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The Effects of Thermal and Non-thermal Processing on Vitamin C, Carotenoids, Phenolic Compounds and Total Antioxidant Capacity in Orange Juice

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ABSTRACT

The abundance of fresh drinks based on fruit juices, especially citrus juices, and minimally processed products allow consumers to ingest a wide variety of antioxidants in the diet, such as vitamin C, carotenoids, flavonoids and other phenolic compounds. Pulsed electric fields (PEF) and high hydrostatic pressure (HHP) are emerging technologies in the field of food preservation. They have the potential to pasteurize various foods non-thermally, and it has been verified that these new technologies guarantee the safety (death of microorganisms) and stability (PME) of juices, with less quality loss in the final product. The effect of non-thermal processing (PEF, HHP) and pasteurization on total phenolic compounds, total antioxidant activity, vitamin C and carotenoids of orange juice was studied. There was a statistically significant reduction ($p<0.05$) in Trolox equivalent antioxidant capacity (TEAC) when juice was processed by either of these treatments, but the decrease was higher after pasteurization. TEAC decreased during refrigerated storage (4°C) of the samples analysed. Vitamin C concentration did not change significantly after pasteurization and non-thermal treatment; during refrigerated storage. However, vitamin C decreased more in pasteurized juice than in juice treated by PEF and HHP. The vitamin A concentration in the refrigerated orange juice was affected less by non-thermal treatments than by conventional thermal treatments. Total phenolic compounds were always higher in the untreated orange juice, followed by juice treated by PEF and HHP and finally by pasteurized juice, although the differences were not statistically significant ($p>0.05$), and during refrigerated storage they remained practically constant in all samples. Furthermore, a juice with similar characteristics as fresh juice, while preserving the bioactive compounds which provide it with its wealthy properties, is obtained.

Keywords: emerging technology, high hydrostatic pressure, pulsed electric fields, refrigerated storage

INTRODUCTION

Fruit and vegetables contain compounds which have a protective effect against degenerative diseases. They are known as phytochemical or bioactive compounds (fibre, phenolic compounds, carotenoids, vitamins A, C and E, glucosinates, organosulphur compounds), and their biological activity has been studied in numerous *in vitro* and *ex vivo* assays and in tests involving humans. Epidemiological studies also show the great protective effect of consumption of fruit and vegetables against the risk of certain diseases, such as cancer, cataract, macular degeneration and cardiovascular diseases (Granado *et al.* 2004).

Citrus fruit is a major source of bioactive compounds. Orange juice is an important source of carotenoids and ascorbic acid, a nutrient that, apart from its vitamin action, is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported. Also present are various phenolic compounds which include flavonoids and phenolic acids (Balasundram *et al.* 2006). Citrus fruit has a high flavanone content, the major flavanones being hesperidin, narirutin, neohesperidin and naringenin (Peterson *et al.* 2006; Cano *et al.* 2008). The phenolic acids most abundant in citrus fruit occur mainly as hydroxycinnamics (Robbins 2003). Recent studies have demonstrated their antioxidant role (Vinson *et al.* 2001; Klimczak *et al.* 2007).

The antioxidant capacity of orange juice makes it beneficial for health (Morton *et al.* 2000). Some studies show that the antioxidant capacity of citrus fruit is due to ascorbic acid and phenolic compounds, but there is disagreement over which compounds contribute most (Caro *et al.* 2004). Dietary antioxidants are able to neutralize oxygen-free radicals and inhibit LDL oxidation, and they may protect against coronary heart disease, cancer and neurodegenerative diseases (Lako *et al.* 2006).

Growing consumer demand for safe processed foods that require minimum preparation time has led the food industry to increase its output of products of this kind and provide means of ensuring that the nutrients and bioactive compounds are maintained or only minimally altered during processing and storage, until they reach the consumer (Hodgins *et al.* 2002; Señorans *et al.* 2003; Deliza *et al.* 2005).

During heat treatment, in addition to inactivation of microorganisms, varying percentages of desirable constituents, such as nutrients, colour, aroma and texture, are destroyed (Manso *et al.* 2001; Lee and Coates 2003; Blasco *et al.* 2004). Sterilization involves the application of very high temperatures (135–150°C) with very short heating times (4–15 s). Pasteurization is a mild heat treatment which produces minimal losses of organoleptic characteristics and nutritional value. There are two main groups of pasteurization technologies, those that use low temperatures (60–65°C) for fairly long times and those that use higher temperatures (75–99°C) for short times. Fruit and vegetable juices are currently subjected to temperatures in the range 90–99°C for 15–60 seconds and heat-filled aseptically. They are then cooled and stored refrigerated for subsequent marketing (Braddock 1999). The aim that many researchers share is to find the best preservation conditions and techniques to obtain orange juice that retains its nutritional value and the beneficial health effects described above. Numerous authors

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have shown that heat degrades anthocyans and vitamin C, and decreases the nutritional quality and antioxidant properties of juices (Klopotek *et al.* 2005). Gil-Izquierdo *et al.* (2002) found that pasteurization of orange pulp produces a decrease in certain phenolic compounds (caffeic acid and derivatives, apigenin 6,8-di-C-glucoside and 5,7,4'-thrihydroxyflavanone-7-rutinoside) and does not affect the juice's antioxidant quality. They found that the ascorbic acid concentration contributes 77–96% to the total antioxidant capacity of orange juice.

