gradient) (table 1), and a temperature gradient (table 1) was applied with retinol palmitate as an internal standard. Data

Carotenoids were identified by UV-vis spectra and retention times in HPLC in the juices analyzed using the system HP Chemstation-A.06.03 (*34*). Carotenoids were expessed as µg/100g.

Time (min)	MeOH + AA (%)	H_2O (%)	TBME (%)	Temperature (ºC)
0	95	5	0	20
3	100	0	0	20
5	95	0	5	20
6	95	0	5	30
10	86	0	14	30
15	75	0	25	30
22	95	0	5	20
23	100	0	0	20

AA: 0.1 M ammonium acetate.

Table 1: Mobile phase and temperature gradient for determination of carotenoids by HPLC

Determination of vitamin A

Vitamin A was expressed as retinol activity equivalents (RAE), using the following conversion (*36, 37*): RAE = (μg of β-carotene)/12 + (μg β-cryptoxanthin + μg α-carotene)/24

Statistical Analysis

Carotenoid contents were compared using one-way analysis of variance (ANOVA). An LSD test ($p < 0.05$) was applied. The computer program employed was SPSS (Statistical Package for the Social Sciences) 12.0 for Windows. By means of simple regression, carotenoids concentration versus storage time (weeks) have been related, obtaining with the equation slope the total carotenoid degradation rate (μg/100g*week).

RESULTS AND DISCUSSION

Changes in total carotenoid content and carotenoid profile due to High Intensity Pulsed Electric Fields (HIPEF) and thermal pasteurization of orange juice were studied. Table 2 shows the concentrations of the various carotenoids identified, obtained after applying the pasteurization and HIPEF treatments to the untreated orange juice. The concentration of total carotenoids was significantly lower ($p < 0.05$) in the pasteurized orange juice than in the untreated juice (decrease of 12.57%), whereas in the juice treated with HIPEF, although the concentration of total carotenoids decreased in comparison with the untreated juice (6.73%), the decrease was not significant ($p > 0.05$). These results coincide with those obtained by Lee et al. (*7*) for pasteurized orange juice, in which the total carotenoid content loss was significant ($p < 0.05$) after thermal pasteurization at 90ºC for 30 s. Similarly, the concentration of carotenoids with vitamin A activity was greatest in the untreated orange juice, followed by the orange juice treated with HIPEF (decrease of 7.52%) and, lastly, the pasteurized orange juice (decrease of 15.62%). The only significant differences found ($p < 0.05$) were between untreated orange juice and pasteurized orange juice. Consequently, the non-thermal treatments affected the vitamin A concentration in the refrigerated orange juice less than the conventional thermal treatments did. Sánchez-Moreno et al. (*38)* also studied the variation in total carotenoid and vitamin contents after applying various types of conservation treatments to tomato juice and obtained results similar to those found in the present study.

The study of storage at different temperatures (2 and 10ºC) of the untreated orange juice, juice treated with HIPEF, and pasteurized juice showed that the total carotenoid concentration decreased during this period.

Table 3 shows the values for total carotenoids and vitamin A in untreated, HIPEF-treated, and pasteurized orange juice stored at 2ºC and 10ºC. The degradation rates for total carotenoid concentration in juices stored a 2ºC were

59.10 µg/100g*week (r^2=0.891), 55.47 µg/100g*week (r^2=0.762) and 36.85 µg/100g*week (r^2=0.643), for untreated, pasteurized and HIPEF juices, respectively. In the same way, in the juices stored at 10ºC, the carotenoid concentration degradation rates were 69.94 µg/100g*week (r^2=0.719), 52.70 µg/100g*week (r^2=0.824) and 29.92 µg/100g*week (r^2=0.545) for untreated, pasteurized and HIPEF juices, respectively.

The decrease was greater and faster in the untreated juice, followed by the pasteurized juice, and finally by the juice treated with HIPEF. The decrease in vitamin A was also greater in the orange juice not subjected to any kind of treatment and in the pasteurized juice than in the juice treated with HIPEF. Thereby, the vitamin A degradation rates were 1.08, 0.84 and 0.71 RAE/100g*week for untreated, pasteurized and HIPEF orange juice stored at 2ºC and 1.90, 0.79 and 0.69 RAE/100g*week for untreated, pasteurized and HIPEF orange juice stored at 10ºC.

Table 2 shows that the total of all the carotenoids was less in the pasteurized orange juice than in the juice treated with HIPEF, although not all these decreases were statistically significant. The thermal conservation treatment produced a statistically significant decrease in the concentrations of zeaxanthin, phytoene + phytofluene, and neoxanthin + 9-*cis*-violaxanthin, ((p=0.006, p=0.022, p=0.035, respectively), whereas with HIPEF treatment there was no significant reduction in any of the carotenoids with respect to the untreated orange juice. Lee et al. (*7*) obtained similar results for pasteurized orange juice.

Carotenoid	λ (nm)	Untreated juice	HIPEF juice	Pasteurized juice
neoxanthin + 9-*cis*-violaxanthin	430	444.57 ± 23.21	388.57 ± 14.57	351.11 ± 10.74
antheraxanthin	450	161.93 ± 10.14	140.91 ± 8.96	137.73 ± 4.26
lutein	450	27.28 ± 1.89	25.42 ± 1.13	23.99 ± 0.89
zeaxanthin	450	68.26 ± 0.95	57.28 ± 3.29	56.42 ± 0.85
isolutein	430	61.99 ± 2.76	59.70 ± 1.87	57.57 ± 0.45
β-cryptoxanthin	450	206.42 ± 14.35	185.97 ± 2.15	175.57 ± 8.09
α-carotene	450	49.91 ± 1.82	46.44 ± 4.99	32.94 ± 4.67
9-*cis*-α-carotene	430	31.65 ± 0.21	30.88 ± 0.23	23.97 ± 4.61
phytoene + phytofluene	290	23.34 ± 0.19	22.54 ± 0.72	21.53 ± 0.27
7,8,7',8'-tetrahydrolycopene	430	28.21 ± 0.84	26.49 ± 0.54	25.45 ± 0.38
β-carotene	450	37.21 ± 1.44	35.72 ± 0.95	33.65 ± 0.02
ζ-carotene	430	21.36 ± 0.33	21.71 ± 0.58	21.33 ± 0.02
9-*cis*-β-carotene	450	12.61 ± 0.55	10.56 ± 0. 33	10.38 ± 0.12
Total Carotenoids		1367.17 ± 64.67	1275.16 ± 56.36	1195.37 ± 31.56
Vitamin A		14.63 ± 0.65	13.53 ± 0.65	12.35 ± 0.70

Table 2: Carotenoid concentration (μg/100 g) and Vitamin A (RAE/100 g) in fresh orange juice, HIPEF orange juice, and pasteurized orange juice

The concentration of 13-*cis*-violaxanthin in the orange juice increased with storage, and this increase was greater in the juice stored at 10ºC than in the juice stored at 2ºC (Figure 1). The concentration of this carotenoid increased in both HIPEF-treated juice and in pasteurized juice.

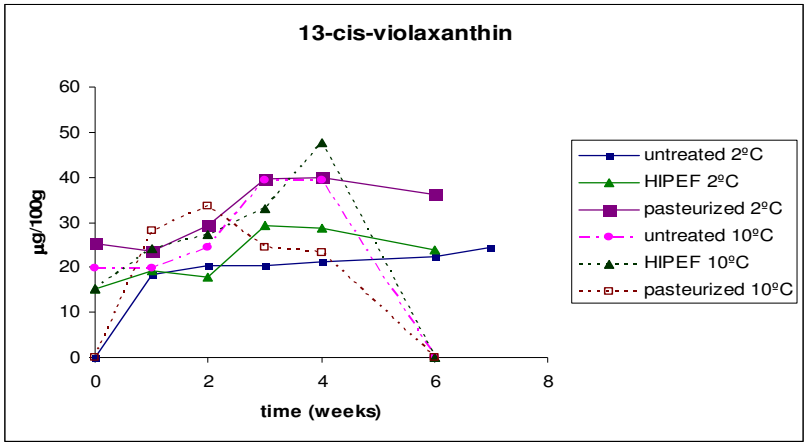
There was a sharp decrease in the neoxanthin + 9-*cis*-violaxanthin mixture with storage (Figure 1), and it was not detected at sixth week onwards in the untreated orange juice, and the juice treated with HIPEF stored at 10ºC. Similar results were obtained in a study of an orange-carrot juice, in which these carotenoids were not detected after three weeks of storage in refrigeration at 10ºC (*40*). It has

been verified that this also happens in the storage of orange-carrot juice frozen at –40ºC (*7*). 9-*cis*-violaxanthin was transformed into its 13-*cis*-violaxanthin form, so that the decrease in the 9-*cis* isomer coincided with the increase in the concentration of the 13-*cis* isomer. The presence of another carotenoid, a rearrangement product of violaxanthin (5,6-epoxycarotenoid), was verified in the untreated orange juice and the juice treated by HIPEF, both stored at 10ºC. It was a furanoid isomer called auroxanthin (5,8,5',8'-diepoxycarotenoid), which appears as a result of the degradation of juice that occurs during storage (*40, 41*). This transformation was only observed at the sixth week of storage. Auroxanthin was not detected in the juices stored at 2ºC, although there was degradation of 9-*cis*-violaxanthin, producing an unidentified compound.

Similarly, as Figure 2 shows, the concentration of antheraxanthin decreased abruptly.

The decrease was greater in the untreated, pasteurized, and HIPEF-treated orange juices stored at 10ºC, and this carotenoid was not detected from the 6th week onwards. Similar results have been reported by Torregrosa (*39*) in a study of pasteurized and HIPEF-treated orange-carrot juice stored in refrigeration, with antheraxanthin not being detected from the 3rd week onwards in storage at 10ºC. The same result was observed in the HIPEF-treated juice stored at 2ºC. However, in the untreated and pasteurized juices stored at 2ºC, this carotenoid was detected throughout the storage period, although its concentration decreased.

(A)



(B)

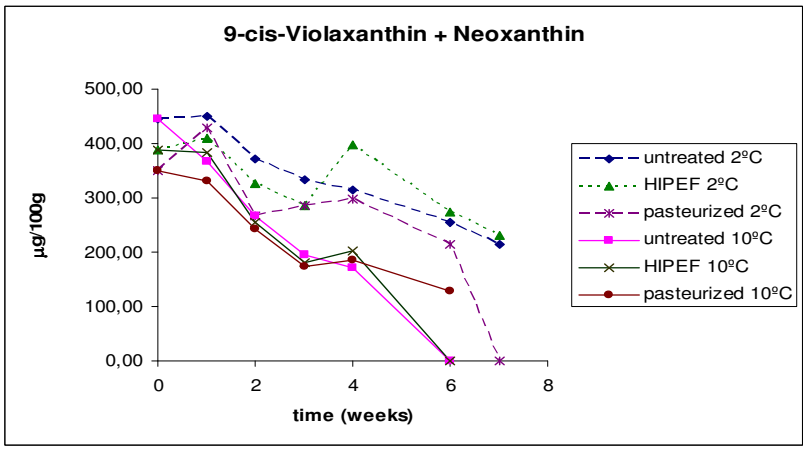
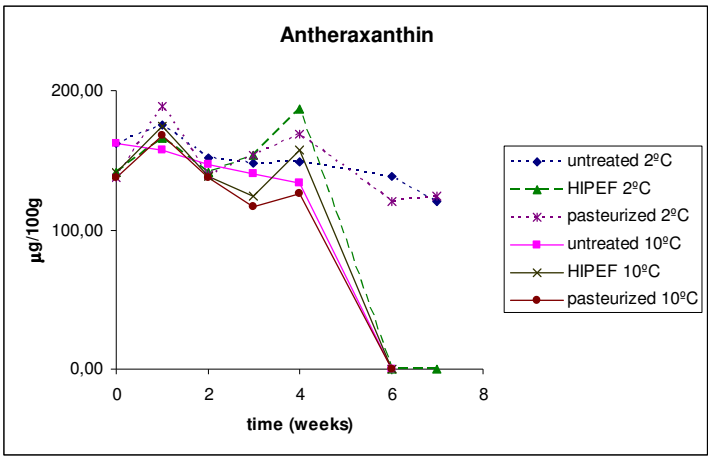


Figure 1. Concentration (µg/100 g) of 13-*cis*-Violaxanthin (A) and 9-*cis*-Violaxanthin + Neoxanthin (B) during storage in untreated, HIPEF-treated, and pasteurized orange juice stored at 2ºC and 10ºC.

(A)



(B)

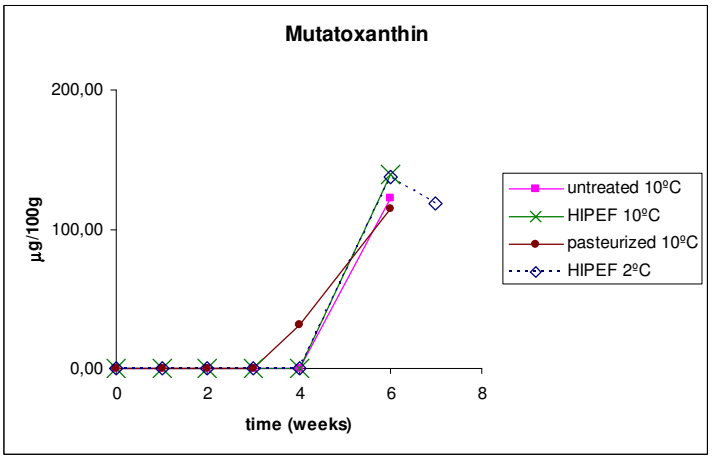


Figure 2. Concentration (µg/100 g) of Antheraxanthin (A) and Mutatoxanthin (B) during storage time in untreated, HIPEF-treated, and pasteurized orange juice stored at 2ºC and 10ºC.

Another carotenoid, mutatoxanthin (Figure 2), was detected in the 6th week of storage, appearing in the cases in which antheraxanthin disappeared. This was not observed in the untreated or pasteurized orange juices stored at 2ºC, in which antheraxanthin continued to be detected. Carotenoid 5,6-epoxides may be transformed to carotenoid 5,8-epoxides under acidic conditions, so antheraxanthin can be converted into the furanoid structure mutatoxanthin (*40*).

As Tables 4, 5 and 6 show, the concentration of lutein increased after the first week of storage in all the orange juices studied, and thereafter the concentration remained constant and in some cases decreased in the seventh week of storage. Application of statistical analysis showed that in the pasteurized juice lutein had a negative tendency, statistically non-significant, throughout storage at both temperatures, whereas in the HIPEF-treated juice this carotenoid had a non-significant increase during the storage period studied. These results do not coincide with those observed by Lin and Chen (*42*) for tomato juice stored at 4ºC for 12 weeks, in which the concentration of lutein decreased and it was no longer detected in the 5th week of storage. In a result similar to the present study, Torregrosa (*39*) also detected a slight increase in lutein during storage in pasteurized orange-carrot juice. The concentrations of zeaxanthin and isolutein decreased during the first week of storage in all the juices studied, followed by an increase during the rest of the storage period (Tables 4 to 6). In the pasteurized juice there was a statistically significant decrease in the concentrations of zeaxanthin and isolutein (r^2=-0.388, p=0.05 and r^2=-0.613, p=0.01, respectively), whereas in the HIPEF-treated juice the changes in the concentrations of these carotenoids during storage were not statistically significant ($p > 0.05$).

			Storage time (weeks)						
Temperature	**Item**	**Juice**	**0**	**1**	**2**	**3**	**4**	**6**	**7**
2ºC	Total carotenoid	Fresh	1367.17 (64.67)	1272.24 (11.70)	1200.80 (29.78)	1184.18 (60.04)	985.23 (80.20)	1023.10 (47.37)	934.56 (23.36)
		HIPEF	1275.16 (56.36)	1174.44 (54.85)	1129.42 (106.73)	1237.42 (95.90)	1234.27 (52.09)	1018.28 (15.99)	964.23 (12.05)
		Pasteurized	1195.37 (31.56)	1305.72 (38.39)	1117.57 (57.55)	1237.42 (95.90)	1020.62 (84.71)	881.99 (13.23)	913.31 (72.33)
	Vitamin A	Fresh	14.63 (0.65)	11.14 (0.23)	11.24 (0.24)	11.89 (0.61)	9.15 (0.71)	9.78 (0.52)	9.97 (0.35)
		HIPEF	13.53 (0.65)	10.21 (0.55)	10.56 (1.02)	12.29 (1.20)	11.19 (0.67)	9.42 (0.14)	10.04 (0.57)
		Pasteurized	12.35 (0.70)	10.84 (0.23)	10.61 (0.76)	12.29 (1.20)	9.78 (1.07)	8.64 (0.17)	9.55 (0.81)
10ºC	Total carotenoid	Fresh	1367.17 (64.67)	1118.32 (73.59)	1138.81 (16.97)	1120.09 (84.45)	908.42 (64.95)	964.65 (52.19)	*
		HIPEF	1275.16 (56.36)	1258.01 (92.21)	1097.98 (61.49)	1082.87 (0.11)	1101.82 (30.08)	1110.26 (65.17)	*
		Pasteurized	1195.37 (31.56)	1122.58 (0.22)	1087.53 (11.42)	919.66 (48.01)	845.15 (39.47)	854.20 (41.94)	*
	Vitamin A	Fresh	14.63 (0.65)	9.99 (0.23)	11.69 (1.04)	9.69 (0.65)	9.53 (0.93)	7.61 (0.23)	*
		HIPEF	13.53 (0.65)	11.37 (0.84)	10.65 (0.53)	12.20 (0.33)	11.63 (0.43)	11.39 (0.29)	*
		Pasteurized	12.35 (0.70)	9.53 (0.11)	10.19 (0.13)	9.58 (0.79)	9.00 (0.38)	9.54 (0.39)	*

**: spoiled orange juice. Not analyzed. The standard deviation is shown in parentheses.*

Table 3: Total carotenoid concentration (μg/100g) and vitamin A (RAE/100g) in untreated, HIPEF-treated, and pasteurized orange juice stored at 2ºC and 10ºC.

		Storage time (weeks)						
Temperature	**Carotenoid**	**0**	**1**	**2**	**3**	**4**	**6**	**7**
2ºC	lutein	27.28 (1.89)	33.31 (0.33)	31.37 (0.38)	31.19 (1.80)	33.59 (3.13)	32.72 (0.96)	27.61 (2.99)
	zeaxanthin	68.26 (0.95)	46.69 (1.07)	50.91 (0.95)	55.91 (4.12)	57.01 (6.27)	50.19 (0.49)	47.77 (1.54)
	isolutein	61.99 (2.75)	45.41 (0.40)	52.04 (2.05)	57.59 (3.48)	46.56 (0.20)	46.39 (1.15)	48.80 (0.53)
	β-cryptoxanthin	206.42 (14.35)	143.88 (0.36)	143.27 (2.32)	157.84 (7.75)	150.70 (12.36)	122.78 (1.15)	133.39 (4.87)
	phytoene-phytofluene	23.34 (0.19)	24.54 (1.04)	23.06 (0.94)	29.68 (1.63)	23.65 (1.56)	29.50 (0.94)	26.88 (1.53)
	7,7',8,8'-tetrahydrolycopene	28.21 (0.84)	29.44 (0.17)	29.93 (1.04)	35.47 (1.77)	24.97 (1.98)	37.27 (1.01)	32.35 (0.93)
	β-carotene	37.21 (1.44)	36.47 (0.53)	36.80 (2.19)	40.67 (1.98)	23.87 (1.55)	38.47 (2.29)	34.86 (1.29)
	ζ-carotene	21.36 (0.33)	20.68 (0.02)	20.95 (0.46)	25.17 (1.39)	21.60 (1.60)	35.30 (6.69)	36.67 (0.99)
10ºC	lutein	27.28 (1.89)	31.80 (2.03)	34.36 (0.98)	34.29 (0.65)	35.80 (1.38)	31.86 (0.39)	*
	zeaxanthin	68.26 (0.95)	45.31 (4.90)	53.29 (0.77)	63.08 (0.32)	62.18 (5.71)	50.69 (1.79)	*
	isolutein	61.99 (2.76)	43.65 (1.05)	56.31 (1.14)	59.62 (2.24)	52.29 (4.25)	48.92 (2.07)	*
	β-cryptoxanthin	206.42 (14.35)	133.45 (4.70)	148.97 (1.70)	154.14 (8.31)	151.93 (9.54)	125.19 (1.59)	*
	phytoene-phytofluene	23.34 (0.19)	22.82 (0.97)	24.88 (0.82)	28.93 (1.13)	23.01 (1.27)	30.69 (2.42)	*
	7,7',8,8'-tetrahydrolycopene	28.21 (0.84)	26.78 (0.24)	26.72 (4.71)	37.73 (3.13)	27.86 (1.92)	21.79 (0.32)	*
	β-carotene	37.21 (1.44)	33.37 (0.20)	37.42 (0.15)	39.43 (3.66)	26.94 (1.21)	ND	*
	ζ-carotene	21.36 (0.33)	19.81 (0.62)	38.09 (0.60)	26.89 (2.43)	23.52 (1.74)	28.14 (2.03)	*

The results are the mean of two determinations. The standard deviation is shown in parentheses.

**: spoiled orange juice. Not analyzed. ND: not detected.*

Table 4: Carotenoid concentration (μg/100 g) in untreated orange juice during refrigerated storage.

		Storage time (weeks)						
Temperature	**Carotenoid**	**0**	**1**	**2**	**3**	**4**	**6**	**7**
2ºC	lutein	25.42 (1.13)	29.94 (2.26)	28.07 (2.50)	31.45 (4.48)	45.23 (0.27)	32.29 (0.71)	27.74 (0.30)
	zeaxanthin	57.28 (3.29)	43.71 (2.14)	45.88 (4.85)	58.98 (8.15)	74.74 (2.14)	47.63 (0.80)	46.83 (2.93)
	isolutein	59.70 (1.87)	46.24 (1.78)	51.35 (4.35)	59.57 (7.74)	62.84 (0.09)	45.26 (0.43)	49.52 (0.91)
	β-cryptoxanthin	185.97 (8.09)	132.72 (7.54)	133.36 (8.75)	159.68 (16.85)	152.32 (2.82)	124.63 (2.06)	135.85 (1.34)
	phytoene-phytofluene	22.54 (0.72)	22.99 (0.90)	23.27 (0.37)	30.92 (3.08)	30.45 (0.24)	30.05 (0.65)	27.71 (0.05)
	7,7',8,8'-tetrahydrolycopene	26.49 (0.76)	27.47 (1.97)	28.15 (2.05)	36.71 (5.40)	26.85 (3.83)	35.63 (0.63)	33.77 (0.68)
	β-carotene	35.72 (0.95)	32.13 (2.89)	35.94 (2.64)	43.03 (4.44)	30.17 (0.89)	37.29 (0.38)	36.55 (3.08)
	ζ-carotene	21.71 (0.58)	20.47 (1.50)	21.64 (1.98)	29.73 (3.38)	19.23 (2.41)	27.25 (0.68)	32.59 (6.25)
10ºC	lutein	25.42 (1.13)	35.63 (2.43)	29.83 (2.29)	30.13 (2.08)	42.63 (1.74)	35.26 (0.49)	*
	zeaxanthin	57.28 (3.29)	52.76 (5.79)	51.45 (7.06)	55.38 (1.36)	74.99 (2.77)	60.63 (2.35)	*
	isolutein	59.70 (1.87)	56.02 (5.45)	56.05 (3.41)	57.95 (0.53)	64.80 (1.74)	53.97 (1.55)	*
	β-cryptoxanthin	185.97 (8.09)	150.63 (13.38)	137.14 (8.98)	144.80 (1.36)	142.55 (8.02)	137.49 (4.91)	*
	phytoene-phytofluene	22.54 (0.72)	26.79 (2.64)	23.84 (0.32)	29.24 (0.80)	28.95 (0.89)	35.20 (3.60)	*
	7,7',8,8'-tetrahydrolycopene	26.49 (0.76)	32.50 (1.22)	31.25 (1.77)	38.17 (0.14)	33.30 (0.27)	ND	*
	β-carotene	35.72 (0.95)	37.90 (1.91)	36.83 (1.47)	40.73 (2.66)	32.10 (1.26)	49.84 (3.59)	*
	ζ-carotene	21.71 (0.58)	24.89 (1.40)	27.73 (0.58)	49.29 (1.92)	35.46 (0.18)	59.72 (3.49)	*

The results are the mean of two determinations. The standard deviation is shown in parentheses.

