JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of High-Intensity Pulsed Electric Fields Processing and Conventional Heat Treatment on Orange−Carrot Juice Carotenoids

FRANCISCO TORREGROSA, CLARA CORTÉS, MARÍA J. ESTEVE, AND ANA FRÍGOLA*

Área de Nutrición y Bromatología, Facultat de Farmàcia, Universitat de València, Avenida Vicent Andrés Estellés s/n, 46100 Burjassot (Valencia), Spain

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

KEYWORDS: Carotenoid; high-intensity pulsed electric field; heat treatment; orange−carrot juice

INTRODUCTION

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

In addition to the excellent intrinsic sensory and nutritive characteristics of orange juice, the incorporation of a proportion of carrot makes a valuable contribution to the health of the consumer because oranges have a high vitamin C content and carrots have a high content of carotenes. Stern (*3*) discussed the development of vitamin-enriched fruit and vegetable juice mixtures, and among the suitable juices, he highlighted orange−carrot juice because of its greater consumer acceptance.

The carotenoids present in citrus fruits are a complex mixture of >115 natural substances (*4*), but not all of them are precursors of vitamin A. Various carotenoids, including α-carotene, *β*-carotene, and *β*-cryptoxanthin, have provitamin A activity, being transformed into retinal by mammals. The xanthophylls (oxocarotenoids) lutein and zeaxanthin are also known to provide protection against macular degeneration connected with age, through their ability to capture free oxygen and blue light in the retina (*5*−*8*). Carotenoids have a range of important and well-documented biological activities. They are potent antioxidants and free-radical scavengers, and they modulate the pathogenesis of cancers and coronary heart disease (*9*−*12*).

Vitamin A deficiencies are widespread in developing countries, influencing the growth of young children severely (*13*, *14*). UNICEF and WHO consider that improving the vitamin A status of young children with marginal deficiency may reduce the mortality by 23% on average. Dietary approaches are needed to replace supplementation programs, ensuring sustainability and an adequate coverage of children in need (*15*). Although fruits and vegetables containing carotenoids are available in developing countries, deficiencies are often found during the off season (*16*). The application of various industrial treatments can lead to the formation of *cis* isomers, which do not have the same vitamin activity as all-*trans* isomers.

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

High-intensity pulsed electric field (HIPEF) treatment has gained increasing interest because it offers some attractive advantages over thermal methods currently used in processing raw materials and foods (*17*). HIPEF has been most successful with fluid products; however, some semisolids and powders have also been treated (*18*). HIPEF processing inactivates microorganisms and enzymes without significant adverse effects on flavor and nutrients (*19*−*26*). An increase in electric field intensity or in treatment time, defined as the product of the number of pulses and the pulse length, increases microbial inactivation. Application of pulses, quantified as energy input,

* To whom correspondence should be addressed. Telephone: +34-963544955. Fax: +34-963544954. E-mail: ana.frigola@uv.es.

