

Vitamin C, Provitamin A Carotenoids, and Other Carotenoids in High-Pressurized Orange Juice during Refrigerated Storage

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Vitamin C, provitamin A carotenoids, and other carotenoids were measured in freshly squeezed juices from oranges (*Citrus sinensis* L. var. Valencia late) that were subjected to high-pressure (HP) treatment. Also, the stability of these compounds was studied during refrigerated storage at 4 °C. HP treatment is an alternative to heat preservation methods for foods; therefore, it is essential to assess the impact of HP on bioactive compounds. Several processes that combine HP treatment with heat treatment for various time periods were assayed: T0, fresh juice (without treatment); T1, 100 MPa/60 °C/5 min; T2, 350 MPa/30 °C/2.5 min; T3, 400 MPa/40 °C/1 min. Fresh and treated samples were kept refrigerated (4 °C) over 10 days. After application of HP and during the refrigeration period, the qualitative and quantitative determination of vitamin C, provitamin A carotenoids (β - and α -carotene; β - and α -cryptoxanthin), and the xanthophylls zeaxanthin and lutein was achieved by high-performance liquid chromatography. T1 and T3 juices showed a decrease in ascorbic acid and total vitamin C just after HP treatment (D0) compared with T0 juices. On the contrary, T2 juices, just after HP treatment (D0), had the same levels of both compounds compared to untreated juices. T1, T2, and T3 treatments led to an increase in the extraction of carotenoids and provitamin A carotenoids. Total carotenoid content after the 10-day refrigerated storage period resulted in no significant quantitative changes in T1 juices, whereas in T2 and T3 juices small losses were found at the end of the storage period (20.56 and 9.16%, respectively). These losses could be influenced by the depleted protection of vitamin C toward carotenoid oxidation during the same period. A similar trend was found in provitamin A carotenoids for the different treated juices.

KEYWORDS: Orange juice; bioactive compounds; vitamin C; carotenoids; provitamin A; high pressure; refrigerated storage

INTRODUCTION

Diets with a high proportion of fruits and vegetables are protective against a variety of diseases, particularly cardiovascular and epithelial cancers. On this basis, the National Research Council of the United States currently recommends consumption of five portions of fruits and vegetables daily, especially citrus fruits (1). If these benefits are a result of the intake of antioxidants, then the obvious protective substances may be vitamin C and carotenoids (2–5), along with bioactive plant-secondary metabolites (6).

Epidemiological data link vitamin C intake with reduced risk of several cancers, especially oral cavity, esophagus, stomach, and, to lesser extent, colon and lung (7). Likewise, the epidemiological evidence clearly shows a strong inverse association between the intake of antioxidant carotenoids, especially α - and β -carotene, lycopene, and, to a minor extent, lutein

and the risk of several type of cancers, especially prostate, lung, and stomach (2, 8–10). Also, a negative association has been reported between dietary intake of vitamin C (11) and provitamin A carotenoids and the risk of cardiovascular disease (12). Evidence of protection against eye-related macular degeneration by certain carotenoids such as lutein and zeaxanthin have been reported (13).

Citrus juices, especially orange juice, are rich sources of vitamin C (6). Orange juice is one of a number of dietary sources of carotenoids (β - and α -carotene, β - and α -cryptoxanthin, zeaxanthin, and lutein), some of them are provitamin A carotenoids (14). A wide physiological role for carotenoids, beyond the provitamin A activity, has been increasingly recognized in recent years (15–18), leading to the elucidation of food content data for all carotenoids. Also, some adverse results obtained with intervention studies with oral supplementation of β -carotene (19, 20) are not in accordance with the beneficial effects found in some epidemiological studies carried out with food carotenoids (16–18). Therefore, more specific

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studies focused on individual carotenoids and, more specifically, on the quantification of dietary antioxidant carotenoids are now of maximum importance.

Orange juices are probably the most recognized and globally accepted fruit juices. In 1996, worldwide orange juice consumption had reached 13 billion liters, and all of the economical forecasts indicate a constant increase in consumer demand (21). Among the sweet oranges, the so-called Valencia variety remains the highest priced and is acknowledged as the highest quality level (21).

Orange juice is traditionally pasteurized by heat treatment at 95 °C for 15 s or at 90 °C for 1 min (22). The effect on ascorbic acid degradation during storage (23) and conventional heating (24) in orange juice has been fixed. However, consumers demand new technologies that minimize the conservation processes for foods. High hydrostatic pressure has certain advantages when applied to fresh fruit/vegetable juices. This process guarantees microbiological safety and aims to produce stable food products that are additive free (25). Until now, the evaluation of the effects of high pressure (HP) on food constituents from enzymatic and safety points of view has been the object of study (26, 27). Nienaber and Shellhammer (28) have shown that this processing technique is an effective nonthermal procedure for the stabilization of freshly squeezed orange juice, and in consequence the shelf life of freshly squeezed orange juice can be greatly extended. Our research group has studied the use of HP as a nonthermal technology to preserve fresh orange juice as freshly squeezed (29). Also, we obtained favorable results, in terms of enzyme inactivation and microbial reduction, in strawberry and orange products (30, 31) and in the carotenoid extraction in persimmon fruit purees (32). Therefore, due to the evident potential of this technology to preserve juices, especially orange juice, more studies are needed to evaluate the nutritional and health-promoting properties of bioactive compounds in HP orange juices. The aim of this work was to evaluate the effect on vitamin C, provitamin A carotenoids, and other antioxidant carotenoids in HP orange juice during refrigerated storage, taking into account the synergic effects of these bioactive compounds in the juice matrix.

