TITLE

Effects of β-cyclodextrins on Antioxidant activity, carotenoids profile and vitamin C in organic and traditional ultrafrozen mandarin juice.

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ABSTRACT

Traducir Se realizó un estudio de zumos de mandarina ecológica y tradicional ultracongelados, recién exprimidos y sin pasteurizar, para determinar las pérdidas de ácido ascórbico, carotenoides, color, capacidad antioxidante y efecto de las β-ciclodextrinas durante un periodo de almacenamiento de 145 días. El contenido de Vitamina C se redujo de un valor inicial de 540 y 470 mg L^{-1} a 515 y 447 mg L^{-1} para ecológico y tradicional, respectivamente. En relación al perfil de carotenoides, se observó una gran estabilidad de los mismos, el carotenoide mayoritario fue β-criptoxantina (7,21± 0,31 mg L^{-1}) seguido de luteína y zeaxantina. Las pérdidas totales fueron del 10% para β-criptoxantina y del 5% para luteína y zeaxantina. β-caroteno fue el carotenoide minoritario para todos los casos. Las medidas de color cielab* provocaron variaciones de color las cuales se detectaron mediante un analisis sensorial. La capacidad antioxidante fue de 0,04 y 0,06 mMT mL^{-1} para orgánico y convencional, con pérdidas que no superaron el 18%. La adicción de ciclodextrinas no provocó ningún efecto sobre los parámetros analizados. Estos datos demuestran que el tratamiento por ultracongelación mantiene estables la calidad nutricional y organoléptica zumos de mandarina.

Key Words: β-cryptoxanthin; color; carotenoids; citrus; antioxidant, mandarin juice

INTRODUCTION

Actually, fruit juices have experienced high growth due, among other things, to progress in the cultivation and improvement on fruit processing, transport and distribution.[1] Mandarin juice production in Spain is low compared with the production of orange juice, but is expected to be higher due to market saturation in fresh and to the pleasant taste, fresh aroma and its great nutritional value. Spain currently produces 2.5 million tons of mandarins per year and production is increasing. The most widespread crop is clementines mandarins cultivar (1.6 million tons) whose about 60% of production is exported to other countries, particulary to North of Europe and United States of America.[2]

Mandarin is a fruit which has more carotenoids in their composition, providing beta-cryptoxanthin and β-carotene in amounts outstanding.[3] The antioxidant activity of these phytochemicals and vitamin C, provide the food physiological properties which go beyond the proper nutrition. Antioxidants fight harmful action of free radicals, substances responsible for the development of cardiovascular diseases, degenerative diseases and cancer.[4] The synthesis and composition of these compounds can oscillate depending on the type of species, variety of fruit, climatic factors, industrial processing, storage conditions and others.[5]

Cultivation practices such as the presence or absence of certain pesticides, fertilizer or type of irrigation can also affect the phytochemical composition of the fruit, both qualitatively and quantitatively.[5] Therefore, now is encouraging the development of products with improved nutritional quality, such as fruits and vegetables in ecological systems.

Organic farming can be defined as a set of agricultural techniques that exclude the use of synthetic chemicals, including fertilizers, pesticides, antibiotics, etc.., With the objective of preserving the environment, maintain or increase soil fertility and provide food with all its natural properties.[6]

Organic farming has become one of the most dynamic agricultural sectors of the European Union, with sales of organic products 13 to 14 billion euros in 2005. In the European Union, the area for to organic crops is 3.5 million hectares, 3% of the total agricultural area, Spain being one of the countries with the largest organic area of the EU. The orange and mandarin are one of the most demanded by the consumer, Spain is the second producer of organic citrus worldwide, Community of Valencia and Murcia are the leaders in the industry.[7]

In recent years it has developed a new type of industrial juice, which, once processed, the juice is ultrafrozen without being subjected to any treatment, concentration or thermal treatment. The deep freeze is one of the food preservation techniques more effective and used. The quick-freezing is a method of preservation that ensures food safety and the maintenance of sensory and organoleptic quality of them.[8]

Besides to organic food, is currently promoting the use of new substances with high added value foods that give a high nutritional value and taste, that's the case of so-called cyclodextrins, which can help the one hand to improve the quality of foodstuffs and also to promote the development of new products such as functional foods.

Cyclodextrins have a hydrophilic outer superficies therefore are soluble in water and a hydrophobic internal cavity,[9] may complexed molecules with different degree of hydrophobicity, resulting in inclusion complexes.

Cyclodextrins are used in the food industry for stabilize the light-sensitive compounds or oxygen, volatiles compounds, increase the water solubility of compounds poorly soluble compounds protect against microbial degradation and to mitigate undesirable odors or flavors.[10-11-9]

There are three types of cyclodextrins: γ, β and α ciclodextrins. Since 1998 are considered as GRAS (safe food ingredients) to a maximum of 2% in many food products.