**: spoiled orange juice. Not analyzed. ND: not detected.*

Table 5: Carotenoid concentration (µg/100 g) in orange juice treated by HIPEF during refrigerated storage

		Storage time (weeks)						
Temperature	**Carotenoid**	**0**	**1**	**2**	**3**	**4**	**6**	**7**
2ºC	lutein	23.99 (0.89)	33.47 (0.13)	27.96 (1.10)	31.45 (4.58)	36.08 (3.73)	28.90 (0.33)	27.92 (2.78)
	zeaxanthin	56.42 (0.85)	52.56 (1.15)	48.76 (4.23)	58.98 (2.25)	60.50 (10.24)	39.43 (1.58)	48.47 (3.82)
	isolutein	57.57 (0.45)	51.62 (3.72)	52.50 (2.58)	59.57 (3.62)	52.21 (3.55)	39.75 (1.38)	47.29 (3.26)
	β-cryptoxanthin	175.57 (2.14)	139.88 (5.19)	131.32 (6.19)	159.68 (17.55)	151.48 (21.07)	111.82 (13.98)	129.05 (10.23)
	phytoene-phytofluene	21.53 (0.27)	29.31 (0.11)	22.68 (0.31)	30.92 (6.17)	26.96 (2.87)	28.79 (0.90)	28.26 (1.81)
	7,7',8,8'-tetrahydrolycopene	25.45 (0.16)	31.22 (1.31)	29.37 (1.63)	36.71 (5.40)	22.99 (1.82)	33.29 (0.02)	31.23 (2.51)
	β-carotene	33.65 (0.02)	36.85 (2.56)	38.55 (2.64)	43.03 (8.30)	25.75 (2.44)	35.54 (0.94)	32.72 (2.97)
	ζ-carotene	21.33 (0.02)	25.88 (1.65)	26.11 (3.80)	29.73 (3.38)	21.66 (1.04)	24.75 (1.00)	35.29 (3.40)
10ºC	lutein	23.99 (0.89)	32.28 (2.74)	29.75 (2.33)	31.46 (3.28)	32.72 (0.06)	30.56 (1.81)	*
	zeaxanthin	56.42 (0.85)	50.69 (2.17)	47.42 (3.13)	49.50 (3.13)	46.60 (9.20)	46.47 (2.66)	*
	isolutein	57,57 (0.45)	52.10 (0.35)	51.01 (2.86)	50.95 (3.55)	53.01 (2.57)	43.23 (2.01)	*
	β-cryptoxanthin	175.57 (2.14)	138.06 (0.84)	128.60 (1.77)	131.51 (10.82)	140.99 (9.00)	112.25 (2.69)	*
	phytoene-phytofluene	21.53 (0.27)	24.42 (0.75)	22.60 (0.08)	26.77 (2.45)	23.42 (0.79)	28.46 (0.89)	*
	7,7',8,8'-tetrahydrolycopene	25.45 (0.16)	28.56 (0.50)	29.59 (1.32)	31.86 (2.16)	25.52 (0.59)	36.83 (1.07)	*
	β-carotene	33.65 (0.02)	34.46 (0.83)	35.06 (1.80)	36.76 (4.10)	25.20 (0.14)	37.16 (0.55)	*
	ζ-carotene	21.33 (0.02)	22.15 (1.72)	26.84 (1.63)	25.30 (0.03)	24.36 (1.61)	42.81 (0.72)	*

The results are the mean of two determinations. The standard deviation is shown in parentheses.

**: spoiled orange juice. Not analyzed.*

Table 6: Carotenoid concentration (µg/100g) in pasteurized orange juice during refrigerated storage.

The concentration of β-cryptoxanthin decreased during storage, especially in the first week, and the decrease was greater in the orange juices stored at 10ºC. Also, the decrease was greater (r^2=–0.543, p=0.04) in the pasteurized orange juice than in the juice treated with HIPEF (p > 0.05) (Tables 4 to 6). However, orange-carrot juice stored in refrigeration did not show the same behavior (*39*), although β-cryptoxanthin did decrease when the juice was stored at –40ºC (*6*).

There was a decrease in the concentration of α-carotene during the first weeks of storage at both temperatures (Figure 3). It was not detected from the 3rd week onwards in the pasteurized juice, and from the 4th week in the untreated and HIPEF-treated orange juices. The concentration of this carotenoid also decreased in refrigerated orange-carrot juice, although it was detected throughout the storage period (*39*). Oxidation is the major cause of carotenoid loss, and it depends on the carotenoid involved.

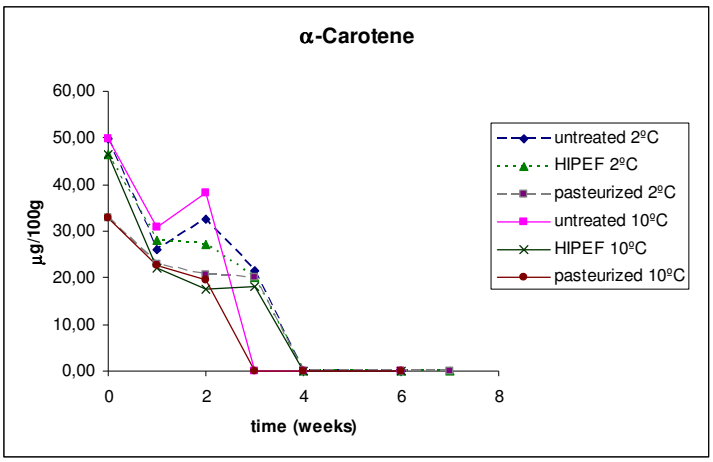


Figure 3. Concentration (µg/100 g) of α-Carotene during storage time in untreated, HIPEF-treated, and pasteurized orange juice stored at 2ºC and 10ºC.

There was a slight but significant increase (p<0.05) in 7,8,7',8'-tetrahydrolycopene and the phytonene-phytofluene mixture during storage, in

both the pasteurized juice (r^2=0.412, p=0.037 and r^2= 0.460, p=0.018, respectively) and the HIPEF-treated juice (r^2 = 0.526, p=0.008 and r^2 = 0.724, p=0.000) (Tables 4 to 6). There were no statistically significant changes in the concentration of β-carotene during storage in all the juices studied.

There was a slight increase ($p<0.05$) in the concentration of ζ-carotene during storage (r^2 = 0.562, p=0.003 and r^2=0.452, p=0.027, for the pasteurized orange juice and HIPEF-treated juice, respectively), except in the untreated juice stored at 10ºC, in which this carotenoid disappeared during the first weeks.

CONCLUSIONS

Non-thermal treatments had less effect than conventional thermal treatments on the concentrations of total carotenoids and vitamin A in refrigerated orange juice. With HIPEF treatment, there was no significant decrease in the concentration of any carotenoid with respect to the untreated juice. During storage in refrigeration, total carotenoids and vitamin A were maintained for a longer time in the juice treated with HIPEF than in the juice conserved using conventional pasteurization treatments.

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LITERATURE CITED

(1) Rodriguez-Amaya, D. B. Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed and stored foods. The Opportunities for Micronutrient Interventions Project (OMNI), USAID. **1997**.

(2) van den Berg, H.; Faulks, R.; Granado, H. F.; Hirschberg, J.; Olmedilla, B.; Sandmann, G.; Southon, S.; Stahl, W. The potential for the improvement of carotenoid levels in foods and the likely systemic effects, *J. Sci. Food Agric.* **2000**, *80*, 880–912.

(3) Marco, D. B. F.; Joseph, V.; John, K. Mechanisms of disease: antioxidants and atherosclerotic heart disease, *New Eng. J. Med.* **1997**, *337*, 408–416.

(4) Simpson, K. L.; Chichester, C. O. Metabolism and nutritional significance of carotenoids, *An. Rev. Nutr.* **1981,** 351–371.

(5) Cortés, C.; Esteve, M. J.; Rodrigo, D.; Torregrosa, F.; Frígola, A. Changes of color and carotenoid contents during high intensity pulsed electric field treatment in orange juices. *Food Chem. Toxicol.* **2006**, in press.

(6) Cortés, C.; Esteve, M. J.; Frígola, A.; Torregrosa, F. Changes in carotenoids including geometrical isomers and ascorbic acid content in orange-carrot juice during frozen storage. *Eur. Food Res. Technol.* **2005**, *221*, 125–131.

(7) Lee, H. S.; Coates, G. A. Effect of thermal pasteurization on Valencia orange juice color and pigments. *Lebensm.-Wiss. u. –Technol.* **2003**, *36*, 153–156.

(8) Lee, H. S.; Coates, G. A. Characterization of color fade during frozen storage of red grapefruit juice concentrates. *J. Agric. Food Chem.* **2002**, *50*, 3988–3991.

(9) Bendicho, S.; Arántegui, J.; Martín, O. Procesado de alimentos mediante pulsos eléctricos de alta intensidad de campo. II. Efectos sobre microorganismos y componentes de los alimentos. *Alimentaria.* **2001**, *323*, 37–44.

(10) Abram, F.; Smelt, J. P. P. M.; Bos, R.; Wouters, P. C. Modelling and optimization of inactivation of Lactobacillus plantarum by pulsed electric field treatment. *J. Appl. Microbiol.* **2003**, *94*, 571–579.

(11) Cortés, C.; Esteve, M. J.; Frígola, A.; Torregrosa, F. Quality characteristics of horchata (a Spanish vegetable beverage) treated with pulsed electric fields during shelf-life. *Food Chem.* **2004**, *91* (2), 319–325.

(12) Rivas, A.; Rodrigo, D.; Martínez, A.; Barbosa-Cánovas, G. V.; Rodrigo, M. Effect of PEF and heat pasteurization on the physical-chemical characteristics of blended orange and carrot juice. *LWT-Food Sci. Technol.* **2005**, in press, available online 10 August 2005.

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

(14) Torregrosa, F.; Esteve, M. J.; Frígola, A.; Cortés, C. Ascorbic Acid stability during refrigerated storage of orange-carrot juice treated by High Pulsed Electric Fields and comparison with pasteurized juice. *J. Food Eng.* **2006**, *73*, 339–345.

(15) Barsotti, L.; Cheftel, J. C. Food processing by pulsed electric fields. II. Biological aspects. *Food Rev. Int.* **1999**, *5*, 181–213.

(16) Calderón-Miranda, M. L.; Barbosa-Cánovas, G. V.; Swanson, B. G. Transmission electron microscopy of *Listeria innocua* treated by pulsed electric fields and nisin in skimmed milk. *Int. J. Food Microbiol.* **1999**, *51*, 31–39.

(17) Fernandez-Díaz, M. D.; Barsotti, L.; Dumay, E.; Cheftel, J. C. Effects of Pulsed Electric Fields on ovalbumin solutions and on liquid egg white. *J. Agric. Food Chem.* **2000**, *48*, 2332–2339.

(18) Rodrigo, D.; Martínez, A.; Harte, F.; Barbosa-Cánovas, G. V.; Rodrigo, M. Study of inactivation of Lactobacillus plantarum in orange-carrot juice by means of pulsed electric fields: comparison of inactivation kinetics. *J. Food Prot.* **2001**, *64*, 259–263.

(19) Rodrigo, D.; Barbosa-Cánovas, G. V.; Martínez, A.; Rodrigo, M. Pectin Methyl Esterase and Natural Microbial Flora of Fresh Mixed Orange and Carrot Juice treated with Pulsed Electric Fields. *J. Food Prot.* **2003**, *66*, 2336–2342.

(20) Spilimbergo, S.; Dehghani, F.; Bertucco, A.; Foster, N. R. Inactivation of bacteria and spores by pulse electric field and high pressure CO_2 at low temperature. *Biotechnol. Bioeng.* **2003**, *82* (1), 118–125.

(21) Selma, M. V.; Salmerón, M. C.; Valero, M.; Fernández, P. S. Control of Lactobacillus plantarum and Escherichia coli by pulsed electric fields in MRS Broth, Nutrient Broth and orange-carrot juice. *Food Microbiol.* **2004**, *21*, 519–525.

(22) Gómez, N.; García, D.; Álvarez, I.; Raso, J.; Condón, S. A model describing the kinetics of inactivation of *Lactobacillus plantarum* in a buffer system of different pH and in orange and apple juice. *J. Food Eng.* **2005**, *70*, 1, 7–14.

(23) Yeom, H. W.; Streaker, C. B.; Zhang, Q. H.; Min, D. B. Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *J. Agric. Food Chem.* **2000***, 48*, 4597–4605.

(24) Ayhan, Z.; Yeom, H. Y.; Zhang, Q. H.; Min, D. B. Flavour, colour, and vitamin C retention of PEF processed orange juice in different packaging materials. *J. Agric. Food Chem.* **2001**, *49*, 669–674.

(25) Min, D. B.; Zhang, Q. H. Pulsed electric field processing to improve the freshness of orange and tomato juices. In *The Institute of Food Technology meeting: Book of abstracts.* 2002.

(26) Min, S.; Jin, Z. T.; Yeom, H.; Min, S. K.; Zhang, Q. H. Commercial-scale pulsed electric field processing of orange juice. *Food Chem. Toxicol.* **2003**, *68* (4), 1265–1271.

(27) Cserhalmi, Zs.; Sass-Kiss, Á.; Tóth-Markus, M.; Lechner, N. Study of pulsed electric field treated citrus juices. *Inn. Food Sci. Emerg. Technol*. **2006**, 7 (1-2), 49-54.

(28) Kidmose, U.; Knuthsen, P.; Edelenbos, M.; Justesen, U.; Hegelung, E. Carotenoids and flavonoids in organically grown spinach (Spinacia oleracea L.) genotypes after deep frozen storage, *J. Sci. Food Agric.* **2001**, *81*, 918–923.

(29) De Sio, F.; Servillo, L.; Loiuduce, R.; Laratta, B.; Castaldo, D. A chromatographic procedure for the determination of carotenoids and chlorophylls in vegetable products. *Acta Alimentaria.* **2001**, *30*, 395–405.

(30) Humphries, J. M.; Khachik, F. Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat and pasta products. *J. Agric. Food Chem,* **2003**, *51*, 1322–1327.

(31) Mendes-Pinto, M. M.; Ferreira, A. C.; Oliveira, M. B.; Guedes de Pinho, P. Evaluation of some carotenoids in grapes by reversed- and normal-phase liquid chromatography: a qualitative analysis. *J. Agric. Food Chem,* **2004**, *52* (10), 3182–3188.

(32) Rouseff, R.; Raley, L.; Hofsommer, H. J. Application of diode-array detection with a C30 reversed-phase column for the separation and identification of saponified orange juice carotenoids. *J. Agric. Food Chem.* **1996**, *44*, 2176–2181.

(33) Kimura, M.; Rodriguez-Amaya, D. B. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. *Food Chem.* **2002**, *78* (3), 389–398.

(34) Cortés, C.; Esteve, M. J.; Frígola, A.; Torregrosa, F. Identification and quantification of carotenoids including geometrical isomers in fruit and vegetable juices by liquid chromatography with ultraviolet-diode array detection. *J. Agric. Food Chem.* **2004**, *52*, 2203–2212.

(35) Braddock, R. J. Single strength orange juices and concentrate. In *Handbook of Citrus by-products and Processing Technology*; Wiley, J.; New York, 1999; 53–83.

(36) Institute of Medicine (IOM). Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press, Washington, DC, 2001.

(37) Trumbo P. R.; Yates A. A; Schlicker-Renfo S.; Suitor, C. Dietary Reference Intakes: revised nutritional equivalents for folate, vitamin E and provitamin A carotenoids. *J. Food Comp. Anal.* **2003**, *16*, 379–382.

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

(39) Torregrosa, F. Determinación de Vitamina C y Carotenoides en zumos de frutas y hortalizas tratados con calor o por HIPEF. Doctoral thesis. **2005**, Universitat de València. Valencia. Spain.

(40) Giger, A. Chemical synthesis project. A new yellow carotenoid. *Pure Appl. Chem.* **2002**, *74*, 1383–1390.

(41) Asai, A.; Terasaki, M.; Nagao, A. An epoxide-furanoid rearrangement of spinach Neoxanthin occurs in the gastrointestinal tract of mice and in vitro: formation and cytostatic activity of neochrome stereoisomers. *J. Nutr.* **2004**, *134*, 2237–2243.

(42) Lin, C. H.; Chen, B. H. Stability of carotenoids in tomato juice during storage. *Food Chem.* **2005**, *90* (4), 837–846.

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Ascorbic acid content in refrigerated orange juice after HIPEF and thermal treatment

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ABSTRACT

Application of high intensity pulsed electric fields (HIPEF) can lead to longer shelf life of fruit juices with minimal product quality loss and good retention of fresh-like flavour. The aim of this study was to evaluate the effect of HIPEF and conventional pasteurization on vitamin C content of orange juice, and to assess modifications in vitamin C concentration of orange juice stored in refrigeration at 2 and 10 ºC for 7 weeks.

The ascorbic acid degradation rate (k) obtained was –0.0003, –0.0006, –0.0009 and –0.0010 μs^{-1} for fields of 25, 30, 35 and 40 kV/cm, respectively.

The ascorbic acid degradation rate (k) for the HIPEF-treated juices was 0.0070 $days^{-1}$ (r^2 = 0.9987) and 0.0058 $days^{-1}$ (r^2 = 0.9882) at 10 ºC and 2 ºC, respectively, and in the pasteurized juices was 0.0195 $days^{-1}$ (r^2 = 0.9638) in the juice stored at 10 ºC and 0.0078 $days^{-1}$ (r^2 = 0.9718) in the juice stored at 2 ºC. Shelf life of the juice increases when the storage temperature is 2 ºC.

Keywords: Ascorbic acid, ºBrix, HIPEF, orange juice, storage

INTRODUCTION

Consumption of fruit and vegetables can prevent certain degenerative chronic diseases, such as cancer and cardiovascular illnesses, because these foods are rich in antioxidant compounds such as vitamins C and E, phenolic compounds and carotenoids [1-5]. Among fruits and fruit products, oranges and orange juice can be highlighted as important sources of vitamin C and carotenoids because of their relatively high consumption. Vitamin C is considered the most water-soluble antioxidant. It is a nutrient which, in addition to its vitamin action, is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported, such as inhibition of the formation of cancer-causing N-nitroso compounds in the stomach [6-7]. Orange juice suffers a considerable number of deterioration reactions during storage (degradation of ascorbic acid, cloud loss, microbial spoilage, development of off-flavour, changes in colour, texture and appearance), which result in a loss of product quality [8-14]. Vitamin C is thermolabile and therefore in fruit and vegetables it provides an indication of the loss of other vitamins and acts as a valid criterion for other organoleptic or nutritional components, such as natural pigments and aromatic substances. Its concentration decreases during storage, depending on storage conditions, such as temperature, oxygen content and light [13-19].

Although it has been shown that conventional heat treatments provide safety in food products and lengthen their shelf life, in many cases they are accompanied by nutritional and sensory losses, leading above all to a loss of vitamin C and antioxidants and flavour compounds [14, 20-21].

Owing to consumer demand, the citrus industry has been exploring innovative processing methods with minimal heat treatment, to increase markets by improving the nutritional and flavour qualities of orange juice [22]. High Intensity Pulsed Electric Fields (HIPEF) is one of these innovative technologies in which flavour and nutritional quality are usually unaffected. HIPEF

processing is an emerging technology in the field of food preservation. HIPEF has the potential to pasteurize various foods non-thermally via exposure to short high-voltage pulses while the material is between the electrodes of a treatment chamber. The electric field affects cell membranes [23] and can cause irreversible breakage of membranes [24-25], alteration in ion transport [26] and changes in enzyme structure [27-28].

Application of HIPEF can lead to longer shelf life of fruit juices with minimal product quality loss and good retention of fresh-like flavour [14, 29-32].

The effect of this emerging technology on the vitamin C concentration of the orange juice is an important topic to be studied. The aim of this study was to evaluate the effect of HIPEF on the vitamin C content of orange juice in comparison with conventional pasteurization, and to assess modifications in vitamin C content when the juice was refrigerated at two temperatures (2 and 10 ºC) and stored for 7 weeks.

MATERIALS AND METHODS

Sampling of orange juice

After appropriate washing and hygienization of the fruits, they were subjected to an extraction process (FMC juice extractors with a 2-mm-diameter sieve) and the juice was introduced into a tank.

HIPEF treatment system

Sample treatments were carried out in a continuous HIPEF treatment system designed by the University of Ohio and located in the Instituto de Agroquímica y Tecnología de los Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS

210, Tektronix, OR U.S.A.). The flow was set at 60 ml/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL). In order to decide on the most appropriate HIPEF conditions, a number of alternative processing conditions were tested (four electric fields and six treatment times for each field). Treatment time ranged from 30 to 340 μs and the electric field was set at 25, 30, 35 and 40 kV/cm. Samples were collected after each treatment. The experiments were performed in duplicate.

Thermal treatment

To treat the samples ARMFIELD FT74P equipment with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (90 ºC, 20 s) are reached. Heating of orange juice at 90–99 ºC for 15–30 s is normal in commercial practice [33]. After treatment, the juice was cooled with cold water from a cooler (Armfield FT61), and it was packed and stored at 2 ºC and 10 ºC until analysis. The experiments were performed in duplicate.

Polarographic determination of Ascorbic acid

5 ml of juice was diluted to 25 ml with the extraction solution: oxalic acid (Panreac, Barcelona, Spain) 1%, w/v, trichloroacetic acid (Baker, Deventer, Holland) 2%, w/v, sodium sulphate (Baker, Deventer, Holland) 1%, w/v. After vigorous shaking the solution was filtered through a folded filter (Whatman no. 1). 9.5 ml of 1% (w/v) oxalic acid and 2 ml of 2M acetic acid/sodium acetate (Panreac, Barcelona, Spain) buffer (pH = 4.8) were added to an aliquot of 0.5 ml of filtrate and the solution was transferred to the polarographic cell. The following instrumental conditions were applied: DP_{50}, mode DME, drop size 2, drop time 1 s, scan rate 10 mV/s, initial potential –0.10 V. Determinations were carried out using the peak heights and standard additions method [34].

Storage conditions

The juice was packaged in Elopack packages (Pure-Pack®) and stored in refrigeration and darkness at 2 ºC and 10 ºC (±1 ºC) with controlled humidity.

Samples were analyzed in duplicate immediately after processing, then after 1, 2, 3, 4, 6 and 7 weeks of storage.

Physicochemical determinations

pH determination was based on the potentiometric measurement at 20 ºC. It was determined in a Crison GLP 21 pH meter equipped with a temperature compensation sensor.

ºBrix was determined by measurement of the refraction index with an Atago model RX-1000 digital refractometer. In citrus juices, ºBrix is used to indicate the percentage of soluble solids, and it is one of the most important factors for grading the quality of a citrus juice [35].

RESULTS AND DISCUSSION

We studied different electric fields (25, 30, 35 and 40 kV/cm), and six treatment times (from 30 to 340 μs) in each field. The results obtained appear in Table 1, which shows a decrease in the concentration of ascorbic acid as treatment time increases for a given electric field intensity.