MATERIALS AND METHODS

Reagents. Potassium hydroxide (KOH) 85%, sodium sulfate anhydrous, sulfuric acid (H₂SO₄), acetonitrile, and methanol were purchased from Panreac Química, S.A. (Barcelona, Spain). Dichloromethane, diethyl ether, and tetrahydrofuran (THF) were obtained from Labscan Ltd. (Dublin, Ireland). Butylated hydroxytoluene (BHT), DL-dithiothreitol, and *trans*- β -apo-8'-carotenol were purchased from Sigma Chemical Co. (St. Louis, MO). Acetic acid was obtained from Hopkin and Williams (Essex, U.K.). L-(+)-Ascorbic acid and metaphosphoric acid were purchased from Merck KGaA (Darmstadt, Germany). β -Carotene, β -cryptoxanthin, lutein, and zeaxanthin were kindly provided by Hoffman-La Roche (Basel, Switzerland).

Orange Juice. Fresh-squeezed orange juice was obtained from orange fruits [*Citrus sinensis* L. variety Valencia late (Valencia, Spain)], purchased in local supermarket, using a domestic squeezer (Lomi model 4, Madrid, Spain) and filtered through 2 mm steel sieves.

High-Pressure Treatment. Three samples of each orange juice were vacuum packed in plastic bags (Doypack) and then introduced into the pressure unit filled with pressure medium (water).

HP treatments were performed in a hydrostatic pressure unit with a 2350 mL capacity at a maximum pressure of 500 MPa and a potential maximum temperature of 95 °C (Gec Alsthom ACB 900 HP, type ACIP 665, Nantes, France). The pressures employed in the treatments selected were 100, 350, and 400 MPa. Pressure was increased and released at 2.5 MPa/s. Several processes that combine HP treatment with heat treatment for various time periods were followed: T0, fresh (without

treatment); T1, 100 MPa/60 °C/5 min; T2, 350 MPa/30 °C/2.5 min; T3, 400 MPa/40 °C/1 min. According to previous results (30, 31) these treatments (T1, T2, and T3) were selected due to the highest enzymatic inactivation and microbial reduction achieved in orange juice. After treatments, orange juices were stored at 4 °C over a 10-day period. Ascorbic acid, total vitamin C, provitamin A carotenoids, and other carotenoids were measured in untreated (T0) and HP-treated (T1, T2, and T3) orange juice samples. Analysis was carried out immediately after the HP treatment (D0) and after 1 (D1), 3 (D3), 6 (D6), and 10 (D10) storage days at 4 °C.

pH, Titratable Acidity, and Soluble and Total Solids. These parameters were measured by conventional methods in freshly squeezed orange juice, as described in Cano et al. (30).

Color Measurements. A tristimulus reflectance Colorimeter (HunterLab, model D25, Hunter Associates Laboratory, Inc., Reston, VA) calibrated with a white standard tile ($X = 82.51$; $Y = 84.53$; $Z = 101.23$) was used. Samples were placed in Petri dishes, and color was recorded using the CIE- L^* , a^* , b^* uniform color space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (–) to red (+) axis, and b^* indicates hue on a blue (–) to yellow (+) axis. Two CIE-Lab values were used to express the sample extract color: hue angle $h = \tan^{-1}(b^*/a^*)$ and saturation (or chroma) $C = (a^{*2} + b^{*2})^{1/2}$. Analysis was carried out in the freshly squeezed orange juice.

Determination of Vitamin C. Ascorbic acid and total vitamin C (ascorbic acid plus dehydroascorbic acid) were determined by high-performance liquid chromatography (HPLC). The procedure employed to determine total vitamin C was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as reductant reagent, according to a modification in the procedure of Sánchez-Mata et al. (33). A volume of 50 mL of each orange juice was homogenized with 40 mL of an extraction solution (3% metaphosphoric acid plus 8% acetic acid). The resulting mixture was centrifuged, filtered, and adjusted to 100 mL with distilled water. Samples were filtered through a 0.45- μ m membrane filter, and duplicates of 20 μ L for each extract were analyzed by HPLC. Results were expressed as milligrams of ascorbic acid per 100 mL.

An aliquot of the mixture was taken to react with 2.0 mL of a solution 20 mg/mL DL-dithiothreitol for 2 h at room temperature and in darkness. During this time the reduction of dehydroascorbic acid to ascorbic acid has been placed. Samples were filtered through a 0.45- μ m membrane filter, and duplicates of 20 μ L for each extract were analyzed by HPLC. Results were expressed as milligrams of total vitamin C per 100 mL.