Cyclodextrins are used to encapsulate hydrophobic nutrients (carotenoids and flavonols compounds) of some foods like juices and nectars hydrophilic increasing its stability in the stages of processing and storage, as well as its bioavailability.[12]

This work had as its main objectives, study the complexation of phytochemicals such as carotenoids, found in mandarin juice, for β-cyclodextrins, along the product life, and to study differences between organic and traditional mandarin juice and other potential benefits of treatment for deep-freezing conservation of the product.

MATERIALS AND METHODS

Fruit Material

Both traditional and organic mandarin oranges (*Citrus reticulata* L.), var. Clemenules, were grown in the same farm and under identical conditions of soil, irrigation, and illumination in eastern Spain (Librilla, Murcia). The citrus rootstock was the same for both mandarin trees, Cleopatra mandarin trees, and all selected trees were about 10-year-old and free of diseases. Fruits were collected in winter (second week of Febrery, 2008). Fruits were selected on the basis of their diameter, pH, total soluble solids content (SSC, º Brix) and maturity index (total soluble content / titratable acidity, SSC / TA).

Clemenules Mandarin oranges were studied on traditional and organic farming. Organic production means that no synthetic chemicals were used in the production of these fruit trees and that only natural substances were used to control pest, weeds, etc. Farming of organic mandarin trees followed all rules established by the Board of Organic Agriculture of the Murcia Region.[13] A complete list of the materials used in both traditional and organic farming is included in **Table 1**.

Sample Preparation

Mandarins were collected and processed the same day, 15 Febrery 2009. Mandarin juices were processed in a commercial plant (Murcia, Spain) and were obtained using a Premium Juice Extractor (FMC Corporation, Florida, USA).[14] This machinery leads to a juice with a low content of essential oils.[15]

Freshly squeezed juices, traditional and organic, were processed in a citrus processing plant. Once squeezed and before ultrafrozen treatment, β- cyclodextrins from TCI (Europe) were added to the traditional mandarin juices samples. Se adicionaron Para las muestras que contenían beta-ciclodextrinas se disolvió 1,5 % de las mismas (13mM) en un volumen conocido de zumo (2 litros) y se agitó durante 2 horas. Se diseño un estudio de almacenamiento de 145 días de duración, almacenándolas a -80ºC, en oscuridad, analizando las muestras a los 7, 30, 60, 100 y 145 days, respectively . La descongelación se llevo acabo a temperatura de refrigeración durante 8 horas. En cada medida se determinó los grados Brix, acidez, pH, Vitamina C, color por espectrorradiometría,y esfera difusa, contenido en carotenos y capacidad antioxidante de las muestras.

Se analizaron 3 tipos de zumo de mandarina, zumo de mandarina tradicional, zumo de mandarina tradicional con beta-ciclodextrinas y zumos de mandarina ecológica.

Se realizaron 6 análisis de cada uno de los 3 tipos de zumo a lo largo de todo el periodo de almacenamiento, iniciándose el primer análisis antes de ser ultracongelados y finalizando a los 145 dias de almacenamiento. El analisis de las muestras se realizaron por triplicado.

Physico-Chemical Analyses

The soluble solids content, SSC (ºBrix), was determined using a portable refractometer Comecta, S.A., model C3 (Barcelona, Spain). Titratable acidity, TA (% citric acid), was determined in 10 mL of juice by titration to pH 8.2 ± 0.1 with a 0.1N NaOH solution. The maturity index, MI, was calculated for each mix and expressed as the percentage of the ratio between the SSC and TA.

Vitamin C

Reduced ascorbic acid) was measured following the AOAC Official Method 985.33.[16] Ascorbic acid was estimated by titration with colored oxidation-reduction indicator, 2,6-dichloroindophenol. EDTA was added as chelating agent to remove Fe and Cu interferences.

All physico-chemical analyses were analyzed in 20 fruits of each agricultural practice.

Instrumental Measurement of Color

Color determinations were made, at 25 ± 1 ºC, using a Hunterlab Colorflex® (Hunterlab, Reston, Virginia, U.S.A.). This spectrophotometer uses an illuminant D_{65} and a 10º observer as references. A sample cup for reflectance measurements was used (5.9 cm internal diameter × 3.8 cm height) with a path length of light of 10 mm. Blank measurements were made with the cup filled with distilled water against a reference white background.[17]

Color data are provided as CIE*L*a*b** coordinates, which define the color in a three-dimensional space.[18] Finally, the color differences (ΔE*) between two points in the CIEL*a*b* space are worked out as the Euclidean distance between their localizations in the three dimensional space defined by L*, a*, and b*. Mathematically, it is therefore calculated by applying the formula $\Delta E^* = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$. Color analyses were run in 6 replicates.

Antioxidant Activity

All the reagents used for the determination of the antioxidant capacity, fluorescein (FL), 2,2–azobis–[2–aminopropane] dihydrochloride (AAPH) and 6–hydroxyl–2,5,7,8-tatramethylchroman–2-carboxilic acid (Trolox C) were purchased from Sigma (Madrid, Spain).