Recent studies on the effect of heat on the quality of various kinds of fruit and vegetables, including orange juice, have indicated a relative loss of total vitamin A content resulting from the loss of certain carotenes present in orange juice. The reduction was estimated at 36% (Lessin *et al.* 1997), although the same study did not reveal loss of pigment in black grapes in pasteurization conditions, which indicates that the carotene profile of orange juice is much more complicated. Lee *et al.* (2003) reported a total carotenoid concentration of 6.25 ± 0.11 mg/mL in fresh juice from oranges of the sweet variety, whereas the same study reported a total concentration of 5.70 ± 0.08 mg/mL in pasteurized juices, which represents a loss of 10%. After pasteurization treatment, carotenoids of the 5,6-epoxide type, such as the violaxanthin, *cis*-violaxanthin and antheraxanthin present in oranges, were seen to be affected. The authors did not find significant losses in the concentration of carotenes that have provitamin A activity (β-carotene, α-carotene and β-cryptoxanthin). However, the concentration of other carotenes, those of the 5,6-epoxide type, such as lutein and mutatoxanthin, increased. This is due to the fact that violaxanthin is one of the most labile carotenoids and is easily isomerized in the presence of acids to luteoxanthin and then to auroxanthin (Rodríguez-Amaya 2000). Cortés *et al.* (2006a) found that the total concentration of carotenoids in orange juice pasteurized at 90°C for 20 s decreased by 12.6%, with significant reductions ($p < 0.05$) in the concentrations of zeaxanthin, phytoene+phytofluene and neoxanthin+9-*cis*-violaxanthin. There was also a decrease (15.6%) in the concentration of carotenoids with vitamin activity.

Apart from the improvements that have been made in thermal preservation processes, such as continuous HTST and UHT treatments and aseptic canning, new non-thermal technologies are emerging, such as pulsed electric fields (PEF) and high hydrostatic pressure (HHP), in order to provide a response to the need for greater nutritional and sensory quality in some manufactured foods in which the characteristics of freshness are especially affected by thermal treatments, and to obtain microbiologically safe foods with physicochemical, nutritional and quality characteristics that are more like those of the fresh product.

The first use of PEF was in the USA in the late 1920s, when attempts were made to pasteurize milk by the use of electric pulses. The technique involves the application of high-voltage electric pulses, normally in the range 20–50 kV/cm, which are generated between two electrodes, between which the food is situated. PEF treatment is an emerging technology which promises the preservation of the organoleptic and nutritional characteristics of the treated product, the production of lethal effects on microorganisms and inhibition of the activity of enzymes that participate in the deterioration of food (Ayhan *et al.* 2001; Cortés *et al.* 2005; Fernández-Molina *et al.* 2005; Torregrosa *et al.* 2005, 2006; Garde-Cerdan *et al.* 2007). As such, interest in this technology is rapidly increasing (Clark 2006). The electric field affects cell membranes, causing irreversible damage, alteration in ion transport and changes in the structure of enzymes (Barsotti and Cheftel 1999). The products that are to be treated must have low viscosity, great homogeneity and a low risk of dielectric rupture, so that fruit juices are suitable for the application of this technology (Góngora-Nieto *et al.* 2002).

High hydrostatic pressure (HHP) has been studied as a food preservation technique for over a century. It is used to treat both solid and liquid foods. The temperatures reached during the treatment can range from 0 to 100°C. Exposure times vary between a few seconds and 20 minutes. Foods treated by this technology preserve their original properties, colour, flavour and aroma. The advantage is that the treatment is uniform irrespective of the size, type or composition of the product. Water activity and pH are critical factors for processing and inactivation of microorganisms by HHP. An increase in temperature during high pressure treatment helps the inactivation of microorganisms. Temperatures between 45 and 50°C are sufficient to produce inactivation. In addition to the destruction of microorganisms, changes are produced in the product involving denaturation/modification of proteins, inactivation/activation of enzymes, changes in enzyme-substrate interactions and changes in the properties of carbohydrates and fat (Polydera *et al.* 2003).

