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Carotenoid Profile Modification during Refrigerated Storage in Untreated and Pasteurized Orange Juice and Orange Juice Treated with High-Intensity Pulsed Electric Fields

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A comparative study was made of the evolution and modification of various carotenoids and vitamin A in untreated orange juice, pasteurized orange juice (90 °C, 20 s), and orange juice processed with high-intensity pulsed electric fields (HIPEF) (30 kV/cm, 100 μs), during 7 weeks of storage at 2 and 10 °C. The concentration of total carotenoids in the untreated juice decreased by 12.6% when the juice was pasteurized, whereas the decrease was only 6.7% when the juice was treated with HIPEF. Vitamin A was greatest in the untreated orange juice, followed by orange juice treated with HIPEF (decrease of 7.52%) and, last, pasteurized orange juice (decrease of 15.62%). The decrease in the concentrations of total carotenoids and vitamin A during storage in refrigeration was greater in the untreated orange juice and the pasteurized juice than in the juice treated with HIPEF. During storage at 10 °C, auroxanthin formed in the untreated juice and in the juice treated with HIPEF. This carotenoid is a degradation product of violaxanthin. The concentration of antheraxanthin decreased during storage, and it was converted into mutatoxanthin, except in the untreated and pasteurized orange juices stored at 2 °C.

KEYWORDS: Carotenoids; orange juice; pulsed electric fields; vitamin A; pasteurized; storage

INTRODUCTION

Orange juice is an excellent source of vitamin A and is a product desired by many consumers who are interested in maintaining a healthy diet. Carotenoids are among the most abundant bioactive compounds in vegetables and fruits, and on a worldwide basis ≈60% of vitamin A is estimated to come from provitamin A carotenoids, whereas in developing countries they provide up to 82% (*1*). The importance of carotenoids and the foods that contain them is based on their two most important properties, their antioxidant capacity and their vitamin A activity, with β-carotene having the highest activity of them all, and they are associated with anticancer, antiaging, and antiulcer properties (*2*, *3*). Not all carotenoids present in fruits and vegetables are vitamin A precursors. Several of them, including α-carotene, β-carotene, and β-cryptoxanthin, have provitamin A activity, being transformed into retinal by mammals (*4*). The main problems associated with carotenoids come from the instability of these pigments, because they are highly unsaturated molecules and are subject to isomerization. The stability of carotenoids during storage is very important for consumer acceptance of the end product. For many years orange juice has been produced in numerous forms, such as frozen concentrate, orange juice from concentrate, and pasteurized juice. Thermal processing is one of the methods by which appropriate foods are preserved and made available to the consumer. During thermal treatment, in addition to the inactivation of microorganisms, various percentages of desirable constituents such as nutrients, color, flavor, and texture are destroyed. Although these products must conform to strict guidelines that prevent unnatural changes in the juice, concern about diet and nutrition has led consumers to seek a more natural product (*5*−*8*).

The citrus industry has been exploring innovative methods with minimal heat treatment to increase markets by improving nutritional and flavor qualities. High-intensity pulsed electric field (HIPEF) processing, a nonthermal method, inactivates microorganisms without significant adverse effects on flavor and nutrients (*9*−*14*).

HIPEF has the potential to pasteurize various foods nonthermally by exposure to short high-voltage pulses while the material passes between electrodes in a treatment chamber. The electric field affects the cell membranes (*15*) and may cause irreversible membrane breakage (*16*), alteration in transport of ions, and changes in enzyme structure (*17*). There are many studies on the effect that this new technology has on microorganisms and their half-lives (*18*−*22*).

A number of authors have studied the evolution of quality factors in orange juice after HIPEF treatment, in some cases making a comparison with the evolution after heat treatment (*12*, *23*−*27*).

Liquid chromatography (LC) is considered to be the method of choice for the separation, identification, and quantification

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of carotenoids found in biological tissues (*28*−*31*), and the use of photodiode array detection in the identification of carotenoids by LC is a valuable tool for the characterization of cis and trans isomers in vegetable and citric juices (*32*−*34*).

The aim of this work is to study carotenoid profile modifications when natural orange juice is treated by means of pasteurization or HIPEF and also its evolution during 7 weeks of storage in refrigeration at two different temperatures (2 and 10 °C) and the transformations of some carotenoids as a result of that storage. A study is also made of the evolution of vitamin A in the different orange juices during the storage period.

MATERIALS AND METHODS

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

Sampling of Orange Juice. After appropriate washing and hygienization of the fruits, they were subjected to an extraction process (FMC juice extractors with a 2-mm-diameter sieve), and the juice was introduced into a tank.