Chromatographic Procedure. A Hewlett-Packard model 1050 quaternary solvent delivery controller pump was used for analysis. Samples were introduced onto the column via a manual injector (Rheodyne) equipped with a sample loop (20 μ L). Separation of ascorbic acid was performed by HPLC using a reversed-phase C18 Hypersil ODS (5 μ m) stainless steel column (250 \times 4.6 i.d. mm) (Technochroma). The solvent system used was an isocratic gradient of a 0.01% solution of H₂SO₄, adjusted to pH 2.5–2.6. The flow rate was fixed at 1.0 mL/min. A Hewlett-Packard 1040A UV–visible photodiode array detector was set at 245 nm; chromatographic data and UV–visible spectra were collected, stored, and integrated using a Hewlett-Packard Chem Station and related software. Identification of the ascorbic acid was carried out by HPLC by comparing the retention time and UV–visible absorption spectrum with those of the standard previously referred to. Calibration curves were built with a minimum of four concentration levels of ascorbic acid standard; the straight-line equations and their coefficients of correlation were calculated.

Extraction, Separation, Identification and Quantification of Carotenoids of Saponified Extract. The extraction, separation, and HPLC method have been described in detail elsewhere (32, 34, 35). Briefly, triplicates of each sample (50 mL) were extracted with 50 mL of THF containing 0.01% BHT until the extracts were colorless. The combined THF extracts were concentrated on a rotatory evaporator at 35 °C and partitioned between diethyl ether and saltwater and transferred to a separating funnel. The organic layer was washed with water until the diethyl ether extract was colorless. The organic layers were combined and dried over sodium sulfate anhydrous. All steps were performed under diminished light. The ethereal solution was reduced in the evaporator to ~30 mL and was treated with 30% methanolic

Table 1. Physical and Physicochemical Characteristics of Freshly Squeezed Valencia Orange Juice^a

characteristic	value
pH	3.22 ± 0.12
soluble solids (°Brix at 20 °C)	11.30 ± 0.12
total solids (g/100 g of fw ^b)	11.37 ± 0.06
titratable acidity (g of citric acid/100 g of fw)	1.12 ± 0.01
CIELab color parameters	
<i>L</i> *	34.76 ± 0.26
<i>a</i> *	-2.48 ± 0.15
<i>b</i> *	35.37 ± 0.61
<i>h</i> [tan ⁻¹ (<i>b</i> [*] / <i>a</i> [*])]	85.99 ± 0.22
<i>C</i> (<i>a</i> ² + <i>b</i> ²) ^{1/2}	35.46 ± 0.61

^a Values are the mean of three independent determinations ± standard deviation.
^b fw, fresh weight.

potassium hydroxide under nitrogen atmosphere at room temperature for 16 h in darkness. The solution was partitioned into a saturated aqueous solution of sodium chloride and diethyl ether and the organic layer removed. The organic layer was washed several times with water until KOH was completely removed (pH 7.0). The solvent was evaporated to dryness and the residue dissolved in 1.0 mL of dichloromethane. Samples were filtered through a 0.45- μ m membrane filter, and duplicates of 20 μ L for each extract were analyzed by HPLC.

Chromatographic Procedure. A Hewlett-Packard model 1050 quaternary solvent delivery controller pump was used for analysis. Samples were introduced onto the column via a manual injector (Rheodyne) equipped with a sample loop (20 μ L). Separation of carotenoids was performed by HPLC using a reversed-phase C18 Hypersil ODS (5 μ m) stainless steel column (250 × 4.6 i.d. mm) (Technochroma). The solvent system used was a gradient of methanol/water (75:25) (solution A) and acetonitrile/dichloromethane/methanol (70:5:25) (solution B). The gradient was as follows: 0 min, 0% B; 5 min, 5% B; 15 min, 85% B; 35 min, 85% B; 50 min, 100% B; 60 min, 100% B; 70 min, 0% B. The flow rate was fixed at 1.0 mL/min. A Hewlett-Packard 1040A UV-visible photodiode array detector was set at 450 nm; chromatographic data and UV-visible spectra were collected, stored, and integrated using a Hewlett-Packard Chem Station and related software. Identification of the carotenoids was carried out by HPLC by comparing the retention time and UV-visible absorption spectrum with those of the standards previously referred to. Quantification of the carotenoids was achieved according to the procedure of Hart and Scott (36), using *trans*- β -apo-8'-carotenal as external standard. Calibration curves were built with a minimum of four concentration levels of each carotenoid standard; the straight-line equations and their coefficients of correlation were calculated.

Determination of Vitamin A. The vitamin A value has been shown as retinol equivalents (RE). To calculate RE on the basis of carotenoids, the following conversion has been employed: RE = μ g of β -carotene/6 + μ g of other provitamin A carotenoid (α -cryptoxanthin + β -cryptoxanthin + α -carotene)/12. This system of vitamin A standardization is according to the FAO/WHO Joint Expert Consultation (37).