The ORAC assay were carried out on a Synergy HT multidetection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, VT), using 96-well polystyrene microplates with black sides and clear bottom, purchased from Nalge Nunc International. Fluorescence was read through the clear bottom, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The oxygen radical absorbance capacity was determined as described by Dávalos et al. (2004) with slight modifications.[19-20] A blank with FL and AAPH using sodium phosphate buffer instead of the antioxidant sample and eight calibration solutions using Trolox C (6.25, 12.5, 15, 18.75, 21.25, 25, 27.5, and 31.25 *μ*M) as antioxidant were also used in each assay (**Figure 1**). All reaction mixtures were prepared in triplicate, and at least three independent assays were performed for each sample. In order to avoid a temperature effect, only the inner 60 wells were used for experimental purposes, while the outer wells were filled with 200 *μ*L of distilled water.

The results were expressed as relative fluorescence with respect to the initial reading. The area under the fluorescence decay curve (AUC) was calculated by the equation:

$$AUC = 1 + \sum_{i=1.14}^{i=120} f_i / f_0$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time *i*. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. The results of antioxidant capacity were defined as mM of Trolox C *per* μL of juice. Data processing was performed using SigmaPlot software package (Jandel Scientific, Germany).

Carotenoid Compounds Extraction and Quantification

Extracción y saponificación de los pigmentos carotenoides del zumo. 350μL de zumo se le añadió 600μL de solvente (Metanol/Acetona/Diclorometano, 25:25:50, v:v:v) y se centrifugó 10 minutos, separando la fase coloreada. A la fase residual se le añadió 300 μL de Diclorometano, se centrifugó y la fase coloreada se añadió a la primera. Finalmente se lavó con agua destilada para eliminar restos de acetona. Para saponificar los extractos se adicionó una solución etanólica de KOH (15%) y se lavó con agua destilada para eliminar restos de base. Los extractos obtenidos se concentraron en sequedad durante 15 minutos a 30ºC y se mantuvieron en el congelador a una temperatura de -18ºC bajo atmósfera de nitrógeno hasta su utilización.

Cromatografía líquida de alta resolución

Los extractos concentrados fueron filtrados antes de su inyección en el sistema HPLC. Los análisis fueron realizados por triplicado. El HPLC utilizado fue un Agilent 1100, que consiste en una bomba cuaternaria, un detector de serie de fotodiodo, un módulo de control de temperatura de la columna y un autodesechado que fue programado para dispensar 20µL de las muestras (Agilent, Palo Alto, CA). Los pigmentos fueron separados mediante una columna C_{30} (5µm, 250 x 4,6 mm) (YMC, Wilmington, NC) mantenida a 17ºC. Para las fases móviles se utilizó metanol (MeOH), metil-ter-butil éter (MTBE) y agua. El gradiente de elución utilizado para el análisis fue el siguiente:0 min, 90% MeOH + 5% MTBE + 5% agua; 12 min, 95% MeOH + 5% MTBE; 25 min, 89% MeOH + 11% MTBE; 40 min, 75% MeOH + 25% MTBE; 50 min, 40% MeOH + 85% MTBE; 56 min, 15% MeOH + 85% MTBE, 62 min, 90% MeOH + 5 % MTBE + 5% agua.[21] Metanol y MTBE contenían una pequeña proporción de BHT (0,1%) y TEA (0,05%) para proteger a los pigmentos durante el análisis.[22] El bombeo de la fase móvil fue de 1mL min^{-1}, los cromatográmas fueron supervisados a 450nm.

Identificación de carotenoides

La identificación fue realizada por comparación cromatográfica con los estándares obtenidos de fuentes naturales mediante los procedimientos apropiados.[23] Así los estándares de Luteína, β- criptoxantina y β-caroteno fueron obtenidos de hojas de espicanas (*Spinacia oleracea* L.), la purificación se realizó por cromatografía en columna utilizando una mezcla de éter de petróleo, éter etílico y acetona en distintas concentraciones.

Análisis Cuantitativo

Luteína, β-criptoxantina y β-caroteno fueron calculados mediante curvas de calibración externas elaboradas mediante las normas y pautas correspondientes.[24] Los niveles de zeaxantina fueron calculados mediante la curva de calibración del β-caroteno, debido a que presentan el mismo cromóforo y por tanto espectros prácticamente idénticos. debido a ellos teniendo el mismo cromóforo y por lo tanto prácticamente espectros idénticos.[25]

Sensory Evaluation with Trained Panel

Sensory evaluation by a trained panel was used to evaluate the quality of mandarin orange juices. A panel of 10 panelists, ages 20 to 50 years (8 female and 2 male, all members of the Catholic University San Antonio of Murcia), with sensory evaluation experience, was trained in descriptive evaluation of citrus juice.[26]

The panel was selected and trained following the ISO standard 8586-1.[27-28] Further details on selection, training and validation of the panel can be found in Pérez-López et al. (2006).[17]

Measurements were performed in individual booths with controlled illumination (750-1000 lux) and temperature (23 ± 2 ºC).[27-28]

The individual products were scored for the intensities of color, sweetness, acidity, fresh mandarin juice aroma and off-flavor using a scale of 0-10, where: 0 = extremely slight intensity, and 10 = extremely high intensity.