HIPEF Treatment System. Sample treatments were carried out in a continuous HIPEF treatment system designed by the The Ohio State University and located in the Instituto de Agroquímica y Tecnología de Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers, immersed in a refrigerated bath to keep the temperature within the designated range. The temperature, wave form, voltage, and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, Beaverton, OR). Flow was set at 60 mL/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, Vernon Hills, IL). Treatment time was 100 μs, and the electric field was set at 30 kV/cm. These treatment conditions were selected on the basis of the results on carotenoid concentration, color, vitamin C, enzymes, and microorganisms, obtained when the orange juice was treated using different fields (25, 30, 35, and 40 kV/cm) and different times (30−340 μs) (*5*, *18*, *19*). Samples were collected after treatment. The experiments were performed in duplicate.

Thermal Treatment. To treat the samples, an Armfield FT74P unit with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger, where the treatment conditions (90 °C, 20 s) were reached. Heating of orange juice at 90−99 °C for 15−30 s is normal in commercial practice (*35*). After treatment, the juice was cooled with cold water from a cooler (Armfield FT61), and it was packed and stored until analysis. The experiments were performed in duplicate.

Storage Conditions. Juices were packaged in Elopack packages (Pure-pack), and they were stored in refrigeration and darkness at 2 and 10 °C (±1 °C) with controlled humidity. Samples were analyzed in duplicate immediately after processing and then after 1, 2, 3, 4, 6, and 7 weeks of storage.

Determination of Carotenoids. The carotenoids (including geometrical isomers) were identified and quantified by LC with a UV−vis diode array detector (Hewlett-Packard, 1100 series) and a column thermostat (Agilent 1100 series).

An extraction process (ethanol/hexane, 4:3, v/v) was performed, followed by saponification with diethyl ether/methanolic KOH (0.1%, w/v, BHT) (1:1, v/v) for 0.5 h at room temperature.The injection volume used was 20 μL.

Table 1. Mobile Phase and Temperature Gradient for Determination of Carotenoids by HPLC

time (min)	MeOH + AA[a] (%)	H_2O (%)	TBME (%)	temp (°C)
0	95	5	0	20
3	100	0	0	20
5	95	0	5	20
6	95	0	5	30
10	86	0	14	30
15	75	0	25	30
22	95	0	5	20
23	100	0	0	20

[a] AA, 0.1 M ammonium acetate.

A 250 × 4.6 mm Vydac 201TP54 reverse phase C_{18} column with a particle size of 5 μm and a Vydac 201TP precolumn (guard column) (4.6 mm i.d. cartridge with 5-μm particles) (Hesperia, CA) were used. The mobile phase used was methanol (0.1 M ammonium acetate), *tert*-butyl methyl ether, and water (in a concentration gradient) (**Table 1**), and a temperature gradient (**Table 1**) was applied with retinol palmitate as an internal standard.

Carotenoids were identified by UV−vis spectra and retention times in HPLC in the juices analyzed using the system HP Chemstation-A.06.03 (*34*). Carotenoids were expessed as micrograms per 100 g.

Determination of Vitamin A. Vitamin A was expressed as retinol activity equivalents (RAE), using the following conversion (*36*, *37*): RAE = (μg of β-carotene)/12 + (μg of β-cryptoxanthin + μg of α-carotene)/24.

Statistical Analysis. Carotenoid contents were compared using one-way analysis of variance (ANOVA). An LSD test ($p < 0.05$) was applied. The computer program employed was SPSS (Statistical Package for the Social Sciences) 12.0 for Windows. By means of simple regression, carotenoids concentration versus storage time (weeks) has been related, obtaining with the equation slope the total carotenoid degradation rate (μg/100 g·week).

RESULTS AND DISCUSSION

Changes in total carotenoid content and carotenoid profile due to HIPEF treatment and thermal pasteurization of orange juice were studied. **Table 2** shows the concentrations of the various carotenoids identified, obtained after the pasteurization and HIPEF treatments had been applied to the untreated orange juice. The concentration of total carotenoids was significantly lower ($p < 0.05$) in the pasteurized orange juice than in the untreated juice (decrease of 12.57%), whereas in the juice treated with HIPEF, although the concentration of total carotenoids decreased in comparison with the untreated juice (6.73%), the decrease was not significant ($p > 0.05$). These results coincide with those obtained by Lee et al. (*7*) for pasteurized orange juice, in which the total carotenoid content loss was significant ($p < 0.05$) after thermal pasteurization at 90 °C for 30 s. Similarly, the concentration of carotenoids with vitamin A activity was greatest in the untreated orange juice, followed by the orange juice treated with HIPEF (decrease of 7.52%) and, last, the pasteurized orange juice (decrease of 15.62%). The only significant differences found ($p < 0.05$) were between untreated orange juice and pasteurized orange juice. Consequently, the nonthermal treatments affected the vitamin A concentration in the refrigerated orange juice less than the conventional thermal treatments did. Sánchez-Moreno et al. (*38*) also studied the variation in total carotenoid and vitamin contents after applying various types of conservation treatments to tomato juice and obtained results similar to those found in the present study.