The concentration of ascorbic acid in the fresh orange juice not subjected to any treatment was 47.56 ± 1.36 mg/100 ml. When the remaining concentration of ascorbic acid versus treatment time in each of the fields was adjusted by least squares, we observed that it followed a zero-order kinetics (see Figure 1): $C = C_0 - kt$, where C is the concentration of ascorbic acid after treatment (mg/100 ml), C_0 is the concentration in the untreated juice, k is the ascorbic acid loss rate (μs^{-1}), and t is the treatment time (μs). The ascorbic acid degradation rate (k) obtained was $-0.0003\ \mu s^{-1}$ ($r^2 = 0.840$), $-0.0006\ \mu s^{-1}$ ($r^2 = 0.921$), $-0.0009\ \mu s^{-1}$ ($r^2 = 0.900$) and $-0.0010\ \mu s^{-1}$ ($r^2 = 0.928$) for fields of 25, 30, 35 and 40 kV/cm, respectively. The fit was significant at 95% ($p<0.05$) in all cases.

Field (kV/cm)	Time (µs)	Ascorbic acid ± SD (mg/100 ml)
25	40	47.60 ± 0.33
	100	45.24 ± 0.72
	170	45.67 ± 1.17
	240	44.73 ± 0.75
	300	43.77 ± 2.50
	340	38.82 ± 1.63
30	40	45.31 ± 0.59
	80	45.03 ± 0.56
	100	44.78 ± 1.31
	150	43.74 ± 0.78
	190	42.54 ± 0.67
	240	41.64 ± 0.71
35	40	45.42 ± 0.73
	60	44.32 ± 1.78
	80	43.28 ± 1.54
	100	43.17 ± 0.81
	140	42.01 ± 0.97
	170	41.41 ± 1.34
40	30	46.19 ± 1.53
	40	45.12 ± 0.97
	60	44.02 ± 0.76
	80	44.23 ± 1.21
	100	43.69 ± 1.20
	130	41.54 ± 0.37

SD: standard deviation.

Table 1. Ascorbic acid concentration (mg/100 ml) in orange juice for different fields and treatment times.

The degradation of ascorbic acid studied by other authors, when thermal treatments are applied, fits first-order kinetics. This can be explained by taking into account the fact that in HIPEF the treatment time lasts for μs, whereas in thermal processes the time during which heat is applied is longer, and in this case the degradation curves divide clearly into two linear sections which correspond to two types of degradation, one aerobic and the other anaerobic [19, 36-41].

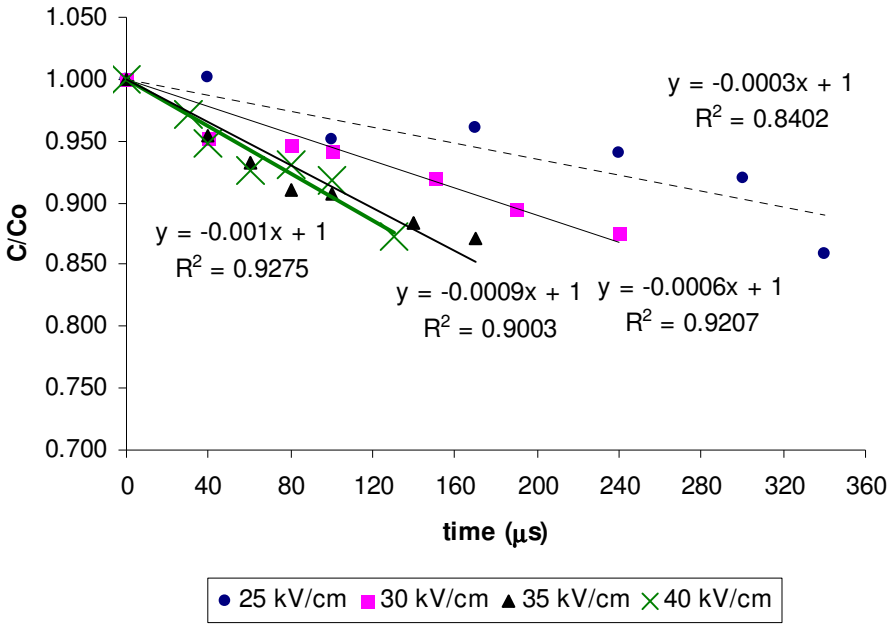

Figure 1. Degradation (k) of ascorbic acid in orange juice in each of the HIPEF treatments studied.

When HIPEF is applied, the treatment times are so short (μs) that anaerobic conditions are not achieved.

	Storage time (weeks)													
	0		1		2		3		4		6		7	
	pH	ºBrix	pH	ºBrix	pH	ºBrix	pH	ºBrix	pH	ºBrix	pH	ºBrix	pH	ºBrix
Fresh 10ºC	3.35	11.8	3.43	12.2	3.46	11.5	3.42	11.0	3.49	11.5	3.49	10.6	*	*
Fresh 2ºC	3.35	11.8	3.45	12.0	3.46	12.0	3.45	12.0	3.52	12.0	3.60	12.0	3.53	11.8
HIPEF 10ºC	3.34	12.0	3.44	12.0	3.43	11.2	3.37	11.2	3.54	11.2	3.52	11.0	*	*
HIPEF 2ºC	3.34	12.0	3.44	12.0	3.43	12.0	3.40	12.0	3.51	12.0	3.50	12.0	3.46	12.0
Pasteur. 10ºC	3.32	11.4	3.45	12.0	3.44	12.0	3.38	11.2	3.50	11.8	3.48	12.0	*	*
Pasteur. 2ºC	3.32	11.4	3.45	12.0	3.44	12.0	3.38	11.8	3.53	12.0	3.51	12.0	3.49	12.0

* spoiled samples, not analysed.

Table 2. Values of pH and ºBrix in fresh, HIPEF-treated and pasteurized orange juice during refrigerated storage (2–10 ºC).

The results obtained indicated that the ascorbic acid degradation rate was similar in the HIPEF treatment studied. 30 kV/cm was the field chosen for preservation of the orange juice and subsequent estimation of its shelf life, and treatment time was set at 100 μs. These treatment conditions were selected on the basis of the results obtained for carotenoid concentration, colour, enzymes and microorganisms when a juice was treated using different fields (25, 30, 35 and 40 kV/cm) and different times (30–340 μs) [30, 42-44].

After selecting the HIPEF treatment conditions, we studied refrigerated storage of pasteurized orange juice (90 ºC, 20 s) and of the same juice treated with HIPEF (30 kV/cm, 100 μs), at 2 and 10ºC for 7 weeks.

Table 2 shows the values obtained for pH and ºBrix. After the pasteurization treatments there was a statistically significant decrease ($p<0.01$) in pH with respect to the pH of the untreated juice, whereas after application of HIPEF (30 kV/cm, 100 μs) there was no statistically significant alteration in pH. Cserhalmi et al. [32] obtained similar results. In their study there was no statistically significant alteration in the pH of citrus fruits (grapefruit, lemon, orange, tangerine) after application of HIPEF treatment to preserve them. Sánchez-Moreno et al. [21] studied the impact of HIPEF (35 kV/cm, 750 μs), high pressure and pasteurization on orange juice, and they observed that except in the orange juice low pasteurized (in which pH increase), the pH values did not change. There was a statistically significant increase ($r = 0.627$; $p = 0.00$) with storage in all the juices. This result agree with Del Caro et al. [44] who study the changes of pH in citrus segments and juices during storage at 4 ºC and also obtain an increase of this parameter. On the other hand, Esteve et al. [46] and Rodrigo et al. [47], observe no statistically significant alteration in the pH of different orange juices and carrot-orange juices pasteurized, during refrigerated storage at 4 and 10 ºC. Bull et al. [48] also obtained no modifications ($p>0.05$) in pH values in a pasteurized or high pressure treated orange juice stored in refrigeration during 12 weeks at 4 and 10ºC.

The ºBrix value remained stable throughout the storage period, with no statistically significant differences between the different types of orange juice studied. The same results for ºBrix were reported by Rivas et al. [30] when they compared pasteurization and HIPEF treatment of orange–carrot juice. Similarly, HIPEF-treated orange juice (35 kV/cm, 59 μs) stored for 112 days at 2 and 22 °C did not show any variation in °Brix [11]. Cserhalmi et al. [32] after processing by HIPEF different citrus juices (lemon, orange, tangerine, grapefruit), also obtained no modifications in ºBrix values. These results agree with Bull et al. [48], who studied the effect of high pressure and pasteurization treatment on pH and ºBrix of orange juice, obtaining that these parameters were no significantly different from fresh untreated orange juice.

The initial concentration of vitamin C in orange juice was 48.03±1.12 mg/100ml. After pasteurization and HIPEF treatment of the orange juice, the vitamin C concentration was maintained without statistically significant alterations (48.88±2.53 and 48.32±1.35 mg/100ml for pasteurized and HIPEF treated orange juice, respectively). These results agree with those reported by other authors [32, 49-50]. However, in a study of an orange–carrot juice mixture, Torregrosa et al. [14] observed that after pasteurization the remaining concentration of vitamin C was 83% of the concentration of the untreated juice, while after HIPEF treatment it was 90% of the concentration of the untreated juice.

During storage, the concentration of vitamin C in the juice decreases according to the storage conditions. In order to study the degradation of ascorbic acid, Ln (C/C_0) was plotted against storage time for both treatments applied (Figure 2). Taking previous studies [15] into account, it is considered that ascorbic acid follows first-order degradation kinetics: $C=C_0\exp(-kt)$ where C_0 is the total initial ascorbic acid content (mg/100ml), k is the rate constant ($days^{-1}$), C is ascorbic acid at time t (mg/100ml) and t is the storage time (days). It can be seen that in the juices treated with HIPEF the vitamin C concentration was stable

throughout the storage period, decreasing very slowly when stored at either 2 ºC or 10 ºC. The ascorbic acid degradation rate for the HIPEF-treated juices was 0.0070 $days^{-1}$ (r^2 = 0.9987) and 0.0058 $days^{-1}$ (r^2 = 0.9882) at 10 ºC and 2 ºC, respectively. In the pasteurized juices the vitamin C concentration decreased more quickly, especially in the juice stored at 10 ºC. The ascorbic acid degradation rate in the pasteurized juices was 0.0195 $days^{-1}$ (r^2 = 0.9638) in the juice stored at 10 ºC and 0.0078 $days^{-1}$ (r^2 = 0.9718) in the juice stored at 2 ºC.

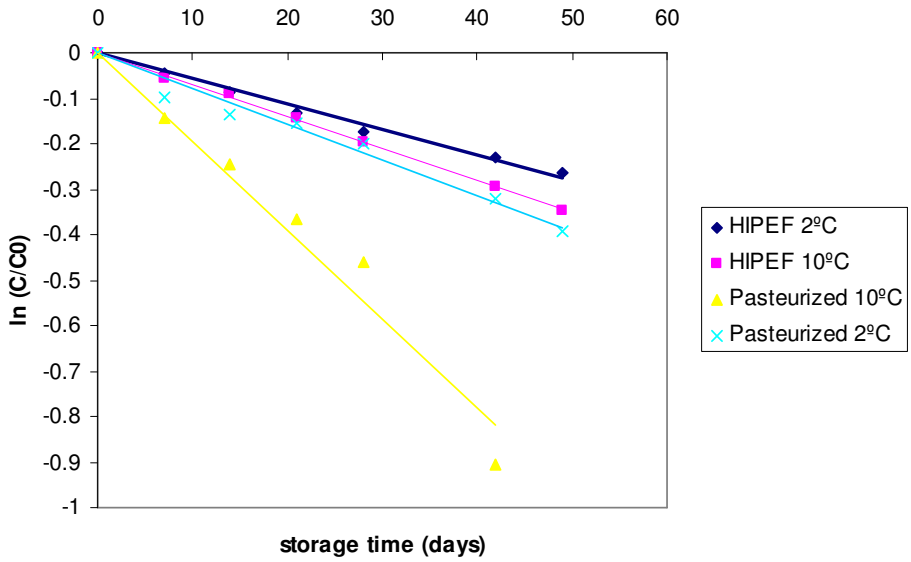

Figure 2. Degradation kinetics of ascorbic acid in pasteurized and HIPEF-treated (30 kV/cm, 100 µs) orange juice stored in refrigeration at 2 and 10 ºC.

The shelf life (days) of HIPEF-treated and pasteurized orange juice stored at 2 ºC and 10 ºC was calculated as the time taken for the ascorbic acid concentration to reduce to 50%, taking into account the value of the degradation rate (k), obtained applying the Arrhenius equation. The results obtained were 120 and 99 days for orange juice treated by HIPEF, stored at 2 and 10 ºC, respectively, and

88 and 35 days for orange juice pasteurized, stored at 2 and 10 ºC, respectively. It is clear that the HIPEF treatment succeeds in extending the shelf life of the orange juice in comparison with the pasteurized juice. Similar results were obtained in a study of HIPEF treatment and pasteurization of an orange–carrot juice mixture [14]. Rodrigo et al. [47] also establish the shelf life of different samples of pasteurized orange-carrot juice, and they consider that a period of 32 to 43 days at 10 ºC and 50 days at 4 ºC would be recommended. The lower degradation rate in the HIPEF-treated juices causes the shelf life of the juice to be much greater than that of pasteurized juice. There is also a considerable increase in the shelf life of the juice when the preservation temperature is 2 ºC, and it is therefore recommendable to store the juice at this temperature. Qiu et al. [51] observed similar results during storage of orange juice, with HIPEF-treated samples suffering less vitamin C loss than heat-treated samples.

Klimczak et al. [52] studied the effect of time and temperature of storage on vitamin C content of commercial orange juices obtaining that after 6 months of storage at 18 ºC, 28 ºC and 38 ºC, the content of vitamin C decreased by 21%, 31% and 81%, respectively. Esteve et al. [53] studied the stability of ascorbic acid in fresh orange juice and commercial orange juices maintained at 4 and 10 ºC, finding that at 4 ºC the loss of ascorbic acid was less than 10% after 7 days of storage. Choi et al. [54] found that, for pasteurized juice (90 ºC, 90 s), during refrigerated storage (4.5 ºC) more than 50% of the ascorbic acid was lost within 3 weeks of storage, and it was completely degraded after 5 weeks of storage. Shivashankara et al. [55] studied the ascorbic acid content of Irwin mango fruits stored at 5 ºC after a high electric field pretreatment, observing that the ascorbic acid decreased after 20 days of storage. Yeom et al. [50] studied the effects of HIPEF (35 kV/cm, 59 μs) and heat (94.6 ºC, 30 s) on the concentration of ascorbic acid in orange juice during storage at 4 ºC and 22 ºC. HIPEF-treated orange juice retained significantly higher ascorbic acid content than heat-pasteurized orange juice during 112 days of storage at 4 ºC.

CONCLUSIONS

The nutritional quality of orange juice (vitamin C) is maintained longer in HIPEF-treated juice than in juice preserved by means of conventional pasteurization treatments. HIPEF treatment allows the shelf life (based on the 50% reduction of ascorbic acid content) of the juice to reach 99 days when stored at 10 ºC, while with pasteurization treatment orange juice only reaches 35 days at 10 ºC. There is a substantial increase in the shelf life of the juice when the preservation temperature is 2 ºC, and it is therefore recommendable to store the juice at this temperature to ensure that it reaches the consumer with a high nutritive value.

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References

[1] Gardner PT, White TAC, McPhail DB, Duthie GG (2000) Food Chem 68:471–474

[2] Block G, Norkus E, Hudes M, Mandel S, Helzlsouer K (2001) Am J Epidem 154:1113–1118

[3] John JH, Ziebland S, Yudkin P, Roe LS, Neil HAW (2002) Lancet 359:1969–1974

[4] Burns J, Frase PD, Bramley PM (2003) Phytochem 62:939–947

[5] Sánchez-Moreno C, Plaza L, de Ancos B, Cano P (2003) J Sci Food Agric 83:430–439

[6] Kim KN, Pie JE, Park JH, Park YH, Kim HW, Kim MK (2006) J Nutr Biochem 17:454-462

[7] Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Chem Biol Interact 160:1–40

[8] Ewaidah EH (1992) Food Chem 44(2):103–111

[9] Goyle A, Ojha P (1998) J Food Sci Technol 35(4):346–348

[10] Roig MG, Bello JF, Rivera ZS, Kennedy JF (1999) Food Res Int 32:609–619

[11] Ayhan Z, Yeom HW, Zhang Q H, Min DB (2001) J Agric Food Chem 49(2):669–674

[12] Bezman Y, Rouseff RL, Naim M (2001) J Agric Food Chem 49(1):5425-5432

[13] Polydera AC, Stoforos NG, Taoukis PS (2003) J Food Eng 60:21–29

[14] Torregrosa F, Esteve MJ, Frígola A, Cortés C (2006) J Food Eng 73:339-345

[15] Esteve M J, Farré R, Frígola A (1995) J Agric Food Chem 43:2058–2061

[16] Murata M, Shinoda Y, Homma S (2002) Int Congress Series 1245:459–460

[17] Alwazeer D, Delbeau C, Divies C, Cachon R (2003). Int J Food Microbiol 89:21–29

[18] Zerdin K, Rooney ML, Vermuë J (2003) Food Chem 82:387–395

[19] Blasco R, Esteve MJ, Frígola A, Rodrigo M (2004) Lebens Wissen Technol 37:171–175

[20] Fiore A, La Fauci L, Cervellati R, Guerra MC, Speroni E, Costa S, Galvano G, De Lorenzo A, Bacchelli V, Fogliano V, Galvano F (2005) Mol Nutr Food Res 49:1129–1135

[21] Sánchez-Moreno C, Plaza L, Elez-Martínez P, de Ancos B, Martín-Belloso O, Cano P (2005) J Agric Food Chem 53:4403-4409

[22] Deliza R, Rosenthal A, Silva ALS (2003) Trends Food Sci Technol 14:43–49

[23] Barsotti L, Cheftel JC (1999) Food Rev Int 5:181–213

[24] Pothakamury UR, Barbosa-Cánovas GV, Swanson BG, Spence KD (1997) Food Sci Technol Int 3:113–121

[25] Calderón-Miranda M L, Barbosa-Cánovas GV, Swanson BG (1999) Int J Food Microbiol 51:31–39

[26] Kim SJ, Kim S (2001) Int J Sol Struct 38(8):1311-1325

[27] Vega-Mercado H, Powers JR, Barbosa-Cánovas GV, Swanson BG (1995) J Food Sci 60:1132–1136.

[28] Fernández-Díaz MD, Barsotti L, Dumay E, Cheftel JC (2000) J Agric Food Chem 48:2332–2339

[29] Selma MV, Salmerón MC, ValeroM, Fernández PS (2004) Food Microbiol 21(5):519-525

[30] Rivas A, Rodrigo D, Martínez A, Barbosa-Cánovas GV, Rodrigo M (2006) LWT – Food Sci Technol 10:1163–1170

[31] Torregrosa F, Cortés C, Esteve MJ, Frígola A (2005) J Agric Food Chem 53(24):9519-9525

[32] Cserhalmi Zs, Sass-Kiss Á, Tóth-Markus M, Lechner N (2006) Innov Food Sci Emerg Technol 7:49–54

[33] Braddock RJ (1999) Handbook of citrus by-products and processing technology. Wiley, New York, pp 53–83

[34] Aparicio P, Farré R, Frígola A (1992) An Bromatol 44:257–261

[35] McAllister JW (1980) Citrus nutrition and quality. American Chemical Society, Washington DC, pp 291-377

[36] Nagy S (1980) J Agric Food Chem 25:602–604

[37] Robertson GL, Samaniego CML (1986) J Food Sci 51:184–187

[38] Kennedy JF, Rivera ZS, Lloyd LL, Warner FP, Jumel K (1992) Food Chem 45:327–331

[39] Ariahu CC, Adekunle DE, Nkpa NN (1997) J Food Proc Preserv 21:21–32

[40] Esteve MJ, Frígola A, Martorell L, Rodrigo C (1998) J Food Prot 61:1518–1521

[41] Esteve MJ, Frígola A, Martorell L, Rodrigo C (1999) Z lebensm unters forsch A Eur food res technol 208:144–147

[42] Rodrigo D, Martínez A, Harte F, Barbosa-Cánovas GV, Rodrigo M (2001) J Food Prot 64:259–263

[43] Rodrigo D, Barbosa-Cánovas GV, Martínez A, Rodrigo M (2003) J Food Prot 66:2336–2342

[44] Cortés C, Esteve MJ, Rodrigo D, Torregrosa F, Frígola A (2006) Food Chem Toxicol, in press

[45] Del Caro A, Piga A, Vacca V, Agabbio M (2003) Food Chem 84:99-105

[46] Esteve MJ, Frígola A, Rodrigo C, Rodrigo D (2005) Food Chem Toxicol 43:1413-1422

[47] Rodrigo D, Arranz JI, Koch S, Frígola A, Rodrigo MC, Esteve MJ, Calvo C, Rodrigo M (2003) J Food Sci 68(6):2111-2116

[48] Bull MK, Zerdin K, Howe E, Goicoechea D, Paramanandhan P, Stockman R, Sellahewa J, Szabo EA, Johnson RL, Stewart CM (2004) Innov Food Sci Emerg Technol 5:135-149

[49] Min S, Jin ZT, Zhang QH (2003) J Agric Food Chem 51:3338–3344

[50] Yeom HW, Streaker CB, Zhang QH, Min DB (2000) J Agric Food Chem 48:4597–4605

[51] Qiu X, Sharma S, Tuhela L, Jia M, Zhang Q (1998) Am Soc Agric Eng 41(4):1069-1074

[52] Klimczak I, Malecka M, Szlachta M, Gliszczynska-Swiglo A (2006) J Food Comp Anal, in press

[53] Esteve MJ, Farré R, Frígola A (1996) J Food Qual 19:243–249

[54] Choi MH, Kim GH, Lee HS (2002) Food Res Int 35:753–759

[55] Shivashankara KS, Isobe S, Al-Haq MI, Takenaka M, Shiina T (2004) J Agric Food Chem 52:1281–1286

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Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice

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Abbreviations: HIPEF, high intensity pulsed electric field. HMF, hydroxymethylfurfural

Keywords: color, browning, furfural, orange juice, pulsed electric field, storage

ABSTRACT

Increased consumer demand for fresh-like products with minimal vitamin and flavour losses stimulated the search for new mild preservation techniques. High Intensity Pulsed Electric Field (HIPEF) is one of the nonthermal minimal processing technologies interesting for scientists and food industry as a new, alternative (preservation) process for liquid food. We have evaluated the effect on color, browning and HMF of a pasteurized orange juice and the same orange juice treated by HIPEF, during seven weeks stored in refrigeration at 2ºC and 10ºC.

Pasteurized orange juice presents greater yellow tendency (b*) and less red tendency (a*) than the untreated orange juice, while HIPEF orange juice presents a coloration more similar to the untreated orange juice. Color variations (ΔE) during storage are greater in orange juice pasteurized than in HIPEF treated orange juice. Non thermal treated orange juice has less non enzymatic browning than the pasteurized one. There is a significant increase in this parameter from the forth week of storage in all the juices stored at 10ºC, while in the ones stored at 2ºC the browning index values are maintained during more time. There are no significant variations in the HMF content of the juices pasteurized or treated by HIPEF respect the untreated orange juice. During refrigerated storage, HMF is always below the maximum values established.

INTRODUCTION

Citrus juices are highly consumed in many countries and nowadays, consumers desire high quality foods, with freshly flavour, texture and color, with minimal or no chemical preservatives, and above all safe.

Annually, more than 63 million of tons of oranges are produced around the world [1]. Orange juice is the predominant juice manufactured by the beverage processing industry worldwide. Conventional thermal processing ensures safety and extends the shelf life of orange juice, but it often leads to detrimental changes in the sensory qualities of the product. Consumers desire high quality foods that are nutritious, with freshly prepared flavor, texture and color, with minimal or no chemical preservatives, and above all safe [2-4]. Consequently, newly developed food technologies usually focus on preservation while keeping food quality attributes.