Samples were presented in 50 mL plastic cups with lids. The entire experiment was repeated 3 times (all judges scored 2 juice samples on each session for a total of 3 sessions) and the sensory scores were presented as the overall means.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and the Tukey's least significant difference multi-comparison test to determine significant differences among mandarin orange juices. Significance of differences was represented as $p \leq 0.001$. The statistical analyses were done using SPSS 14.0 (SPSS Science, Chicago, USA) and figures using Sigma Plot 9.0 (SPSS Science, Chicago, U.S.A).

RESULTS AND Y DISCUSSION.

Se eligió la variedad Clemenules pues es la variedad más adecuada para la producción de zumo, diversos estudios han demostrado que contienen mayor contenido en vitamina C, aporta los aromas más intensos y produce el zumo más anaranjado.[29]

Physico-chemical Analyses

Todos las muestras de zumo tanto ecologico como tradicional, con o sin ciclodextrinas, mantuvieron unos valores medios de pH de 3,2 ± 0,2 y un indice de madurez de 13,3 ± 0,1 a lo largo de la duracion de este ensayo.

Vitamin C.

El nivel de vitamina C depende de diferentes factores, entre los que se incluye la variedad, tipo de cultivo y estadío de maduración.[30-31-32]

El contenido inicial en vitamina C tanto para zumo ecologico como tradicional fue de 540 y 470 mg L^{-1} respectivamente. Posteriormente, a los 7 dias de almacenamiento de los zumos de mandarina ultracongelados (-80 ºC) , disminuyó su contenido en vitamina C un 5% para todas las muestras analizadas, con unos valores 515 mg L^{-1} para zumos ecologicos y 447 ± 5 mg L^{-1} para zumos de mandarina con y sin ciclodextrinas. Posteriormente, desde este dia hasta el final del ensayo, 145 dias, todas las muestras de zumo de mandarina analizadas experimentaron perdidas menores del 3%, tanto apra zumo ecologico como tradicional. Aun asi, se encontró que el nivel de vitamina C en mandarinas cultivadas bajo producción ecológica fue más alto que en sistemas de tipo tradicional. Así el contenido en vitamina C fue un 13% más alto en producción ecológica que en tradicional.Estos resultados son similares a los descritos para fresa y máiz.[33]

Respecto al proceso de congelacion de los zumos, las perdidas en vitamina C, como se ha mencionado anteriormente, fueron menores del 3% hasta el final del ensayo, 145 dias, por lo que el proceso de congelacion mantiene los niveles de vitamina C de los zumos de mandarina. Estos resultados concuerdan con otros estudios realizados en zumos de naranja ultracongelados con niveles de acidos ascórbico similares a los encontrados en este estudio.[34-35-36]

La adicion de ciclodextrinas produjo un efecto escaso o nulo en el contenido de Vitamina C de las muestras analizadas.

CIELAB* Color coordenates.

The pulp content has a direct effect on the measurement of reflectance and to avoid this effect all samples must have similar pulp content.[37] The fiber contents of all mandarin juice samples were equilibrated by using a FMC FoodTech Quick Fiber device (FMC Corporation, Florida, USA); in this way, juice samples presented a mean value for the centrifugable pulp content of 15.0±1.1 g L^{-1} (no significant differences were found among juice samples).

The main color characteristics of the fresh squeezed mandarin juice analyzed (day 0) in this study were: L^* =75,88 ± 0,31; a^* = 22,64 ±0,15; b^* = 62,37±0,19 and were indicative of a deep orange color. Finally (day 145), the cielab* color coordenates for the samples of organic, tradicional and tradicional with cyclodextrins were respectively: L*= 73,64 ± 0,25 a*= 23,12 ± 0,42 b*= 66,76 ± 0,37; L*= 72,34 ± 0,29 a*= 20,27 ± 0,51 b*= 67,35 ±0,44; L*= 72,15 ± 0,62 a*= 19,92 ± 0,23 b*= 67,78 ± 0,52. The instrumental color data showed differences among mandarin juices. The color of organic mandarin juice was reddish (+a) than traditional. The addition of β-cyclodextrins to the mandarin juices didn´t show lower variations in total color differences. As is shown in **figure 2** the total color difference (ΔE*) of the mandarin juices increased from day 0 to day 145.

Color data showed differences among mandarin juices of 0,70 value total color difference (ΔE), at storage day 145, for the samples analized. Según Melgosa et.al, ΔE superior a 2 dan lugar a diferencias visuales perceptibles en determinadas situaciones.[38] Estudios realizados en zumos tratados térmicamente observaron diferencias de color (ΔE) ,entorno a 3, entre zumos frescos y pasteurizados.[39] Por otro lado, estudios realizados por Meléndez et.al en zumos ultracongelados concluyeron que estos presentaban un color naranja intenso (L * = 63,23 ± 1,27; un *= 16,18 ± 0,56; b * = 64,64 ± 3,78) gracias a que los jugos no fueron sometidos a altas temperaturas ni procesos de concentración durante la producción, indicando que la ultracongelación es una técnica de conservación óptima para preservar el color de los zumos.[5]