The study of storage at different temperatures (2 and 10 °C) of the untreated orange juice, juice treated with HIPEF, and pasteurized juice showed that the total carotenoid concentration decreased during this period.

Table 2. Carotenoid Concentration (Micrograms per 100 g) and Vitamin A (Retinol Activity Equivalents per 100 g) in Fresh Orange Juice, HIPEF Orange Juice, and Pasteurized Orange Juice

carotenoid	λ (nm)	untreated juice	HIPEF juice	pasteurized juice
neoxanthin + 9-*cis*-violaxanthin	430	444.57 ± 23.21	388.57 ± 14.57	351.11 ± 10.74
antheraxanthin	450	161.93 ± 10.14	140.91 ± 8.96	137.73 ± 4.26
lutein	450	27.28 ± 1.89	25.42 ± 1.13	23.99 ± 0.89
zeaxanthin	450	68.26 ± 0.95	57.28 ± 3.29	56.42 ± 0.85
isolutein	430	61.99 ± 2.76	59.70 ± 1.87	57.57 ± 0.45
β-cryptoxanthin	450	206.42 ± 14.35	185.97 ± 2.15	175.57 ± 8.09
α-carotene	450	49.91 ± 1.82	46.44 ± 4.99	32.94 ± 4.67
9-*cis*-α-carotene	430	31.65 ± 0.21	30.88 ± 0.23	23.97 ± 4.61
phytoene + phytofluene	290	23.34 ± 0.19	22.54 ± 0.72	21.53 ± 0.27
7,8,7′,8′-tetrahydrolycopene	430	28.21 ± 0.84	26.49 ± 0.54	25.45 ± 0.38
β-carotene	450	37.21 ± 1.44	35.72 ± 0.95	33.65 ± 0.02
ζ-carotene	430	21.36 ± 0.33	21.71 ± 0.58	21.33 ± 0.02
9-*cis*-β-carotene	450	12.61 ± 0.55	10.56 ± 0. 33	10.38 ± 0.12
total carotenoids		1367.17 ± 64.67	1275.16 ± 56.36	1195.37 ± 31.56
vitamin A		14.63 ± 0.65	13.53 ± 0.65	12.35 ± 0.70

Table 3. Total Carotenoid Concentration (Micrograms per 100 g) and Vitamin A (Retinol Activity Equivalents per 100 g) in Untreated, HIPEF-Treated, and Pasteurized Orange Juice Stored at 2 and 10 °C

			storage time						
temp (°C)	item	juice	0 weeks	1 week	2 weeks	3 weeks	4 weeks	6 weeks	7 weeks
2	total carotenoid	fresh	1367.17 (64.67)[a]	1272.24 (11.70)	1200.80 (29.78)	1184.18 (60.04)	985.23 (80.20)	1023.10 (47.37)	934.56 (23.36)
		HIPEF	1275.16 (56.36)	1174.44 (54.85)	1129.42 (106.73)	1237.42 (95.90)	1234.27 (52.09)	1018.28 (15.99)	964.23 (12.05)
		pasteurized	1195.37 (31.56)	1305.72 (38.39)	1117.57 (57.55)	1237.42 (95.90)	1020.62 (84.71)	881.99 (13.23)	913.31 (72.33)
	vitamin A	fresh	14.63 (0.65)	11.14 (0.23)	11.24 (0.24)	11.89 (0.61)	9.15 (0.71)	9.78 (0.52)	9.97 (0.35)
		HIPEF	13.53 (0.65)	10.21 (0.55)	10.56 (1.02)	12.29 (1.20)	11.19 (0.67)	9.42 (0.14)	10.04 (0.57)
		pasteurized	12.35 (0.70)	10.84 (0.23)	10.61 (0.76)	12.29 (1.20)	9.78 (1.07)	8.64 (0.17)	9.55 (0.81)
10	total carotenoid	fresh	1367.17 (64.67)	1118.32 (73.59)	1138.81 (16.97)	1120.09 (84.45)	908.42 (64.95)	964.65 (52.19)	*b*
		HIPEF	1275.16 (56.36)	1258.01 (92.21)	1097.98 (61.49)	1082.87 (0.11)	1101.82 (30.08)	1110.26 (65.17)	*b*
		pasteurized	1195.37 (31.56)	1122.58 (0.22)	1087.53 (11.42)	919.66 (48.01)	845.15 (39.47)	854.20 (41.94)	*b*
	vitamin A	fresh	14.63 (0.65)	9.99 (0.23)	11.69 (1.04)	9.69 (0.65)	9.53 (0.93)	7.61 (0.23)	*b*
		HIPEF	13.53 (0.65)	11.37 (0.84)	10.65 (0.53)	12.20 (0.33)	11.63 (0.43)	11.39 (0.29)	*b*
		pasteurized	12.35 (0.70)	9.53 (0.11)	10.19 (0.13)	9.58 (0.79)	9.00 (0.38)	9.54 (0.39)	*b*

[a] The standard deviation is shown in parentheses. [b] Spoiled orange juice; not analyzed.