10.1021/jf051171w CCC: $30.25
Published on Web 10/29/2005

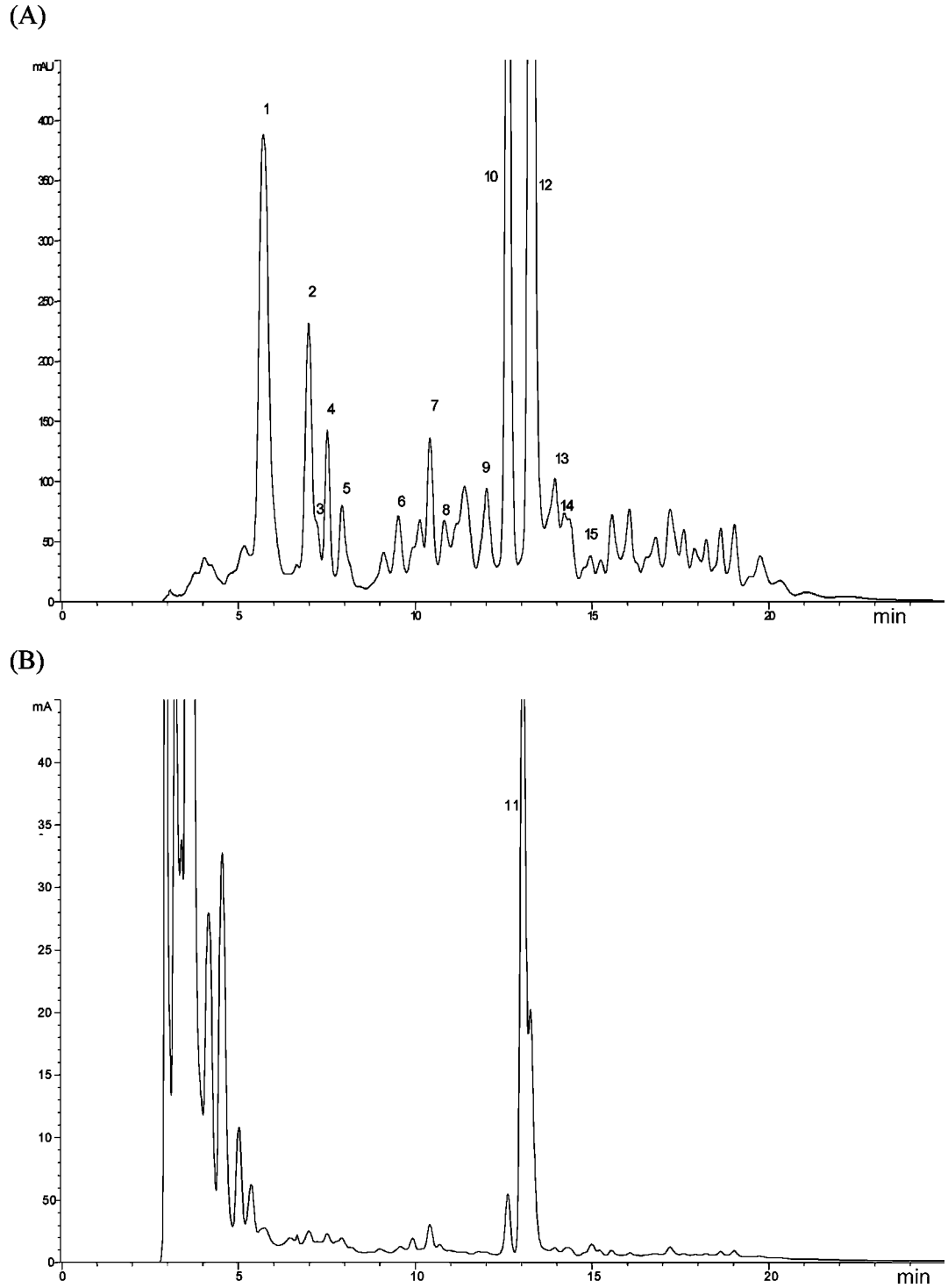

Figure 1. Chromatogram of the carotenoids of untreated orange–carrot juice mixture at $\lambda = 450$ nm (A) and $\lambda = 290$ nm (B).

results in thermal energy dissipation and consequently in an increase of the temperature of the product (*17*). Optimal treatment parameters depend upon the specific food matrix, temperature, pH, presence of antimicrobial compounds, and conductivity of media (*27*). However, no reference about the effects of HIPEF on carotenoid changes in orange−carrot juice have been found in the literature.

Liquid chromatography (LC) is considered to be the method of choice for the separation, identification, and quantification of carotenoids found in biological tissues (*27*−*36*). The use of photodiode array detection in the identification of carotenoids by liquid chromatography is a valuable tool for characterization of *cis* and *trans* isomers in vegetable and citric juices (*37*−*42*).

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

MATERIALS AND METHODS

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

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

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

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

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

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

Thermal Treatment. The thermal treatment intensity given to *T* samples (98 °C, 21 s) was similar to the one given by manufacturers of refrigerated juice. To treat the samples, an ARMFIELD FT74P equipment with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (98 °C, 21 s) are reached. Heating of orange juice at 90−99 °C for 15−30 s is normal in commercial practice (*43*). After treatment, the juice was cooled with cold water from a cooler (ARMFIELD FT61), and it was packed and stored until analysis. Experiments were performed in duplicate.

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

A 250 × 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 carotenes (including geometric isomers) were identified and quantified by HPLC with a ultraviolet/visible diode array detector. The mobile phase used was *tert*-butyl methyl ether, methanol with ammonium acetate (0.1 M) and water (in a concentration gradient), and a temperature gradient was applied with retinol palmitate as an internal standard. An extraction process (4:3 ethanol/hexane, v/v) was performed, followed by saponification with diethyl ether/methanolic KOH (0.1%, w/v, BHT) (1:1, v/v) for 0.5 h at room temperature. The carotenoids were identified by UV−vis spectra, and retention times in HPLC in the juices were analyzed (*42*).