Análisis de carotenoides

Identificación. Luteína, zeaxantina, β-criptoxantina y β-caroteno fueron identificados por comparación de su comportamiento espectral y cromatográfico con las normas vigentes de identificación. Análisis Cuantitativo. La exactitud para determinar el contenido en carotenoides de las muestras de zumo depende de los coeficientes de absorción usados para calcular la concentración de las soluciones estándares.[40-41-23] La cuantificacion de este tipo de compuestos implica cierto grado de inexactitud en parte debido a que los coeficientes de absorción son difíciles de determinar.[42] Initially, para los zumos de mandarina analizados, β-Cryptoxanthin content was higher than Lutein, Zeaxanthin and β-Carotene. Esto es normal ya que la β-Cryptoxanthin es el carotenoide mayoritario en zumo de mandarina.[43] Sin embargo, fue el carotenoide analizado que tuvo mayor variacion de su concentracion a lo largo de los 145 dias de analisis, teniendo unas perdidas de un 10% (**Table 1**). Las perdidas de β-Cryptoxanthin pueden correlacionarse con la variacion de color total mediante coordenadas CIELab* que pueden verse en la **figura 2**. Para el resto de carotenoides analizados, luteina, zeaxantina y criptoxantina, las perdidas producidas fueron menores del 5 %. Por lo tanto el proceso de ultracongelacion produjo unas perdidas maximas de un 10% en estos carotenoides, sin embargo los procesos de conservacion mediante pasterizacion alta provocan el doble de perdidas de estos carotenos en zumos de naranja y por consiguiente una mayor variacion en el color.[44] El uso de β-ciclodextrinas no produjo ningun efecto beneficioso respecto a la estabilidad inicial de los carotenoides de las muestras analizadas. Otros autores demostraron que el uso de β-ciclodextrinas en zumo de melocoton no evitaba el pardeamiento de estos y por lo tanto provocaba una variacion de color de los zumos.[45]

Equivalentes de retinol

The retinol activity equivalents (RAEs) of the samples analysed and discussed here are referred to 1L mandarin juice. The bioavailability of carotenoids is influenced by many factors, such as amount, food matrix, age, existence of certain diseases , intake of fat, vitamin E and fibre, protein and zinc status.[46] In this study, α-carotene was not found in mandarin juices, calculations were performed, considering new guidelines according to the following formula:[46]

$$RAE = \frac{C_{\beta-car}}{12} + \frac{C_{\beta-crip}}{24}$$

where $C_{\beta-car}$ and $C_{\beta-crip}$ are the concentrations (μg L^{-1}) of β-carotene and β-criptoxanthin, respectively. Initially the RAE values found for organic and traditional, with and without β-cycodextrins, mandarin juices was 310,42 and 261,25 μg L^{-1} respectively; these high values of RAE L^{-1} support the general fact that mandarin products are a very good source of vitamin A and this statement is especially true for organic mandarin juices (Beltran-Gonzalez et al., 2008; Beltran- Gonzalez F., Perez-Lopez Antonio J., Lopez-Nicolas J.M., Carbonell-Barrachina A.A., 2008. Effects of Agricultural Practices on instrumental colour, mineral content, carotenoid compostion and sensory quality of mandarin orange juice, cv. Hernandina. J. Sci. Food Agric. 88:1731:1738. Finally, the RAE values lost for organic and traditional was about 11% so the adittion of β-cycodextrins didn´t increase the RAE values at the end of the assay, day 145. These experimental values fit perfectly within the range previosly described by Melendez-Martinez et al. for the RAE L^{-1} contents of different cultivars of mandarin and orange juices marketed in Spain ranging from 9,7 to 359. This RAE L^{-1} range was significantly reduced when only oranges juice were considered, 9,7 - 94,8. Without any doubt the high levels of RAE in mandarin juices compared to others citrus juice are related to their higher concentrations of β-cryptoxanthin.

Carotenoids content

The carotenoids Lutein, zeaxanthin, Criptoxanthin and β-carotene were measured due to their highest concentrations in mandarin juice.[43] These carotenoids analized in this study, showed higher concentrations in organic mandarin juices than in traditional (**table 2**). Initially, day 0, results of this study showed highest concentrations for total carotenoids organic mandarin juice, 8,54 ± 0,35, than for traditional, 6,97 ± 0,26. No differences were found for convencional + cyclodextrins samples, 6,99 ± 0,22. Finally, day 145, there was a lost of 10% of total carotenoids on every samples analized so the addition of β-cyclodextrins no provoco ningún efecto beneficioso a nivel cuantitativo respecto a la cantidad de carotenos en estos zumos de mandarina (**Table 2**). No se observo el efecto de las β-cyclodextrinas debido a que las bajas temperaturas a las que fueron sometidas y almacenadas las muestras fueron suficientes para mantener la concentracion de carotenoides mientras las muestras estuvieron ultracongeladas . No se han encontrado estudios relacionados con el proceso de ultracongelacion en la capacidad de encapsulacion de estas β-cyclodextrinas. Otherwise, otros autores demostraron el efecto beneficioso de estas β-cyclodextrinas sobre los carotenos sometidas a tratamientos termicos de pasteurizacion y esterilizacion, los cuales are susceptibles to isomerization and oxidation during processing and storage of foods.[47-48-49]