Food color afect our perception of its quality, thus, the color of the orange juice is an important attribute in the preferences of the consumers and has been implemented in the quality control of the food industries in the European Union [5]. Also in the USA, the color of citrus juices is one of the parameters evaluated for the commercial classification of the product in relation to its quality [6].

The color of a fruit is the first quality factor that the consumer appreciates and has a remarkable influence on its acceptance. Color of foods dramatically influences consumers' preferences [2, 7-9] and in this sense, some studies revealed that colour of citric beverages in general, are related to the consumer's perception of flavour, sweetness and other characteristics in relation to the quality of these products [2, 6], and it's also an indicator of the natural transformation of a fresh food (ripeness) or of changes that occur during its storage or processing. Color of orange juice is mainly due to carotenoid pigments [10-13] and it's related with the ripening of the product, the presence of impurities, the technological treatments, the storage conditions, microorganisms alterations, browning reactions, etc. During storage, the orange

juice suffers an important number of deterioration reactions (ascorbic acid degradation, cloud loss, microbial spoilage, development of off-flavor, changes in color, texture, appearance), that produce an important quality loss [14-19]. The presence of furfural and 5-hidroxymethylfurfural (HMF) in stored citric products is an indicator of their quality loss; furfural and HMF are related with the browning of the juice and they are also good indicators of the excess of temperature and storage time in some food [20-21]. Consequently, the analysis of these compounds has special importance in the food industry. A few inadequate conditions during thermal treatment and during storage of the juice reflect on an increase of the concentration of the different derivatives of furfural, formed by degradation of the reducer sugars or by Maillard's reaction, and in addition changes in the color.

Pasteurization is important to the stability of citrus juice during transportation and marketing. Increased consumer demand for fresh-like products with minimal vitamin and flavour losses stimulated the search for new mild preservation techniques [22]. The development of alternatives to heat processing is also motivated by the real need for environmental friendly technologies.

High Intensity Pulsed Electric Field (HIPEF), one of the non thermal minimal processing technologies that stand in the interest of scientists and food industry as a new, alternative (preservation) process for liquid food, meets these criteria [23-28].

A number of authors have studied the evolution of quality factors in orange juice after HIPEF treatment in some cases making a comparison with the evolution after heat treatment [16, 29-30].

HIPEF technology needs further evaluation, because there is not enough information about its effect on physical and chemical properties of fruit juices.

The aim of this study was to evaluate the effect on color, browning and HMF of an orange juice treated by HIPEF and the same juice pasteurized, during seven weeks stored in refrigeration at 2ºC and 10ºC.

MATERIALS AND METHODS

Sampling of orange juice

Juice was extracted (FMC juice extractors with a 2-mm-diameter perforated plate) after appropriate washing of the oranges and placed in a tank.

HIPEF treatment system

Sample treatments were carried out in continuous HIPEF treatment system designed by the University of Ohio and located in the Instituto de Agroquímica y Tecnología de Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage, and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, OR U.S.A.). The flow was set at 60 ml/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL).

Treatment time was 100 μs and the electric field was set at 30 kV/cm. These treatment conditions were selected on the basis of the results on carotenoid concentration, color, vitamin C, enzymes and microorganisms, obtained when the orange juice was treated using different fields (25, 30, 35, and 40 kV/cm) and different times (30–340 μs) [31-33]. Samples were collected after each treatment. The experiments were performed in duplicate.

Thermal treatment

To treat samples ARMFIELD FT74P equipment with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (90ºC, 20 s) are reached. Heating of orange juice at 90–99ºC for 15–30 s is normal in commercial practice [34]. After treatment, the juice was cooled with cold water from a cooler (ARMFIELD

FT61), and it was packed and stored at 2ºC and 10ºC until analysis. Experiments were performed in duplicate.

Storage conditions

Juices were packaged in Elopack packages (pure-pack®), and they were stored in refrigeration and darkness at 2ºC and 10ºC (±1ºC) with controlled humidity. Samples were analyzed in duplicate immediately after processing, then after 1, 2, 3, 4, 6, and 7 weeks of storage.

Color, browning index and hidroxymethylfurfural determination

Color was determined using a Hunter Labscan II spectrophotometric colorimeter (Hunter associates laboratory, INC. Reston. Virginia. USA) controlled by a computer that calculated colour ordinates from the reflectance spectrum [35] and calibrated with a white standard tile. Samples were placed in Petri dishes and filled to the top, and colour was recorded using the CIELab uniform colour space. Colour determined by CIE (Commission Internationale l'Eclairage) classifies colour in three dimensions; L*, brightness, a*, red to green colour and b*, yellow to blue colour. Chroma $((a^{*2} + b^{*2})^{1/2})$, that quantify color intensity, and total color differences $(\Delta E = \Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})$, which indicate the magnitude of the color difference between juices at initial time and after the storage period, were also determined. The results were expressed in accordance with the CIELAB system with reference to illuminant D65 and with a visual angle of 10º. The measurements were made in triplicate throught of the product.

Browning index: Non enzymatic browning index is determined with an absorbance measure at 420 nm. Three mL of orange juice are centrifuged at 2000 rpm during 20 min at 18 ºC, and the supernatant is mixed equally distributed with ethanol 95 %. After filtering the mixture, the absorbance is measured at 420nm [36].

Hidroxymethylfurfural (HMF): Hidroxymethylfurfural content is measured using the method described by IFFJP [37]. This method is based in that HMF

reacts with barbituric acid and p-toluidin, forming a red compound. This reaction has a maximum at 3-4 minutes.

The Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community [5] has included the amount of HMF among the absolute parameters of quality (≤ 20 mg/ L) in the code of practice for the evaluation of fruits and vegetable juices.

Physicochemical determinations

pH determination was based on the potentiometric measurement at 20ºC. It was determined in a Crison GLP 21 pH meter equipped with a temperature compensation sensor at 20ºC.

ºBrix was determined by measurement of the refraction index with an Atago model RX-1000 digital refractometer. In citrus juices, ºBrix is used to indicate the percentage of soluble solids, and is one of the most important factors for grading the quality of a citrus juice [38].

Statistical Analysis

Results were compared using one-way analysis of variance (ANOVA). Tukey test ($p < 0.05$) was applied to compare the average values obtained. The computer program employed was SPSS® (Statistical Package for the Social Sciences) 12.0 for Windows.

RESULTS AND DISCUSSION

The values obtained for pH and ºBrix are shown in table 1. After pasteurization treatments there is a statistically significant decrease ($p<0.01$) of the pH values (3.32±0.01) with regard to the pH of the untreated juice (3.35±0.00), whereas after applying HIPEF treatment (30kV/cm, 100μs), pH is not statistically modified (3.34±0.01). These results agree the ones described by Cserhalmi et al [30] after applying HIPEF treatments (28 kV/cm, 100 μs) for the conservation of diverse citrus fruits (grapefruit, lemon, orange, tangerine). Similar results are described by Bull et al [3] for a pasteurized orange juice and the same juice processed with non thermal technologies (high pressure).

Table 1. pH and ºBrix values of orange juices treated by HIPEF and pasteurized during refrigerated storage (2-10ºC).

	Storage time (weeks)													
	0		1		2		3		4		6		7	
	pH	ºBrix	pH	ºBrix	pH	ºBrix	pH	ºBrix	pH	ºBrix	pH	ºBrix	pH	ºBrix
Fresh 10ºC	3.35	11.8	3.43	12.2	3.46	11.5	3.42	11.0	3.49	11.5	3.49	10.6	*	*
Fresh 2ºC	3.35	11.8	3.45	12.0	3.46	12.0	3.45	12.0	3.52	12.0	3.60	12.0	3.53	11.8
HIPEF 10ºC	3.34	12.0	3.44	12.0	3.43	11.2	3.37	11.2	3.54	11.2	3.52	11.0	*	*
HIPEF 2ºC	3.34	12.0	3.44	12.0	3.43	12.0	3.40	12.0	3.51	12.0	3.50	12.0	3.46	12.0
Pasteur. 10ºC	3.32	11.4	3.45	12.0	3.44	12.0	3.38	11.2	3.50	11.8	3.48	12.0	*	*
Pasteur. 2ºC	3.32	11.4	3.45	12.0	3.44	12.0	3.38	11.8	3.53	12.0	3.51	12.0	3.49	12.0

*spoiled samples, not analyzed

Sánchez-Moreno et al [39] obtain the same results when orange juices were processed using non thermal technologies, both high pressure (400 MPa, 40ºC, 1 min) and HIPEF (35 kV/cm, 750 μs), describing that there are not significant differences for pH among the untreated juice and the treated ones.

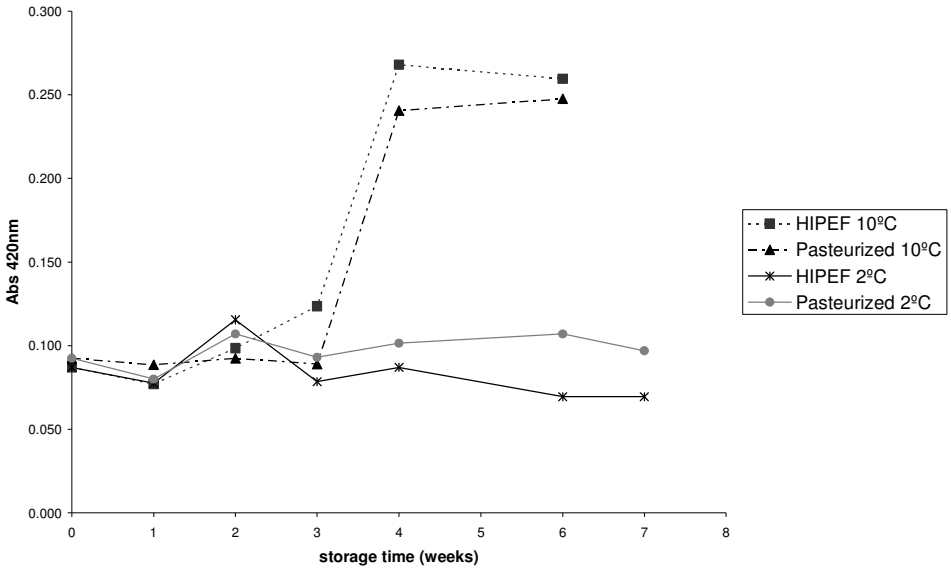
There was a statistically significant increase in pH values ($r = 0.627$; $p = 0.00$) with storage in all the juices analyzed. This result agree with Del Caro et al [40] who study the changes of pH in citrus segments and juices during storage at 4 ºC and also obtain an increase of this parameter. Contrary, Esteve et al. [41] and Rodrigo et al [21] don't observe significant modifications in the pH values of different pasteurized orange and orange-carrot juices, during their storage in refrigeration at 4 and 10 ºC. Neither Bull et al [3] obtain significant modifications ($p>0.05$) in pH of pasteurized or high pressurized orange juices, stored during 12 weeks at 4 and 10ºC.

ºBrix in untreated (11.8±0.1), HIPEF (12.0±0.1) and pasteurized (11.4±0.2) orange juice don't present significant differences at initial time. ºBrix values are stable during the storage of HIPEF and pasteurized orange juice, not presenting statistically significant differences between them. The same results for ºBrix were described by Rivas et al [4], that studied a pasteurized orange-carrot juice and an orange-carrot juice treated by HIPEF. Also Bull et al. [3] obtained similar results when they study a pasteurized orange juice and an orange juice treated by high pressure. Similarly, HIPEF-treated orange juice (35 kV/cm, 59 μs) stored for 112 days at 2 and 22 °C did not show any variation in ° Brix [16].

The browning index is one of the parameters that indicate us the non enzymatic browning of the orange juices after being pasteurized or treated by HIPEF, as well as during their refrigerated storage at 2 and 10ºC. Values obtained are shown in figure 1. After pasteurization of the orange juice, the browning index (0.093±0.001) increases in a statistically significant way with regard to the untreated orange juice (0.086±0.001), whereas between untreated and HIPEF orange juice (0.087±0.001), don't exist significant differences. Cserhalmi et al

[30], obtain similar results when they applied HIPEF's treatments (28 kV/cm, 100μs) for the conservation of diverse citrus fruits (grapefruit, lemon, orange, tangerine), although the increase of the browning index with regard to the untreated juice is not statistically significant. Bull et al [3] describe that the browning index of the orange juice increases after thermal treatments (65 ºC, 1 min) or high pressure treatment (600 MPa, 20 ºC, 60 s), although it was not significantly different from untreated juice.

Figure 1: Browning index (Abs 420nm) of orange juices treated by HIPEF and pasteurized during refrigerated storage (2-10ºC).



When we study the evolution of this parameter during the refrigerated storage of the orange juices analyzed, we have observed a significant increase ($p<0.05$) from the third week of storage at 10ºC, whereas at 2ºC the values of the browning index are stable during more time in HIPEF treated juices or pasteurized juices. When we applied a tukey test, we obtained that in the sixth week of storage there are significant differences ($p<0.05$) for the browning index value of the juice treated by HIPEF and the pasteurized juice, both at 2 and 10 ºC.

The control of furanic aldehydes is important in the evaluation of non-enzymatic browning, adulterations, heating, or incorrect storage conditions. The main decomposition product of the hydrolysis of sugars catalysed by acid is 5-(hydroxymethyl)-2-furfuraldehyde (HMF).

The mean values for HMF content are 0.088 ± 0.019 mg/L, 0.089 ± 0.023 mg/L and 0.115 ± 0.023 mg/L for untreated, HIPEF and pasteurized orange juice, respectively. There are not statistically significant variations between HMF contents of the HIPEF treated juice or the pasteurized juice with regard to the untreated orange juice, immediately after the treatments, though the content in HMF is greater in the pasteurized orange juice. HMF values obtained at the last week of analysis (week 7) in the juices stored at 2ºC are 0.169 ± 0.054 and 0.196 ± 0.022 mg/L for HIPEF and pasteurized orange juice, respectively. In orange juices stored at 10ºC these values are 0.197 ± 0.034 and 0.223 ± 0.021mg/L for HIPEF and pasteurized juice, respectively.

During refrigerated storage at both temperatures, HMF increase slightly in both kinds juices (pasteurized and HIPEF) (data not shown), although it remains always below the maximum values allowed [5].

Rivas et al [4], studying an orange-carrot juice, obtain that the HMF content was very small (0.013 ± 0.001 mg/L) and did not vary with the treatments applied (HIPEF and pasteurized) and also during storage at 2 °C no variation was found in the content of HMF. Similar results were found by Martín et al. [42] in orange

juice. Cserhalmi et al [30] also observe that there are not significant differences in HMF content with regard to the untreated orange juice when they treated the juice by HIPEF (28 kV/cm, 100 μs), obtaining that the content of HMF was 0.22 and 0.25 mg/L for untreated and treated orange juice, respectively. These values are greater than the ones obtained in the present work. Rodrigo et al [21] obtain similar results when they analyze different orange-carrot juices stored in refrigeration during 6 weeks at 4 and 10 ºC. These authors observe a slight increase in HMF during the storage of the juices, being higher in the ones stored at 10 ºC, but in all cases the values did not reach the tolerable limit for orange juices subjected to mild heat treatment (5 mg/L of HMF).

Burdulu et al [43], when they stored a concentrated orange juice at different temperatures (28, 37 and 45 ºC), obtained that at 28ºC, HMF values didn't reach the limits established for orange juices among all the storage time (8 weeks).

Solomon et al [44] studied the values of HMF in orange juice reconstituted from frozen concentrate and pasteurized (95 ºC, 15 s), and they obtained that the HMF content in the juice increased slightly (but no significant) during 50 days of refrigerated storage a 8ºC.

To measure the color, we have obtained the parameters L*, a*, b*, chroma (($a^{*2} + b^{*2})^{1/2}$) and total color differences (($\Delta E = \Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}$)).

L*, that indicates the luminosity of the sample, is higher in the juice treated by HIPEF (52.23±0.05) and in the pasteurized one (52.41±0.12) than in the untreated orange juice (51.36±0.54), showing statistically significant differences. This result agrees the results obtained by Cserhalmi et al [30], in which the parameter L* also increases on the juice treated by HIPEF, although in this case there aren't statistically significant differences, and the values obtained were 14.48±0.58 and 14.73±0.11 for the untreated and HIPEF orange juice, respectively.

Lee et al. [10] also report a small increase in CIE L* value from 40.22 to 41.22 for pasteurized orange juice. A similar observation of small increases of CIE L*

values was also reported with thermal processing of red grapefruit juices by Lee and Coates [45]. Contrary, Rivas et al [4] don't obtain significant variations in the parameter L* after applying conventional pasteurization treatments (98ºC, 21 s) or HIPEF treatments (25 kV/cm, 280 μs) to an orange-carrot juice (62.80±0.03, 62.65±0.20 and 63.08±0.09, for untreated, pasteurized and HIPEF juice, respectively).

L* values increase in a significant way after one week of refrigerated storage, and afterwards they decrease significantly in all the samples analyzed, being always higher than initial values (figure 2). This decrease can probably be attributed to partial precipitation of unstable, suspended particles in juice as described by Genovese et al [46]. Rivas et al [4], described a decrease of the parameter L* on pasteurized orange-carrot juice during refrigerated storage. Esteve et al [41] analyzed different commercial orange juices stored in refrigeration, and they obtain slight decreases in L * (not significant) at 4ºC, whereas during storage at 10ºC they obtain significant increases in three of the juices studied. In the same way, Rodrigo et al [21] describe an increase with time when they stored an orange-carrot juice mildly pasteurized (77 ºC, 20 s) at 10 ºC, whereas at 4 ºC the variations observed were minimal and not significant.

The mean CIE a*, that indicates the variation between red and green color, is significantly higher ($p \leq 0.05$) in untreated orange juice (4.56±0.40) than in the processed juices. The value of this parameter for the HIPEF orange juice is 2.99±0.08, and for the pasteurized juice is 1.57±0.03, observing significant differences ($p \leq 0.05$) between them. Sánchez-Moreno et al [39] describe similar results (decrease of the parameter a*) after pasteurization or high pressure treatment of orange juice. Parameter b *, that indicates the variation between yellow and blue color, is significantly higher ($p \leq 0.05$) in the pasteurized orange juice samples (57.61±0.56) than in the HIPEF ones (53.62±0.57) and in the untreated orange juice (50.73±0.67). These results coincide with the ones described by Lee and Coates [10] when they study CIE a* and CIE b* in

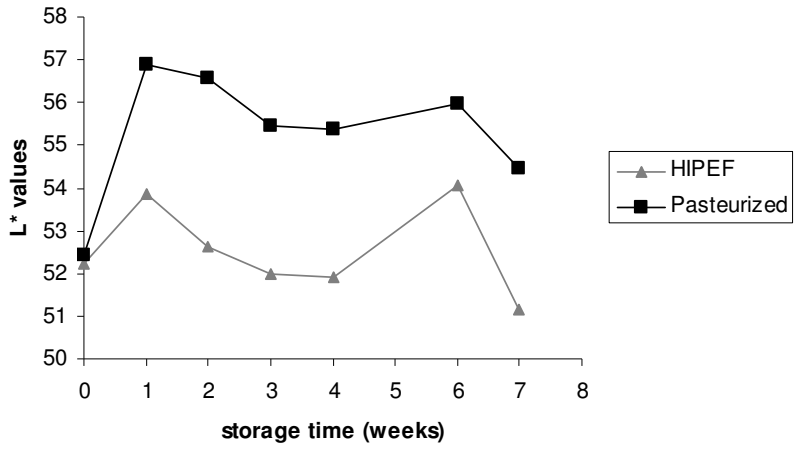
untreated and pasteurized orange juices. These authors obtain that CIE b* values increase with the pasteurization of the juice, whereas CIE a* values decrease. Thus a color shift toward positive b* and negative a* directions indicate more yellow and less red in pasteurized orange juices.

CIE a* values decrease significantly ($p<0.05$) during the first weeks of refrigerated storage in the juices studied, although they increase again in all of them from the sixth week of storage (table 2). CIE b* decrease significantly ($p<0.05$) with the refrigerated storage of the processed juices (table 2). Rodrigo et al [21] describe a significant decrease of the parameter b* when they analyze different pasteurized orange-carrot juices (77º C, 20 s) stored at 10 ºC, whereas CIE a* don't present significant variations during the refrigerated storage.

Chroma $[(a^2+b^2)^{1/2}]$, that quantifies the intensity of color, is higher in the pasteurized orange juice (57.63±0.55), followed by HIPEF juice (53.70±0.57) and finally by the untreated juice (50.93±0.69). A similar observation in increases in chroma (increases in brightness) due to the pasteurization of orange juice has been described by Lee et al [10] and Sánchez-Moreno et al [39]. During refrigerated storage of the samples analysed in the present study, color intensity decrease because chroma (C*) decrease (figure 3). Other authors describe similar results after refrigerated storage of orange juice [47] or after frozen storage of red grapefruit juice concentrates [48].

Figure 2: L* values of orange juices treated by HIPEF and pasteurized during refrigerated storage (2-10ºC).

(A)



(B)

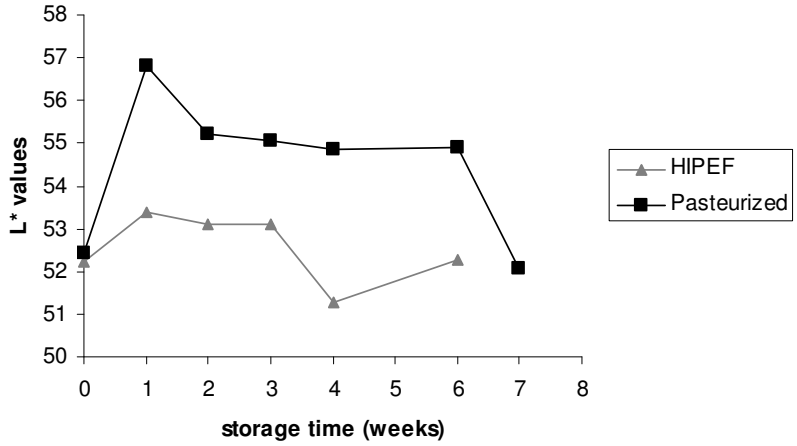
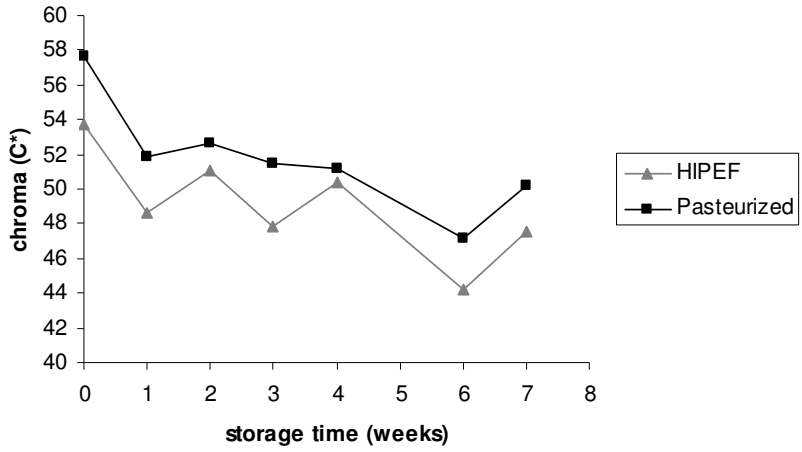
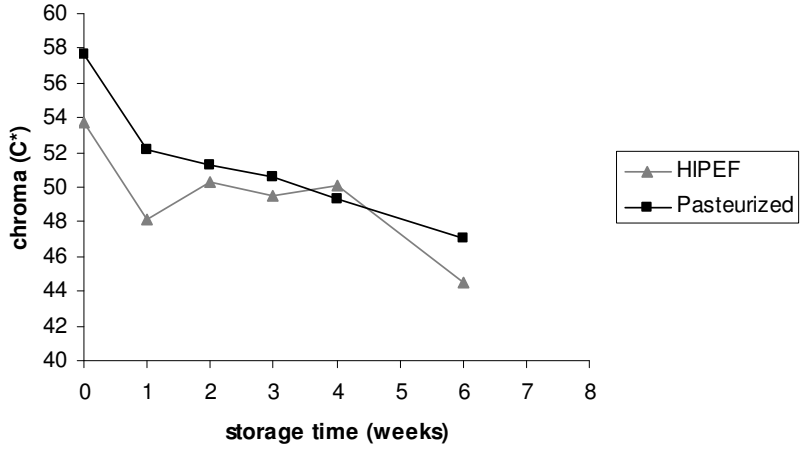


Figure 3: Chroma (C*) of orange juices treated by HIPEF and pasteurized during refrigerated storage (2-10ºC).

