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EFFECTS OF FARMING PRACTICES ON THE QUALITY OF ULTRA-FROZEN MANDARIN JUICE

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ABSTRACT

The effects of the farming type (organic or conventional) and cyclodextrin addition on the quality (loss of ascorbic acid, carotenoids composition, color and antioxidant activity) of freshly squeezed, nonpasteurized, ultra-frozen mandarin juices were studied during a shelf life of 145 days. The vitamin C content was reduced from 540 and 470 mg/L to 515 and 447 mg/L in organic and conventional juices, respectively. Carotenoids were stable during the storage time (decreases being in the range 5–10%), with β-cryptoxanthin (7.21 6 0.31 mg/L) being the most abundant compound, followed by lutein and zeaxanthin. The juices color was not affected by thermal treatment and these data were provided by both instrumental *Commission internationale de l'éclairage L**, *a**, *b** data and the sensory studies conducted. The antioxidant activity was 0.04 and 0.06 mMT/mL, at day 0, for the organic and conventional juices; the losses during the storage were below 18%. The addition of cyclodextrins did not have any significant effect on the parameters under study. This study showed that the nutritional and organoleptic quality of ultra-frozen juices is quite stable during the storage period.

PRACTICAL APPLICATIONS

The application of cyclodextrins in the food industry is very important as solubilizing agents, stabilizers and emulsifiers. The molecular encapsulation of lipophilic food ingredients with cyclodextrin improves the stability of flavors, vitamins, colorants and unsaturated fats, etc., both in physical and chemical sense leading to extended product shelf life, accelerated and long-term storage stability.

INTRODUCTION

Nowadays, both the consumption and therefore the manufacturing of fruit juices have experienced high growth because, among other things, of progress in the farming and improvement on fruit processing, transport and distribution (Schieber *et al.* 2001). Mandarin juice production in Spain is low compared with the production of orange juice, but is expected to be higher because of market saturation in fresh and to the pleasant taste, fresh aroma and the great nutritional value of mandarin. Spain currently produces 2.5 million tons of mandarins per year and production is increasing. The most widespread cultivar is Clementine (1.6 million tons), from which about 60% of production is exported to other countries, particularly to Northern European countries and the United States (Agroinformacion 2010).

Mandarin is the fruit that has more carotenoids in its composition, providing β-cryptoxanthin and β-carotene in outstanding amounts (Meléndez-Martínez *et al.* 2010). The antioxidant activity of these phytochemicals and vitamin C provide the food physiological properties that go beyond the proper nutrition. Antioxidants fight the harmful action of free radicals, substances responsible for the development of cardiovascular diseases, degenerative diseases and cancer (Sánchez-Moreno *et al.* 2003a). The synthesis and composition of these compounds can oscillate depending on the type of species, fruit variety, climatic factors, industrial processing and storage conditions (Meléndez-Martínez *et al.* 2010).

Farming 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 (Meléndez-Martínez *et al.* 2010). Therefore, the high demand by health-concerned consumers is encouraging the development of products with improved nutritional quality, such as fruits and vegetables grown under organic 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, maintaining or increasing soil fertility and provide food with all its natural properties (Willer and Yussefi 2004).

Organic farming has become one of the most dynamic agricultural sectors of the European Union, with sales reaching 13–14 billion euros in 2005. In the European Union, the area of organic crops is 3.5 million hectares, 3% of the total agricultural area. Spain is one of the European countries with the largest organic area. Oranges and mandarins are one of the most demanded fruits by the consumer; Spain is the second producer of organic citrus worldwide, with the regions of Valencia and Murcia being the leaders in this industry (Mapa 2005).

In the recent years, a new type of industrial juice has been developed; after processing, the juice is ultra-frozen without being subjected to any concentration or thermal treatments. Deep freezing is one of the most effective and used preservation techniques. Quick freezing is a method of preservation that ensures food safety and the maintenance of the food sensory and nutritional properties (Meléndez-Martínez *et al.* 2009).