El carotenoide predominante en todos los zumos de mandarina fue la β-criptoxantina, representando más del 80% del total de los carotenoides identificados. Las concentraciones iniciales, tanto para producto ecologico como tradicional, fueron de 7,21 ± 0,31 y 6,09 ± 0,25, respectivamente. Las perdidas, al final del estudio, 145 dias, fueron de un 10% para ambas muestras (**table 2**). La β-criptoxantina es uno de los principales carotenoides encontrados en zumos de naranja y mandarina.[5] La β-criptoxantina tiene actividad de provitamina A, otorga al zumo un color naranja oscuro, casi rojo, propiedad distintiva de los zumos de mandarina respecto a los de naranja, que suelen presentar cantidades inferiores en este tipo de carotenoide.

Luteina y zeaxantina presentaron menores concentraciones que el caroteno mayoritario β-criptoxantina, tanto en cultivo ecologico como tradicional, siendo mayores las concetraciones en cultivo ecologico y no detectando diferencias significativas a los 145 dias respecto al uso de β-ciclodextrinas. Las perdidas de estos dos carotenoides fueron de un 5% al final del estudio (**table 2**), presentando una gran estabilidad respecto al proceso de ultracongelacion. Some researchers showed that canning does not decrease these carotenoids content, in corn and some vegetables, and that freezing may increase carotenoid content in corn which can further influence bioavailability and health benefits.[50-51]

Finally, β-Carotene fue el carotenoide analizado que presento menor concentracion, 0,12 ± 0,03 y 0,09 ± 0,01, inicialmente, para zumos de mandarina ecologicos y tradicionales, representando el 1,4% de los carotenoides medidos. Una vez mas, este carotenoide obtuve el mismo comportamiento, al final del ensayo, 145 dias, y obtuvo unos valores de 0,10 ± 0,02 y 0,07 ± 0,01, por lo tuvo unas perdidas del 15% aproximadamente (**table 2**). Estas perdidas fueron mayores que las obtenidas para los carotenoides luteina y zeaxantina debido a que el β-caroteno es mas vulnerable a la degradacion ante determinados antioxidantes que otros carotenos.[52]

Actividad Antioxidante

Todas las muestras ultracongeladas de zumo de mandarina ultracongeladas mostraron una gran estabilidad a lo largo del periodo de almacenamiento, 145 dias. La **figure 3** muestra los valores de la actividad antioxidante para cada uno de los zumos analizados, expresados como equivalentes de Trolox.

La agricultura ecológica causó una actividad antioxidante considerablemente superior a la agricultura convencional, con valores que oscilaron entre 0,06 ± 0.004 y

0.04 ± 0.005 mMTrolox mL^{-1}, respectivamente. La agricultura ecológica no utiliza productos químicos de síntesis, como fertilizantes, plaguicidas, antibióticos, etc., lo que da como resultado la inducción de compuestos de protección natural por la propia planta, dentro de estos compuestos de protección se encuentran carotenoides y compuestos fitoquímicos que presentan capacidad antioxidante. Otros autores han comparado los efectos de las prácticas tradicionales respecto a las ecológicas, obteniendo resultados positivos para estas últimas.[53-54-55-37-56]

La capacidad antioxidante de las muestras analizadas se mantuvo estable durante todo el estudio, tanto en presencia como en ausencia de β-Cyclodextrins, por lo que la adiccion de este tipo de ciclodextrinas no provoco ningun efecto sobre la capacidad antioxidante **figura 3**.

Finally, El tratamiento térmico de ultracongelacion al que fueron sometidas las muestras de zumo provocó una pérdida, en todos los casos, inferior al 18% a os 145 dias de almacenamiento (**figura 3)**.

Sensory Evaluation

The trained panel established that the quality of the mandarin juices analized was high but organic juice had higher intensities for the sensorial parameters color, sweetness, fresh mandarin juice and aroma than traditional mandarin juices. Other hand, no sensorial differences were detected for the traditional mandarin juice with or without β-cyclodextrins added.

CONCLUSIONS

Estos datos demuestran que la conservación de zumos de mandarina mediante ultracongelación es una alternativa a los tratamientos tradicionales (altas temperaturas) que si bien reduce la carga microbiológica y elimina la actividad enzimática, afecta a la calidad sensorial y nutricional del producto asi como la capacidad antioxidante de este. El tratamiento de ultracongelación dan como resultado zumos de gran calidad, que mantienen todas sus propiedades durante largos periodos de tiempo . Por otro lado, el uso de β-ciclodextrinas no es necesario al no povcar ningun efecto beneficioso en estos zumos de mandarina conservados bajo ultracongelacion.