Determination of Vitamin A. Vitamin A was expressed as retinol activity equivalents (RAE), using the following conversion (*44*): RAE= (μg of β-carotene)/6 + (μg of β-cryptoxanthin + α-cryptoxanthin + α-carotene + ζ-carotene)/12.

Statistical Analysis. Carotenoid contents were compared using one-way analysis of variance (ANOVA). LSD test ($p < 0.05$) was applied. The computer program employed was Statgraphics Plus for Windows 3.0.

Table 1. Wavelengths and Retention Times of Orange–Carrot Juice Mixture

peak number	carotenoids	λ (nm)	orange–carrot t_R[a] (min) ± SD
1	9-*cis*-violaxanthin and neoxanthin	430	5.75 ± 0.04
2	antheraxanthin	450	7.01 ± 0.03
3	mutatoxanthin	430	7.20 ± 0.01
4	lutein	450	7.54 ± 0.02
5	zeaxanthin	450	7.96 ± 0.04
6	α-cryptoxanthin	450	9.53 ± 0.03
7	β-cryptoxanthin	450	10.45 ± 0.04
8	*cis*-β-cryptoxanthin	450	10.83 ± 0.05
9	9-*cis*-α-carotene	430	12.06 ± 0.04
10	α-carotene	450	12.63 ± 0.03
11	phytoene and phytofluene	290	13.06 ± 0.02
12	β-carotene	450	13.28 ± 0.03
13	13-*cis*-β-carotene	450	13.95 ± 0.02
14	ζ-carotene	430	14.28 ± 0.04
15	9-*cis*-β-carotene	450	14.96 ± 0.03

[a] t_R = retention time.

Table 2. Concentration of Carotenoids (μg/100 g) and Vitamin A (RAE) in Untreated and Pasteurized Orange–Carrot Juice

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

RESULTS AND DISCUSSION

Carotenoid Profile and Effect of Heat Treatment. The cromatographic profile of the saponified extract of fresh orange−carrot juice is shown in **Figure 1**, and the carotenoids identified appear in **Table 1**.

Table 2 shows the mean concentration of each of the carotenoids quantified in the orange−carrot juice and their vitamin activity expressed as retinol activity equivalents. It also gives the concentration of each of the carotenoids in the heat-treated orange−carrot juice. Of all of the carotenoids studied, only five decreased significantly ($p < 0.05$): 9-*cis*-violaxanthin and neoxanthin (32.9%), antheraxanthin (3.9%), α-cryptoxanthin (15.5%), 9-*cis*-α-carotene (25.9%), and 9-*cis*-β-carotene (27.3%), while there was a significant increase in the others, with the exception of lutein, mutatoxanthin, β-carotene, and ζ-carotene. The decrease in violaxanthin and neoxanthin and antheraxanthin after pasteurization coincide with an increase of mutatoxanthin concentration. This can be explained because violaxanthin and antheraxanthin under slightly acidic conditions can be converted to mutatoxanthin and other compounds such as auroxanthin, not identified in this case (*45*). The greatest increase in concentration was in 13-*cis*-β-carotene (163.9%), followed by

Table 3. Concentration of Carotenoids (μg/100 g) and Vitamin A (RAE) in Orange–Carrot Juice Treated by HIPEF with a Field of 25 kV/cm

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

Table 4. Concentration of Carotenoids (μg/100 g) and Vitamin A (RAE) in Orange–Carrot Juice Treated by HIPEF with a Field of 30 kV/cm

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

zeaxanthin (46.5%) and *cis*-β-cryptoxanthin (46.3%). Thermal processing implies an increase in the carotenoid concentration, perhaps owing to greater stability, enzymatic degradation, and unaccounted losses of moisture and soluble solids, which concentrate the sample per unit weight (*46*). Heat induces cis/trans isomerization (13-*cis*-β-carotene), and different carotenoid byproducts can be formed (*47*−*49*). Lee and Coates (*50*) studied the effect of heat treatment (90 °C, 30 s) on the color and pigments of oranges and found that the carotenoids that decreased were violaxanthin (46.4%), *cis*-violaxanthin (19.7%), and antheraxanthin (24.8%). They found a clear increase in the concentration of luteoxanthin (30.9%), mutatoxanthin (74.2%), lutein (13.4%), and ζ-carotene (23.1%). Doering et al. (*51*) reported that when β-carotene is exposed to temperatures below 100 °C the carotenoids formed are mainly 13- and 15-*cis*-β-carotene and that 9-*cis*-β-carotene is formed when the temperature is above 100 °C. Similar results were obtained by Kuki et al. (*52*), who found that when β-carotene is heated to 80 °C for 30 min 13- and 15-*cis*-β-carotene are formed. However, Chen et al. (*53*) and Johnsson (*54*) found that β-carotene in carrot juice was stable to cis isomerization when pasteurized at 100 °C. Marx et al. (*55*) studied the effects of heat treatment on trans−cis isomerization of β-carotene in carrot juice and found that pasteurization at 95 °C and sterilization at 121 °C caused a lower percentage of 13-*cis*-β-carotene to be formed than when the juice was sterilized at 130 °C. Bull et al. (*1*) report that high-pressure processing and thermal treatment of orange juice do not significantly reduce its β-carotene content.