Besides to organic food, the market is requiring the use of new substances with high added value, which are able to lead to high nutritional value and taste, which is the case of the so-called cyclodextrins (CDs). These compounds can be used to improve the quality of current foodstuffs and also to promote the development of new products. There are three types of CDs: γ, β and α. β-CDs are considered as GRAS (safe food ingredients) up to a maximum content of 2% in food products since 1998.

CDs have a hydrophilic outer surface and are therefore soluble in water but at the same time have a hydrophobic internal cavity (Szejtli 1998), that may complex molecules with different degree of hydrophobicity, resulting in inclusion complexes.

In general, CDs are used in the food industry to: (1) stabilize light- or oxygen-sensitive compounds, volatile compounds; (2) increase the water solubility of poorly soluble compounds; (3) protect against microbial degradation; and (4) mitigate undesirable odours or flavors (Loftsson and Stefasson 1997; Szejtli 1998; Buschmann and Schollmayer 2002).

In the recent years, the effects of CDs on color of fruit juices has been widely studied, however, only few studies have evaluated the effects of CDs on juice aroma (López-Nicolás *et al.* 2009). In this particular study, β-CD (the most reported type of CD to complex different guest molecules) was used to encapsulate hydrophobic nutrients (carotenoids and flavonols) and aroma compounds of hydrophilic juices, perhaps increasing its stability in the stages of processing and storage, as well as their bioavailability (Mercader-Ros *et al.* 2009).

The main objective of this work is to study the effects of: (1) the complexation of phytochemicals, such as carotenoids, by β-CD; and (2) the farming type (organic or conventional) on quality properties, such as antioxidant activity, carotenoids profile and vitamin C, of ultra-frozen mandarin juices during their shelf life.

MATERIALS AND METHODS

Fruit Material

Both conventional 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 February 2008). Fruits were selected on the basis of their diameter, pH, total soluble solids content ([SSC] °Brix) and maturity index (MI) (total soluble content/titratable acidity [SSC/TA]).

Clemenules mandarin oranges were studied under conventional and organic farming. Organic production means that no synthetic chemicals were used in the cultivation 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 (Boam 2009).

Sample Preparation

Mandarin juices were processed in a commercial plant (Murcia, Spain) and were obtained using a Premium Juice Extractor (FMC Corporation, Jacksonville, FL) (Kimball 2002). This machinery leads to a juice with a low content of essential oils (FMC 2010). After the mechanical extraction, the juice was counter-currently treated with nitrogen to remove air and prevent oxidative processes and microbial contamination. Next, the content of pulp was adjusted and the juice was cooled to around 0C. Finally, the product was bottled in high-density propylethylene plastic containers (1.15 L total capacity, 50 mL headspace) and passed through a freezing tunnel using liquid nitrogen as cryo-protective fluid, with which a rapid freezing is achieved. The mandarin juice manufactured did not contain additives, was not enriched with vitamins or any other kind of permitted substance, and was kept at a temperature equal to or below −18C over storage, delivery and retailing (Meléndez-Martínez *et al.* 2009).

β-CD from TCI (Europe) was added to the conventional mandarin juices samples. β-Cyclodextrin was added at a concentration of 1.5% to 2 L of freshly squeezed juice. Three types of mandarin juices were studied: conventional, conventional plus β-CD and organic. [6]

Samples were stored at −18C in darkness and analyzed after 7, 30, 60, 100 and 145 days. Samples were thawed at 5C for about 8 h and in each sample the following parameters were analyzed: total soluble solids, titratable acidity, pH, vitamin C, color, carotenoids content and composition and antioxidant activity. All juices were prepared in triplicate.

Physicochemical Analyses

SSC (°Brix) was determined using a portable refractometer Comecta, S.A., model C3 (Barcelona, Spain). TA (% citric acid), was determined in 10 mL of juice by titration to pH 8.2 6 0.1 with a 0.1 N NaOH solution. 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 (Horwitz 2000). Ascorbic acid was estimated by titration with colored oxidation-reduction indicator, 2,6-dichloroindophenol. Ethylenediaminetetraacetic acid was added as chelating agent to remove Fe and Cu interferences.