Statistical Analysis. Significant differences between the results were calculated by one-way analysis of the variance (ANOVA) on the triplicate measurements. Significant differences were considered at the 95% confidence level ($P < 0.05$). All statistical analyses were performed using Statgraphics Plus 2.1 (Statistical Graphics Corp., Rockville, MD) and SPSS (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

To characterize the freshly squeezed Valencia orange juice obtained, physical and physicochemical parameters were measured (Table 1). The characterization of the tested juices by these parameters was in agreement with literature data for orange juice (38, 39).

Vitamin C Content. Effect of High-Pressure Treatment on the Extraction of Vitamin C in Orange Juices. T1 juices (100 MPa/60 °C/5 min) showed a decrease in ascorbic acid (10.63%)

Table 2. Vitamin C Content of Orange Juices (Milligrams/100 mL)^a

treatment/storage days ^{b,c}	ascorbic acid	total vitamin C
T0/D0	40.64 ± 1.56abB	41.95 ± 1.04aB
T0/D1	39.63 ± 0.52aB	41.61 ± 0.68aAB
T0/D3	41.01 ± 0.75bA	42.77 ± 0.35aB
T0/D6	41.04 ± 0.23bB	42.74 ± 1.16aB
T0/D10	40.86 ± 0.89abC	42.13 ± 0.35aC
T1/D0	36.32 ± 1.49aA	37.64 ± 2.01aA
T1/D1	39.49 ± 0.58bB	41.71 ± 1.17cAB
T1/D3	38.63 ± 3.23abA	40.86 ± 0.64bcA
T1/D6	36.55 ± 3.48abA	39.07 ± 3.28abA
T1/D10	37.74 ± 0.63abA	39.64 ± 0.47abcA
T2/D0	40.62 ± 2.04abB	41.31 ± 1.86aB
T2/D1	41.09 ± 0.95abC	42.30 ± 0.74aB
T2/D3	40.81 ± 1.69abA	42.43 ± 0.27aB
T2/D6	41.31 ± 0.37bB	42.18 ± 0.33aB
T2/D10	39.55 ± 1.29aBC	41.96 ± 0.65aC
T3/D0	37.84 ± 2.29aA	38.56 ± 2.22aA
T3/D1	38.46 ± 0.53aA	40.83 ± 0.41bA
T3/D3	39.38 ± 0.98aA	41.10 ± 0.53bA
T3/D6	38.08 ± 1.04aA	40.39 ± 0.56bAB
T3/D10	38.74 ± 0.96aAB	40.33 ± 1.48abB

^a Values are the mean of three independent determinations ± standard deviation. Different lower case letters for each carotenoid and treatment during storage period indicate significant differences ($P < 0.05$). Different capital letters for each carotenoid in different treatments but for the same storage day indicate significant differences ($P < 0.05$). ^b Treatments: T0, fresh (without treatment); T1, 100 MPa/60 °C/5 min; T2, 350 MPa/30 °C/2.5 min; T3, 400 MPa/40 °C/1 min. ^c Storage days: D0, immediately after treatment; D1, D3, D6, and D10, 1, 3, 6, and 10 storage days at 4 °C.

and total vitamin C (10.27%) just after HP treatment (D0) compared with T0 juices (untreated). T3 juices (400 MPa/40 °C/1 min) also showed a decrease in ascorbic acid (6.89%) and vitamin C (8.08%) just after HP treatment (Table 2). On the contrary, T2 juices (350 MPa/30 °C/2.5 min), just after HP treatment (D0), had the same levels of both compounds compared to untreated juices (T0 juices). Putting these findings together, we observe that among the three HP treatments assayed, even though the losses were <10%, HP treatments with the higher temperatures showed a decrease in the content of vitamin C, which could be due to a thermal degradation of this vitamin. Therefore, the mild temperature tested in the T2 HP treatment was better to preserve the vitamin C level in pressurized orange juices. Ascorbic acid and total vitamin C levels found in this study in T0 and T2 juices were the same. These results are in agreement with Fernández García et al. (40), who reported that the vitamin C content of orange and orange-carrot-lemon juices processed at 500 and 800 MPa was not, or only insignificantly, reduced compared to that of unprocessed juices. Also, our results confirm those reported by Donsi et al. (41) and Van den Broeck et al. (42) about the stability of ascorbic acid in orange juice when pressurized at mild temperatures.

Effect of High-Pressure Treatment on the Vitamin C Content in Orange Juices at Refrigeration Temperature during Storage and at the End of Storage. The ascorbic acid content, during 10 days of refrigerated storage, averaged 40.64 mg/100 mL (39.63–41.04 mg/100 mL) for T0 juices, 37.75 mg/100 mL (36.32–39.49 mg/100 mL) for T1 juices, 40.68 mg/100 mL (39.55–41.31 mg/100 mL) for T2 juices, and 38.50 mg/100 mL (37.84–39.38 mg/100 mL) for T3 juices (Table 2).