Effect of HIPEF Treatment on Carotenoid Content of Orange−Carrot Juice. To establish the effect of HIPEF treatment, different field intensities (25, 30, 35, and 40 kV/cm) were applied for different times (from 30 to 340 μs), and in all cases, the results were compared with the results for untreated orange−carrot juice. **Tables 3**−**6** show the results obtained. The tables give the maximum temperature attained in each treatment, which did not exceed 65 °C in any case.

It can be seen that the concentration of the 9-*cis*-violaxanthin and neoxanthin mixture increases with treatment time; simple regression analysis is done, and although the fit is not significant in all cases, it can be seen that the rate of formation of these carotenoids increases with treatment intensity: 0.73 ± 0.18 (correlation coefficient of 0.89, $p < 0.05$), 0.97 ± 0.35 (correlation coefficient of 0.81, $p < 0.05$), 1.63 ± 0.79 (correlation coefficient of 0.68, $p > 0.05$), and 1.86 ± 0.69 (correlation coefficient of 0.805, $p > 0.05$) μg (100 g of juice)$^{-1}$ μs^{-1}, for juice treated at 25, 30, 35, and 40 kV/cm, respectively. Something similar happens with the concentration of antheraxanthin, which increases with treatment time, and its rate of formation increases with treatment intensity: 0.26 ± 0.07 (correlation coefficient of 0.90, $p < 0.05$), 0.42 ± 0.08 (correlation coefficient of 0.93, $p < 0.05$), 0.75 ± 0.12 (correlation coefficient of 0.95, $p < 0.05$), and 0.94 ± 0.27 (correlation coefficient of 0.87, $p < 0.05$) μg (100 g of juice)$^{-1}$ μs^{-1}, for juice treated at 25, 30, 35, and 40 kV/cm, respectively. When the HIPEF treatment applied is 25 kV/cm, the concentration of β-cryptoxanthin increases slightly in the first 110 μs

Table 5. Concentration of Carotenoids (μg/100 g) and Vitamin A (RAE) in Orange–Carrot Juice Treated by HIPEF with a Field of 35 kV/cm

	treatment time (μs)	0	60	90	110	130	150
	maximum temperature (°C)		44	50	54	59	64
1	9-*cis*-violaxanthin and neoxanthin	261.00	501.00	313.75	383.64	537.22	552.20
2	antheraxanthin	93.73	148.99	157.14	204.03	194.79	198.43
3	mutatoxanthin	16.38	18.00	14.89	15.52	13.43	15.31
4	lutein	38.56	61.48	64.98	78.33	84.48	81.43
5	zeaxanthin	33.39	47.6	53.89	53.13	70.88	76.09
6	α-cryptoxanthin	29.11	33.92	29.04	28.28	27.03	29.21
7	β-cryptoxanthin	35.71	45.08	53.47	71.49	105.24	92.70
8	*cis*-β-cryptoxanthin	25.20	36.13	30.79	27.83	31.39	36.21
9	9-*cis*-α-carotene	222.69	137.15	161.81	143.87	153.04	133.3
10	α-carotene	867.62	472.61	577.91	571.76	721.70	489.43
11	phytoene and phytofluene	423.15	447.64	348.67	368.46	387.53	375.32
12	β-carotene	2253.27	1086.74	1564.3	1644.78	2102.18	1000.86
13	13-*cis*-β-carotene	157.95	118.04	115.41	112.51	103.72	41.51
14	ζ-carotene	205.50	132.83	122.48	116.38	145.25	126.47
15	9-*cis*-β-carotene	70.66	40.44	45.11	48.89	46.68	50.74
	vitamin A (RAE)	467.95	235.33	323.54	337.43	431.38	225.86