All physicochemical analyses were analyzed in 20 fruits of each agricultural practice.

Instrumental Color

Color measurement was made, at 25 6 1C, using a Hunterlab Colorflex® (Hunterlab, Reston, VA). This spectrophotometer uses an illuminant D65 and a 10° 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 (Pérez-López *et al.* 2006).

Color data are provided as CIE L^*, a^*, b^* coordinates, [7] which define the color in a three-dimensional space (Minolta 1994). Finally, the color differences (ΔE^*) between two points in the CIE L^*, 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 six 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 was 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 (Rochester, NY). 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 (Lucas-Abellán *et al.* 2008). 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 μM) as antioxidant were also used in each. 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, whereas the outer wells were filled with 200 μL 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:

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

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 millimolar of Trolox C per milliliter of juice. Data processing was performed using SigmaPlot software package (Jandel Scientific, Erkrath, Germany).

Carotenoid Compounds Extraction and Quantification

The analysis of the carotenoid composition requires an extraction with organic solvent followed by de-esterification of the carotenoid fraction. Mandarin orange juices (350 μL) were extracted with 600 μL of solvent (methanol [MeOH]/acetone/dichloromethane, 25:25:50 v : v : v), centrifuged retaining the colored phase. The final residue was finally extracted with 300 μL of dichloromethane, centrifuged and again keeping the colored phase, which was mixed with the previous one. This mixture was washed several times with deionised water to remove any trace of acetone. This phase containing the pigments, in various stages of esterification with fatty acids, was saponified with 15% KOH-MeOH for 1h at room temperature and washed with water to remove any trace of the base. The pigments were subsequently evaporated in a rotary evaporator at 30C, keeping the samples under nitrogen atmosphere at –18C until analyzed.

The high-performance liquid chromatography system consisted of an HP-1100 series unit with a photodiode array detector equipped with HP ChemStation software (Hewlett Packard, Palo Alto, CA). The column used was 250 ∞4.6 mm in internal diameter, YMC C30, S-5 μm (YMC, Wilmington, NC), and column temperature was kept at 17C. The mobile phase for this column was MeOH, methyl tertiary butyl ether (MTBE) and water; MeOH and MTBE contained BHT (0.1%) and TEA (0.05%) to protect carotenoids during analysis (Hart and Scott 1995). The gradient elution was: 0 min, 90% MeOH + 5% MTBE + 5% water; 12 min, 95% MeOH + 5% MTBE; 25 min, 89% MeOH + 11% MTBE; 40 min, 75% MeOH + 25% MTBE; 50 min, 40% MeOH + 60% MTBE; 56 min, 15% MeOH + 85% MTBE; 62 min, 90% MeOH + 5% MTBE + 5% water, at a flow rate of 1.0 mL/min (Mouly *et al.* 1999). Carotenoids were monitored at 450 nm and analyses were carried out in triplicate.

Identification of carotenoids was conducted by comparison with standards isolated from natural sources. Lutein, β-cryptoxanthin and β-carotene were obtained from spinach leaves (*Spinacia oleracea* L.) (Britton *et al.* 1995).

Quantification of lutein, β-cryptoxanthin and β-carotene was carried out using calibration curves (Rodriguez-Amaya 2001). Zeaxanthin concentration was quantified using the β-carotene calibration curve because they have the same spectral properties (Meléndez-Martínez *et al.* 2010).

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 panellists, ages 20 to 50 years (eight females and two males, all members of the Catholic University San Antonio of Murcia), with sensory evaluation experience, was trained in descriptive evaluation of citrus juice (Serrano-Megías *et al.* 2005).

The panel was selected and trained following the ISO standard 8586-1 (Aenor 1997; Meilgaard *et al.* 1999). Further details on selection, training and validation of the panel can be found in Pérez-López *et al.* (2006).