Regarding total vitamin C content, during 10 days of refrigerated storage, T0 juices averaged 42.24 mg/100 mL (41.61–42.77 mg/100 mL), T1 juices averaged 39.78 mg/100 mL (37.64–41.71 mg/100 mL), T2 juices averaged 42.04 mg/100 mL (41.31–42.43 mg/100 mL), and T3 juices averaged 40.24 mg/100 mL (38.56–41.10 mg/100 mL) (Table 2).

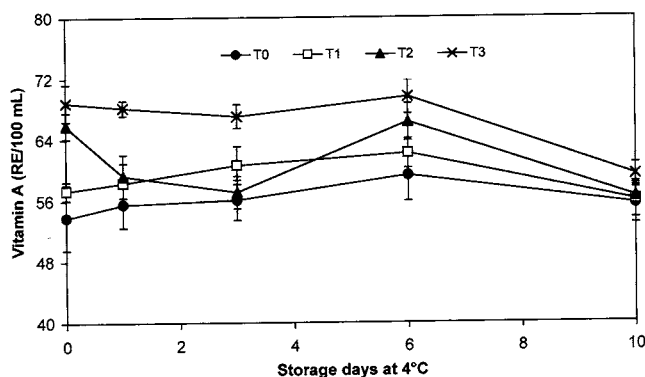


Figure 1. Profile of total carotenoid content in orange juices during storage at 4 °C for each high-pressure treatment assayed: T0, fresh (without treatment); T1, 100 MPa/60 °C/5 min; T2, 350 MPa/30 °C/2.5 min; T3, 400 MPa/40 °C/1 min. D0, immediately after treatment; D1–10, storage days at 4 °C. RE, retinol equivalents.

In the same manner that occurs with ascorbic acid, the total vitamin C content in T1 and T3 juices showed a decrease compared with that in T0 or T2 juices. The decrease occurred during the whole storage period (D0–D10) and was in accordance with the decrease occurring just after the HP treatments (D0) in these juices, which means that the degradation of vitamin C happened at the beginning, due to the HP treatment, and remained constant during the 10-day storage period, with small oscillations.

Nienaber and Shellhammer (28) studied the ascorbic acid content in orange juice treated at 800 MPa and 25 °C for 1 min. They stored the juice at different temperatures (4, 15, 26, and 37 °C) and observed, as expected, that the highest ascorbic acid retention occurred in juices stored at 4 °C and progressively diminished with the increase in the storage temperature. During further storage, ascorbic acid gradually decreased in all samples. These authors concluded that >80% of ascorbic acid was retained after 3 months at 4 °C or after 2 months at 15 °C. In our case, even though there were some losses just after HP treatment (D0) for T1 and T3 juices compared to T0, at the end of the shorter period of 10 days at 4 °C (D10) there were no losses in ascorbic acid and vitamin C compared with D0, with the exception of T2 juices for ascorbic acid, but the loss was insignificant (2.63%).

Carotenoid Content. *Effect of High-Pressure Treatment on the Extraction of Carotenoid in Orange Juices.* The major carotenoids in Valencia orange juice, β - and α -carotene, β - and α -cryptoxanthin, zeaxanthin, and lutein, were identified and quantified by HPLC after extraction with THF. Because the amount of carotenoids increases according to fruit maturity and varies with variety (14), the values obtained for individual carotenoids in the tested orange juices were in reasonable agreement with those described by Mouly et al. (21) in orange juices. Also, these values agree with those obtained in orange juices treated with different preservation processes (43).

T1, T2, and T3 orange juices showed just after HP treatment (D0) an increase in total carotenoid content (10.03, 24.11, and 31.73%, respectively) compared with T0 juices (Figure 1). Thus, among the tested treatments, the lowest influence on the extractability of total carotenoids was found in T1 HP treatment (100 MPa/60 °C/5 min), whereas the influence of T2 (350 MPa/30 °C/2.5 min) and T3 (400 MPa/40 °C/1 min) HP treatments on the extractability of total carotenoids was similar, although slightly higher in the T3 HP treatment. Consequently, pressure combined with temperature in the treatments applied to orange juices seemed not to cause any loss in carotenoids compared to

untreated orange juices. These results were in accordance with those reported by Fernández-García et al. (44), showing no losses in β -carotene and lycopene extraction with THF in HP-treated tomato puree (600 MPa/20 °C/60 min) compared with untreated tomato puree. Moreover, the major increase in total carotenoids had been obtained in the upper range of pressure applied. Thus, differences in carotenoids extractability with different treatments implied that extraction of carotenoids was pressure-dependent, because the level of total compound recovered was lower after 100 MPa than after 350 or 400 MPa (Figure 1). Butz et al. (45) reported that pressures >100 MPa damaged cell structures in onions. Therefore, the more damage to membranes caused by upper range pressure might induce the better release of carotenoids. Also, it has been evidenced that HP strongly affects food macromolecular structures, including proteins (46). In this sense, our group have reported an increase in carotenoid extraction from HP treated orange juice compared with untreated orange juice (29), being attributed to the denaturation of the carotenoid-binding protein induced by pressure. Such changes might also result in altered bioavailability with possible nutritional consequences (47).