Table 3 shows the values for total carotenoids and vitamin A in untreated, HIPEF-treated, and pasteurized orange juice stored at 2 and 10 °C. The degradation rates for total carotenoid concentration in juices stored a 2 °C were 59.10 μg/100 g·week ($r^2 = 0.891$), 55.47 μg/100 g·week ($r^2 = 0.762$), and 36.85 μg/100 g·week ($r^2 = 0.643$) for untreated, pasteurized, and HIPEF juices, respectively. In the same way, in the juices stored at 10 °C, the carotenoid concentration degradation rates were 69.94 μg/100 g·week ($r^2 = 0.719$), 52.70 μg/100 g·week ($r^2 = 0.824$), and 29.92 μg/100 g·week ($r^2 = 0.545$) for untreated, pasteurized, and HIPEF juices, respectively.

The decrease was greater and faster in the untreated juice, followed by the pasteurized juice and finally by the juice treated with HIPEF. The decrease in vitamin A was also greater in the orange juice not subjected to any kind of treatment and in the pasteurized juice than in the juice treated with HIPEF. Thereby, the vitamin A degradation rates were 1.08, 0.84, and 0.71 RAE/100 g·week for untreated, pasteurized, and HIPEF orange juice stored at 2 °C and 1.90, 0.79, and 0.69 RAE/100 g·week for untreated, pasteurized, and HIPEF orange juice stored at 10 °C.

Table 2 shows that the total of all the carotenoids was less in the pasteurized orange juice than in the juice treated with HIPEF, although not all of these decreases were statistically significant. The thermal conservation treatment produced a statistically significant decrease in the concentrations of zeaxanthin, phytoene plus phytofluene, and neoxanthin plus 9-*cis*-violaxanthin ($p = 0.006$, 0.022, and 0.035, respectively), whereas with HIPEF treatment there was no significant reduction in any of the carotenoids with respect to the untreated orange juice. Lee et al. (*7*) obtained similar results for pasteurized orange juice.

The concentration of 13-*cis*-violaxanthin in the orange juice increased with storage, and this increase was greater in the juice stored at 10 °C than in the juice stored at 2 °C (**Figure 1**). The

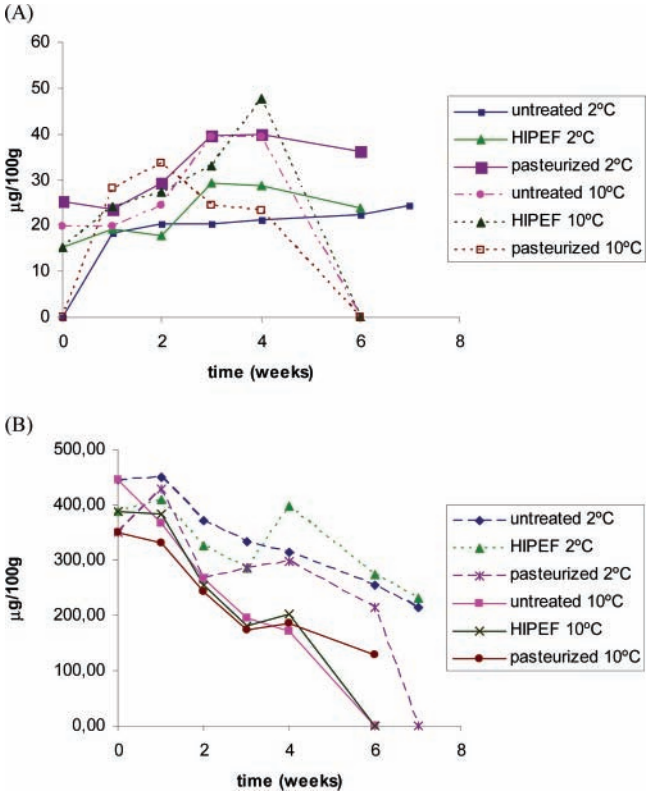

Figure 1. Concentration (micrograms per 100 g) of 13-*cis*-violaxanthin (**A**) and 9-*cis*-violaxanthin plus neoxanthin (**B**) during storage in untreated, HIPEF-treated, and pasteurized orange juice stored at 2 and 10 °C.