Measurements were performed in individual booths with controlled illumination (750–1,000 lux) and temperature (23 6 2C) (Aenor 1997; Meilgaard *et al.* 1999).

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 three times (all judges scored two juice samples on each session for a total of three sessions) and the sensory scores were presented as the overall means.

Statistical Analysis

All data were subjected to analysis of variance and the Tukey's least significant difference multicomparison test to determine significant differences among mandarin orange juices. Significance of differences was represented as *P* # 0.001. The statistical analyses were done using SPSS 14.0 (SPSS Science, Chicago, IL) and figures using Sigma Plot 9.0 (SPSS Science).

RESULTS AND DISCUSSION

Clemenules mandarin oranges were chosen for this experiment because several studies have proved they are the best ones for juice manufacturing because of their high vitamin C content, intense orange color and aroma (Beltrán *et al.* 2008).

Physicochemical Analyses

All samples under assay presented the same pH (3.2 6 0.2) and MI (13.3 6 0.1°Brix/% citric acid) and these values remained stable throughout the experiment.

Vitamin C

The vitamin C content of fruits depends on several factors, including cultivar, farming type and MI (Howard *et al.* 2000; Lee and Kader 2000; Tudela *et al.* 2002).

The initial contents of vitamin C in organic and conventional juices were 540 and 470 mg/L, respectively. After 7 days of storage of the ultra-frozen mandarin juices vitamin C contents decreased about 5% and were 515 and 447 mg/L for juices nontreated and treated with β-CD, respectively. After the first week and until the end of the storage period (145 days), all samples experienced losses of vitamin C lower than 3%. Always organic juices had higher vitamin C than conventional samples and at the end of the experiment the difference was about 13%; these results agree with data previously published for strawberry and corn (Wang *et al.* 1996).

The fact that only a 3% loss of vitamin C was found proved that the ultra-freezing treatment was effective in maintaining the quality of mandarin juices. These results also agree with previous studies conducted with orange juices (Lee and

