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Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and *gazpacho*, a cold vegetable soup

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Abstract

Orange juice and *gazpacho*, a cold vegetable soup, were submitted to high intensity pulsed electric fields (HIPEF). The effects of electric field strength, treatment time, pulse frequency, width and polarity, as process parameters, on vitamin C retention and antioxidant capacity of both products were evaluated and compared to those in a heat pasteurization. Vitamin C was determined by HPLC and antioxidant capacity through the inhibition of the DPPH$^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl) radical. Orange juice and *gazpacho* retained a 87.5–98.2% and 84.3–97.1% of vitamin C, respectively, after HIPEF treatments. Pulses applied in bipolar mode, as well as a lower electric field strength, treatment time, pulse frequency and width, led to higher levels of vitamin C retention ($p < 0.05$). HIPEF-treated orange juice and *gazpacho* always showed a vitamin C retention higher than that of the heat-pasteurized products. There were no differences ($p < 0.05$) in antioxidant capacity between HIPEF-treated and untreated products, whereas heat-treated foods showed lower values of antioxidant capacity.

Keywords: High intensity pulsed electric fields; Orange juice; *Gazpacho*; Vitamin C; Antioxidant capacity

1. Introduction

Vegetables provide antioxidant compounds and a complex mixture of other natural substances that promote antioxidant capacity (Arnao, Cano, & Acosta, 2001). The presence of bioactive compounds in fruits and vegetables has recently been considered to be of nutritional importance in the prevention of chronic diseases, such as cardiovascular disease, various cancers, diabetes, and neurological diseases (Kalt, Forney, Martin, & Prior, 1999; Willet, 1994). Vitamin C is an essential vitamin found in fruits and vegetables. Its antioxidant properties have been studied and they may prevent free radical-induced damage to DNA (Fraga et al., 1991), quenching oxidants which can lead to the development of cataracts (Mares-Perlman, 1997), thus overcoming endothelial cell dysfunction (Levine et al., 1996) and decreasing LDL-induced leukocyte adhesion (Lehr, Frei, Olofsson, Carew, & Arfors, 1995). Vitamin C also contributes to a healthy vasculature through the regulation of collagen synthesis, prostacyclin production, and maintenance of nitric oxide levels (Ness, Khaw, Bingham, & Day, 1996a, 1996b; Simon, 1992). Moreover, several studies have shown that vitamin C plays an important role in human health, affecting the immune system (Grimble, 1997), the risk of Alzheimer disease (Engelhart et al., 2002) and the efficiency with which lysosomes in brain glial cells degrade modified proteins (Martin, Joseph, & Cuervo, 2002).

The Mediterranean diet has been postulated as protective against different diseases, including coronary heart disease, stroke and neurological disorders. It also reduces cholesterol levels, and cancer risk (Covas, Marrugat, Fito, Elosua, & De la Torre-Boronat, 2002; Esposito & Giugliano, 2002). Orange juice, as well as *gazpacho*, a ready-to-use cold vegetable soup, are included in the typical

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Mediterranean diet. Orange juice is a rich source of vitamin C and the antioxidant capacity of orange juice is attributed to vitamin C (Gardner, White, McPhail, & Duthie, 2000; Miller & Rice-Evans, 1997; Rapisarda et al., 1999). *Gazpacho* contains tomato, cucumber and pepper as main components. Other minor ingredients of *gazpacho* are olive oil, onion, garlic, wine vinegar and sea salt (Aguilera, Brotons, Rodríguez, & Valverde, 2003; Arnao et al., 2001). The important contribution of vitamin C to *gazpacho* antioxidant activity was reported by Arnao et al. (2001).

Thermal processing is the most common method for extending the shelf life of fruit and vegetable products, by inactivating microorganisms and enzymes. However, thermal processing can diminish the sensory and nutritional qualities of juices (Braddock, 1999). Consumer demands for healthy and nutritious food products with a fresh-like appearance has undergone a continuous rise during recent years (Linneman, Meerdink, Meulenberg, & Jongen, 1999). Consequently, emerging technologies for food processing and preservation, such as high intensity pulsed electric fields (HIPEF), are being investigated (Deliza, Rosenthal, & Silva, 2003). Until now, most of the studies on the evaluation of the effects of HIPEF on juice have been focussed on enzymatic and safety aspects (Espachs-Barroso, Barbosa-Cánovas, & Martín-Belloso, 2003). Nonetheless, the literature on the effects of HIPEF technology on bioactive compounds is limited and information about the impact of HIPEF-processing on the antioxidant food properties is scarce. Promising results have been obtained about the levels of vitamin C retention in orange, apple and tomato juices processed by HIPEF compared to thermal treatment (Evrendilek et al., 2000; Min, Jin, Min, Yeom, & Zhang, 2003a; Min, Jin, & Zhang, 2003b; Yeom, Streaker, Zhang, & Min, 2000). However, no information has been found in the literature regarding the effects of HIPEF-process parameters on vitamin C retention in any food. In addition, at the stage of development of HIPEF technology, evaluating the influence of process variables on the antioxidant properties of foods is a key factor in defining treatment conditions necessary to avoid the loss of these important properties of foods.