The cause of instability, and consequent degradation, of carotenoids is due to the susceptibility to oxidation and geometric isomerization of its polyene chain (48). It is suggested that geometric isomerization goes through covalent bonding rupture, and covalent bonding should not be affected by pressure (46). Therefore, it seems that high pressure might not affect to any extent carotenoid content.

Regarding individual carotenoid content just after HP treatment (D0), zeaxanthin content increased in T1, T2, and T3 juices compared with T0 juices (32.53, 41.02, and 59.26%, respectively), whereas β - and α -carotene, β -cryptoxanthin, and lutein contents were significantly increased compared with T0 juices only in T2 juices (29.57, 16.90, 31.93, and 18.44%, respectively) and T3 juices (43.50, 37.51, 28.92, and 23.47%, respectively). On the contrary, α -cryptoxanthin content increased only in T3 juices (11.34%) compared with T0 juices (Table 3). In all of the tested treatments, among individual carotenoids the greater increase in the extraction was found in zeaxanthin. Moreover, T1 HP treatment (100 MPa/60 °C/5 min) might influence only zeaxanthin extraction, but not the rest of the carotenoids. A hypothetical explanation is that the grade of binding of each carotenoid in a protein–carotenoid complex or to the cell membrane depends on the kind of carotenoid study (49). In general, HP treatment between 50 and 100 MPa can affect the nuclear membrane (50), producing disruption of the chromoplasts where the carotenoids are located. Pressures >300 MPa cause irreversible protein denaturation and could increase the amount of extractable carotenoids in orange juice, perhaps owing to the release of more carotenoids from the food matrix (orange juice cloud) after the denaturation of protein–carotenoid complexes induced by pressure (26). This HP effect could increase the amount of antioxidant carotenoids available for absorption, improving the bioavailability of the nutritional and antioxidant carotenoids in HP-treated orange juice as happened with lycopene in thermally treated tomato products (51).

Effect of High-Pressure Treatment on Carotenoid Content at Refrigeration Temperature during Storage. During storage, the averaged total carotenoid content in T1, T2, and T3 orange juices remained higher (11.16, 14.08, and 23.41%, respectively) compared with T0 juices (Figure 1). The same pattern was observed in almost all of the individual carotenoids: a significant difference was found in averaged zeaxanthin content in T1, T2, and T3 juices (17.31, 19.18, and 43.10%, respectively) compared

Table 3. Individual Carotenoid Content of Orange Juices (Micrograms/100 mL)^a

treatments/ storage days ^{b,c}	β -cryptoxanthin	α -cryptoxanthin (as β -cryptoxanthin)	zeaxanthin	lutein	β -carotene	α -carotene (as β -carotene)
T0/D0	314.09 \pm 19.07aA	161.82 \pm 35.01aA	345.38 \pm 42.03aA	765.72 \pm 68.63aA	60.73 \pm 4.26aA	48.57 \pm 4.03aA
T0/D1	335.37 \pm 23.76abA	130.11 \pm 28.88aA	355.96 \pm 28.51aA	768.59 \pm 61.81aA	69.04 \pm 0.70bcA	63.00 \pm 8.40bAB
T0/D3	351.74 \pm 14.54bcA	130.56 \pm 3.87aA	390.18 \pm 52.13aA	793.51 \pm 74.45aA	69.61 \pm 6.18bcA	51.42 \pm 1.83aA
T0/D6	377.02 \pm 24.30cA	135.71 \pm 2.90aA	398.93 \pm 43.18aA	764.36 \pm 89.80aA	72.38 \pm 5.72cAB	54.03 \pm 2.20aA
T0/D10	351.11 \pm 10.11bcA	142.49 \pm 19.53aA	396.01 \pm 18.91aAB	737.39 \pm 63.02aAB	62.10 \pm 1.95abB	47.38 \pm 2.72aAB
T1/D0	343.03 \pm 19.02aA	162.52 \pm 17.54cA	457.72 \pm 54.22bB	786.70 \pm 64.94aAB	65.38 \pm 3.63abA	51.04 \pm 2.02aA
T1/D1	341.86 \pm 21.53aA	140.13 \pm 4.36aA	358.48 \pm 69.23aA	1082.31 \pm 61.81bC	79.67 \pm 8.31dAB	58.36 \pm 0.30bA
T1/D3	361.82 \pm 20.27abA	151.45 \pm 13.37abcB	463.29 \pm 37.54bA	859.18 \pm 74.45aAB	76.63 \pm 7.22cdAB	60.72 \pm 5.22bB
T1/D6	390.31 \pm 20.03bA	157.90 \pm 5.85bcB	474.82 \pm 34.49bAB	827.93 \pm 89.80aAB	69.49 \pm 0.55bcA	58.93 \pm 2.23bAB
T1/D10	357.18 \pm 15.44aA	144.45 \pm 7.91abA	458.65 \pm 19.16bB	769.88 \pm 90.96aAB	59.48 \pm 8.44aB	50.43 \pm 3.02aB
T2/D0	414.39 \pm 16.04bB	161.44 \pm 15.68bA	487.05 \pm 82.45bBC	906.90 \pm 88.69bBC	78.69 \pm 4.35bB	56.78 \pm 1.16abB
T2/D1	353.70 \pm 25.56aA	134.26 \pm 5.58aA	423.75 \pm 41.59abAB	947.40 \pm 144.91bBC	81.84 \pm 11.92bAB	59.08 \pm 5.16bA
T2/D3	394.89 \pm 8.27aB	131.36 \pm 4.03aA	476.05 \pm 74.42bA	969.29 \pm 76.93bBC	68.03 \pm 4.24aA	55.01 \pm 2.40abAB
T2/D6	418.28 \pm 9.92bB	169.22 \pm 7.91bB	488.34 \pm 25.19bBC	924.71 \pm 94.02bB	73.52 \pm 5.64abAB	60.61 \pm 4.24bB
T2/D10	351.30 \pm 6.22aA	144.41 \pm 7.21aA	373.16 \pm 23.89aA	686.87 \pm 40.05aA	63.88 \pm 4.94aB	52.84 \pm 2.49aB
T3/D0	404.94 \pm 17.93aB	180.17 \pm 7.83cA	550.05 \pm 27.46aC	945.45 \pm 87.29abC	87.15 \pm 12.21bB	66.79 \pm 3.07cC
T3/D1	394.89 \pm 8.27aB	173.80 \pm 6.49bcB	480.85 \pm 57.95aB	861.73 \pm 78.02aAB	90.16 \pm 5.99bB	58.75 \pm 6.39cB
T3/D3	413.33 \pm 12.93aB	161.61 \pm 6.55abB	569.52 \pm 64.12aB	1027.72 \pm 139.33bC	81.01 \pm 6.40bB	67.63 \pm 2.64cC
T3/D6	419.30 \pm 15.41aB	198.58 \pm 21.35dC	549.39 \pm 72.44aC	820.77 \pm 84.73aAB	79.74 \pm 8.12bB	57.62 \pm 5.38bAB
T3/D10	411.40 \pm 31.02aB	155.37 \pm 3.55aA	549.81 \pm 92.53aC	818.82 \pm 47.24aB	49.50 \pm 3.79aA	45.03 \pm 4.59aA