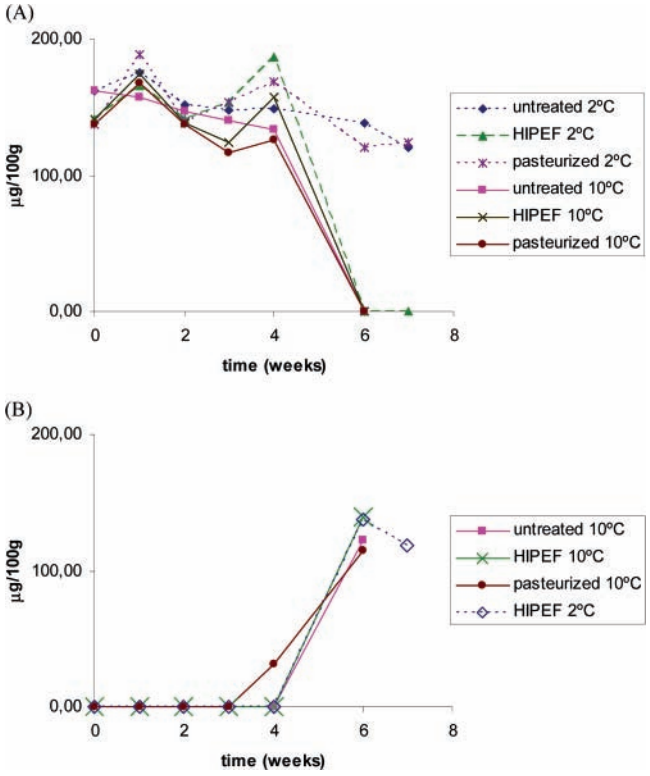

Figure 2. Concentration (micrograms per 100 g) of antheraxanthin (**A**) and mutatoxanthin (**B**) during storage time in untreated, HIPEF-treated, and pasteurized orange juice stored at 2 and 10 °C.

Table 4. Carotenoid Concentration (Micrograms per 100 g) in Untreated Orange Juice during Refrigerated Storage[a]

		storage time						
temp (°C)	carotenoid	0 weeks	1 week	2 weeks	3 weeks	4 weeks	6 weeks	7 weeks
2	lutein	27.28	33.31	31.37	31.19	33.59	32.72	27.61
		(1.89)	(0.33)	(0.38)	(1.80)	(3.13)	(0.96)	(2.99)
	zeaxanthin	68.26	46.69	50.91	55.91	57.01	50.19	47.77
		(0.95)	(1.07)	(0.95)	(4.12)	(6.27)	(0.49)	(1.54)
	isolutein	61.99	45.41	52.04	57.59	46.56	46.39	48.80
		(2.75)	(0.40)	(2.05)	(3.48)	(0.20)	(1.15)	(0.53)
	β-cryptoxanthin	206.42	143.88	143.27	157.84	150.70	122.78	133.39
		(14.35)	(0.36)	(2.32)	(7.75)	(12.36)	(1.15)	(4.87)
	phytoene–phytofluene	23.34	24.54	23.06	29.68	23.65	29.50	26.88
		(0.19)	(1.04)	(0.94)	(1.63)	(1.56)	(0.94)	(1.53)
	7,7′,8,8′-tetrahydrolycopene	28.21	29.44	29.93	35.47	24.97	37.27	32.35
		(0.84)	(0.17)	(1.04)	(1.77)	(1.98)	(1.01)	(0.93)
	β-carotene	37.21	36.47	36.80	40.67	23.87	38.47	34.86
		(1.44)	(0.53)	(2.19)	(1.98)	(1.55)	(2.29)	(1.29)
	ζ-carotene	21.36	20.68	20.95	25.17	21.60	35.30	36.67
		(0.33)	(0.02)	(0.46)	(1.39)	(1.60)	(6.69)	(0.99)
10	lutein	27.28	31.80	34.36	34.29	35.80	31.86	*b*
		(1.89)	(2.03)	(0.98)	(0.65)	(1.38)	(0.39)	
	zeaxanthin	68.26	45.31	53.29	63.08	62.18	50.69	*b*
		(0.95)	(4.90)	(0.77)	(0.32)	(5.71)	(1.79)	
	isolutein	61.99	43.65	56.31	59.62	52.29	48.92	*b*
		(2.76)	(1.05)	(1.14)	(2.24)	(4.25)	(2.07)	
	β-cryptoxanthin	206.42	133.45	148.97	154.14	151.93	125.19	*b*
		(14.35)	(4.70)	(1.70)	(8.31)	(9.54)	(1.59)	
	phytoene–phytofluene	23.34	22.82	24.88	28.93	23.01	30.69	*b*
		(0.19)	(0.97)	(0.82)	(1.13)	(1.27)	(2.42)	
	7,7′,8,8′-tetrahydrolycopene	28.21	26.78	26.72	37.73	27.86	21.79	*b*
		(0.84)	(0.24)	(4.71)	(3.13)	(1.92)	(0.32)	
	β-carotene	37.21	33.37	37.42	39.43	26.94	ND[c]	*b*
		(1.44)	(0.20)	(0.15)	(3.66)	(1.21)		
	ζ-carotene	21.36	19.81	38.09	26.89	23.52	28.14	*b*
		(0.33)	(0.62)	(0.60)	(2.43)	(1.74)	(2.03)	

[a] The results are the mean of two determinations. The standard deviation is shown in parentheses. [b] Spoiled orange juice; not analyzed. [c] Not detected.