(A)



(B)



Total color differences (ΔE), which indicate the magnitude of the color difference between orange juices at initial time and after the storage period, is

shown in table 2. It has been considered that ΔE of two would be a noticeable visual difference for a number of situations [47, 49]. In all the cases ΔE values higher than 2 are obtained, although the orange juices stored at 10ºC present higher color differences than the ones stored at 2ºC, and the pasteurized orange juice always presents higher color differences than the juice treated by HIPEF (table 2).

Table 2. a*, b* and ΔE values of orange juices treated by HIPEF and pasteurized during refrigerated storage (2-10ºC).

	week	2ºC			10ºC		
		a	b	ΔE	a	b	ΔE
HIPEF juice	0	2.99±0.08	53.62±0.57	0	2.99±0.08	53.62±0.57	0
	1	1.73±0.13	48.64±0.55	5.39	1.23±0.06	48.13±0.28	5.88
	2	2.82±0.20	50.97±0.30	2.69	3.32±0.04	50.23±0.07	3.52
	3	2.19±0.10	47.83±0.41	5.85	2.01±0.06	49.51±0.23	4.31
	4	0.66±0.13	50.40±0.12	3.99	0.51±0.06	50.08±0.38	4.42
	6	3.46±0.17	44.12±0.67	9.69	2.63±0.14	44.44±0.43	9.19
	7	2.71±0.05	47.51±0.14	6.21	*	*	*
Pasteurized juice	0	1.57±0.03	57.61±0.56	0	1.57±0.03	57.61±0.56	0
	1	1.34±0.07	51.88±0.31	7.29	0.87±0.02	52.12±0.05	7,07

2	2.90±0.04	52.55±0.24	6.67	2.77±0.03	51.20±0.31	7,10
3	0.12±0.13	51.51±0.30	6.97	-0.09±0.15	50.61±0.28	7,66
4	1.22±0.08	51.17±0.31	7.09	0.88±0.15	49.28±0.70	8.71
6	3.54±0.22	47.02±0.86	11.35	4.68±0.07	46.82±0.42	11,50
7	2.77±0.19	50.09±0.54	7.89	*	*	*

*spoiled samples, not analyzed

When statistical analysis is applied, a positive correlation between browning index and CIE b* (r^2=0.970; p=0.001) and L* (r^2=0.821; p=0.045) is obtained, and contrary, there is a negative correlation between this index and CIE a* (r^2=-0.928; p=0.008). There are no correlations between these parameters and the HMF.

CONCLUDING REMARKS

Orange juice treated by HIPEF presents similar color than the untreated orange juice. Color differences (ΔE) during refrigerated storage are higher in the pasteurized juice than in the juice treated by HIPEF. Orange juice processed by non thermal treatments presents less browning index than the pasteurized one. There are no significant differences on HMF content between orange juices treated by HIPEF or pasteurized and untreated orange juice. HMF remains in all cases below the maximum values established.

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References

[1] FAOSTAT (FAO Statistical Databases), 2004.

[2] Chan, M.M., Kane-Martinelli, C., *J. Am. Diet. Assoc.* 1997, *97*, 657-659.

[3] Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E. A., Johnson, R. L., Stewart, C. M., *Innov. Food Sci. Emerg. Technol.* 2004, *5*, 135-149.

[4] Rivas, A., Rodrigo, D., Martínez, A., Barbosa-Cánovas, G.V., Rodrigo, M., *Lebens-Wiss Technol.* 2006, *10*, 1163-1170.

[5] Association of the Industry of Juices and Nectars of the European Economic Community Code of Practice for Evaluation of Fruit and Vegetable Juices (AIJN), Brussels 1996.

[6] Tepper B. J., *J. Sens. Stud.* 1993, *8*, 145-154.

[7] Hutchings J.B., Food colour and appearance, in: *Blackie Academic and Professional Publication*, London 1994.

[8] Calvo, C., Salvador, A., Fiszman, S.M., *Eur. Food Res. Technol.* 2001, *213***,** 99–103.

[9] Gamble, J., Jaeger, S.R., Harker, F. R., *Postharvest Biol. Technol*. 2006, *41 (1),* 38-47.

[10] Lee, H. S., Coates, G. A., *Lebensm.-Wiss. Technol.* 2003, *36,* 153-156.

[11] Meléndez-Martínez, A.J., Viacario, I.M., Heredia, F., *J. Agric. Food Chem.* 2003, *51*, 7266-7270.

[12] Meléndez-Martínez, A. J., Britton, G., Vicario, I. M., Heredia, F. J., *Food Res. Int.* 2005, *38 (8-9),* 931-936.

[13] Vikram, V.B., Ramesh, M.N., Prapulla, S.G., *J. Food Eng.* 2005, *69 (1),* 31-40.

[14] Goyle, A., Ojha, P., *J. Food Sci. Technol*. 1998, *35 (4),* 346-348.

[15] Roig, M.G., Bello, J.F., Rivera, Z.S., Kennedy, J.F., *Food Res. Int.* 1999, *32,* 609-619.

[16] Ayhan, Z., Yeom, H.W., Zhang, Q.H., Min, D.B., *J. Agric. Food Chem.* 2001, *49 (2),* 669-674.

[17] Bezman, Y., Rouseff, R.L., Naim, M., *J. Agric. Food Chem.* 2001, *49 (1),* 5425-5432.

[18] Polydera, A.C., Stoforos, N.G., Taoukis, P.S., *J. Food Eng*. 2003, *60,* 21–29.

[19] Torregrosa, F., Esteve, M. J., Frígola, A., Cortés, C., *J. Food Eng.* 2006, *73,* 339-345.

[20] Nagy, S., Randall, V., *J. Agric. Food Chem.* 1973, 21, 272.

[21] Rodrigo, D., Arranz, J.I., Koch, S., Frígola, A., Rodrigo, M.C., Esteve, M.J., Calvo, C., Rodrigo, M., *J. Food Sci.* 2003, *68 (6),* 2111-2116.

[22] Mertens, B., Knorr, D., *Food Technol.* 1992, *46,* 124-133.

[23] Butz, P., Tauscher, B., *Food Res. Int.* 2002, *35*, 279-284.

[24] Spilimbergo, S., Dehghani, F., Bertucco, A., Foster, N. R., *Biotechnol. Bioeng.* 2003, *82***,** 118-125.

[25] Shivashankara, K. S., Isobe, S., Al-Haq, M. M., Takenaka, M., Shiina, T., *J. Agric. Food Chem.* 2004, *52 (5),* 1281-1286.

[26] Elez-Martínez, P., Escolá-Hernández, J., Soliva-Fortuny, R. C., Martín-Belloso, O., Food Microbiol. 2005, *22,* 311-319.

[27] Torregrosa, F., Cortés, C., Esteve, M. J., Frígola, A., *J. Agric. Food Chem.* 2005, *53(24)*, 9519-9525.

[28] Torregrosa, F., Esteve, M. J., Frígola, A., Cortés, C., *Eur. Food Res. Technol*. 2005, *221,* 125-131.

[29] Yeom, H. W., Streaker, C. B., Zhang, Q. H., *J. Agric. Food Chem.* 2000, *48,* 4597-4605.

[30] Cserhalmi, Zs., Sass-Kiss, Á., Tóth-Markus, M., Lechner, N., *Innov. Food Sci. Emerg. Technol.* 2006, *7 (1-2),* 49-54.

[31] Cortés, C., Esteve, M. J., Rodrigo, D., Torregrosa, F., Frígola, A., *Food Chem. Toxicol.* 2006, *in press.*

[32] Rodrigo, D., Martínez, A., Harte, F., Barbosa-Cánovas, G. V., Rodrigo, M., *J. Food Prot.* 2001, *64*, 259–263.

[33] Rodrigo, D., Barbosa-Cánovas, G. V., Martínez, A., Rodrigo, M., *J. Food Prot.* 2003, *66*, 2336–2342.

[34] Braddock, R. J., Single strength orange juices and concentrate, in: Wiley, J. (Ed.), *Handbook of Citrus by-products and Processing Technology*, New York 1999, pp. 53-83.

[35] Calvo, C., Duran L., Z. *Lebensm. Unters Forsch A. Eur. Food Res. Technol*. 1998, *207 (2),* 101-104.

[36] Meydav, S., Saguy, I., Kopelman, I. J., *J. Agric. Food Chem.*1977*, 25,* 602-604.

[37] International Federation of Fruit Juice Producers Methods (IFFJP). Analysen-analyses. Zug. Switzerland. Fruit Union Suisse Assoc. Svizzera Frutta. 1984, 12, 1-2.

[38] Mc Allister, J. W., Methods of determining the quality of citrus juice, in S. Nagyand, J. A. (Ed.), Attaway, *Citrus nutrition and quality,* American Chemical Society, Washington DC 1980, pp. 291-377.

[39] Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., Cano, P., *J. Agric. Food Chem.* 2005*, 53,* 4403-4409.

[40] Del Caro, A., Piga, A., Vacca, V., Agabbio, M., *Food Chem.* 2004, *84*, 99-105.

[41] Esteve, M.J., Frígola, A., Rodrigo, C., Rodrigo, D., *Food Chem. Toxicol.* 2005, *43 (9)*, 1413-1422.

[42] Martín, J.J., Solanes, E., Bota, E., Sancho, J., *Alimentaria,* 1995, 59–63.

[43] Burdulu, H. S., Koca, N., Karadeniz, F., *J. Food Eng.* 2006, *74* (2), 211-216.

[44] Solomon, O., Svanberg, U., Sahlström, A., *Food Chem.* 1995, *53* (4), 363-368.

[45] Lee, H. S., Coates, G. A., *J. Food Sci.* 1999, *64*, 663-666.

[46] Genovese, D. B., Elustondo, M. P., Lozano, J. E., *J. Food Sci.* 1997, *62*, 1171-1175.

[47] Choi, M. H., Kim, G. H., Lee, H. S., *Food Res. Int.* 2002, *35 (8),* 753-759.

[48] Lee, H. S., Coates, G. A., *J. Agric. Food Chem.* 2002, *50*, 3988-3991.

[49] Francis, F.J., Clydesdale, F.M. *Food colorimetry: Theory and applications*, AVI Publishing Company, Westport 1975.

(artículo enviado para su publicación)

Determination of Total Antioxidant Capacity and Phenolic compounds of orange juice refrigerated treated by High Intensity Pulsed Electric Fields

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Abstract

Effect of High Intensity Pulsed Electric Fields (HIPEF) and pasteurization on total phenolic compounds, total antioxidant activity, vitamin C and vitamin A of orange juice, as well as the effect of refrigerated storage was studied. Trolox equivalent antioxidant capacity (TEAC) diminishes in a statistically significant way ($p<0.05$) when the juice is processed by both treatments, but this decreases is higher after pasteurization (12.9% after HIPEF and 38.21% after pasteurization). TEAC decreases during refrigerated storage in the samples analyzed, being this decrease higher in samples stored at 10 ºC. Vitamin C concentration after pasteurization and HIPEF treatment isn't modified significantly, but during refrigerated storage, vitamin C of the pasteurized juice decrease more than in the orange juice treated by HIPEF.

Total phenolic compounds are always higher in the untreated orange juice, followed by juice treated by HIPEF and finally by pasteurized juice, although these differences are not statistically significant ($p>0.05$), and during refrigerated storage remain practically invariable in all samples.

Keywords: Antioxidant capacity, High Intensity Pulsed Electric Fields, orange juice, total phenolic compounds, vitamin A, vitamin C

INTRODUCTION

Nowadays there is an increasing interest in minimally processed food due to its similarity with fresh food, overcoat when it is a question of fruits and vegetables. Phenolic compounds, especially phenolic acids and flavonoids, are ubiquitously present in vegetables, fruits, seeds, tea, wines and juices; thus they are an integral part of the human diet. Moreover, the benefits of fruit and vegetable consumption are now widely reported in the literature. Dietary antioxidants are able to neutralise oxygen free radicals and inhibit LDL oxidation, and they may protect against coronary heart disease, cancer and neurodegenerative diseases (Ames, Shigenaga & Hagen, 1993; Miquel, Ramírez-Bocá, Ramirez-Boscá & Díaz-Alperi, 2006, Lako, Trenerry, Wahlqvist, Wattanapenpaiboon, Sotheeswaran, & Premier, 2006; Kiran, Ahuja, Pittaway & Ball, 2006).

Orange juice is an important source of carotenoids and ascorbic acid, a nutrient that besides its vitamin action is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported, such as inhibition of formation of cancer-causing N-nitroso compounds in the stomach (Kim, Pie, Park, Park, Kim & Kim, 2006; Valko, Rhodes, Moncol, Izakovic & Mazur, 2006). During processing and/or storage, orange juice bears an important number of deterioration reactions, which result in a loss of quality (Ayhan, Yeom, Zhang & Min, 2001). As ascorbic acid, carotenoids are very capable of deterioration during the storage of the food.

Fruits and vegetables contain also phenolic compounds, that in recent studies has been demostrated to have antioxidant capacity (Dillard & German, 2000; Vinson, Su, Zubik & Bose, 2001; Chaovanalikit & Wrolstad, 2004) although, their nutritional importance is uncertain, as they may be poorly absorbed and rapidly metabolized, and have limited antioxidant ability in vivo (Gadner, Tasmin, McPhail, & Duthie, 2000). So it's important to determine the content of total phenols to be able to evaluate the possible effect synergic or antagonistic on its contribution to the total antioxidant capacity.

Seasonality and perishability of fruits and vegetables explain the necessity of applying preservation technologies. The aim is to combine shelf life extension with maintenance of sensory and nutrient characteristics.

The abundance of fresh drinks based on fruit and/or vegetables juices, and the minimally processed products, allow the consumers to ingest a wide variety of antioxidants in the diet, such as vitamin C, carotenoids, flavonoids and other phenolic compounds.

Nowadays, thermal treatments are the methods most used by the food industry for food conservation. During the thermal treatments, microorganisms present in food are inactivated, but also there are produced undesirable effects on some nutrients, antioxidant compounds, color, flavor and texture (Alwazeer Cachón & Divies, 2002; Cortés, Esteve, Frígola & Torregrosa, 2004; Torregrosa, Cortés, Esteve & Frígola, 2005; Torregrosa, Esteve, Frígola & Cortés, 2006).

High intensity pulsed electric fields (HIPEF) is an emerging technology in the field of food preservation. HIPEF has the potential to pasteurize several foods non-thermally, via exposure to high voltage short pulses while the material is between the electrodes of a treatment chamber. It has been checked that this new technology produce microorganism inactivation with less quality lose of the final product (Ayhan et al., 2001; Min, Jin, Yeom, Min & Zhang, 2002; Abram, Smelt, Bos, Wouters, 2003; Torregrosa et al., 2005; Torregrosa et al., 2006).

The aim of this manuscript is to study antioxidant parameters (total antioxidant capacity, total phenolic compounds, vitamins A and C) of the orange juice treated by HIPEF and its comparison with an orange juice treated by conventional thermal treatments, as well as the evolution of these parameters in the orange juices stored in refrigeration at 2 and 10ºC for seven weeks.

MATHERIAL AND METHODS

Sampling of orange juice

After appropriate washing and hygienisation of the fruits, they were subjected to an extraction process (FMC juice extractors with a 2-mm-diameter sieve) and the juice was introduced into a tank.

HIPEF treatment system

Sample treatments were carried out in continuous HIPEF treatment system designed by the University of Ohio and located in the Instituto de Agroquímica y Tecnología de los Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage, and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, OR U.S.A.). Flow was set at 60 ml/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL). Treatment time was 100 μs and the electric field was set at 30 kV/cm. These treatment conditions were selected on the basis of the results on carotenoid concentration, color, vitamin C, enzymes and microorganisms, obtained when the orange juice was treated using different fields (25, 30, 35 and 40 kV/cm) and different times (30-340μs) (Cortés, Esteve, Rodrigo, Torregrosa & Frígola, 2006). Samples were collected after treatment. The experiments were performed in triplicate.

Thermal treatment

To treat samples Armfield FT74P equipment with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (90ºC, 20 s) are reached. Heating of orange juice at 90–99ºC for 15–30 s is normal in commercial practice (Braddock, 1999). After treatment, the juice was cooled with cold water from a cooler (Armfield FT61), and it was packed and stored until analysis. Experiments were performed in triplicate.

Storage conditions

Juices were packaged in Elopack packages (pure-pack®), and they were stored in refrigeration and darkness at 2ºC and 10ºC (±1ºC) with controlled humidity. Samples were analyzed in duplicate immediately after processing, then after 1, 2, 3, 4, 6, and 7 weeks of storage.

Total Antioxidant Capacity

Method adapted from Rice-Evans and Miller (1994). This method is based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2´-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS), wich has a characteristic long-wavelenght absorption spectrum showing maxima at 734 nm. The ABTS radical cation is formed by the interaction of ABTS (150 μM) with the ferrylmyoglobin radical species, generated by the activation of metamyoglobin (2.5 μM) with H_2O_2 (75 μM). Antioxidant compounds suppress the absorbance of the ABTS radical cation to an extent and on a time scale dependent on the antioxidant capacity of the substance under investigation.

This inhibition assay with a fixed time point on 3 minutes, ABTS, myoglobin and a sample are mixed, the reaction is initiated by the addition of hydrogen peroxide. After a fixed time the absorbance of the solution is read, along with a buffer blank (which have a greater absorbance value). Results are obtained by difference of absorbance before and after adding the oxidant compound (H_2O_2), and interpolating the value obtained in a curve of calibration prepared every day with trolox standard in the interval of 0.5-2 mM.

Total Phenol content

Total phenol content of the samples was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965) reading samples on a spectrofotometer UV/Vis Perkin- Elmer Lambda 2 at 750 nm. Results were expressed as galic acid equivalents (mg/100 mL).

Vitamin C content

Five mL of juice were diluted to 25 mL with the extraction solution (oxalic acid 1%, w/v, trichloroacetic acid 2 %, w/v, sodium sulfate 1%, w/v). After vigorous

shaking the solution was filtered through a folded filter (Whatman nº 1). The 9.5 mL of oxalic acid 1% (w/v) and 2 mL of acetic acid/sodium acetate 2M buffer (pH= 4.8) were added to an aliquot of 0.5 mL of filtrate and the solution transferred to the polarographic cell. The following instrumental conditions were applied: DP_{50}, mode DME, drop size 2, drop time 1s, scan rate 10 mV/s, initial potential -0.10 V. Determinations were carried out using the peak heights and standard additions method (Aparicio, Farré & Frígola, 1992).

Carotenoid and vitamin A content

Carotenoid pigments were extracted, saponified and analysed by HPLC method, according to a procedure described by Cortés et al. (2004).

The carotenoids were identified by UV-vis spectra and retention times in HPLC in the juices analyzed (Cortés et al, 2004).

Determination of vitamin A: it was expressed as retinol equivalents (RAE), using the following conversion (IOM, 2001): RAE = (μg β-carotene)/12 + (μg β-cryptoxanthin + μg α-carotene)/24.

Statistical Analysis

Results were compared using one-way analysis of variance (ANOVA). Tukey test ($p<0.05$) was applied to compare the average values obtained. The computer program employed was SPSS® (Statistical Package for the Social Sciences) 12.0 for Windows.

RESULTS AND DISCUSSION

The trolox equivalent antioxidant capacity (TEAC) of the samples after applying the conservation treatments are 4.03±0.04 mmol trolox/L for untreated orange juice, 3.51±0.04 mmol trolox/L for HIPEF treated juice and 2.49±0.20 mmol trolox/L for pasteurized orange juice. As it can be observed, TEAC decrease in a significant way ($p<0.05$) after processing the orange juice with both types of treatment, but it decreases more in the pasteurized juice than in he HIPEF treated juice (decrease 12.9 % after HIPEF and 38.21 % after pasteurization). Thus,

HIPEF treatment of orange juice has an antioxidant capacity more similar to the one that has the untreated juice. HIPEF technology was more effective than pasteurization in preserving the antioxidant capacity in orange juice. Sánchez-Moreno, Plaza, Elez-Martínez, De Ancos, Martín-Belloso and Cano (2005), obtain that after applying different non thermal treatments, high pressure and HIPEF (35 kV/cm, 750 μs), total antioxidant capacity values are not modified significantly, while pasteurization treatments makes a decrease with regard to the untreated orange juice. Similarly, Polydera, Stoforos and Taoukis (2004) observe a higher decrease of the Total Antioxidant Capacity in the pasteurized orange juice, whereas when the same juice is processed by non thermal treatments (high pressure) the decrease of this parameter is slighter.

Fiore et al. (2005), after determining total antioxidant capacity in orange juice, they obtain that this parameter decrease after thermal treatments (sterilized and pasteurized orange juice), obtaining similar results than the ones described in the present study for the pasteurized orange juice refrigerated (2.12-3.53 mmol trolox/L).

Total antioxidant capacity of samples during refrigerated storage is shown in table 1, observing a decrease of this parameter in the three types of samples analyzed, having a higher decrease the samples stored at 10 ºC.

Compared to conventional pasteurization, HIPEF treatment led to higher total antioxidant activity of orange juice immediately after processing (time 0 of storage), as well as during storage at 2–10 °C. This is in agreement with results in fresh navel juice and in orange juice reconstituted from frozen concentate after high pressure treatment (Polydera et al., 2004; Polydera, Stoforos and Taoukis, 2005).

Table 1: Total antioxidant capacity (mmol trolox/L) in untreated, HIPEF and pasteurized orange juice, during refrigerated storage (2 and10ºC).

storage temperature	weeks	Fresh juice	HIPEF juice	Pasteurized juice
2ºC	0	4.03±0.04	3.51±0.04	2.49±0.20
	1	3.51±0.22	3.22±0.16	1.93±0.26
	2	3.39±0.02	2.98±0.07	2.10±0.07
	3	3.07±0.02	2.94±0.07	2.40±0.16
	4	2.37±0.18	2.33±0.02	1.83±0.30
	6	2.24±0.07	2.23±0.02	1.80±0.04
	7	2.10±0.08	2.21±0.02	1.80±0.01
10ºC	0	4.03±0.04	3.51±0.04	2.49±0.20
	1	2.57±0.46	2.37±0.15	2.02±0.02
	2	2.55±0.33	2.10±0.07	2.15±0.77
	3	2.77±0.05	2.37±0.18	2.52±0.27
	4	1.34±0.18	1.54±0.09	1.74±0.20
	6	1.25±0.10	1.53±0.04	1.70±0.06
	7	*	*	*

*spoiled samples, not analyzed.

Klimczak, Maleka, Szlachta and Gliszczynska-Swiglo (2006) obtain similar results in an orange juice stored during six months at 18, 28 and 38 ºC, observing that at higher storage temperature, total antioxidant values decrease to a greater extent (18%, 45% and 84% after 6 months at 18, 28 and 38 ºC, respectively) and more quickly.