A study by the Institute of Food Technology (IFT 2000) reported research requirements for emerging preservation technologies, highlighting the need to identify how changes in critical factors in processes or the introduction of new factors may affect bacterial inactivation and the quality, nutritional value and shelf life of foods.

The consumption of foods rich in antioxidant substances may contribute to the prevention of "oxidative stress" situations. Knowledge about the concentrations of these substances in foods or their total antioxidant power may contribute to a better diet and a decrease in chronic diseases. In recent years, there has been considerable advances in the study and knowledge of the properties of free radical acceptors and the estimation of the antioxidant power of various substances, such as certain vitamins and phenolic compounds. All this shows the importance of evaluating citrus juices as sources of antioxidants, estimating the content of ascorbic acid, carotenoids, phenolic compounds and total antioxidant power after the application of preservation treatments, either traditional treatments (heat) or emerging technologies, such as PEF and HHP.

MATERIALS AND METHODS

Sampling of orange juice

Oranges (*Citrus sinensis* L., 'Navel' variety) were purchased in a supermarket in 'Valencia'. Orange juice was obtained by squeezing (FMC juice extractor with 2-mm perforated plates) and passed through a filter with a pore diameter of 0.23 mm. The filtrate was divided into four aliquots: one to be treated by heat, one by PEF, one by HHP, and one was not treated and that was used to ascertain the value of each of the parameters in the fresh juice. Each of the treatments was applied in duplicate.

PEF treatment system

Sample treatments were carried out in a continuous PEF treatment system designed by the University of Ohio 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. The system was immersed in a refrigerated bath in order 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 U.S.A.). Flow was set at 60 mL/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, 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 for carotenoid concentration, colour, vitamin C, enzymes and microorganisms obtained when orange juice was treated using different fields (25, 30, 35 and 40 kV/cm) and different times (30–340 μs) (Cortés *et al.* 2006b). Samples were collected after treatment.

HHP treatment

Orange juice was placed in a 50-mL PE-LD flask and treated at

4000 bars for 5 min in an high pressure unit (EPSI NV, Belgium). After the treatment, the samples were quickly cooled and then analysed.

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. Treatment conditions were comparable to those used with orange juice in commercial practices; heating 90–99°C for 15–30 s (Braddock 1999). After treatment, the juice was cooled with cold water from a cooler (Armfield FT61), and it was packed and stored (4 ± 2°C) until analysis, which is realized in the same day.

Storage conditions

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

Vitamin C content

Five mL of juice was diluted to 25 mL with the extraction solution (oxalic acid 1%, w/v, trichloroacetic acid 2%, w/v, sodium sulphate 1%, w/v). After vigorous shaking the solution was filtered through a folded filter (Whatman no. 1). Then 9.5 mL of oxalic acid 1% (w/v) and 2 mL of acetic acid/sodium acetate 2M buffer (pH = 4.8) were added to an aliquot of 0.5 mL of filtrate and the solution was transferred to the polarographic cell. The following conditions were applied: DP_{50}, mode DME, drop size 2, drop time 1 s, scan rate 10 mV/s, initial potential –0.10 V. Vitamin C concentration were determined by the peak heights and standard additions method (Aparicio *et al.* 1992).

Carotenoid content

Carotenoid pigments were extracted, saponified and analysed by HPLC, according to a procedure described by Cortés *et al.* (2004). The carotenoids in the juices were identified by UV-Vis spectra and retention times in HPLC.

Total phenol content

Total phenol content of the samples was determined using the Folin-Ciocalteu method (Singleton and Rossi 1965), reading samples on a Perkin-Elmer Lambda 2 UV/Vis spectrophotometer at 750 nm. Results were expressed as gallic acid equivalents (mg/100 mL).

Total antioxidant capacity

The method, adapted from Rice-Evans and Miller (1994), was based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2'-azinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS), which has a characteristic long-wavelength absorption spectrum showing maxima at 734 nm. The ABTS radical cation is formed by the interaction of ABTS (150 μM) with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin (2.5 μM) with H_2O_2 (75 μM). Antioxidant compounds suppress the absorbance of the ABTS radical cation to an extent and on a time scale dependent on the antioxidant capacity of the substance under investigation.

This inhibition assay used a fixed time point of 3 min. ABTS, myoglobin and a sample were mixed, and the reaction initiated by the addition of hydrogen peroxide. After a fixed time, the absorbance of the solution was read, together with a buffer blank (which has a greater absorbance value). Results were obtained by calculating the difference in absorbance before and after the addition of the oxidant compound (H_2O_2) and interpolating the value obtained from a calibration curve prepared every day with Trolox standard in the range of 0.5–2 mM.