Table 6. Concentration of Carotenoids (μg/100 g) and Vitamin A (RAE) in Orange–Carrot Juice Treated by HIPEF with a Field of 40 kV/cm

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

and then remains stable until 340 μs. In fields of 30, 35, and 40 kV/cm, the concentration of this carotenoid increases with longer treatment times and the rate of formation increases with treatment intensity: 0.24 ± 0.07 (correlation coefficient of 0.88, $p < 0.05$), 0.45 ± 0.11 (correlation coefficient of 0.94, $p < 0.05$), and 0.49 ± 0.07 (correlation coefficient of 0.97, $p < 0.05$) μg (100 g of juice)$^{-1}$ μs^{-1}, for treatments of 30, 35, and 40 kV/cm, respectively. Similar behavior can be seen in the evolution of the concentration of *cis*-β-cryptoxanthin and 9-*cis*-α-carotene. In the 25 kV/cm field, there is a significant increase ($p < 0.05$) in the first 110 μs of treatment, decreasing when the treatment time increases. When the field intensity is increased (30, 35, and 40 kV/cm), the concentration of *cis*-β-cryptoxanthin increases as the treatment time increases, whereas the concentration of 9-*cis*-α-carotene increases when the field intensity applied is 30 kV/cm but decreases when the intensity is higher. The concentration of α-carotene increases when 25 and 30 kV/cm fields are applied; it is maintained in treatments of 40 kV/cm; and it decreases significantly ($p < 0.05$) when a field of 35 kV/cm is applied. It can be seen that this result is not influenced by treatment time. The concentration of β-carotene is higher when a 30 kV/cm field is applied, although the increase is not significant ($p > 0.05$). When the field is 25, 35, or 40 kV/cm, no significant differences ($p > 0.05$) are observed when the treatment time increases. The concentration of 13-*cis*-β-carotene, ζ-carotene, and 9-*cis*-β-carotene increases when a 25 kV/cm field is applied. With a field of 30 or 35 kV/cm, no significant differences are observed in the concentrations of 13-*cis*-β-carotene and ζ-carotene at the various treatment times, but they decrease significantly ($p < 0.05$) when the treatment intensity increases (40 kV/cm). The concentration of 9-*cis*-β-carotene decreases in fields of 35 and 40 kV/cm and increases in fields of 25 and 30 kV/cm.

In 25 kV/cm, all carotenoids identified an increase in their concentration with regard to the orange−carrot fresh juice. It can be pointed out that the carotenoid concentrations increase when shorter treatment times are applied and their concentrations decrease or are mainteined when the longer treatment times are applied (**Tables 3−6**), with reference to the concentration of the first treatment time. In 30 kV/cm, an increase in the carotenoid concentration is also observed, and it can emphasize a very significant increase when a 200 μs time is applied.

HIPEF processing is an alternative to conventional thermal treatment of orange−carrot juice. To select the best HIPEF treatment conditions, it is necessary to bear in mind not only microbiological and enzymatic inactivation but also organoleptic characteristics and nutritive value.

Effect of HIPEF Treatment on Vitamin A. Vitamin A increases in orange−carrot juice with regard to untreated orange−carrot juice when a 25 kV/cm field is applied, enhancing the three first times. After 280 μs, the increase in the vitamin A concentration is smaller. When 200 μs is applied, it produces an increase of 26.6% with regard to fresh juice. When the field is 30 kV/cm, vitamin A increases with treatment times with regard to untreated orange−carrot juice (an increase of 58.2% with the last time applied, 220 μs), except for the first time

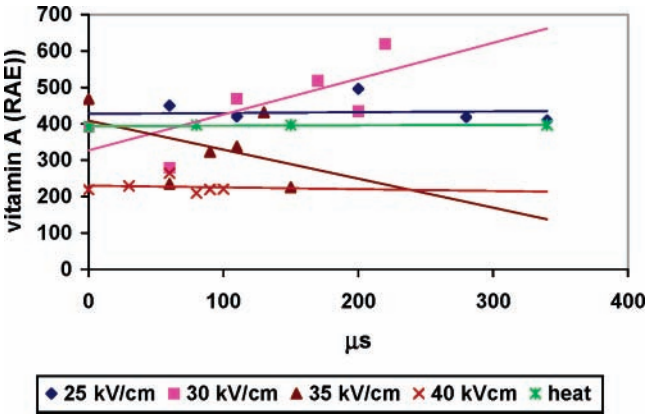

Figure 2. Variation of vitamin A (RAE) with HIPEF treatment and heat.

applied, which produces a great decrease in vitamin A (29%). In 35 kV/cm, vitamin A decreases in all cases regarding untreated orange−carrot juice. Vitamin A increases when a 40 kV/cm field is applied (4.5 and 20% in the first and second treatment times applied, respectively), but afterward, its decrease is similar to fresh juice. Vitamin A increases in pasteurized orange juice with regard to untreated orange−carrot juice (7.8%).