Table 5. Carotenoid Concentration (Micrograms per 100 g) in Orange Juice Treated by HIPEF during Refrigerated Storage[a]

		storage time						
temp (°C)	carotenoid	0 weeks	1 week	2 weeks	3 weeks	4 weeks	6 weeks	7 weeks
2	lutein	25.42	29.94	28.07	31.45	45.23	32.29	27.74
		(1.13)	(2.26)	(2.50)	(4.48)	(0.27)	(0.71)	(0.30)
	zeaxanthin	57.28	43.71	45.88	58.98	74.74	47.63	46.83
		(3.29)	(2.14)	(4.85)	(8.15)	(2.14)	(0.80)	(2.93)
	isolutein	59.70	46.24	51.35	59.57	62.84	45.26	49.52
		(1.87)	(1.78)	(4.35)	(7.74)	(0.09)	(0.43)	(0.91)
	β-cryptoxanthin	185.97	132.72	133.36	159.68	152.32	124.63	135.85
		(8.09)	(7.54)	(8.75)	(16.85)	(2.82)	(2.06)	(1.34)
	phytoene–phytofluene	22.54	22.99	23.27	30.92	30.45	30.05	27.71
		(0.72)	(0.90)	(0.37)	(3.08)	(0.24)	(0.65)	(0.05)
	7,7′,8,8′-tetrahydrolycopene	26.49	27.47	28.15	36.71	26.85	35.63	33.77
		(0.76)	(1.97)	(2.05)	(5.40)	(3.83)	(0.63)	(0.68)
	β-carotene	35.72	32.13	35.94	43.03	30.17	37.29	36.55
		(0.95)	(2.89)	(2.64)	(4.44)	(0.89)	(0.38)	(3.08)
	ζ-carotene	21.71	20.47	21.64	29.73	19.23	27.25	32.59
		(0.58)	(1.50)	(1.98)	(3.38)	(2.41)	(0.68)	(6.25)
10	lutein	25.42	35.63	29.83	30.13	42.63	35.26	*b*
		(1.13)	(2.43)	(2.29)	(2.08)	(1.74)	(0.49)	
	zeaxanthin	57.28	52.76	51.45	55.38	74.99	60.63	*b*
		(3.29)	(5.79)	(7.06)	(1.36)	(2.77)	(2.35)	
	isolutein	59.70	56.02	56.05	57.95	64.80	53.97	*b*
		(1.87)	(5.45)	(3.41)	(0.53)	(1.74)	(1.55)	
	β-cryptoxanthin	185.97	150.63	137.14	144.80	142.55	137.49	*b*
		(8.09)	(13.38)	(8.98)	(1.36)	(8.02)	(4.91)	
	phytoene–phytofluene	22.54	26.79	23.84	29.24	28.95	35.20	*b*
		(0.72)	(2.64)	(0.32)	(0.80)	(0.89)	(3.60)	
	7,7′,8,8′-tetrahydrolycopene	26.49	32.50	31.25	38.17	33.30	ND[c]	*b*
		(0.76)	(1.22)	(1.77)	(0.14)	(0.27)		
	β-carotene	35.72	37.90	36.83	40.73	32.10	49.84	*b*
		(0.95)	(1.91)	(1.47)	(2.66)	(1.26)	(3.59)	
	ζ-carotene	21.71	24.89	27.73	49.29	35.46	59.72	*b*
		(0.58)	(1.40)	(0.58)	(1.92)	(0.18)	(3.49)	

[a] The results are the mean of two determinations. The standard deviation is shown in parentheses. [b] Spoiled orange juice; not analyzed [c] Not detected.

concentration of this carotenoid increased in both HIPEF-treated juice and pasteurized juice.

There was a sharp decrease in the neoxanthin plus 9-*cis*-violaxanthin mixture with storage (**Figure 1**), and it was not detected at the sixth week onward in the untreated orange juice or in the juice treated with HIPEF stored at 10 °C. Similar results were obtained in a study of an orange−carrot juice, in which these carotenoids were not detected after 3 weeks of storage in refrigeration at 10 °C (*40*). It has been verified that this also happens in the storage of orange−carrot juice frozen at −40 °C (*7*). 9-*cis*-Violaxanthin was transformed into its 13-*cis*-violaxanthin form, so that the decrease in the 9-cis isomer coincided with the increase in the concentration of the 13-cis isomer. The presence of another carotenoid, a rearrangement product of violaxanthin (5,6-epoxycarotenoid) was verified in the untreated orange juice and the juice treated by HIPEF, both stored at 10 °C. It was a furanoid isomer called auroxanthin (5,8,5′,8′-diepoxycarotenoid), which appears as a result of the degradation of juice that occurs during storage (*40, 41*). This transformation was observed only at the sixth week of storage. Auroxanthin was not detected in the juices stored at 2 °C, although there was degradation of 9-*cis*-violaxanthin, producing an unidentified compound.