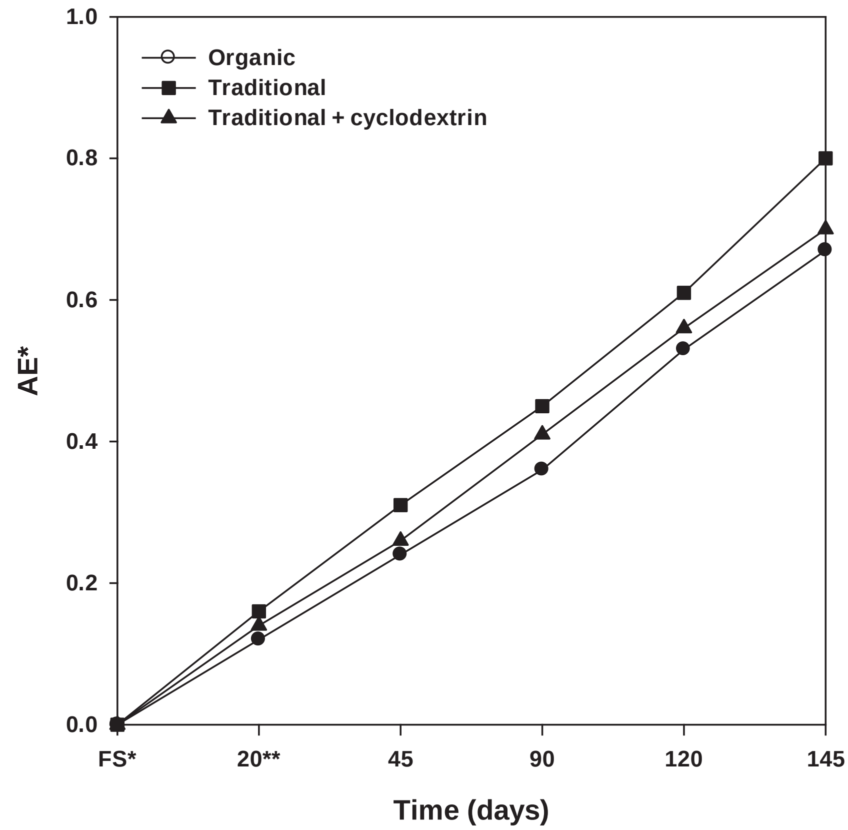

FIG. 1. CHANGES OF ΔE^* (TOTAL COLOR) OF ULTRA-FROZEN MANDARIN JUICES THROUGHOUT 145 DAYS OF STORAGE
*Freshly squeezed. **Storage time (−18C)

Coates 1999; Farnworth *et al.* 2001; Sánchez-Moreno *et al.* 2003b; Meléndez-Martínez *et al.* 2009).

The addition of β-CD had no significant effect on vitamin C changes with time.

CIE L^*, a^*, b^* Color Coordinates

The pulp content has a direct effect on the measurement of reflectance and to avoid this effect all samples must have similar pulp content at the beginning of any experiment (Arena *et al.* 2001). The fiber contents of all mandarin juice samples were equilibrated by using a FMC FoodTech Quick Fiber device (FMC Corporation). In this way, juice samples presented a mean value for the centrifugable pulp content of 15.0 6 1.1 g/L (no significant differences were found among juice samples).

The main color characteristics of the freshly squeezed mandarin juice (day 0) analyzed in this study were: L^* = 75.88 6 0.31; a^* = 22.64 6 0.15; b^* = 62.37 6 0.19 and were indicative of a deep orange color. The color coordinates at the end of the study (day 145) in organic, conventional and β-CD-treated conventional juices were: L^* = 73.64 6 0.25, a^* = 23.12 6 0.42, b^* = 66.76 6 0.37; L^* = 72.34 6 0.29, a^* = 20.27 6 0.51, b^* = 67.35 6 0.44; and L^* = 72.15 6 0.62, a^* = 19.92 6 0.23, b^* = 67.78 6 0.52, respectively. The instrumental color data showed significant differences among mandarin juices as affected by farming type. The color of organic mandarin juice was more reddish (+a) than that of conventional samples. However, the addition of β-CD to the mandarin juices had not significant effects on the values of ΔE^*. As shown in Fig. 1, the values of ΔE^* increased from day 0 to day 145.

Color data showed differences among mandarin juices of 0.70 units in ΔE at day 145 of storage. Studies on the effects of thermal treatment on juices reported differences of about 3 units between fresh and pasteurized juices (Lee and Coates 2003).

On the other hand, studies by Meléndez *et al.* (2009) in 8
ultra-frozen juices indicated that these products had an intense orange color (L^* = 63.23 6 1.27; a^* = 16.8 6 0.56; b^* = 64.64 6 3.78), because of the fact that juices were not subjected to neither high temperature nor concentration steps.

Carotenoids

Identification of carotenoids was based on their spectral parameters using the current rules. The precision of the carotenoids quantification is based on the absorption coefficients [9] used to calculate the concentration of the standards (Davies 1976; Britton 2002).

Lutein, zeaxanthin, β-cryptoxanthin and β-carotene were measured because of their predominance in mandarin juice (Beltrán-Górnzalez *et al.* 2008). All the studied carotenoids showed higher concentrations in organic mandarin juices than in conventional ones (Table 1). Initially, day 0, results of this study showed higher concentration of total carotenoids in organic mandarin juice, 8.54 6 0.35 mg/L, than in conventional samples, 6.97 6 0.26 mg/L. No differences were found between conventional and conventional + β-CD, 6.99 6 0.22 mg/L. Finally, at day 145, there was a loss of approximately 10% of total carotenoids on all samples under analysis. The addition of β-CD did not represented any beneficial effect on the total concentration of carotenoids (Table 1); it is possible that the effects of the low temperatures used during the manufacturing of juices (ultra-freezing) were much higher than those from the addition of CDs and therefore, the effects of CDs was masked. On the other hand, some studies have previously reported beneficial effects of β-CD on the quality of juices subjected to pasteurization and sterilization [10] protocols (Meier *et al.* 2001; Rodríguez-Amaya 2003; Jun 2009).