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Figure 1. (A) Regression of net AUC of Trolox C on differents concentrations of Trolox C. The net AUC =AUC_{sample} – AUC_{blank}. (B) FL Fluorescence decay curves induced by AAPH in the presence of Trolox C.

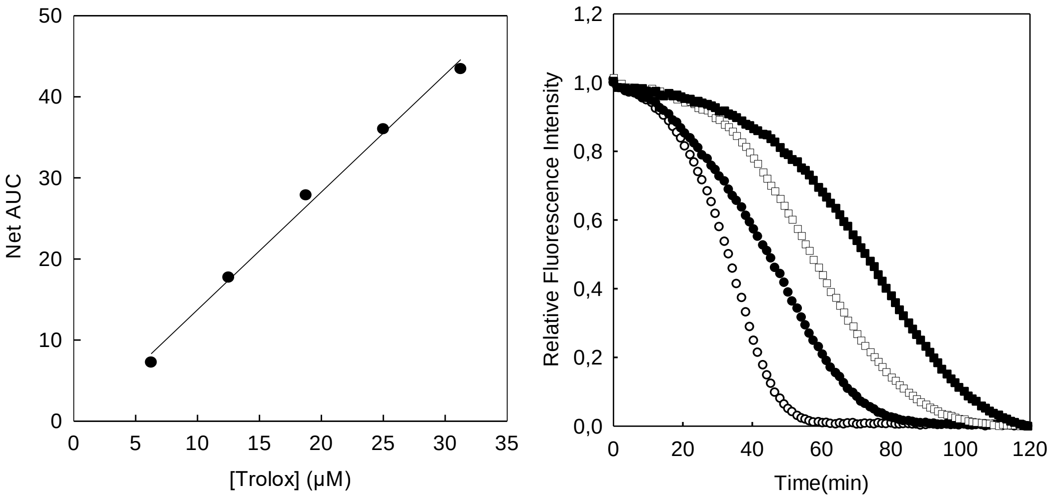


Figure 2. Evolucion de total de color (ΔE) on ultrafrozen mandarin juices along 145 days of storage.

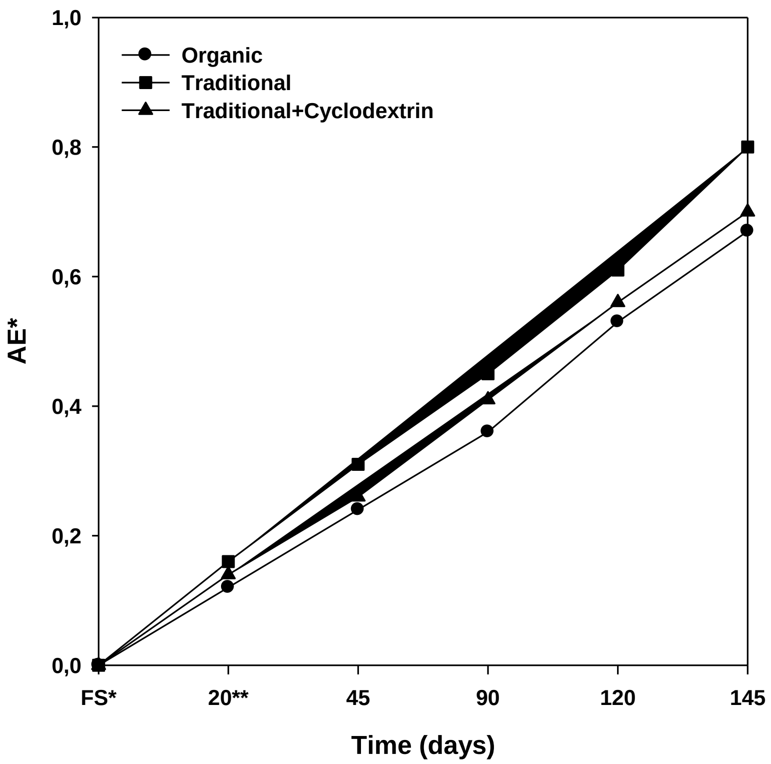
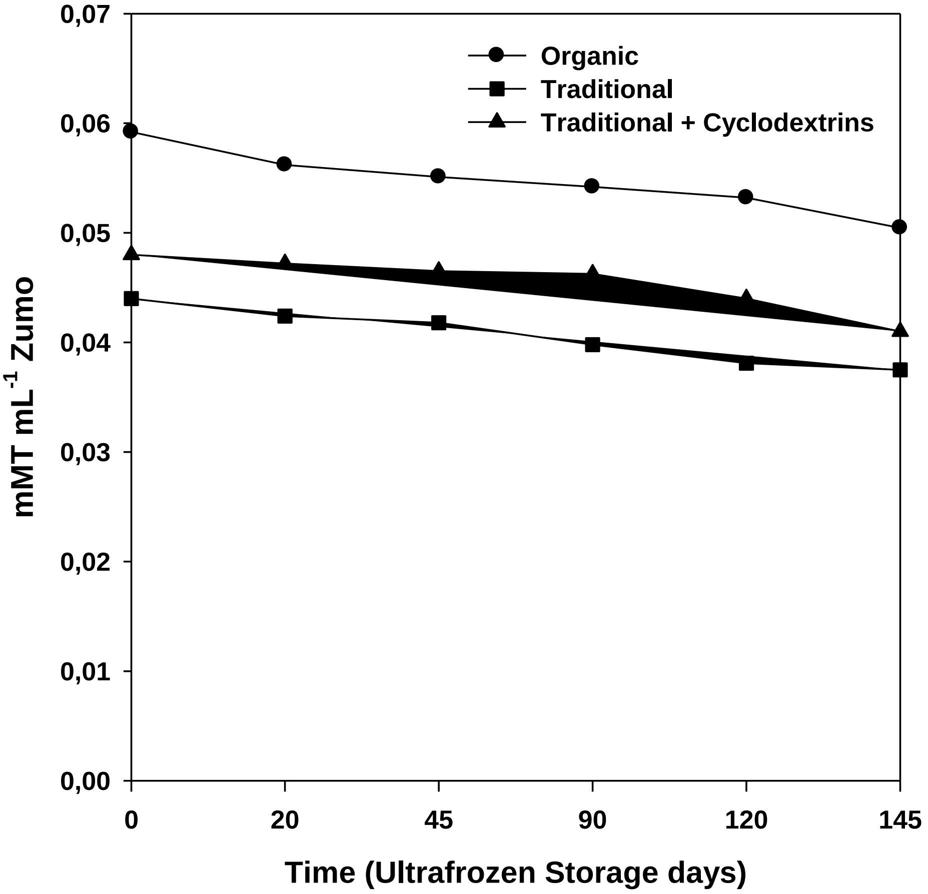