Statistical analysis

The results were compared by one-way analysis of variance (ANOVA). To determine differences between during storage of each of the treatments, the LSD test ($p < 0.05$) was applied, and a multiple regression analysis was performed to study the influence of time on vitamin C content. Finally, Pearson's correlation test was conducted to determine the linear correlations among variables. The computer program employed was SPSS® (Statistical Package for the Social Sciences) Ver. 12.0 for Windows.

RESULTS AND DISCUSSION

Tables 1–4 summarize the results obtained for each of the parameters studied (vitamin C, carotenoids, total phenols and total antioxidant capacity) in fresh juice and in juice treated by heat, PEF and HHP and stored at 4 ± 2 and 10 ± 2°C.

Vitamin C

Orange juice is a rich source of vitamin C, which is an important antioxidant in this juice. Vitamin C concentration is a significant indicator of orange juice quality, and it may serve as an indicator that all processes which ensure the high quality of the product have been applied in the produc-

Table 1 Vitamin C (mg/100 mL) in untreated, PEF and pasteurized orange juice, during refrigerated storage (4 ± 2 and 10 ± 2°C).

Storage		Fresh juice	Treated juice		
T	Weeks		PEF♦	HHP	Pasteurized♦
4 ± 2°C	0	48.33 ± 1.12	48.32 ± 1.35 a	48.30 ± 1.03 a	48.29 ± 2.53 a
	1	*	47.60 ± 0.15 a	47.76 ± 0.87 a	44.35 ± 1.18 b
	2	*	43.52 ± 0.03 bc	44.02 ± 0.96 b	42.73 ± 1.21 c
	3	*	44.69 ± 0.42 b	43.87 ± 0.87 b	43.35 ± 0.99 c
	4	*	43.73 ± 0.70 b	43.03 ± 0.65 b	41.56 ± 0.01 c
	6	*	43.82 ± 0.64 b	42.98 ± 0.77 b	35.41 ± 0.83 d
	7	*	42.66 ± 0.17 c	42.59 ± 0.39 b	35.58 ± 0.15 d
10 ± 2°C	0	48.33 ± 1.12	48.32 ± 1.35 a	48.30 ± 1.03 a	48.88 ± 2.53 a
	1	*	43.71 ± 0.50 b	44.02 ± 0.66 b	42.42 ± 0.46 b
	2	*	42.52 ± 0.52 b	43.76 ± 0.89 b	38.26 ± 0.45 c
	3	*	44.56 ± 1.82 b	43.02 ± 0.47 b	33.94 ± 0.81 d
	4	*	43.81 ± 1.32 b	43.47 ± 1.01 b	30.89 ± 0.47 d
	6	*	43.03 ± 0.63 b	42.98 ± 1.13 b	18.74 ± 1.28 e
	7	*	*	*	*

T: temperature.
♦ Published by Cortes *et al*. (2008)
*spoiled samples, not analysed.
Mean ± standard deviation for two samples.
Differences in letters within a column at same temperature (4 or 10°C) indicate significant (P<0.05) differences (LSD test)

Table 2 Total carotenoids (μg/100 g) in untreated, PEF and pasteurized orange juice, during refrigerated storage (4 ± 2 and 10 ± 2°C).

Storage		Fresh juice	Treated juice		
T	**weeks**		**PEF**	**HHP**	**Pasteurized**
4 ± 2°C	0	1367.2 ± 64.7	1275.2 ± 56.3 a	1309.2 ± 46.7 a	1195.4 ± 31.6 ab
	1	*	1174.4 ± 54.9 b	1214.8 ± 32.2 ab	1305.7 ± 38.4 a
	2	*	1129.4 ± 106.7 bc	1167.2 ± 67.9 b	1117.6 ± 57.5 bc
	3	*	1128.5 ± 33.7 bc	1004.2 ± 41.7 c	1237.4 ± 21.6 ab
	4	*	1234.3 ± 52.0 ab	1178.1 ± 38.2 b	1020.6 ± 84.7 cd
	6	*	1018.3 ± 16.0 cd	1032.9 ± 58.8 c	881.9 ± 13.2 e
	7	*	964.2 ± 12.1 d	997.2 ± 81.2 c	913.3 ± 72.3 de
10 ± 2°C	0	1367.2 ± 64.7	1275.2 ± 56.3 a	1309.2 ± 46.7 a	1195.4 ± 31.6 a
	1	*	1258.0 ± 92.2 a	1207.5 ± 39.2 ab	1122.6 ± 0.24 a
	2	*	1097.9 ± 61.5 b	1198.1 ± 65.8 ab	1087.5 ± 11.4 a
	3	*	1082.9 ± 51.3 b	1002.4 ± 69.1 c	919.7 ± 48.0 a
	4	*	1101.8 ± 30.1 b	997.4 ± 72.5 c	845.1 ± 39.5 a
	6	*	1107.8 ± 8.0 b	1087.4 ± 61.2 bc	854.2 ± 41.9 a
	7	*	*	*	*

T: temperature.
*spoiled samples, not analysed.
Mean ± standard deviation for two samples.
Differences in letters within a column at same temperature (4 or 10°C) indicate significant (P<0.05) differences (LSD test)

Table 3 Total phenolic compounds (mg/mL of gallic acid) in untreated, PEF and pasteurized orange juice, during refrigerated storage (4 ± 2 and 10 ± 2°C).