The most abundant carotenoid in all the juices was β-cryptoxanthin, representing more than 80% of the total concentration of carotenoids. The initial concentrations for the organic and conventional juices were 7.21 6 0.31 and 6.09 6 0.25 mg/L, respectively. The losses for both types of juices after 145 days of storage were about 10% (Table 1). β-cryptoxanthin is one of the main carotenoids found in orange and mandarin juices (Meléndez-Martínez *et al.* 2009). This carotenoid has provitamin A activity and provides juices with a dark orange, almost red color, which is a distinctive attribute of mandarin juices compared with orange juices.

Lutein and zeaxanthin presented lower concentration than β-cryptoxanthin, but always their concentrations were higher in organic than in conventional juices; again no significant effects were found after addition of β-CD. The losses caused by the storage period (145 days) were about 5% of the initial concentrations, and therefore their stability was high because of the ultra-freezing treatment. Some researchers showed that canning does not decrease the content of these carotenoids, in corn and some vegetables, and that freezing may increase carotenoid content in corn, which can further influence bioavailability and health benefits (Updike and Schwartz 2003; Scott and Eldreidge 2005).

Finally, β-carotene was the studied carotenoid with the lowest concentration, 0.12 6 0.03 and 0.09 6 0.01 mg/L in organic and conventional juices, respectively; these concentrations only represented about 1.4% of the total concentration of carotenoids. These concentrations decreased about 15% after 145 days of storage, because of the high sensitivity

TABLE 1. CHANGES IN CAROTENOIDS COMPOSITION OF ULTRA-FROZEN MANDARIN JUICES THROUGHOUT 145 DAYS OF STORAGE

Storage time	Carotenoids (mg/L)											
	Lutein			Zeaxanthin			β-Cryptoxanthin			β-Carotene		
(days)	Organic	Tradit.	Tr. + β-CD	Organic	Tradit.	Tr. + β-CD	Organic	Tradit.	Tr. + β-CD	Organic	Tradit.	Tr. + β-CD
0	0.61 6 0.08	0.38 6 0.06	0.38 6 0.11	0.60 6 0.08	0.41 6 0.03	0.43 6 0.09	7.21 6 0.31	6.09 6 0.25	6.09 6 0.32	0.12 6 0.03	0.09 6 0.01	0.09 6 0.01
20	0.60 6 0.12	0.37 6 0.08	0.37 6 0.06	0.59 6 0.03	0.39 6 0.08	0.41 6 0.04	7.14 6 0.22	6.08 6 0.32	6.08 6 0.17	0.12 6 0.01	0.09 6 0.03	0.09 6 0.02
45	0.58 6 0.16	0.37 6 0.11	0.36 6 0.07	0.59 6 0.04	0.37 6 0.05	0.37 6 0.08	7.12 6 0.17	5.98 6 0.41	5.98 6 0.12	0.11 6 0.04	0.08 6 0.02	0.08 6 0.01
90	0.58 6 0.05	0.37 6 0.14	0.36 6 0.02	0.59 6 0.09	0.36 6 0.09	0.36 6 0.03	6.95 6 0.27	5.87 6 0.26	5.87 6 0.29	0.11 6 0.02	0.08 6 0.01	0.08 6 0.02
120	0.58 6 0.15	0.37 6 0.03	0.36 6 0.08	0.59 6 0.06	0.35 6 0.07	0.35 6 0.09	6.75 6 0.19	5.79 6 0.34	5.79 6 0.24	0.10 6 0.03	0.08 6 0.02	0.08 6 0.03
145	0.58 6 0.22	0.37 6 0.10	0.35 6 0.05	0.58 6 0.09	0.34 6 0.05	0.35 6 0.04	6.45 6 0.24	5.47 6 0.39	5.47 6 0.15	0.10 6 0.02	0.07 6 0.01	0.07 6 0.02

Tr. + β-CD, conventional mandarin juice + β-cyclodextrin.

of β-carotene compared with other carotenoids, such as lutein (Siems *et al.* 1999).