After pasteurization and HIPEF treatment of the orange juice, vitamin C concentration doesn't present any significant modification (table 3). These results agree with other authors (Yeom, Streaker, Zhang & Min, 2000; Cserhalmi et al., 2006). According with other data, the content of vitamin C in

different juices decreases during storage, depending on storage conditions, such as temperature, oxygen and light access (Kabasakalis, Siopidou & Moshatou, 2000; Zerdin, Rooney & Vermuë, 2003). At 10 ºC, vitamin C concentration in both types of orange juice decrease more quickly than in the same orange juices stored at 2ºC. Klimczak et al. (2006) describe similar results in an orange juice stored during six months at 18, 28 and 38 ºC, observing that at higher storage temperature, vitamin C concentration decrease to a greater extent and more quickly (21%, 31% and 81% after 6 months at 18, 28 and 38 ºC, respectively).

Results for phenolic compounds are shown in table 3, and as it can be observed, values are higher in untreated orange juice, followed by HIPEF treated orange juice and finally by the pasteurized orange juice, although the differences are not significant ($p>0.05$). Alper, Savas Bahéc and Acar (2005) describe small decrease (7%) in phenolic compounds after pasteurization of a pomegranate juice. We have observed a non significant increase ($p>0.05$) in phenolic compounds of all the juices analyzed, after their storage in refrigeration. Klimczack et al. (2006) also describe a small increase of the phenolic compounds of an orange juice after six months of storage. The authors use to different methods for phenolic compounds determination, and although there is evidence that the spectrophotometric method overstimates the polyphenolic content compared to the chromatographic method, explained by the lack of selectivity of Folin Ciocalteau reagent (Escarpa & González, 2001), the spectrophotometric method (Folin Ciocalteau method) has been shown to be a useful analytical tool for the routine analysis of polyphenols, and its widely used in many laboratories for the determination of differences among fruits and vegetables and their products (Bartolomé, Bengoechea, Sancho, Estrella, Hernández & Gómez-Cordovés, 1998; Klimczak et al., 2006). Similarly, other authors (Ke & Salveit, 1988; Babic, Amiot, Nguyen-The, & Aubert, 1993) observe small increases in phenolic compounds after storage of iceberg lettuce

or carrots exposed to several kinds of stress. These authors indicate that phenols build up as a physiological response to infections and damage.

Table 2: Vitamin C and total carotenoids in untreated, HIPEF and pasteurized orange juice, during refrigerated storage (2 and 10ºC).

weeks	**Vitamin C (mg/100mL)**		**Total carotenoids**	
	HIPEF	**Pasteurized**	**HIPEF**	**Pasteurized**
		10ºC		
0	48.32±1.35	48.88±2.53	1275.2±56.3	1195.4±31.6
1	43.71±0.50	42.42±0.46	1258.0±92.2	1122.6±0.24
2	42.52±0.52	38.26±0.45	1097.9±61.5	1087.5±11.4
3	44.56±1.82	33.94±0.81	1082.9±51.3	919.7±48.0
4	43.81±1.32	30.89±0.47	1101.8±30.1	845.1±39.5
6	43.03±0.63	18.74±1.28	1107.8±8.0	854.2±41.9
7	*	*	*	*
		2ºC		
0	48.32±1.35	48.88±2.53	1275.2±56.3	1195.4±31.6
1	47.60±0.15	44.35±1.18	1174.4±54.9	1305.7±38.4
2	43.52±0.03	42.73±1.21	1129.4±106.7	1117.6±57.5
3	44.69±0.42	43.35±0.99	1128.5±33.7	1237.4±219.6
4	43.73±0.70	41.56±0.01	1234.3±52.0	1020.6±84.7
6	43.82±0.64	35.41±0.83	1018.3±16.0	881.9±13.2
7	42.66±0.07	35.58±0.05	964.2±12.1	913.3±72.3

*spoiled samples, not analyzed.

Total carotenoid concentration (table 2) is significantly minor ($p < 0.05$) in the pasteurized orange juice than in the untreated orange juice (-12.57 %), whereas in the juice treated by HIPEF, although total carotenoid concentration decrease

compared to the untreated juice (-6.73 %), this decrease is not statistically significant ($p > 0.05$). These results coincide with the ones obtained by Lee and Coates (2003) for the pasteurized orange juice, in which total carotenoid content loss was significant ($p<0.05$) after thermal pasteurization at 90ºC for 30 s. In the same way, vitamin A is higher in the untreated orange juice, followed by the orange juice treated by HIPEF (-7.52%) and, finally, by the pasteurized orange juice (-15.62%), only being significant differences ($p<0.05$) between untreated orange juice and pasteurized orange juice. Therefore, in the refrigerated orange juice, non thermal treatments (HIPEF) affect to a lesser extent the concentration of vitamin A than the thermal conventional treatments do. Sánchez-Moreno et al. (2005) also study the vitamin A and total carotenoid content modification after applying different kinds of conservation treatments to the orange juice, obtaining results similar to the ones described in the present study.

Table 3. Total phenolic compounds (mg/mL of Galic acid) in untreated, HIPEF and pasteurized orange juice, during refrigerated storage (2-10ºC).

	Storage time (weeks)						
	0	1	2	3	4	6	7
Fresh 10ºC	1.002	0.873	1.072	0.876	1.025	1.067	*
Fresh 2ºC	1.002	0.919	1.101	0.899	1.062	0.949	1.027
HIPEF 10ºC	0.988	0.954	1.148	0.937	0.955	0.941	*
HIPEF 2ºC	0.988	0.953	1.073	0.877	1.023	1.008	1.045
Pasteurized 10ºC	0.949	1.039	1.032	0.844	1.067	0.985	*
Pasteurized 2ºC	0.949	1.021	1.055	0.871	0.956	1.004	1.07

*spoiled samples, not analyzed.

TEAC presents positive correlations with total carotenoids, vitamin A and vitamin C ($r^2=0.318$, $p=0.013$; $r^2=0.379$, $p=0.003$ and $r^2=0.317$, $p=0.014$,

respectively), so juices with higher total antoxidant value also present higher carotenoid, vitamin C and vitamin A concentration, being these parameters the ones that more influence the total antioxidant capacity of the juice. These parameters can be considered vitamins with antioxidant activity. Nevertheless, total antioxidant capacity doesn't present statistically significant correlations with total phenols, so this parameter doesn't contribute to a great extent to the total antioxidant capacity of the orange juices analyzed.

Similarly, Gardner, White, McPhail and Duthie (2000) and Polydera et al., (2005), describe that vitamin C is the compound with higher antioxidant capacity in different orange juices. Orange juices are a rich source of vitamin C, which is an important antioxidant in these juices. Concentration of vitamin C is a significant indicator of orange juice quality, and it may serve as an indicator that all processes, which ensure a high quality of the product, have been applied in the production process (Post, 1998).

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REFERENCES

Abram, F., Smelt, J. P. P. M., Bos, R., & Wouters, P. C. (2003). Modelling and optimization of inactivation of Lactobacillus plantarum by pulsed electric field treatment. *Journal of Applied Microbiology, 94*, 571–579.

Alper, N., Savas Bahçeci K., & Acar J. (2005). Influence of processing and pasteurization on color values and total phenolic compounds of pomegranate juice. *Journal of Food Processing and Preservation, 29*, 357-368.

Alwazeer, D., Cachon, R., & Divies, C. (2002). Behavior of Lactobacillus plantarum and Saccharomyces cerevisiae in fresh and thermally processed orange juice. *Journal of Food Protection, 65,* 1586–1589.

Ames, B. N., Shigenaga, M. K., Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences, USA, 90*, 7915-7922.

Aparicio, P., Farré, R., & Frígola, A. (1992). Determinación del ácido ascórbico en vegetales por polarografía diferencial de impulsos. *Anales de Bromatología, XLIV*, 257–261.

Ayhan, Z., Yeom, H. Y., Zhang, Q. H., & Min, D. B. (2001). Flavour, colour, and vitamin C retention of PEF processed orange juice in different packaging materials. *Journal of Agricultural and Food Chemistry, 49*, 669–674.

Babic, L., Amiot, L. J., Nguyen-The, C., & Aubert, S. (1993). Changes in phenolic content in fresh ready-to-use shredded carrots during storage. *Journal of Food Science, 58* (2), 351-356.

Bartolomé, B., Bengoechea, M. L., Sancho, A. I., Estrella, I., Hernández, T., Gómez-Cordovés, T. (1998). Differentiation of intermediate products (concentrates and purées) from the fruit industry by means of phenolic content. *Zeitschrift für Lebensmittel-Untersuchung un –Forschung A, 206*, 355-359.

Braddock, R. J. (1999). Single strength orange juices and concentrate. In Wiley, J., Ed., *Handbook of Citrus by-products and Processing Technology*, (pp. 53-83). New York.

Chaovanalikit, A., & Wrolstad, R. E. (2004). Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Food and Chemical Toxicology, 69,* 67-72.

Cortés, C., Esteve, M. J., Frígola, A. & Torregrosa, F. (2004). Quality characteristics of horchata (a Spanish vegetable beverage) treated with pulsed electric fields during shelf-life. *Food Chemistry, 91* (2), 319-325.

Cortés, C., Esteve, M. J., Rodrigo, D., Torregrosa, F., & Frígola, A. (2006). Changes of color and carotenoid contents during high intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology,* in press.

Cserhalmi, Zs., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science and Emerging Technologies, 7* (1-2), 49-54,

Dillard, C. J., & German, J. B. (2000). Phytochemicals: Nutraceuticals and human health. Review. *Journal of Science and Agriculture, 80*, 1744-1756.

Escarpa, A., González, M.C. (2001). Approach to the content of total extractable phenolic compounds from different food simples by comparison of chromatographic and spectrophotometric methods. *Analytica Chimica Acta, 427*, 119-127.

Fiore, A., La Fauci, L., Cervellati, R., Guerra, M.C., Speroni, E., Costa, S., Galvano, G., De Lorenzo, A., Bacchelli, V., Fogliano, V., & Galvano, F. (2005). Antioxidant activity of pasteurized and sterilized commercial red orange juices. *Molecular Nutrition and Food Research, 49*, 1129.

Gardner, P. T., White, T. A. C., McPhail, D. B., & Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry, 68,* 471-474.

Kabasakalis, V., Sioidou, D., & Moshatou, E. (2000). Ascorbic acid content of commercial fruit juices and its rate of loss upon storage. *Food Chemistry, 70* (3), 325-328.

Ke, D., Saltveit, M. E. (1988). Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. *Plant Physiology, 88,* 1136-1140.

Kiran, D., Ahuja, K., Pittaway, J. K., & Ball, M. J. (2006). Effects of olive oil and tomato lycopene combination on serum lycopene, lipid profile, and lipid oxidation. *Nutrition, 22* (3), 259-265.

Klimczak, I., Malecka, M., Szlachta, M., & Gliszczynska-Swiglo, A. (2006). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis,* in press.

Lako, J., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., & Premier, R. (2006). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry, In Press*, Available online 7 March 2006.

Lee, H. S., & Coates, G. A. (2003). Effect of thermal pasteurization on Valencia orange juice color and pigments. *Lebensmittel-Wissenschaft + Technologie, 36,* 153-156.

Min, S., Jin, Z. T., Yeom, H., Min, S. K., & Zhang, Q. H. (2002). Optimisation of processing conditions and effects of commercial scale PEF processing on the microbiological, chemical, physical, and sensory properties of orange juice. *International Meeting, IFT.*

Miquel, J., Ramírez-Boscá, A., Ramírez-Bosca, J. V., & Diaz Alperi, J. (2006). Menopause: A review on the role of oxygen stress and favorable effects of dietary antioxidants. *Archives of Gerontology and Geriatrics, 42* (3), 289-306.

Polydera, C., Stoforos, N. G., & Taoukis, P. S. (2004). Effect of storage on antioxidant activity of high pressure and thermally pasteurised reconstituted orange juice. *International Journal of Food Science and Technology*, *39*, 783–791.

Polydera, C., Stoforos, N. G., & Taoukis, P. S. (2005). Effect of high hydrostatic pressure treatment on post processing antioxidant activity of fresh Navel orange juice. *Food Chemistry, 91,* 3, 495-503.

Post, G. (1998). Technologia produkcji wysokiej jakości soku pomarańczowego z koncentratu przy umiarkowanym koszcie. *Przemysl Fermentacyjny I Owocowo-Warzywny, 12,* 36-39.

Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., & Cano, M. P. (2005). Impact of High Pressure and Pulsed Electric Fields on Bioactive compounds and Antioxidant Activity of Orange Juice in comparison with traditional thermal processing. *Journal of Agricultural and Food Chemistry, 53,* 4403-4409.

Singleton, V. L, & Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16,* 144-158.

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.

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.

Vinson, J. A., Su, X., Zubik, L., & Bose, P. (2001) Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry, 49,* 5315-5321.

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 heat pasteurization. *Journal of Agricultural and Food Chemistry, 48,* 4597-4605.

Zerdin, K., Rooney, M. L., & Vermuë, J. (2003). The vitamin C content of orange juice packed in an oxygen scavenger material. *Food Chemistry, 82* (3), 387-395.

Capítulo 5

Tomate

1. **Thermal and high pressure stability of purified Polygalacturonase and Pectinmethylesterase from four different tomato processing varieties.** Food Research International, 39, 440-448 (2006).

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Thermal and high pressure stability of purified Polygalacturonase and Pectinmethylesterase from four different tomato processing varieties

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Abstratc

Polygalacturonase (PG) and pectinmethylesterase (PME) were extracted and purified from four tomato varieties (Galeón, Malpica, Perfectpeel and Soto) used in the processing industry. The processing stability (thermal and high pressure) of PG and PME from the four varieties was analyzed, and they all showed the same behavior. PG was present in two isoforms, PG1 (inactivated at 90°C, 5 min) and PG2 (inactivated at 65°C, 5 min). In contrast, PG1s and PG2s showed the same pressure stability, both can be inactivated at room temperature in the pressure range of 300-500 MPa. On the other hand, purified PMEs could be thermally inactivated (5 min, 70°C) but 50% of its activity remained after high pressure treatment (850 MPa, 15 min, 25°C). High pressure processing can thus be used for selective inactivation of PG in tomato processing (while keeping PME intact). This fact could open prospectives for improving texture/rheology of processed tomato based products; however further research in the texture/rheology area is needed.

Keywords: tomatoes, polygalacturonase stability, pectinmethylesterase stability, thermal processing, high pressure processing

1. INTRODUCTION

The consumer demand for minimally processed food products has increased remarkably during the last years. Emerging technologies are being investigated to replace or complement conventional technologies employed in food processing. Such technologies among others include high hydrostatic pressure, pulsed electric fields, micro-wave heating, gamma irradiation and ultrasound. High hydrostatic pressure is considered to be one of the most promising technologies for industrial utilization (Farkas, & Hoover, 2000; Hugas, Garriga, & Monfort, 2002). One of the main advantages of this technology is that high pressure at room temperature does not result in Maillard reactions, off-flavor formation and vitamin destruction (reaction that do occur during thermal processing). Therefore there is an improved preservation of the nutritional and sensorial quality of high pressure processed products (Krebbers, Matser, Hoogerwerf, Moezelaar, Tomassen, & van den Berg, 2003)

Tomatoes are often used as a raw material in foods, and besides microbial safety, consistency of tomato based products is an important quality aspect. When processed, the consistency of tomato products is a result of transformations facilitated by a series of pectolytic enzymes (polygalacturonase, pectimethylesterase, polymethylgalacturonase, and pectin and pectate lyases) and chemical breakdown of cell wall material (Krebbers et al. 2003).

Polygalacturonase (EC3.2.1.15) (PG) is present in the tomato fruit in two isoforms, PG1, the heat stable form, and PG2, the heat labile form. All of them are structurally related (Pressey, & Avants, 1973; Ali, & Bradi, 1982; Zheng, Heupel, & DellaPenna, 1992). PG1 is a complex between the catalytic subunit and a group of catalytically inactive proteins called β-subunits (Moshrefi, & Luh, 1983; Moshrefi, & Luh, 1984; Knegt, Veermer, & Bruinsma, 1988; DellaPenna, Watson, Liu, & Schuchman, 1996; Bergey, Orozco-Cárdenas, De Moura, & Ryan, 1999). A model for the interactions of the β-subunits with the PG2 catalytic subunit and with plant pectin, suggests that the β-subunits may

alter the catalytic properties of the enzyme and may play an important regulatory role in pectin degradation (DellaPenna et al. 1996).

Pectinmethylesterase (EC3.1.1.11) (PME) is present mainly in the middle lamella and the cell junctions of tomato tissues as different isozymes that change with the tomato variety, the degree of maturity (Tucker, Robertson, & Grierson, 1982) and may depend on the extraction and purification procedure applied.

PME catalyzes the de-esterification of pectin that subsequently is a substrate for PG catalyzing the hydrolytic cleavage of the α–1,4-glycosidic bonds. The depolymerization of pectin leads to a drastic loss in rheology (decrease in viscosity) during industrial processing and, consequently, diminishes the quality of tomato based products. On the contrary, a controlled activity of PME with selective PG inactivation can result in some benefits; reduction of the β-elimination and a texture improvement through the addition of calcium salts forming calcium pectate with the low-methoxyl pectin groups. Previous enzyme inactivation studies have shown that, under suitable high pressure/temperature conditions, tomato PG can be inactivated, whereas tomato PME can remain active (Crelier, Robert, Claude, & Juillerat, 2001; Fachin, van Loey, Nguyen, Verlent, Indrawati, & Hendrickx, 2003), but there are so far no studies available confirming this principle for tomato varieties used in the food processing industry. Evidences for differences between tomato varieties in some tomato components can be found in literature. For instance, Hackett, Lee, Francis, & Schwartz (2004) describe a faster thermal degradation of tomato lycopene in Tangerine variety than in Roma; Sanchez, Valencia, Gallegos, Ciruelos, & Latorre (2002) found that some rheological properties of tomato pastes (linear viscosity behavior) were highly dependent on the tomato variety (H-282 ctrl, H-282F, 1401-C, 1401-J).

Therefore, the main objective of this research was to study the effect of thermal and high pressure treatments on PG and PME activity extracted and purified from different tomato varieties used in the tomato processing industry, as a basis

for identifying the processing conditions that allow to obtain processed tomato products with improved textural properties.

2. MATERIALS AND METHODS

2.1. Raw Material

Four different varieties (Malpica, Perfectpeel, Soto and Galeón) of ripe tomato fruits (*Lycopersicon esculentum*), used in the tomato processing industry in Spain were obtained from wholesale market. They were washed, cut and stored at -40°C until use.

2.2. Enzyme Extraction

Enzymes were extracted from each of the four tomato varieties following the method described by Pressey (1986) with some modifications (Verlent, van Loey, Smout, Duvetter, & Hendrickx, 2004). Thawed tomatoes (500 g) were blended with cold distilled water (1:1.5 (w/v)) for 2 min. The homogenate was adjusted to pH 3.0 with 0.1 M HCl, stirred for 15 min and centrifuged at 8000 g for 20 min at 4°C. The pellets were dispersed again in cold distilled water (1:1(w/v)), pH was adjusted with 0.1 M HCl and the sample was centrifuged at 8500g, 20 min at 4°C. The washing procedures at pH 3.0 were performed in order to reduce the amount of reducing groups in the final extract. The supernatant was discarded, the pellet was dissolved in cold 1.2M NaCl (1:1(w/v)), and the pH was adjusted to 6.0 with 0.1 M NaOH. After stirring during 3 h, keeping the pH constant by adding NaOH, the solution was centrifuged at 10000g for 20 min. The resulting supernatant was partially purified by ammonium sulfate precipitation. It was brought to 20 % saturation by the addition of solid $(NH_4)_2SO_4$, stirred for 1 h at 4°C, and centrifuged at 12000 g for 10 min. Pellet was discarded. The $(NH_4)_2SO_4$ concentration was increased to 90% saturation, stirred again for 1 h at 4°C and centrifuged at 18000 g for 20 min. The resulting precipitate was dissolved in 40 mL of 0.5 M NaCl and dialyzed for 3 h against distilled water. Finally, the extracted solution was

centrifuged at 10000 g for 20 min and the supernatant was kept at -40°C until further purification by cation exchange chromatography.

2.3. Purification of enzymes

PG and PME where purified as described by Verlent et al. (2004). Briefly, crude tomato PG and PME extract (~ 5 mL) was thawed at room temperature, filtered using 0.45 μm syringe driven filter (Millipore, MA, USA) and loaded on a cation-exchange column (CIEX, Hi Prep 16/10 SP/XL, Amersham Biosciences, Uppsala, Sweden). The column was previously equilibrated with 40 mM Na-acetate buffer (pH 4.4). Bound proteins were eluted with a linear salt gradient to 1 M NaCl in 40 mM Na-acetate buffer (pH 4.4) at a flow rate of 0.5 mL/min using AKTA Prime System (Amersham Biosciences, Uppsala, Sweden). Fractions with PG or PME activity were pooled and stored at 4°C before use.

2.4. Gel Electrophoresis

After purification, the fractions of each variety showing PG activity were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (PhastSystem, Amersham Biosciences, Uppsala, Sweden) under denaturing conditions, using PhastGel homogenous (20%) and PhastGel Tris-tricine SDS buffer strips. Gel staining was performed with silver nitrate according to Heukeshoven, & Dernick (1985). The estimation of the molecular mass was derived by comparing the migration distance with a calibration curve of marker proteins (LMW marker: 14-97 kDa, Amersham Biosciences, Uppsala, Sweden) with known molecular mass: phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa), and α-lactalbumin (14 kDa).

2.5. Maldi-Tof-spec Analysis

PG fractions were analyzed by MALDI (matrix-assisted laser desorption ionization) coupled to a time-of-flight (TOF) tube for mass analysis, for accurate determination of the molecular masses of the peptides present.

MALDI-TOF MS was performed on a Reflex IV (Bruker Daltonic GmbH), equipped with a N_2-laser and pulsed ion extraction accessory. In each case, 1 µl of the sample solution was transferred to a steel target, mixed with 1 µl of a 50-mM solution of α-cyano-4-hydroxycinnamic acid in acetone and air-dried. Positive ion spectra were recorded in the linear mode within a mass range from m/z 3000 to m/z 20000.

2.6. Polygalacturonase Assay

Activity of tomato PG was measured spectrophotometrically based on the formation of reducing groups from polygalacturonic acid and quantifying these with 2-cyanoacetamide (Gross, 1982). The reaction mixture (400 µL) containing 0.2% polygalacturonic acid (350 µL) and enzyme sample (50 µL) was incubated at 40°C for 10 min. The reaction was stopped by the addition of 2 mL of cold 10 mM borate buffer, pH 9.0 and 0.4 mL 1% cyanoacetamide. The samples were mixed, immersed in an oil bath at 100°C for 10 min and cooled immediately in ice. After equilibration at room temperature, the absorbance was measured at 276 nm and 22°C using an UV-spectrophotometer (Ultrospec 1100 pro, Biochrom Ltd. Cambridge, UK). Blanks were prepared in the same way but using 50 µL of 40 mM Na-acetate buffer (pH 4.4). The amount of reducing groups formed was calculated using a standard curve of mono-D-galacturonic acid, assuming that the concentration of this acid is proportional to the concentration of reducing groups. The activity was reported in mM mono-D-galacturonic acid per minute at 40°C.

2.6. Pectinmethylesterase Assay

PME activity was determined by measuring the release of acid per unit time at pH 7.0 and 22°C (Crelier, Tâche, Raemy, Renken, & Raetz, 1995). The reaction mixture consisted of 250 µL of enzyme solution and 30 mL of a 0.35% apple pectin solution (70-75% esterification, Fluka) containing 0.12 mM of NaCl. Before injection of enzyme sample, the pectin solution was adjusted to pH 7.0. During hydrolysis at 22°C, the pH was maintained at 7.0 by the addition of

0.01N NaOH using an automatic pH-stat titrator (Metrohm, Switzerland). Every 15 s, for 10 or 15 min reaction time, the consumption of 0.01N NaOH was recorded. PME activity is proportional to the rate of consumption of NaOH (ΔV_{NaOH})/Δt). One unit of PME activity can be expressed as amount of enzyme that produces one micromole of acid per minute at pH 7 and 22°C:

(1)
$$PME(U/mL) = \frac{V \cdot N \cdot 1000}{V_s \cdot t_r}$$

where V is the volume of NaOH used during titration; N is the normality of NaOH (0.01N); V_s is the sample volume and t_r is the treatment time.