^a Values are the mean of three independent determinations \pm standard deviation. Different lower case letters for each carotenoid and treatment during storage period indicate significant differences ($P < 0.05$). Different capital letters for each carotenoid in different treatments but for the same storage day indicate significant differences ($P < 0.05$). ^b Treatments: T0, fresh (without treatment); T1, 100 MPa/60 °C/5 min; T2, 350 MPa/30 °C/2.5 min; T3, 400 MPa/40 °C/1 min. ^c Storage days: D0, immediately after treatment; D1, D3, D6, and D10, 1, 3, 6, and 10 storage days at 4 °C.

with T0 juices (**Table 3**), as was found just after HP treatment. In T1 and T2 juices, there were no differences between averaged α -cryptoxanthin and lutein contents, compared with T0 juices, whereas in T3 juices a significant increase in averaged α -cryptoxanthin (24.10%) and averaged lutein (17.10%) contents compared with T0 juices was shown (**Table 3**). There was no difference between averaged β -cryptoxanthin content in T1 juices and T0 juices, whereas a significant difference in T2 juices (9.83%) and T3 juices (18.19%) was found compared to T0 juices (**Table 3**). Regarding hydrocarbon carotenoids β - and α -carotene during storage period, there was no difference in averaged contents in T1, T2, and T3 juices compared to T0 juices (**Table 3**). In conclusion, during storage only zeaxanthin content was influenced by T1 HP treatment, and α - and β -cryptoxanthin, zeaxanthin, and lutein contents were influenced by T3 HP treatment. However, T2 HP treatment could influence only zeaxanthin and β -cryptoxanthin compounds.

Effect of High-Pressure Treatment on Carotenoid Content at the End of Storage. Regarding the total carotenoid content, after 10 days of storage at 4 °C, within each type of treated orange juice, in T1 juices there was not a significant quantitative change between total carotenoid content just after treatment (D0) and after the storage period (D10). However, in T2 and T3 juices small losses were found (20.56 and 9.16%, respectively) (**Figure 1**). Among individual carotenoids, comparing within each treated juice, in T2 juices, the xanthophylls lutein and zeaxanthin showed the major losses (24.26 and 23.38%, respectively), whereas in T3 juices, β - and α -carotene showed the major losses (43.20 and 32.58%, respectively). However, after the storage period, the total carotenoid content in T2 juices was equal compared to T0 juices. In T1 and T3 juices, the total carotenoid content remained higher after storage compared to T0 juices.

As a consequence, HP treatments led to higher or equal carotenoid content in orange juice during 10 days of storage at 4 °C. These results are consistent with previous reports, which indicated that HP-processed orange juice (800 MPa/25 °C/1 min) retains its freshlike quality after being refrigerated for 3 months (28).