Figure 3. Actividad antioxidante



Organic
Traditional
Traditional + Cyclodextrins
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0,00
mMT mL-1 Zumo
0
20
45
90
120
145
Time (Ultrafrozen Storage days)

Table 1. Materials used in both traditional and organic farming.(Beltran-Gonzalez et al., 2008 Effects of Agricultural Practices on Instrumental Colour, Mineral Content, Carotenoid Composition, and Sensory Quality of Mandarin Orange Juice, cv. Hernandina," by F. Beltran-Gonzalez, A.J. Perez-Lopez, J.M. Lopez-Nicolas and A.A. Carbonell-Barrachina. ***Journal of the Science of Food and Agriculture*** 88, no. 10 (2008): pp. 1731-1738..)

Compound	**Farming**	
	Traditional	**Organic**
Fertilizers	Ammonium nitrate, calcium nitrate, ammonium sulfate, phosphoric acid, diammonium phosphate, potassium sulfate, potassium nitrate, potassium chloride	Manure, compost, and fulvic and humic acids
Foliar fertilizers	potassium phosphate, magnesium nitrate, urea, mixture of oligoelements	algae extracts, aminoacids
Herbicides	Bromacil, diuron, diquat, fluroxypyr, glyphosate, norflurazon, paraquat, sulfosate, simazine.	None; weeds are removed by mechanical methods
Pesticides	Malathion, dicofol, methidathion, clopidol	Neem oil, pheromone traps

Tabla 2.- xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

Storage Time (Days)	Carotenoids (mg L^{-1})											
	Lutein			Zeaxanthin			β - Cryptoxanthin			β-Carotene		
	Organic	Traditional	Tr.+ β-Cycl*	Organic	Traditional	Tr.+ β-Cycl	Organic	Traditional	Tr.+ β-Cycl	Organic	Traditional	Tr.+ β-Cycl
0	0,61±0,08	0,38±0,06	0,38±0,11	0,60±0,08	0,41±0,03	0,43±0,09	7,21±0,31	6,09±0,25	6,09±0,32	0,12±0,03	0,09±0,01	0,09±0,01
20	0,60±0,12	0,37±0,08	0,37±0,06	0,59±0,03	0,39±0,08	0,41±0,04	7,14±0,22	6,08±0,32	6,08±0,17	0,12±0,01	0,09±0,03	0,09±0,02
45	0,58±0,16	0,37±0,11	0,36±0,07	0,59±0,04	0,37±0,05	0,37±0,08	7,12±0,17	5,98±0,41	5,98±0,12	0,11±0,04	0,08±0,02	0,08±0,01
90	0,58±0,05	0,37±0,14	0,36±0,02	0,59±0,09	0,36±0,09	0,36±0,03	6,95±0,27	5,87±0,26	5,87±0,29	0,11±0,02	0,08±0,01	0,08±0,02
120	0,58±0,15	0,37±0,03	0,36±0,08	0,59±0,06	0,35±0,07	0,35±0,09	6,75±0,19	5,79±0,34	5,79±0,24	0,10±0,03	0,08±0,02	0,08±0,03
145	0,58±0,22	0,37±0,10	0,35±0,05	0,58±0,09	0,34±0,05	0,35±0,04	6,45±0,24	5,47±0,39	5,47±0,15	0,10±0,02	0,07±0,01	0,07±0,02

* Tr.+ β-Cy : Traditional mandarin juice + β - cyclo dextrins