2.7. Thermal Stability of tomato PG and PME of the four different tomato varieties in Crude Extracts

Crude tomato PG and PME extracted from each variety was assayed at temperatures ranging from 25°C to 90°C for PG and from 25°C to 70°C for PME. 200 μL crude extract in 40 mM Na-acetate (pH 4.4) was filled manually using a syringe into capillary tubes (200 μL, 2 mm inner diameter, 140 mm length; BlauBrand IntraMark, Wertheim, Germany). Isothermal inactivation experiments were performed in a water bath with temperature control. After 5 min at constant temperature the samples were withdrawn from the water bath and immediately cooled in ice water. The residual PG and PME activities were measured within one h of storage at room temperature. No reactivation of PG or PME was observed during storage. Experiments were performed in duplicates.

2.8. Thermal and High-Pressure Stability of Purified tomato PGs and PME of the four different tomato varieties studied

Purified tomato PG and PME from the four different varieties in 40 mM Na-acetate (pH 4.4) were assayed after thermal treatment at temperatures ranging from 25°C to 90°C and from 25 to 70°C for PG and PME respectively, as described before for the tomato crude extracts. Experiments were done in duplicates.

Isothermal/isobaric inactivation experiments were performed in a laboratory scale multivessel high-pressure equipment (Resato, Roden, The Netherlands) with a glycol-oil mixture (TR-15, Resato) as pressure medium. A thermostated mantel, which surrounds each vessel, was connected to a cryostat, keeping the temperature constant during the experiment. The purified PGs and PMEs in 40 mM Na-acetate (pH 4.4) were filled in 0.5 mL flexible micro tubes (Biozym, The Netherlands) and enclosed in the pressure vessels already equilibrated at 25°C. The samples were assayed at pressures from 0.1 to 500 MPa and from 0.1 to 850 MPa for PG and PME respectively. Pressure was built up slowly (~100 MPa/min) to minimize adiabatic heating until attaining the desired pressure. After 2 min of equilibration period to ensure isothermal conditions, one vessel was decompressed and the enzyme activity of this sample was considered as a blank (A_0). After 15 min, the other vessels were decompressed and the activity of the samples was measured (A). Experiments were performed in duplicates.

3. RESULTS AND DISCUSSION

3.1. Purification of Tomato Polygalacturonase and Pectinmethylesterase

The elution profile for the enzyme extract of all the tomato varieties studied showed a number of protein peaks detected at 280 nm. All fractions were analyzed for PG and PME activity. The first peak presents PME activity whereas the second and third peak show PG activity (figure 1). It will be confirmed later by the heat treatment that the second peak corresponds to PG2 being totally inactivated after a treatment of 65°C during 5 min while PG from the third peak was stable after the same heat treatment, confirming the presence of PG1.

As far as the different tomato varieties is concerned, purified Perfectpeel and Soto were the ones that showed higher PME and PG activity (Table 1).

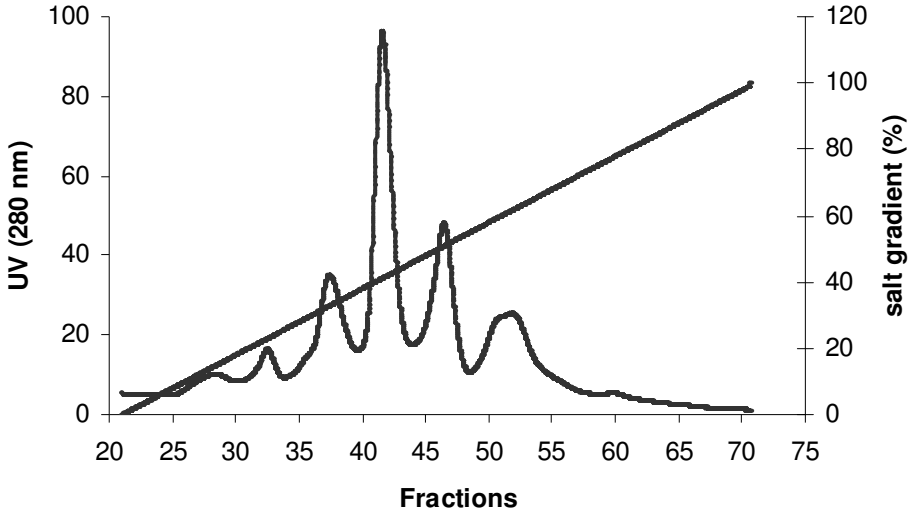


Figure 1. Elution profile for Soto variety PG in tomato crude extract on Hi Prep 16/10 SP/XL. Elution was performed with a linear salt gradient to 1.0 M NaCl in Na-acetate buffer (pH 4.4)

For all varieties, the purified PG2 activity was higher compared to PG1, a result that agrees with previous findings in a tomato variety, cultivated for fresh consumption (Peeters, Fachin, Smout, van Loey, & Hendrickx, 2004).

Table 1: Purified PME and PG activities for the different tomato varieties.

Tomato variety	PME (U/mL)	PG1(mM/min)[1]	PG2(mM/min)[1]
Galeón	46.86±0.96	0.026±0.001	0.068±0.002
Malpica	26.95±0.08	0.104±0.001	0.109±0.001
Perfectpeel	77.26±2.75	0.122±0.001	0.132±0.001
Soto	60.92±0.35	0.086±0.001	0.128±0.005

[1]mM reducing groups/min

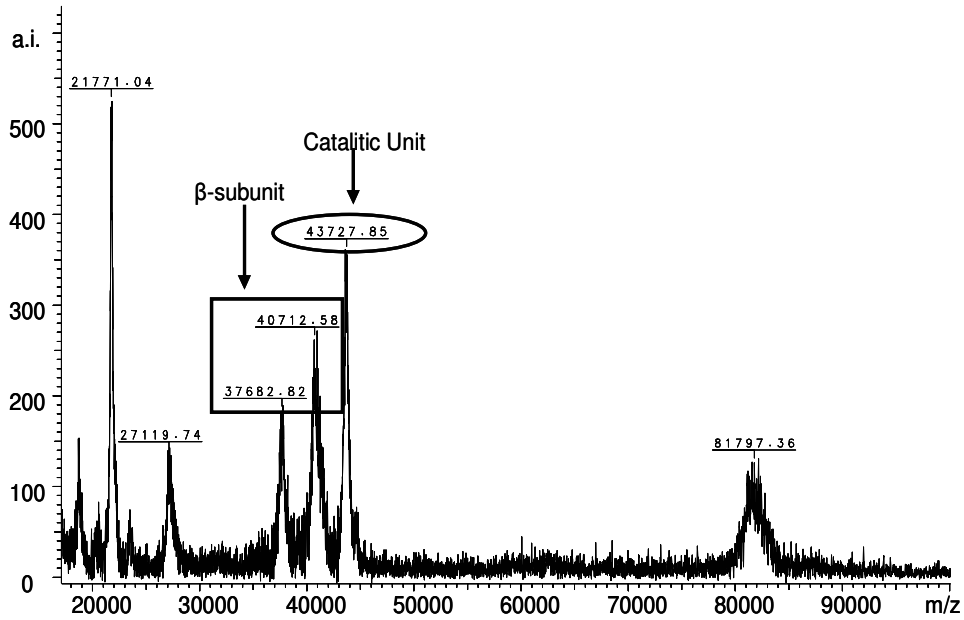
3.2. Gel Electrophoresis

After purification, the two fractions showing PG activity from each tomato variety were analyzed by SDS-PAGE electrophoresis on a 20% homogenous gel. PG1 and PG2 present an estimated molecular mass of about 43 kDa, a result that agrees with data previously reported in literature (Tucker, Robertson, & Grierson, 1981; Ali et al. 1982; Moshrefi et al. 1984; Pogson, Brady, & Orr, 1991; Fachin, 2003).

3.3. Maldi-Tof-spec Analysis

After analyzing all PG fractions by Maldi-Tof-spec analysis, some differences between the PG1 and PG2 samples can be pointed out (figure 2). For each tomato variety, and in both PG samples the same polypeptide, with a molecular mass of 43.7 kDa, representing the catalytic unit, is present. This result agrees with the molar mass obtained by SDS-PAGE electrophoresis and the values reported in literature (Tucker et al. 1981; Ali et al. 1982; Moshrefi et al. 1984; Pogson et al. 1991; Fachin, 2003). For the PG1 fractions we could further identify the proteins involved. The presence of two extra proteins with molecular masses between 37 and 41 kDa, corresponding to β-subunits could be confirmed. It is known that PG1 is a complex between PG2 (the catalytic subunit) and two catalytically inactive glycoproteins called β-subunits (Moshrefi et al. 1983; Moshrefi et al. 1984; Knegt et al. 1988; DellaPenna et al. 1996; Bergey et al. 1999). Zheng et al, (1992) found a similar molecular mass for β-subunit. Figure 2 shows the spectra obtained after Maldi-Tof analysis for PG1 and PG2 from Perfectpeel variety. Similar results were obtained for all the varieties studied.

(A)



(B)

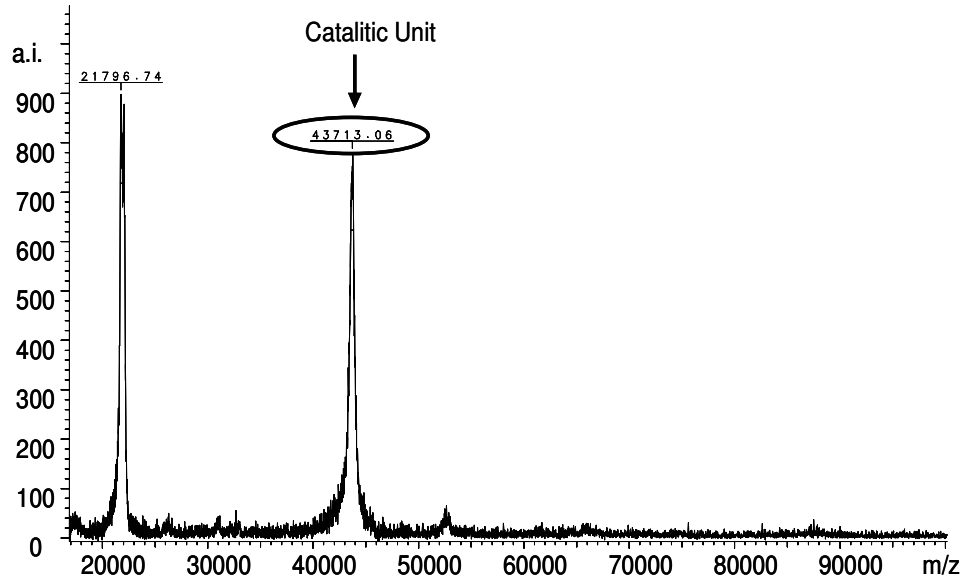


Figure 2. MALDI-TOF spectra of PG1 (A) and PG2 (B) fractions from Perfectpeel tomato variety.

3.4. Thermal Stability of PG and PME of the four different tomato varieties in tomato crude extracts

Crude tomato PG and PME were assayed at pre-set temperatures for 5 min. The relative residual activity is shown in figure 3. For PG two inactivation phases are observed, the first one starts at around 55°C and it corresponds to PG2, the thermolabile enzyme fraction, and the second one starts at around 80°C and it corresponds to PG1, the heat stable enzyme fraction.

After 5 min at 90°C almost complete inactivation is observed. It is known that crude extracts of different varieties contained different levels of PG1 and PG2 (Tucker, Robertson, & Grierson et al. 1980) which is confirmed in this study; each variety treated has a different percentage of PG1 and PG2. Galeón and Soto have about 50% of each enzyme fraction (46% PG1 for Galeón and 54% PG1 for Soto), whereas Malpica and Perfectpeel contained more PG 1 than PG 2 (~75% PG1).

For thermal stability of tomato PME in crude extract, a progressive activity loss was observed from 55°C up to 70°C for all four tomato varieties. Contrary to PG, the heat stability curve of PME does not indicate the presence of a heat stable PME fraction in the crude extract.

Fachin et al. (2003) obtained similar results in a crude extract from another tomato variety (Prince), which presented the same two inactivation phases for PG, being PG1 isoform in a percentage of about 60%.

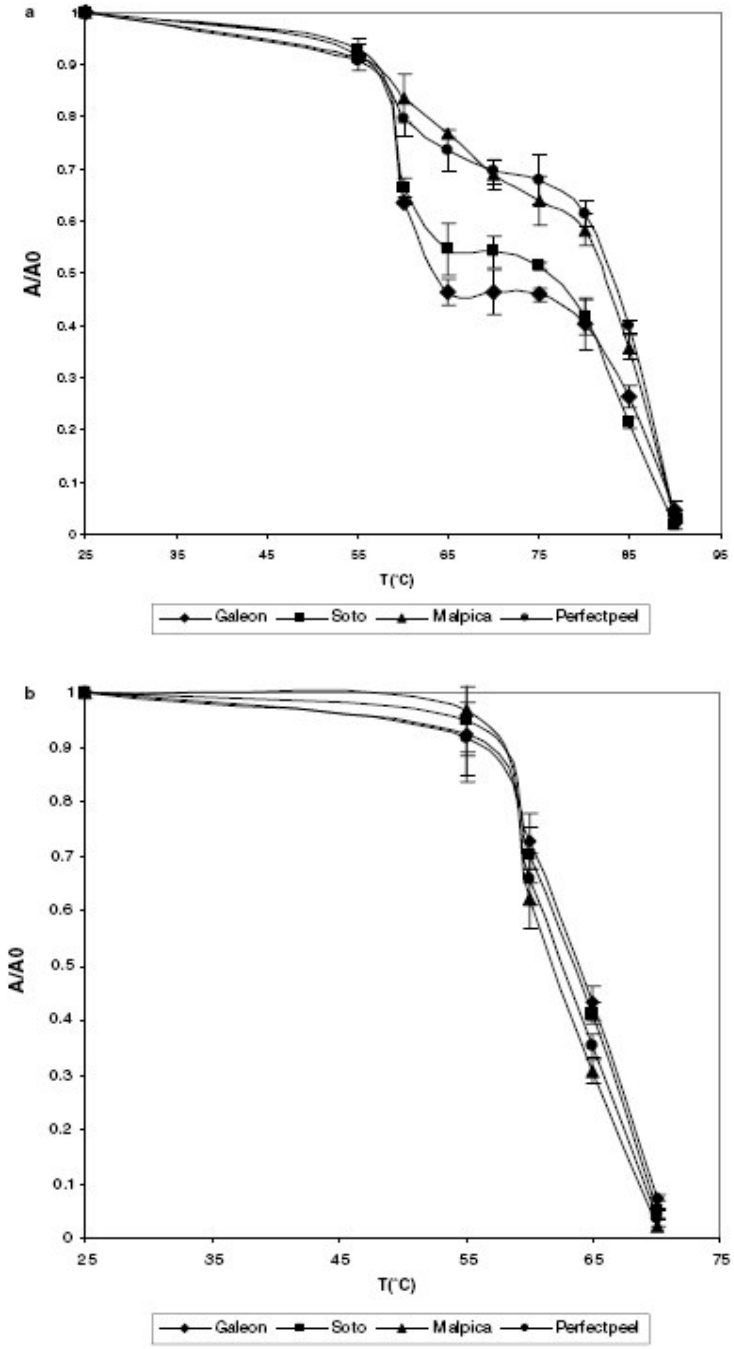


Figure 3. Thermal stability of PG (A) and PME (B) of the four different varieties studied in tomato crude extracts after 5 min at pre-set temperatures.

3.5. Thermal and High-Pressure Stability of Purified PGs and PME of the four different tomato varieties studied

The thermal stability of purified tomato PME, PG1 and PG2 in 40 mM Na-acetate buffer (pH 4.4) was studied at pre-set temperatures for 5 min, and the relative residual PME and PG activity was determined. As can be seen in figure 4, showing the relative residual PME and PG activity in function of temperature for each variety, PG1 and PG2 have large differences in thermal stability (Pressey et al. 1973; Knegt et al. 1988; López, Sánchez, Vercet, & Burgos, 1997; Fachin, 2003; Peeters et al. 2004). All varieties have the same behavior against temperature, all of them show the thermolabile enzyme (PG2) which is completely inactivated after 5 minutes at 65°C, and the heat stable enzyme (PG1) that still retains activity after 5 min at 65°C. Even after 5 min at 90°C, there is still some residual PG activity, indicating that the β-subunit transforms the PG polypeptide in a complex with enhanced thermal resistance. Peeters et al. (2004) also found this behavior for a tomato variety cultivated for fresh consumption. On one hand, they studied the thermal inactivation of β-subunit at 140°C 5 min, and some remaining activity was found. On the other, the residual fractions containing the β-subunit were incubated with an excess PG2 and heat treated at 65°C for 5 min. Under these conditions, PG activity was found, indicating that β-subunit was able to combine in vitro with PG into PG1, increasing the thermal resistance of PG polypeptide and explaing the two inactivation phases observed for thermal inactivation of tomato PG in crude extract (Fig. 3).

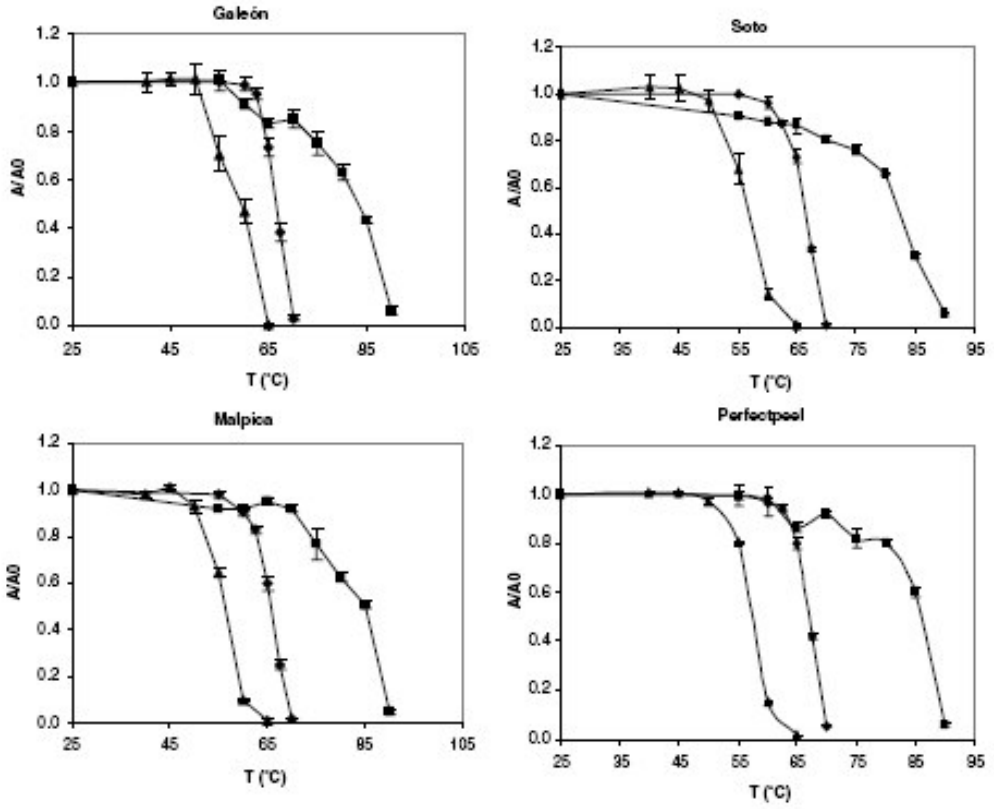


Figure 4: Thermal stability of purified tomato PG1 (■), PG2 (▲) and PME (♦) after 5 min at pre-set temperatures for each variety.

With regard to PME, the enzyme is completely inactivated after 5 min at 70 °C in all the varieties studied. Therefore, purified tomato PME is more thermostable than purified tomato PG2 but less thermostable than purified tomato PG1. These results, for tomato processing varieties, confirm those found by Fachin (2003) in a single tomato variety (Prince).

Purified tomato PG1, PG2 and PME were submitted to a screening study in a pressure range from 0.1 to 500 MPa at 25°C for PG1 and PG2 and from 0.1 to 850 MPa for PME (Fig. 5). The reduction of the activity after 15 min was

compared to the activity at time zero, after the equilibration period of 2 min. In contrast to a clear difference in thermostability, PG1 and PG2 both are pressure-labile in the pressure range studied. At room temperature PG1 and PG2 can both be inactivated in the pressure range from 300 to 500 MPa. Up to 300 MPa there was almost no decrease in the activity of PG 1 and PG 2.

Several studies have pointed out that polymeric proteins, stabilized by non-covalent bounds, are dissociated at low pressures (<150 MPa) (Balny, & Masson, 1993). This can suggest that the complex between PG and β-subunit dissociates under pressure which explains the similar stability of PG1 and PG2 against pressure. Although PG1 is not easily inactivated by thermal treatments, it can be completely inactivated when applying mild pressure treatments.

On the contrary, PME was found pressure stable under all conditions studied. Up to 700 MPa, there was almost no decrease in the activity, and only 50% of the enzyme activity was inactivated after 15 min treatment at 850MPa for all tomato varieties tested.

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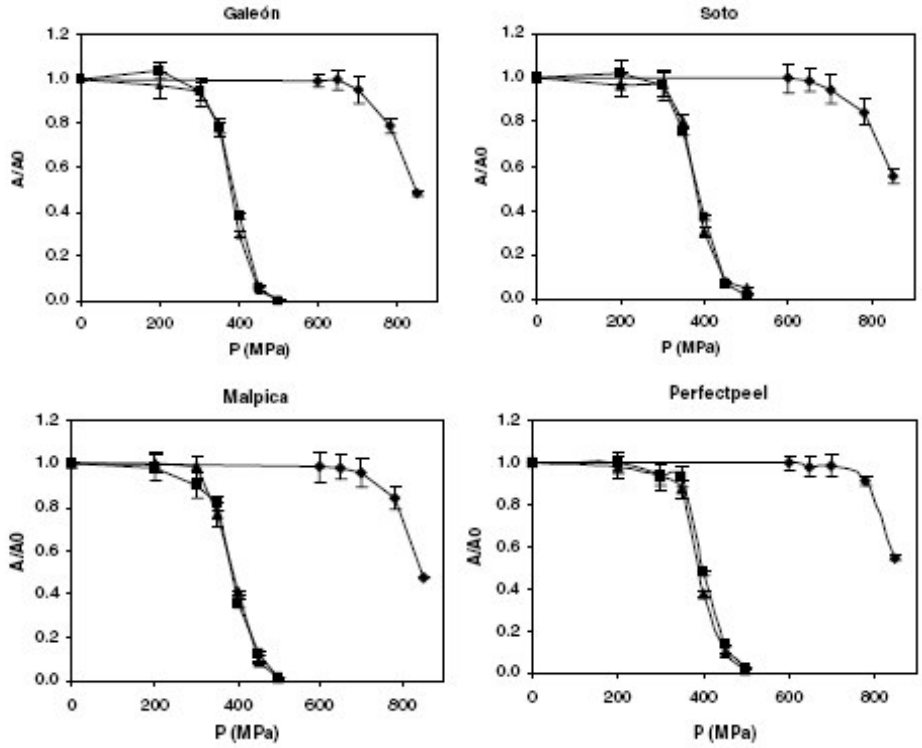

Figure 5: Pressure stability of purified tomato PG1 (■), PG2 (▲) and PME (♦) after 15 min at pre-set pressures for each variety

Although there are significant differences in the activity level of PG and PME between the four tomato varieties studied, no difference is found in the purified PG and PME thermal / high pressure stability of the four varieties. This work, therefore confirms, for a number of tomato varieties typically used in food processing, earlier results obtained for a single tomato variety used for fresh consumption (Fachin et al. 2003; Peeters et al. 2004).