According to literature there are several factors that can justify the fact that the concentrations of carotenoids were higher in organic than in traditional mandarin juices. For instance, Mäder *et al.* (2006) suggested that organic soils are richer in some nutrients and that can result in higher contents of some elements in plants and therefore in the products manufactured from them, such as juices. Asami *et al.* (2003) also indicated that plants not treated with pesticides produce higher concentrations of protective-compounds, such as carotenoids.

Retinol Activity Equivalents (RAEs)

RAEs of the samples analyzed and discussed here are referred to 1 L of 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 fiber, protein and zinc status (FNBIM 2002). In this study, α-carotene was not found in mandarin juices, calculations were performed, considering new guidelines according to the following formula (FNBIM 2002):

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

where $C_{\beta\text{-}car}$ and $C_{\beta\text{-}crip}$ are the concentrations (µg/L) of β-carotene and β-criptoxanthin, respectively. Initially the RAE values found for organic and conventional, with and without β-CD, mandarin juices were 310 and 261 µg/L, respectively; these high values of RAE/L 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 (Beltrán *et al.* 2008). Finally, the RAE values lost for organic and conventional was about 11% after 145 days of storage, independently of the addition of β-CD (which had no effect on this quality parameter). These experimental values agreed well with the range previously described by Meléndez-Martínez *et al.* (2007) for the RAE/L contents of different cultivars of mandarin and orange juices marketed in Spain, ranging from 9.7 to 359. This RAE/L range was significantly reduced when only orange juices were considered, 9.7–94.8. Without any doubt the high levels of RAE in mandarin juices compared with others citrus juice are related to their high concentrations of β-cryptoxanthin.

Antioxidant Activity

The antioxidant activity of all assayed samples was quite high during the 145 days of storage (Fig. 2). Organic farming led to significantly higher values of antioxidant activity than conventional farming, 0.06 6 0.01 and 0.04 6 0.01 mM Trolox/