Similarly, as **Figure 2** shows, the concentration of antheraxanthin decreased abruptly. The decrease was greater in the untreated, pasteurized, and HIPEF-treated orange juices stored at 10 °C, and this carotenoid was not detected from the sixth week onward. Similar results have been reported by Torregrosa (*39*) in a study of pasteurized and HIPEF-treated orange−carrot juice stored in refrigeration, with antheraxanthin not being detected from the third week onward in storage at 10 °C. The same result was observed in the HIPEF-treated juice stored at 2 °C. However, in the untreated and pasteurized juices stored at 2 °C, this carotenoid was detected throughout the storage period, although its concentration decreased.

Another carotenoid, mutatoxanthin (**Figure 2**), was detected in the sixth week of storage, appearing in the cases in which antheraxanthin disappeared. This was not observed in the untreated or pasteurized orange juices stored at 2 °C, in which antheraxanthin continued to be detected. Carotenoid 5,6-epoxides may be transformed to carotenoid 5,8-epoxides under acidic conditions, so antheraxanthin can be converted into the furanoid structure mutatoxanthin (*40*).

As **Tables 4**−**6** show, the concentration of lutein increased after the first week of storage in all of the orange juices studied, and thereafter the concentration remained constant and in some cases decreased in the seventh week of storage. Application of statistical analysis showed that in the pasteurized juice lutein had a negative tendency, statistically nonsignificant, throughout storage at both temperatures, whereas in the HIPEF-treated juice this carotenoid had a nonsignificant increase during the storage period studied. These results do not coincide with those observed by Lin and Chen (*42*) for tomato juice stored at 4 °C for 12 weeks, in which the concentration of lutein decreased, and it was no longer detected in the fifth week of storage. In a result similar to the present study, Torregrosa (*39*) also detected a slight increase in lutein during storage in pasteurized orange−carrot juice. The concentrations of zeaxanthin and isolutein decreased during the first week of storage in all of the juices studied, followed by an increase during the rest of the storage period (**Tables 4**−**6**). In the pasteurized juice there was a statistically significant decrease in the concentrations of zeax-

Table 6. Carotenoid Concentration (Micrograms per 100 g) in Pasteurized Orange Juice during Refrigerated Storage

		storage time						
temp (°C)	carotenoid	0 weeks	1 week	2 weeks	3 weeks	4 weeks	6 weeks	7 weeks
2	lutein	23.99	33.47	27.96	31.45	36.08	28.90	27.92
		(0.89)	(0.13)	(1.10)	(4.58)	(3.73)	(0.33)	(2.78)
	zeaxanthin	56.42	52.56	48.76	58.98	60.50	39.43	48.47
		(0.85)	(1.15)	(4.23)	(2.25)	(10.24)	(1.58)	(3.82)
	isolutein	57.57	51.62	52.50	59.57	52.21	39.75	47.29
		(0.45)	(3.72)	(2.58)	(3.62)	(3.55)	(1.38)	(3.26)
	β-cryptoxanthin	175.57	139.88	131.32	159.68	151.48	111.82	129.05
		(2.14)	(5.19)	(6.19)	(17.55)	(21.07)	(13.98)	(10.23)
	phytoene–phytofluene	21.53	29.31	22.68	30.92	26.96	28.79	28.26
		(0.27)	(0.11)	(0.31)	(6.17)	(2.87)	(0.90)	(1.81)
	7,7′,8,8′-tetrahydrolycopene	25.45	31.22	29.37	36.71	22.99	33.29	31.23
		(0.16)	(1.31)	(1.63)	(5.40)	(1.82)	(0.02)	(2.51)
	β-carotene	33.65	36.85	38.55	43.03	25.75	35.54	32.72
		(0.02)	(2.56)	(2.64)	(8.30)	(2.44)	(0.94)	(2.97)
	ζ-carotene	21.33	25.88	26.11	29.73	21.66	24.75	35.29
		(0.02)	(1.65)	(3.80)	(3.38)	(1.04)	(1.00)	(3.40)
10	lutein	23.99	32.28	29.75	31.46	32.72	30.56	*b*
		(0.89)	(2.74)	(2.33)	(3.28)	(0.06)	(1.81)	
	zeaxanthin	56.42	50.69	47.42	49.50	46.60	46.47	*b*
		(0.85)	(2.17)	(3.13)	(3.13)	(9.20)	(2.66)	
	isolutein	57.57	52.10	51.01	50.95	53.01	43.23	*b*
		(0.45)	(0.35)	(2.86)	(3.55)	(2.57)	(2.01)	
	β-cryptoxanthin	175.57	138.06	128.60	131.51	140.99	112.25	*b*
		(2.14)	(0.84)	(1.77)	(10.82)	(9.00)	(2.69)	
	phytoene–phytofluene	21.53	24.42	22.60	26.77	23.42	28.46	*b*
		(0.27)	(0.75)	(0.08)	(2.45)	(0.79)	(0.89)	
	7,7′,8,8′-tetrahydrolycopene	25.45	28.56	29.59	31.86	25.52	36.83	*b*
		(0.16)	(0.50)	(1.32)	(2.16)	(0.59)	(1.07)	
	β-carotene	33.65	34.46	35.06	36.76	25.20	37.16	*b*
		(0.02)	(0.83)	(1.80)	(4.10)	(0.14)	(0.55)	
	ζ-carotene	21.33	22.15	26.84	25.30	24.36	42.81	*b*
		(0.02)	(1.72)	(1.63)	(0.03)	(1.61)	(0.72)	