Storage		Fresh juice	Treated juice		
T	**weeks**		**PEF**	**HHP**	**Pasteurized**
4 ± 2°C	0	1.002 ± 0.056	0.988 ± 0.036 ab	0.998 ± 0.038 a	0.949 ± 0.055 ab
	1	*	0.953 ± 0.042 ab	1.004 ± 0.028 a	1.021 ± 0.027 ac
	2	*	1.073 ± 0.025 a	1.041 ± 0.077 a	1.055 ± 0.044 bc
	3	*	0.877 ± 0.056 b	1.007 ± 0.052 a	0.871 ± 0.057 b
	4	*	1.023 ± 0.081 a	0.997 ± 0.031 a	0.956 ± 0.028 a
	6	*	1.008 ± 0.066 a	1.032 ± 0.023 a	1.004 ± 0.033 ac
	7	*	1.045 ± 0.041 a	1.051 ± 0.044 a	1.070 ± 0.041 c
10 ± 2°C	0	1.002 ± 0.056	0.988 ± 0.036 a	0.998 ± 0.038 a	0.949 ± 0.055 a
	1	*	0.954 ± 0.047 a	1.014 ± 0.042 a	1.039 ± 0.022 ab
	2	*	1.148 ± 0.036 b	1.005 ± 0.054 a	1.032 ± 0.027 ab
	3	*	0.937 ± 0.067 a	1.021 ± 0.037 a	0.844 ± 0.036 c
	4	*	0.955 ± 0.029 a	1.012 ± 0.048 a	1.067 ± 0.042 ab
	6	*	0.941 ± 0.052 a	1.002 ± 0.067 a	0.985 ± 0.033 b
	7	*	*	*	*

T: temperature.
*spoiled samples, not analysed.
Mean ± standard deviation for two samples.
Differences in letters within a column at same temperature (4 or 10°C) indicate significant (P<0.05) differences (LSD test)

Table 4 Total antioxidant capacity (mmol Trolox/L) in untreated, PEF and pasteurized orange juice, during refrigerated storage (4 ± 2 and 10 ± 2°C).

Storage		Fresh juice	Treated juice		
T	**weeks**		**PEF**	**HHP**	**Pasteurized**
4 ± 2°C	0	4.03 ± 0.04	3.51 ± 0.04 a	3.86 ± 0.13 a	2.49 ± 0.20 a
	1	*	3.22 ± 0.16 b	3.54 ± 0.09 b	1.93 ± 0.26 b
	2	*	2.98 ± 0.07 c	3.26 ± 0.04 c	2.10 ± 0.07 bc
	3	*	2.94 ± 0.07 c	3.96 ± 0.08 a	2.40 ± 0.16 ac
	4	*	2.33 ± 0.02 d	2.46 ± 0.05 d	1.83 ± 0.10 b
	6	*	2.23 ± 0.02 de	2.32 ± 0.04 d	1.80 ± 0.04 b
	7	*	2.21 ± 0.02 e	2.29 ± 0.02 d	1.80 ± 0.01 b
10 ± 2°C	0	4.03 ± 0.04	3.51 ± 0.04 a	3.86 ± 0.13 a	2.49 ± 0.20 a
	1	*	2.37 ± 0.15 b	3.02 ± 0.07 b	2.02 ± 0.02 a
	2	*	2.10 ± 0.07 c	2.79 ± 0.04 c	2.15 ± 0.77 a
	3	*	2.37 ± 0.18 b	2.36 ± 0.03 d	2.52 ± 0.27 a
	4	*	1.54 ± 0.09 d	1.88 ± 0.02 e	1.74 ± 0.20 a
	6	*	1.53 ± 0.04 d	1.50 ± 0.05 f	1.70 ± 0.06 a
	7	*	*	*	*

T: temperature.
*spoiled samples, not analysed.
Mean ± standard deviation for two samples.
Differences in letters within a column at same temperature (4 or 10°C) indicate significant (P<0.05) differences (LSD test)

tion process (Post 1998).