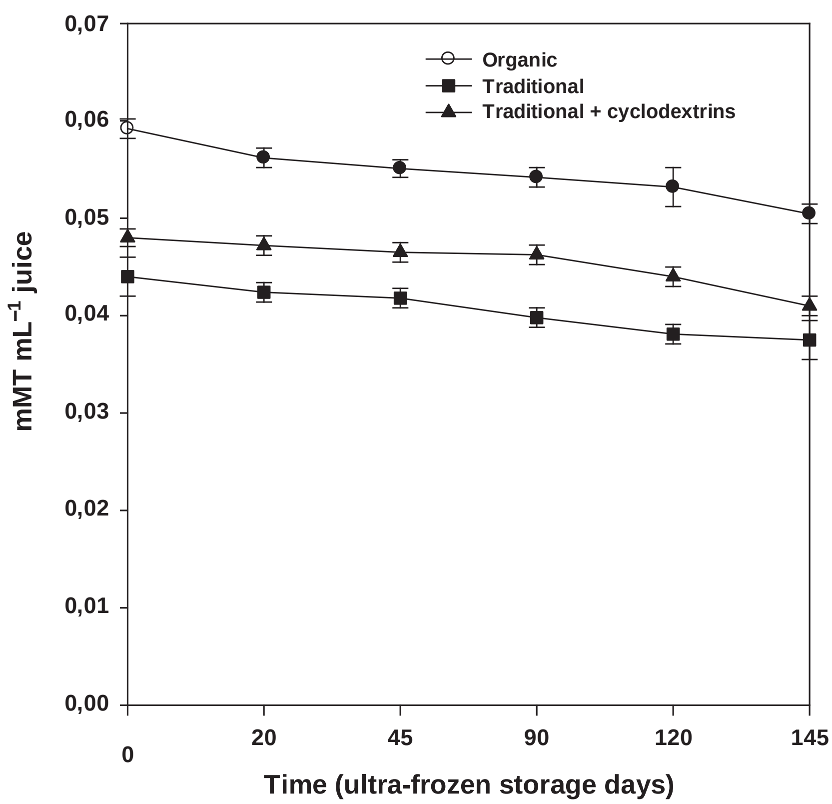

FIG. 2. CHANGES OF ANTIOXIDANT ACTIVITY VALUES OF ULTRA-FROZEN MANDARIN JUICES THROUGHOUT 145 DAYS OF STORAGE

TABLE 2. SENSORY ANALYSIS OF ULTRA-FROZEN MANDARIN JUICES THROUGHOUT 145 DAYS OF STORAGE

	Sensory properties											
	Color			Sweetness			Fresh mandarin aroma			Off-Flavor		
Storage time (days)	Organic	Tradit.	Tr. + β-CD	Organic	Tradit.	Tr. + β-CD	Organic	Tradit.	Tr. + β-CD	Organic	Tradit.	Tr. + β-CD
0	8.2a	7.1b	7.0b	6.5a	5.5b	5.4b	7.2a	6.0b	6.0b	1.0a	2.3b	2.1b
20	8.0a	6.8b	7.0b	6.5a	5.6b	5.5b	7.1a	6.0b	6.0b	1.0a	2.2b	2.3b
45	8.0a	6.7b	6.8b	6.4a	5.4b	5.6b	7.1a	5.9b	5.9b	1.0a	2.3b	2.4b
90	8.0a	6.7b	6.8b	6.5a	5.5b	5.5b	6.9a	5.8b	5.8b	1.0a	2.1b	2.2b
120	8.0a	6.6b	6.8b	6.3a	5.6b	5.5b	6.9a	5.8b	5.7b	1.0a	2.0b	2.2b
145	8.0a	6.6b	6.7b	6.4a	5.5b	5.6b	7.0a	5.9b	5.7b	1.1a	2.0b	1.9b

Samples with the same letters were not significantly different at $P < 0.001$ for the attribute evaluated (Tukey multiple range test).
Tr. + β-CD, conventional mandarin juice + β-cyclodextrin.

mL, respectively. Because of the fact that organic farming does not use synthetic chemicals as fertilizers, pesticides, etc., the plant synthesized natural protecting compounds (e.g., carotenoids), which in general have antioxidant activity. Results agreeing with this last statement have been reported by several authors (Negro *et al*. 2003; Riso *et al*. 2005; Toor and Savage 2006; Rivero-Pérez *et al*. 2008).

Again the addition of β-CD did not have any significant effect on the antioxidant activity of mandarin juices (Fig. 2).

Ultra-freezing of mandarin juices was very effective in protecting the antioxidant activity of juices and only 18% of the initial activity was lost after 145 days of storage (Fig. 2).

Sensory Evaluation

The trained panel established that the quality of the mandarin juices analyzed was high but organic juice had higher intensities than conventional samples for the following sensory attributes: color, sweetness, fresh mandarin juice and aroma. However, the addition of β-CD had no significant effect on the quality of conventional juices (Table 2).

CONCLUSIONS

This study proved that the conservation of mandarin juice by ultra-freezing is an interesting alternative to the heat treatments. Ultra-frozen juices were characterised by having high sensory (intense orange color and fresh mandarin aroma and low presence of off-flavors) and nutritional quality (high content of total carotenoids and antioxidant capacity) and being stable during storage (reduction of vitamin C and total carotenoids contents ranged from 5 to 10%). However, the addition of β-CD, at a concentration of 1.5%, did not resulted in any improvement on the quality of ultra-frozen mandarin juices. However, other concentrations and types of CDs must be used in future studies to determine the role of these protective compounds on ultra-frozen citrus juices.

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