Oxidation is the major cause of carotenoid loss, and it depends on the carotenoid involved. Oxidation is stimulated by light, heat, metals, enzymes, and peroxides, and it is inhibited by antioxidants, such as tocopherols and ascorbic acid (48, 52). In this work, the effects of the HP treatments could be probably mediated because of the remaining enzymatic activity and the presence of vitamin C. The losses in total carotenoids during the storage period occurred between D3 and D10 in T1 juices (6.74%), between D6 and D10 in T2 juices (21.65%), and between D3 and D6 in T3 juices (8.42%) (**Figure 1**). From D3 to D10 the loss in ascorbic acid in T1 juices was 2.30%. From D6 to D10 the loss in ascorbic acid in T2 juices was 4.26%. In the same way, from D3 to D6 the loss in ascorbic acid in T3 juices was 3.30%. Thus, it seems that due to these losses, vitamin C offers less protection to carotenoids from oxidation in T2 and T3 juices during the same period. On the other hand, our group have compared the effects of HP treatments on peroxidase activity in orange juice (30), concluding that the greatest inactivation rate of this enzyme is obtained under an HP range of 300–400 MPa at a range of 20–32 °C during 15 min. Therefore, in T1 juices peroxidase activity could be higher than in T2 and T3 juices, due to the lower pressure (100 MPa) applied in the T1 HP treatment compared to that in the T2 and T3 HP treatments, and this also might explain the losses in total carotenoids occurring in T1 juices between D3 and D10.

Vitamin A Value. *Effect of High-Pressure Treatment on Vitamin A Extraction in Orange Juices.* Vitamin A is provided in the diet as preformed vitamin A (retinyl ester, retinol retinal, and retinoic acid) from foods of animal origin or as provitamin A carotenoids that can be biologically transformed to vitamin A, generally from plant foods (53). Potential precursors of vitamin A are all carotenoids containing at least one unsubstituted β -ionone ring and a polyene side chain attached with at least 11 carbon atoms. The process of conversion to retinol occurs primarily in the enterocytes, although enzyme activity responsible for this conversion is found in other tissues such as the liver (54).

Vitamin A values in the orange juices were determined by the potential precursors β -carotene, which shows the highest

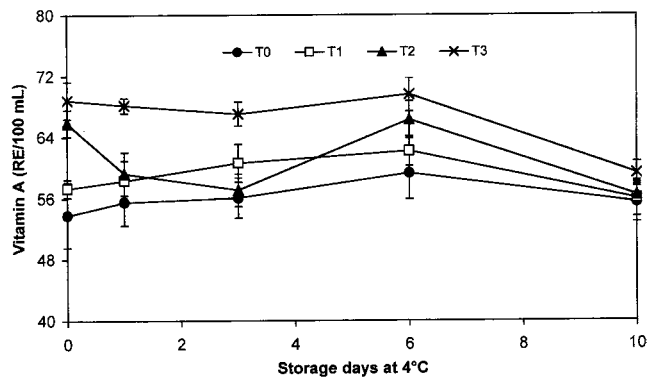


Figure 2. Profile of vitamin A value in orange juices during storage at 4 °C for each high-pressure treatment assayed: T0, fresh (without treatment); T1, 100 MPa/60 °C/5 min; T2, 350 MPa/30 °C/2.5 min; T3, 400 MPa/40 °C/1 min. D0, immediately after treatment; D1–10, storage days at 4 °C. RE, retinol equivalents.

vitamin A activity on a molar basis, and α -cryptoxanthin, β -cryptoxanthin, and α -carotene, which are assumed to have provitamin A activity that is 50% that of β -carotene (37).

T1, T2, and T3 orange juices showed just after HP treatment (D0) an increase in vitamin A value (6.41, 22.29, and 27.90%, respectively) compared to T0 juices (Figure 2), according to the increase in β -cryptoxanthin (9.21, 31.93, and 28.92%) and β -carotene (7.65, 29.57, and 43.50%) in T1, T2, and T3 juices, respectively.

Effect of High-Pressure Treatment on Vitamin A at the End of Storage. Regarding the vitamin A value, within each type of orange juice, in T2 and T3 juices there were significant losses (14.38 and 13.97%, respectively) between contents just after treatment (D0) and after the storage period (D10) (Figure 2). In T2 juices, the losses were due to β -carotene (18.82%) and β -cryptoxanthin (15.22%), whereas in T3 juices the losses were due to β -carotene (43.20%) and α -carotene (32.58%). However, after the storage period, the vitamin A value in T3 juices remained higher compared to that in T0 juices (6.85%).

We conclude that, in general, HP treatment did not improve significantly the vitamin C content. T2 HP treatment (350 MPa/30 °C/2.5 min) preserved the vitamin C level in pressurized orange juices better than T1 and T3 HP treatment. Total carotenoids and vitamin A (expressed as retinol equivalents) showed an increasingly better extraction when the pressure increased from 100 to 400 MPa. Vitamin C content seems to preserve the carotenoid compounds from oxidation in the treated orange juices.

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