The vitamin C concentration in the fresh juice was 48.33 ± 1.12 mg/100 mL, this value is inside of the interval (45.03 ± 7.90 mg/100 mL) mentioned in the biography (Cano *et al* 2008). Although it to be considered that the content of vitamin C can differ from different factors such as genotypic differences, preharvest climatic conditions and cultural practices, maturity and harvesting methods, and postharvest handling procedures (Lee and Kader 2000). After HHP treatment of the orange juice, there was no significant modification in the vitamin C concentration, neither changes are observed after the pasteurization and PEF treatment (Cortés *et al.* 2008). Elez-Martínez *et al.* (2007) studied the effect of PEF treatment on vitamin C concentration

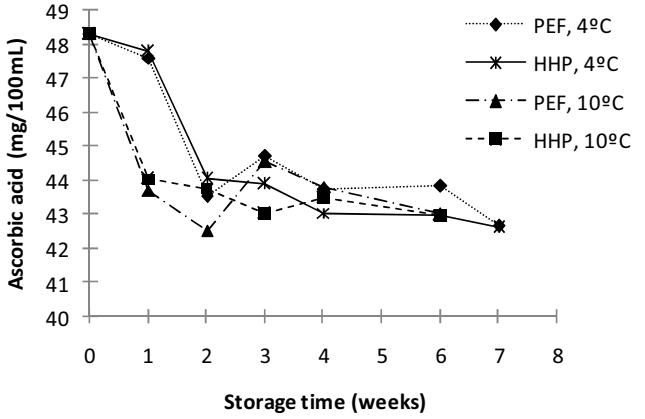

Fig. 1 Evolution of ascorbic acid concentration in juice treated by PEF (Cortés *et al.* 2008) and HHP during storage at 4 ± 2°C and 10 ± 2°C.

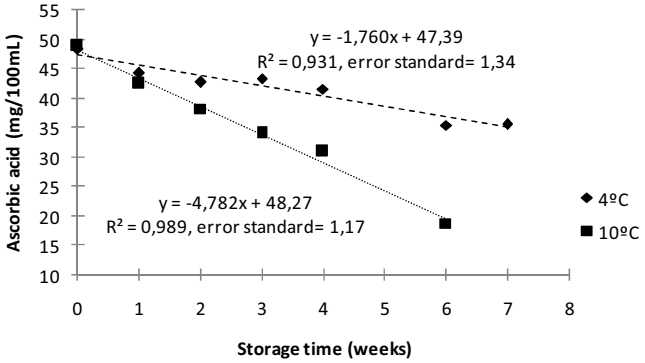

Fig. 2 Evolution of ascorbic acid concentration in pasteurized juice during storage at 4 ± 2°C and 10 ± 2°C (Cortés *et al.* 2008).

in orange juice and found that after treatment by pulses, the retention of vitamin C was between 87.5 and 98.2%, higher than the retention of juice pasteurized at 90°C for 1 min (82.4%). They showed that vitamin C retention is greater when bipolar pulses are applied, and it decreases in accordance with an increase in electric field strength, treatment time and pulse frequency and width. Min *et al.* (2003) also found no significant differences between fresh juice and juice treated by PEF at 40 kV/cm for 97 ms at 2000 Hz with a bipolar pulse of 2.6 μs and a maximum temperature of 45°C, whereas the losses after pasteurization (90°C, 90 s) were greater. Sánchez-Moreno *et al.* (2005) found that after treatment by PEF (35 kV/cm for 750 μs, maximum temperature 50°C), HHP (400 MPa, 40°C, 1 min) and pasteurization (90°C, 1 min) the retention of vitamin C was of the order of 93%, but when a mild pasteurization treatment (70°C, 30 s) was applied they observed no change.

According to other data, vitamin C content in various juices decreases during storage, depending on storage conditions, such as temperature, oxygen and light access (Zerdin *et al.* 2003). When juice treated by HHP was stored at 4°C, the decrease in the ascorbic acid concentration was 9% after two days of storage and 12% after seven days. When the storage temperature was 10°C, however, the rate of degradation of ascorbic acid was greater, with a decrease of 9% in the first 24 h of storage of juices treated by HHP, although after 7 days of storage the remaining concentration was similar to that of the juices stored at 4°C. These main results are obtained in the juice treated by PEF (Cortés *et al.* 2008). This can be explained by the fact that the degradation of ascorbic acid in these samples fits an exponential model (**Fig. 1**), but, as **Fig. 2** shows, in the pasteurized juice stored at 4 and 10°C (Cortés *et al.* 2008), the ascorbic acid follows a linear kinetic, with losses of 26 and 61% by the end of storage at 4 and 10°C, respectively. Fernández-García *et al.* (2001) observed that there were no significant losses of ascorbic acid in juices treated by HHP and stored at 4°C for 21 days. Nienaber and Shellhammer (2001) found that the losses of ascorbic acid in juice treated by HHP (800 MPa, 25°C, 1 min) were less than 20% after being stored for 3 months at 4°C or 2 months at 15°C. Polydera *et al.* (2005a) studied the shelf life of orange juice treated by HHP (600 MPa, 40°C, 4 min) and pasteurization (80°C, 60 s) and stored at different temperatures (0–30°C). The results showed that the degradation of ascorbic acid during storage was less in the juice treated by HHP, so that the shelf life of the juice increased. The shelf life of the juice ranged from 13 days when it was stored at 15°C to 99 days when the storage temperature was 0°C. However, when the juice was stored at 30°C, the shelf life did not differ with the treatment applied. These shelf life values are double the values obtained by the same authors (Polydera *et al.* 2003) with reconstituted orange juice. Klimczak *et al.* (2007) described similar results with orange juice stored for six months at 18, 28 and 38°C. The vitamin C concentration decreased to a greater extent and more quickly (21, 31 and 81% after 6 months at 18, 28 and 38°C, respectively) at a higher storage temperature.