A comparison of these results with pasteurized orange−carrot juice shows that it is only in the HIPEF treatments with an intensity of 25 or 30 kV/cm that the vitamin A content is higher than in the pasteurized juice (**Figure 2**).

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

ACKNOWLEDGMENT

We thank IATA (CSIC) for allowing us to use its installations and equipment.

LITERATURE CITED

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

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

(3) Stern, P. In nature's image. Fruit and vegetable beverages with added ingredients. *Fluessiges Obst.* **1998**, *65*, 126−130.

(4) Klaui, H.; Bauernfeind, J. C. In *Carotenoids as Colorants and Vitamin A Precursors*; Bauernfeind, J. C., Ed.; Academic Press: New York, 1981; chapter 2, p 47.

(5) Granado, F.; Olmedilla, B.; Blanco, I. Carotenoid depletion in serum of young type-1 diabetics fed low-carotenoid diets. *Ann. Nutr. Metab.* **2004**, *48*, 251−258.

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

(7) Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M.; Cajigal, C. Lutein in patients with cataracts and a gel-related macular degeneration: A long-term supplementation study. *J. Sci. Food Agric.* **2001**, *81*, 904−909.

(8) Landrum, J. T.; Bone, R. A. Lutein, zeaxantin, and the macular pigment. *Arch. Biochem. Biophys.* **2001**, *385*, 28−40.

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

(10) van Poppel, G.; Goldbohm, R. A. Epidemiological evidence for β-carotene and cancer prevention. *Am. J. Clin. Nutr.* **1995**, *62*, 1493−1503.

(11) Giovannucci, E. Tomatoes, tomato-based products, lycopene, and cancer: A review of the epidemiological literature. *J. Natl. Cancer Inst.* **1999**, *91*, 317−331.

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

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

(14) Chytil, F. Vitamin A: Not for vision only. *Br. J. Nutr.* **1999**, *82*, 161−162.

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

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

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

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

(19) Grahl, T.; Märkl, H. Killing of microorganisms by pulsed electric fields. *Appl. Microbiol. Biotechnol.* **1996**, *45*, 148−157.

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

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

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

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

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

(25) Bendicho, S.; Espachs, A.; Stevens, D.; Arántegui, J.; Martín, O. Effect of high-intensity pulsed electric fields on vitamins of milk; European Conference of Emerging Food Science and Technology; Tampere, Finland, 1999.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(42) Cortés, C.; Esteve, M. J.; Frígola, A.; Torregrosa, F. Identification and quantification of carotenoids including geometrical isomers in fruit and vegetable juices by liquid chromatography with ultraviolet-diode array detection. *J. Agric. Food Chem.* **2004**, *52*, 2203−2212.

(43) Braddock, R. J. Single strength orange juices and concentrate. In *Handbook of Citrus By-products and Processing Technology*; John Wiley: New York, 1999; pp 53−83.

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

(45) Asai, A.; Terasaki, M.; Nagao, A. An epoxide-furanoid rearrangement of spinash neoxanthin occurs in the gastrointestinal tract of mice and *in vitro*: Formation and cytostatic activity of neochrome stereoisomers. *J. Nutr.* **2004**, *134*, 2237−2243.

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

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

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

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

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

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

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

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

(54) Johnsson, L. Thermal degradation of carotenes and influence on their physiological functions. In *Nutritional and Toxicological Consequences of Food Processing*; Friedman, M., Ed.; Plenum Press: New York, 1991; pp 75−82.

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

Received for review May 20, 2005. Revised manuscript received September 27, 2005. Accepted September 29, 2005. This study was undertaken as part of a multidisciplinary study on HIPEF of orange−carrot juice financed by a technological-FEDER research and development project (1FD97-0575-C03-02), a Ministry of Science and Technology project (AGL-2003-05236-C02-02), and Ayudas a Grupos de Investigación de la Generalitat Valenciana (Grupos 03/147). C. C. holds an award from the Generalitat Valenciana (Spain).

JF051171W