4. CONCLUSION

This study allows to conclude that high pressure treatment of tomato based products allows selective inactivation of PG (e.g. 500 MPa, 15 min, 25°C) followed by, if necessary, a mild heat treatment to inactivate PME (e.g. 70°C, 5 min). Such processing concepts, based on our current know how on the role of pectinases in fruit and vegetable processing, can be a basis for improving texture and rheology of tomato based products.

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REFERENCES

Ali, Z. M., & Brady, C. J. (1982). Purification and characterization of the polygalacturonases of tomato fruits. *Australian Journal Plant Physiology*, 9, 155-169.

Balny, C., & Masson, P. (1993). Effects of high pressure on proteins. *Food Reviews. International,* 9, 611-628.

Bergey, D. R., Orozco-Cardenas, M., De Moura, D. S., & Ryan, C. A. (1999) A wound- and systemin-inducible polygalacturonase in tomato leaves. *Plant Biology,* 96, 1756-1760.

Crelier, S., Tâche, M. C., Raemy, A., Renken, A., & Raetz, E. (1995). High-pressure for the inactivation of enzymes in food products: thermal and HHP treatment of tomato pectin methyl esterase. Poster presentation at 9th World Congress on Food Science & Technology. Budapest, Hungary.

Crelier, S., Robert, M.C., Claude, J., & Juillerat, M. A. (2001). Tomato pectinmethylesterase and polygalacturonase display opposite behaviors regarding heat- and pressure-induced inactivation. *Journal of Agriculture and Food Chemistry*, 49, 5566-5575.

DellaPenna, D., Watson, C., Liu, J. P., & Schuchman, D. (1996). The β-subunit of tomato fruit Polygalacturonase Isoenzyme 1 defines a new class of plant cell proteins involved in pectin metabolism: AroGPs (Aromatic Amino Acid Rich Glyco Proteins). In *Pectins and Pectinesterases*. Eds. Visser, J., & Boragen, A. G. J. Elsevier Science, Amsterdam, The Netherlands, pp 247-262.

Fachin, D., Van Loey A., Nguyen, B. L., Verlent, I., Indrawati, I., & Hendrickx, M. E. (2003). Inactivation kinetics of polygalacturonase in tomato juice. *Innovative Food Sciences Emerging Technologies,* 4, 135-142.

Fachin, D. Temperature and pressure inactivation of tomato pectinases: a kinetic study. Doctoral dissertation, Department of Food Technology, Faculty of Agriculture and Applied Biology Sciences, Katholieke Universiteit Leuven, Leuven, Belgium (2003).

Farkas, D. F., & Hoover, D. G. (2000). High Pressure Processing. *Journal of Food Sciences*, 65, 47-64.

Grahl, T., & Märkl, H. (1996). Killing of microorganisms by pulsed electric fields. Applied Microbiology and Biotechnology, 45, 148-157.

Gross, K. C. (1982). A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide. *HortSciences*, 17, 933-934.

Hackett, M.M., Lee, J.H., Francis, D., & Schwartz, S.J. (2004). Thermal stability and isomerization of lycopene in tomato from different varieties. *Journal of Food Science*, 69, 536-537.

Heukeshoven, J., & Dernick, R. (1985). Simplified method for silver staining of proteins in polyacrylamide gels and the mechanisims of silver staining. *Electrophoresis*, 6, 103-112.

Hugas, M., Garriga, M., & Monfort, J. M. (2002). New mild technology in meat processing: High pressure as a model technology. *Meat Sciences*, 62, 359-371.

Knegt, E., Veermer, E., & Bruinsma, J. (1988). Conversion of the polygalacturonase isoenzymes from ripening tomato fruits. *Physiology Plant*, 72, 108-114.

Krebbers, B., Matser, A. M., Hoogerwerf, S. W., Moezelaar, R., Tomassen, M. M. M., & van den Berg, R. W. (2003). Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innovative Food Sciences and Emerging Technology*, 4, 377-385.

Lado, B. H., & Yousef, A. E. (2002). Alternative food-preservation Technologies: efficacy and mechanisms. Microbes and Infection, 4, 433-440.

López, P., Sánchez, A. C., Vercet, A., & Burgos, J. (1997). Thermal resistance of tomato polygalacturonase and pectinmethylesterase at physiological pH. Zeitschrift fur Lebensmittel-untersuchung und-forschung. A: Food Research and Technology, 204, 146-150.

Marco, D. B. F., Joseph, V., & John, K. (1997). Mechanisms of disease: antioxidants and atherosclerotic heart disease, New England Journal of Medicine, 337, 408–416.

Moshrefi, M., & Luh, B. S. (1983). Carbohydrate composition and electrophoretic properties of tomato polygalacturonase isoenzymes. *European Journal Biochemistry,* 135, 511-514.

Moshrefi, M., & Luh, B. S. (1984). Purification and characterization of two tomato polygalacturonase isoenzymes. *Journal Food Biochemistry*, 8, 39-54.

Peeters, L., Fachin, D., Smout, C., Van Loey, A., & Hendrickx, M. E. (2004). Influence of β-subunit on thermal and high-pressure process stability of tomato polygalacturonase. *Biotechnology Bioengineering,* 86, 543-549.

Pogson, B. J., Brady, C. J., & Orr, G. R. (1991). On the occurrence and structure of subunits of endopolygalacturonase isoforms in mature-green and ripening tomato fruits. *Australian Journal Plant Physiology,* 18, 65-79.

Pressey, R., & Avants, J. K. (1973). Two forms of polygalacturonase in tomatoes. *Biochemistry Biophysics Acta,* 309, 363-369.

Pressey, R. (1986). Extraction and assay of tomato polygalacturonases. *HortSciences,* 21, 490-492.

Sanchez, M.C., Valencia, C., Gallegos, C., Ciruelos, A., & Latorre, A. (2002). Influence of processing on the rheological properties of tomato paste. *Journal of the Science of Food and Agriculture*, 82, 990-997.

Tucker, G. A., Robertson, N. G., & Grierson, D. (1981). The conversion of tomato fruit polygalacturonase isoenzyme 2 into isoenzyme 1 in vitro. *European Journal Biochemistry,* 115, 87-90.

Tucker, G. A., Robertson, N. G., & Grierson, D. (1982). Purification and changes in activities of tomato pectinesterases isoenzymes. *Journal of the Science of Food and Agriculture,* 33, 396-400.

Tucker, G. A., Robertson, N. G., & Grierson, D. (1980). Changes in polygalacturonase isoenzymes during the ripening of normal and mutant tomato fruit. *European Journal Biochemistry,* 112, 119-124.

Verlent, I., Van Loey, A., Smout, C., Duvetter, T., & Hendrickx, M. E. (2004). Purified tomato polygalacturonase activity during thermal and high-pressure treatment. *Biotechnolgy. Bioengineering,* 86 (1), 63-71.

Zheng, L., Heupel, R. C., & DellaPenna, D. (1992). The beta-subunit of tomato fruit polygalacturonase isoenzyme 1: Isolation, characterization, and identification of unique structural features. *Plant Cell*, 4, 1147-1156.

RESUMEN DE LOS RESULTADOS

6. RESUMEN DE LOS RESULTADOS

1. Horchata de chufa.

Para caracterizar los diferentes tipos de horchatas comercializados en España y establecer la influencia de los tratamientos de conservación en sus parámetros nutricionales, se seleccionan distintas muestras de horchata de chufa de varias marcas comerciales, sometidas a diversos tipos de procesado para su conservación. Se observa que existen pocas diferencias entre los distintos tipos de horchata, excepto la horchata condensada, que posee valores más bajos de sólidos solubles, cenizas y azúcares que los demás tipos de horchata. La horchata condensada pasteurizada presenta además valores más bajos de grasa y almidón, y la horchata natural presenta un contenido proteico más elevado que las demás.

La reglamentación técnico-sanitaria se cumple en todos los casos, excepto en el contenido en grasa de todas las horchatas esterilizadas y de una de las horchatas UHT, en las que permanece por debajo de los mínimos establecidos en esta reglamentación.

Para estudiar si los Pulsos Eléctricos de Alta Intensidad pueden ser utilizados para obtener una horchata de calidad, así como aumentar su vida útil, manteniendo sus características organolépticas similares a las de la horchata natural, se determinan diversos parámetros nutritivos y de calidad, tanto en la horchata sometida a tratamientos de PEAI como en la horchata natural. Se observa que sólamente la actividad peroxidásica disminuye de manera estadísticamente significativa tras el procesado no térmico. Además, se estudia la estabilidad de la horchata tratada mediante Pulsos Eléctricos durante su almacenamiento en refrigeración a 2ºC, observando que sólamente la actividad peroxidásica y el pH varían, obteniéndose una correlación negativa entre ellos.

2. Puesta a punto de un método de determinación de carotenoides en zumos de frutas y vegetales.

Ante la necesidad de analizar diversos parámetros nutritivos y de calidad de zumos de frutas y hortalizas sometidos a la tecnología no térmica de PEAI, se pone a punto un método cromatográfico para la separación, identificación y cuantificación de carotenoides y vitamina A en dichos zumos.

Previo a la determinación cromatográfica de los carotenoides, es necesario un proceso de extracción (etanol-hexano, 4:3, v/v) seguido de una saponificación con KOH durante 30 minutos a temperatura ambiente. Se utiliza palmitato de retinol como patrón interno.Durante la determinación cromatográfica se aplica un gradiente de temperatura, y se utiliza una fase móvil ternaria: acetato amónico en metanol, tert-butil-metil-éter y agua (en gradiente de concentración). La columna seleccionada para la separación de los carotenoides es una C18 (Vydac 201TP54) y se utiliza un detector de foto-diodo array, puesto que es una herramienta muy valiosa para la caracterización de *cis*- y *trans*-carotenoides.

Con el método propuesto se pueden caracterizar los carotenoides más importantes a partir de sus espectros y de sus tiempos de retención. Los parámetros analíticos muestran que es un método sensible, exacto y reproducible, además de ser un método sencillo.

3. Zumo de naranja-zanahoria.

Se somete el zumo mezcla de naranja-zanahoria (80:20, v/v) a tratamientos mediante Pulsos Eléctricos de Alta Intensidad, utilizando diferentes intensidades de campo (25, 30, 35, y 40 kV/cm) y diferentes tiempos de tratamiento (30-340 μs). También, con el fin de comparar esta nueva tecnología con la pasteurización

tradicional, el zumo de naranja-zanahoria se procesa a 98ºC durante 21 segundos. Se observa que los tratamientos por PEAI causan un aumento estadísticamente significativo en la concentración de los carotenoides identificados respecto al zumo sin tratar al aumentar el tiempo de tratamiento, mientras que cuando el zumo se procesa por pasteurización, la mayoria de los carotenoides disminuyen su concentración. La concentración de vitamina A es mayor en el zumo de naranja-zanahoria tras los tratamientos por PEAI a los campos 25 y 30 kV/cm que en el zumo pasteurizado.

Del mismo modo, se determina la cinética de degradación del ácido ascórbico en el zumo mezcla de naranja-zanahoria tratado por PEAI para establecer su vida útil. Para ello también se estudian diferentes intensidades de campo (25-40 kV/cm) y distintos tiempos de tratamiento (30-340 μs).

Tras el tratamiento por PEAI del zumo de naranja-zanahoria la concentración remanente de ácido ascórbico es del 90% respecto al zumo de naranja-zanahoria natural, mientras que tras la pasteurización del mismo esta concentración es del 83%.

Para elegir las mejores condiciones de tratamiento por PEAI, es necesario tener en cuenta no solamente la inactivación enzimática y microbiológica, sino también el valor nutritivo y las características organolépticas del zumo. Basándonos en la cinética de degradación del ácido ascórbico, el campo de tratamiento seleccionado fue 25 kV/cm, y de este modo se aplicó esta intensidad de tratamiento para estudiar la evolución de la concentración de vitamina C durante el almacenamiento en refrigeración a 2 y 10ºC del zumo de naranja-zanahoria. También, se observa que la cinética de degradación del ácido ascórbico durante el almacenamiento en refrigeración es mayor en el zumo pasteurizado que en el zumo tratado por PEAI, y a su vez, es mayor en los zumos almacenados a 10ºC que en los almacenados a 2ºC. El tratamiento por PEAI aumenta la vida útil (basándose en una reducción del 50% de ácido ascórbico) a 50 días del zumo de naranja-zanahoria almacenado a 2ºC. Por tanto,

se puede considerar que los PEAI son una alternativa al tratamiento térmico convencional del zumo de naranja-zanahoria.

Se analiza la influencia del almacenamiento en congelación (-40ºC, 132 días) sobre los carotenoides y la vitamina C del zumo de naranja-zanahoria, observándose que este tipo de almacenamiento causa un descenso en la concentración de ácido ascórbico del 4.1%. Por el contrario, la actividad vitamínica A aumenta durante el almacenamiento en congelación del zumo siguiendo un modelo lineal (R^2 = 0.87). Nueve de los 14 carotenoides identificados aumentan significativamente tras 132 días de almacenamiento a -40ºC. Solamente anteraxantina y la mezcla 9-cis-violaxantina + neoxantina disminuyen su concentración durante el período estudiado.

4. Zumo de naranja.

Se estudia el zumo de naranja, y se somete al tratamiento de Pulsos Eléctricos de Alta Intensidad, a diferentes intensidades de campo (25, 30, 35 and 40 kV/cm) y distintos tiempos (30-340 μs). Paralelamente, se aplica al zumo de naranja un tratamiento de pasteurización (90ºC, 20s), con el fin de comparar el efecto que produce el tipo de procesado en la concentración de carotenoides, vitamina A, vitamina C, color, y otros parámetros de calidad del zumo de naranja.

Los tratamientos por PEAI son una alternativa a los térmicos, puesto que aún cuando se aplican los más intensos, la concentración de carotenoides y vitamina A se mantiene similar a la del zumo fresco, y algunos de los carotenoides identificados, poseen incluso mayor concentración tras el procesado del zumo de naranja con los tratamientos de PEAI más intensos. En ningún caso la concentración de los carotenoides identificados supera la de cada uno de ellos en el zumo de naranja natural.

Este resultado es importante puesto que difiere del obtenido en el zumo de naranja-zanahoria, en el que tras los tratamientos mediante PEAI la concentración de la mayoría de los carotenoides identificados aumenta respecto al zumo natural.

Sin embargo, el tratamiento de pasteurización produce un descenso importante en el contenido de carotenoides y de vitamina A. De manera que la concentración de carotenoides decrece un 12.6% tras la pasteurización, mientras que tras los PEAI decrece un 9.6%, 6.3% o 7.8% al aplicar las intensidades de campo de 25, 30 y 40 kV/cm, respectivamente.

Al analizar el color, se puede concluir que está influenciado por la presencia de diferentes carotenoides en el zumo de naranja, obteniéndose correlaciones entre el color y la concentración de carotenoides. El zumo de naranja tratado con Pulsos Eléctricos de Alta Intensidad y el zumo de naranja pasteurizado, muestran mayor tendencia hacia el color amarillo y menor tendencia hacia el color rojo que el zumo de naranja natural, siendo estas variaciones mayores en el zumo de naranja pasteurizado. La luminosidad de los zumos permanece prácticamente invariable al aplicar cualquiera de los tratamientos de conservación estudiados.

La cinética de degradación del ácido ascórbico es similar en todos los tratamientos de PEAI estudiados. Se elige una intensidad de campo de 30 kV/cm y un tiempo de tratamiento de 100 μs para estudiar el almacenamiento en refrigeración (2 y 10ºC) y la vida útil del zumo de naranja.

Se realiza un estudio comparativo de la evolución y modificación de los diferentes carotenoides identificados y de la concentración de vitamina A entre el zumo de naranja tratado mediante PEAI (30 kV/cm, 100 μs) y el zumo de naranja pasteurizado (90ºC, 20 s), almacenados durante 7 semanas a 2 y 10ºC.

A tiempo inicial, la concentración de carotenoides totales en el zumo de naranja pasteurizado se reduce en un 12.6% respecto al zumo de naranja sin tratar,

mientras que en el zumo tratado por PEAI el descenso es del 6.7%, siendo estos resultados similares a los descritos anteriormente al estudiar los diferentes tratamientos de PEAI. De manera similar, la concentración de vitamina A decrece en menor medida en el zumo de naranja tratado por PEAI (7.5%) que en el zumo de naranja pasteurizado (15.6%), respecto al zumo de naranja natural.

Durante el almacenamiento en refrigeración, el descenso de la concentración de carotenoides totales y de vitamina A es mayor en el zumo pasteurizado que en el tratado mediante PEAI. Durante el almacenamiento a 10ºC, se forma el carotenoide auroxantina en el zumo de naranja tratado por PEAI. Este carotenoide es un producto de degradación del carotenoide violaxantina. La concentración de anteraxantina disminuye durante el almacenamiento y se transforma en el carotenoide mutatoxantina, excepto en el zumo de naranja pasteurizado almacenado a 2ºC.

Durante el almacenamiento en refrigeración, los carotenoides totales y la vitamina A se mantienen durante más tiempo en el zumo de naranja tratado mediante pulsos, que en zumo conservado utilizando tratamientos de pasteurización convencionales. Del mismo modo, la calidad nutricional del zumo de naranja (concentración de vitamina C) se mantiene más tiempo en los zumos procesados por pulsos eléctricos que en los zumos pasteurizados.

El tratamiento por PEAI permite que el zumo de naranja alcance una vida útil de 99 días almacenado en refrigeración a 10 ºC (basándose en una reducción del 50% del contenido de ácido ascórbico), mientras que con los tratamientos por pasteurización, el zumo de naranja sólamente alcanza 35 días refrigerado a 10 ºC.

Se observa que un aumento sustancial de la vida útil de zumo de naranja cuando se almacena a 2 ºC, por lo que es recomendable almacenarlo a dicha temperatura para asegurar que llega al consumidor con un alto valor nutritivo.

Se estudia también el efecto que producen los Pulsos Eléctricos de Alta Intensidad en el color, hidroximetilfurfural (HMF) e índice de pardeamiento del zumo de naranja almacenado durante 7 semanas a 2 y 10ºC, comparándolo con el zumo de naranja pasteurizado.

El zumo pasterizado presenta mayor tendencia al color amarillo (b*) y menor tendencia al rojo (a*) que el zumo de naranja natural, mientras que el zumo de naranja tratado mediante PEAI presenta una coloración intermedia, más similar al zumo natural. Las variaciones de color (ΔE) durante el almacenamiento son superiores en el zumo de naranja pasterizado que en el zumo tratado mediante PEAI. El zumo de naranja sometido a tratamientos no térmicos presenta menor pardeamiento no enzimático que el pasteurizado. Se produce un aumento significativo de este parámetro a partir de la semana 4 de almacenamiento en todos los zumos refrigerados a 10ºC, mientras que a 2ºC se mantienen los valores del índice de pardeamiento durante más tiempo. No existen variaciones significativas en los contenidos de HMF de los zumos tratados por HIPEF o pasteurizados respecto al zumo de naranja natural y durante el almacenamiento, el HMF permanece siempre por debajo de los valores máximos establecidos.

También se determina el efecto que producen los tratamientos por PEAI y la pasteurización en el poder antioxidante y los fenoles solubles totales en el zumo de naranja, así como la variación de estos parámetros durante el almacenamiento en refrigeración del zumo.

El poder antioxidante total disminuye de manera estadísticamente significativa ($p<0.05$) al procesar el zumo de naranja por ambos tipos de tratamiento, pero lo hace en mayor medida tras la pasteurización del mismo (disminución del 12.9% tras el procesado por PEAI y 38.21% tras la pasteurización). El poder antioxidante en los zumos de naranja durante el almacenamiento en refrigeración disminuye en las muestras analizadas, siendo este descenso mayor en las muestras almacenadas a 10 ºC. Los fenoles solubles totales son siempre ligeramente superiores en el zumo de naranja natural, seguido del zumo tratado

por PEAI y por último del zumo pasterizado, aunque estas diferencias no son estadísticamente significativas ($p>0.05$).

5. Tomate.

Durante la estancia en la Universidad Católica de Leuven (Bélgica), se estudia la influencia del tratamiento térmico y del tratamiento por Altas Presiones sobre la textura de diferentes variedades de tomate utilizadas en la industria alimentaria para su procesado. Se estudia la inactivación de las enzimas involucradas en la degradación de la pectina del tomate (Polygalacturonasa y Pectinmetilesterasa), responsables de la textura de los productos derivados del mismo

Las enzimas poligalacturoasa (PG) y pectinmetilesterasa (PME) se extraen y purifican a partir de cuatro variedades de tomates (Galeón, Malpica, Perfectpeel y Soto) utilizadas en la industria para su procesado.

El tratamiento por Altas Presiones de los productos derivados del tomate, permite una inactivación selectiva del enzima Poligalacturonasa, manteniendo intacta la Pectinmetilesterasa.

Teniendo en cuenta el papel que juegan las pectinasas en el procesado de frutas y vegetales, esto puede ser la base para mejorar la textura y la reología de estos productos.

CONCLUSIONES

7. CONCLUSIONES

Del estudio realizado se pueden establecer las siguientes conclusiones:

1. El método de cromatografía líquida con gradiente de elución ternario y detector de fotodiodo-array, puesto a punto, permite la separación, identificación y cuantificación de carotenoides y sus isómeros geométricos en zumos de frutas y hortalizas. Como muestran los parámetros analíticos, es un método sensible, fiable, exacto y reproducible.

2. La aplicación de la tecnología de Pulsos Eléctricos de Alta Intensidad (PEAI) permite obtener una horchata de calidad "similar a la horchata natural" y aumentar su vida útil, aunque ligeramente, manteniendo sus características organolépticas.

3. Tras el tratamiento por PEAI, la concentración de carotenoides en el zumo de naranja es similar a la del zumo fresco, mientras que en el zumo de naranja-zanahoria aumenta significativamente. Sin embargo, tras la pasteurización la concentración de carotenoides disminuye en ambos zumos.

4. Durante el almacenamiento en refrigeración del zumo de naranja-zanahoria y del zumo de naranja, la concentración de carotenoides y vitamina A se mantiene durante más tiempo en los zumos procesados por PEAI que en los pasteurizados, aumentando así la vida útil de los mismos.

5. La concentración de ácido ascórbico disminuye en menor medida en el zumo de naranja-zanahoria y en el zumo de naranja tratados por PEAI que en los pasteurizados; la velocidad de degradación del ácido ascórbico durante el almacenamiento en refrigeración es mayor en los zumos pasteurizados que en los tratados por PEAI.

6. Al almacenar en congelación a -40ºC el zumo de naranja-zanahoria, la concentración de ácido ascórbico disminuye y la de vitamina A aumenta.

7. El zumo de naranja tratado mediante PEAI presenta una coloración similar al zumo natural. Las variaciones de color durante el almacenamiento son mayores en el zumo de naranja pasterizado que en el tratado por PEAI. El zumo de naranja sometido a PEAI presenta menor pardeamiento no enzimático que el pasteurizado.

8. En el zumo de naranja, el poder antioxidante total y los fenoles solubles totales disminuyen en menor medida tras los tratamientos por PEAI que por pasteurización

9. El tratamiento por Altas Presiones puede ser la base para mejorar la textura y la reología de los productos derivados del tomate, ya que permite una inactivación selectiva de la enzima Poligalacturonasa. Para inactivar la Pectinmetilesterasa es necesario aplicar tratamientos severos.