[a] The results are the mean of two determinations. The standard deviation is shown in parentheses. [b] Spoiled orange juice; not analyzed.

anthin and isolutein ($r^2 = -0.388$, $p = 0.05$, and $r^2 = -0.613$, $p = 0.01$, respectively), whereas in the HIPEF-treated juice the changes in the concentrations of these carotenoids during storage were not statistically significant ($p > 0.05$).

The concentration of β-cryptoxanthin decreased during storage, especially in the first week, and the decrease was greater in the orange juices stored at 10 °C. Also, the decrease was greater ($r^2 = -0.543$, $p = 0.04$) in the pasteurized orange juice than in the juice treated with HIPEF ($p > 0.05$) (**Tables 4−6**). However, orange−carrot juice stored in refrigeration did not show the same behavior (*39*), although β-cryptoxanthin did decrease when the juice was stored at −40 °C (*6*).

There was a decrease in the concentration of α-carotene during the first weeks of storage at both temperatures (**Figure 3**). It was not detected from the third week onward in the pasteurized juice and from the fourth week in the untreated and HIPEF-treated orange juices. The concentration of this carotenoid also decreased in refrigerated orange−carrot juice, although it was detected throughout the storage period (*39*). Oxidation is the major cause of carotenoid loss, and it depends on the carotenoid involved.

There was a slight but significant increase ($p < 0.05$) in 7,8,7′,8′-tetrahydrolycopene and the phytonene−phytofluene mixture during storage, in both the pasteurized juice ($r^2 = 0.412$, $p = 0.037$, and $r^2 = 0.460$, $p = 0.018$, respectively) and the HIPEF-treated juice ($r^2 = 0.526$, $p = 0.008$, and $r^2 = 0.724$, $p = 0.000$, respectively) (**Tables 4−6**). There were no statistically significant changes in the concentration of β-carotene during storage in any of the juices studied.

There was a slight increase ($p < 0.05$) in the concentration of ζ-carotene during storage ($r^2 = 0.562$, $p = 0.003$, and $r^2 = 0.452$, $p = 0.027$, for the pasteurized orange juice and HIPEF-treated juice, respectively) except in the untreated juice stored at 10 °C, in which this carotenoid disappeared during the first weeks.

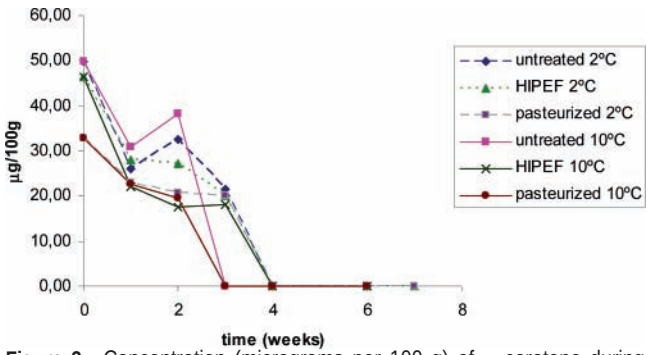

Figure 3. Concentration (micrograms per 100 g) of α-carotene during storage time in untreated, HIPEF-treated, and pasteurized orange juice stored at 2 and 10 °C.

In conclusion, nonthermal treatments had less effect than conventional thermal treatments on the concentrations of total carotenoids and vitamin A in refrigerated orange juice. With HIPEF treatment, there was no significant decrease in the concentration of any carotenoid with respect to the untreated juice. During storage in refrigeration, total carotenoids and vitamin A were maintained for a longer time in the juice treated with HIPEF than in the juice conserved using conventional pasteurization treatments.

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