Carotenoids

The total carotenoid concentration in the pasteurized juice decreased (–12.8%) significantly ($p < 0.05$) in comparison with the fresh juice, and the decrease (–6.7%) was less ($p > 0.05$) in the juice treated by PEF. The decrease in the juice treated by HHP was 4.2%, and no significant changes ($p < 0.05$) were observed in the conditions selected. Férnandez-García *et al.* (2001) also found no losses in carotene content.

This agrees with the results obtained by Lee and Coates (2003) for pasteurized orange juice, in which total carotenoid content loss was significant ($p < 0.05$) after thermal pasteurization at 90°C for 30 s. Similarly, vitamin A was higher in the untreated orange juice, followed by the orange juice treated by PEF (–7.52%) and, finally, by the pasteurized orange juice (–15.62%). Only the differences between the untreated orange juice and the pasteurized orange juice were significant ($p < 0.05$). Thus, in refrigerated orange juice, the concentration of vitamin A is affected less by non-thermal treatment (PEF) than by conventional thermal treatments. Sánchez-Moreno *et al.* (2005) also studied modifications in vitamin A and total carotenoid content after applying various kinds of preservation treatments to orange juice, obtaining results similar to the ones described in the present study. The combined effect of pressure, temperature and treatment time on total carotenoids or individual carotenoids with antioxidant capacity, lutein, zeaxanthin, β-cryptoxanthin, α-cryptoxanthin, β-carotene and α-carotene, studied by de Ancos *et al.* (2002) resulted in a significant increase in total carotene content. Treatment by HHP at 350 MPa, 30°C, 5 min, produced an increase of 50% for β-carotene, 60% for α-carotene, 42% for β-cryptoxanthin and 63% for α-cryptoxanthin in comparison with fresh juice. However, pressures ranging from 50 to 200 MPa did not produce significant changes in the carotenoid profile. Similarly, no direct correlations were found between pressure values and extraction of carotenoids. A possible explanation could be that carotenes do not occur freely in the medium, but form bonds with proteins in the cell membranes. The increase that takes place at certain pressures could be due to the increase in extraction capacity. Treatment at 350 MPa increases the carotene content after denaturation of the carotene-protein bonds induced by pressure. It has been hypothesized, therefore, that HHP may be a suitable treatment to increase extraction of carotenes from the matrix, which might be associated with an increase in nutritional value. The same study also revealed that juices treated at pressures between 200 and 350 MPa at 30°C for 5 min, had a similar carotene content throughout the shelf life (30 days) of the product under conditions of refrigerated storage at 4°C. Férnandez-García *et al.* (2001) observed that storage at 4°C for 21 days did not produce significant changes in carotene content in orange juice.

Phenolic compounds

Results for phenolic compounds are shown in **Table 4**. As can be seen, values are higher in untreated orange juice, followed by PEF and HHP treated orange juice and finally by pasteurized orange juice, although the differences are not significant ($p > 0.05$). Sánchez-Moreno *et al.* (2005) found that HHP treatment increases ($p < 0.05$) the content of naringenin (20.2%) and hesperetin (39.9%). This may be due to changes in the structure of vesicles in the orange juice, permitting a greater extraction of flavanones, whereas PEF treatment does not affect flavanone content, and pasteurization generally reduces it.

We observed a non-significant increase ($p > 0.05$) in phenolic compounds in all the juices analysed, after refrigerated storage. Klimczak *et al.* (2007) also describe a small increase in phenolic compounds in orange juice after six months of storage at 18, 28 and 38°C. They used two different methods for the determination of phenolic compounds. Although there is evidence that the spectrophotometric method overestimates the polyphenolic content in comparison with the chromatographic method (explained by the lack of selectivity of the Folin-Ciocalteu reagent (Escarpa and González 2001), the spectrophotometric method (Folin-Ciocalteu method) has been shown to be a useful analytical tool for the routine analysis of polyphenols, and it is widely used in many laboratories for the determination of differences among fruits and vegetables and their products (Klimczak *et al.* 2007).

Antioxidant capacity

The Trolox equivalent antioxidant capacity (TEAC) of the samples after applying the preservation treatments was 4.03 ± 0.04 mmol Trolox/L for untreated orange juice, 3.51 ± 0.04 mmol Trolox/L for PEF treated juice, 3.86 ± 0.13 mmol Trolox/L for HHP treated juice and 2.49 ± 0.20 mmol Trolox/L for pasteurized orange juice. As can be seen, TEAC decreased significantly ($p < 0.05$) after processing the orange juice with both types of treatments, but the decrease was greater in the pasteurized juice than in the PEF and HHP treated juices (decrease of 12.9 and 4.2% after PEF and HHP, respectively, and 38.21% after pasteurization). Thus, PEF and HHP treated orange juice has an antioxidant capacity more like that of untreated juice. These technologies are more effective than pasteurization in preserving the antioxidant capacity of orange juice. Sánchez-Moreno *et al.* (2005) found that the total antioxidant capacity of orange juice treated by mild pasteurization (70°C, 30s), HHP (400 MPa/40°C/1 min) and PEF (35 $kVcm^{-1}$/750 µs) did not undergo significant changes ($p < 0.05$), whereas pasteurization (90°C, 1 min) produced a decrease. Similarly, Polydera *et al.* (2005b) observed a greater decrease in total antioxidant capacity in pasteurized orange juice (80°C, 60 s), whereas when the same juice was processed by HHP (600 MPa, 40°C, 4 min) the decrease in this parameter was slighter. Similarly, Elez-Martínez and Martín-Belloso (2007) found that PEF treatment of orange juice does not affect its antioxidant capacity, while pasteurization produces a decrease in this parameter.

Fiore *et al.* (2005) determined the total antioxidant capacity in orange juice and found that this parameter decreases after thermal treatments (sterilized and pasteurized orange juice). Results similar to those described in the present study were obtained with refrigerated pasteurized orange juice (2.12–3.53 mmol Trolox/L).

The total antioxidant capacity of the samples during refrigerated storage is summarized in **Table 4**. Decreases in this parameter in the three types of sample analysed were observed, with a greater decrease in the samples stored at 10°C. In comparison with conventional pasteurization, PEF and HHP treatments led to higher total antioxidant activity in orange juice immediately after processing (time 0 of storage), as well as during storage at 4–10°C. This is in agreement with results in fresh navel juice and in orange juice reconstituted from frozen concentrate after high pressure treatment (Polydera *et al.* 2005b). Férnandez-García *et al.* (2001) found that HHP treatment and storage at 4 ± 2°C for 21 days, did not produce significant changes in the total antioxidant capacity of orange juice. Klimczak *et al.* (2007) obtained similar results in orange juice stored for six months at 18, 28 and 38°C, observing that at higher storage temperatures, total antioxidant values decreased to a greater extent (18, 45 and 84% after 6 months at 18, 28 and 38°C, respectively) and more quickly.

TEAC correlates positively with total carotenoids, vitamin A and vitamin C ($r^2 = 0.318$, $p = 0.013$; $r^2 = 0.379$, $p = 0.003$; and $r^2 = 0.317$, $p = 0.014$, respectively), so that juices with a higher total antioxidant value also have higher carotenoid, vitamin C and vitamin A concentrations, these being the parameters that most influence the total antioxidant capacity of the juice. Nevertheless, the total antioxidant capacity did not show statistically significant correlations with total phenols, so this parameter may not contribute greatly to the total antioxidant capacity of the orange juices analysed.

Similarly, Gardner *et al.* (2000), Polydera *et al.* (2005) and Sánchez-Moreno *et al.* (2005) reported that vitamin C was the compound with the highest antioxidant capacity in various orange juices. However, Elez-Martínez and Martín-Belloso (2007) did not find a relationship with vitamin C content in orange juice treated by PEF. They indicated that this might be due to the stability of other antioxidant compounds, such as carotenoids and phenols.

CONCLUSIONS

The results obtained indicate that it is possible to obtain orange juice with a high nutritional value and a content of bioactive compounds similar to that of fresh juice with the emerging technologies studied (PEF and HHP). In comparison with conventional pasteurization, PEF and HHP treatments led to higher total antioxidant activity in orange juice immediately after processing, as well as during storage at 4 ± 2 and 10 ± 2°C.

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