The objective of this work was to study the influence of electric field strength, treatment time, pulse width, frequency and polarity, as HIPEF-processing parameters, on the vitamin C content and the antioxidant capacity of orange juice and a cold vegetable soup, *gazpacho*. The effects of HIPEF on vitamin C and antioxidant capacity were also compared with a thermal treatment.

2. Materials and Methods

2.1. Chemicals

L(+)-ascorbic acid, 2,3 dimercapto-1-propanol (BAL) and 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Metaphosphoric acid, potassium dihydrogen phosphate, acetonitrile and methanol were obtained from Sharlau Chemie, SA (Barcelona, Spain).

2.2. Orange juice and gazpacho preparation

Oranges (*Citrus sinensis* L., var. Navelina) (Valencia, Spain) were bought from a local supermarket, and kept at 4 °C before being processed. The orange juice was obtained using a squeezer (Santos; Lyon, France) and then filtered using a 2 mm steel sieve.

The cold vegetable soup, *gazpacho*, was elaborated with the following ingredients and proportions: tomatoes (*Lycopersicon esculentum* Mill., from Almeria, Spain, 50%), cucumber (*Cucumis sativus* L., short cucumber from Spain, 15%), green pepper (*Capsicum annuum* L., Italian pepper, 10%), onion (*Allium cepa* L., Onion Buti from Valencia, Spain, 3%), garlic (*Allium sativum* L., white garlics from Alicante, Spain, 0.8%), virgin olive oil (Borges from Tarrega, Spain, 2%), wine vinegar (Borges from Tarrega, Spain, 2%), sugar (0.05%) and water (16.4%). Vegetables were cut and all the ingredients were ground and mixed using a domestic blender (Hamilton Beach Blendmaster; Washington, DC, USA). The obtained *gazpacho* was filtered using a 2 mm steel sieve.

Analytical characteristics of freshly squeezed orange juice and freshly made *gazpacho* are shown in Table 1. Electrical conductivity (Testo 240 conductivimeter; Testo GmBh & Co, Lenzkirch, Germany), pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), total acidity (AOAC, 1997), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan), vitamin C content (Soliva-Fortuny, Elez-Martínez, & Martín-Belloso, 2004) and antioxidant capacity (De

Table 1
Analytical characteristics of fresh orange juice and *gazpacho*[a]

	Orange juice	*Gazpacho*
Soluble solids (°Brix)	11.3 ± 0.6	3.77 ± 0.3
Total acidity (g citric acid/100 ml)	0.44 ± 0.05	0.29 ± 0.02
pH	3.58 ± 0.03	4.36 ± 0.06
Electrical conductivity (S/m)	0.450 ± 0.012	0.401 ± 0.015
Vitamin C (mg/100 ml)	56.3 ± 4.1	22.6 ± 3.6
Antioxidant capacity (% inhibition DPPH$^{\cdot}$)	39.3 ± 1.5	45.7 ± 1.6

[a] Results are the means ± SD of three measurements.

Ancos, Sgroppo, Plaza, & Cano, 2002) of orange juice and *gazpacho* were determined.

2.3. HIPEF treatments

HIPEF treatments were carried out in a continuous flow bench scale system (OSU-4F, Ohio State University, OH, USA), using square-wave pulses. The treatment system consisted of eight colinear chambers in series and each one with two stainless steel electrodes separated by a gap of 0.29 cm with a treatment volume of 0.012 cm^3. The flow rate of the process was adjusted to 60 ml/min and controlled by a variable speed pump (model 75210-25, Cole Palmer; Vernon Hills, IL, USA). A cooling coil was connected before and after each pair of chambers and submerged in an ice-water shaking bath. The temperatures of orange juice and *gazpacho* during HIPEF treatments were monitored and never exceeded 35 °C.

Samples of orange juice and *gazpacho* were treated at field strengths of 15, 25 and 35 kV/cm during 100, 400 and 1000 μs, using pulses of 4 μs width at 200 Hz frequency applied in mono- or bipolar mode. Moreover, the effectiveness of different frequencies (50, 200 and 450 Hz) were tested at 25 kV/cm for 400 μs of total treatment time using 4 μs pulses in bipolar mode. Different pulse widths (2, 4 or 10 μs) were evaluated at 200 Hz in bipolar mode, to reach 400 μs of total treatment time at 25 kV/cm, to study the effect of pulse width on vitamin C content and antioxidant capacity of orange juice and *gazpacho*.

2.4. Thermal treatment

Orange juice and *gazpacho* were heat-pasteurized (90 °C, 1 min) to compare the effects of HIPEF versus a conventional thermal treatment on vitamin C content and antioxidant capacity. Typical heat treatments of juices vary from 95 °C to 90 °C for 15–60 s (Nagy, Chen, & Shaw, 1993).

Products were thermally processed in a continuous flow tubular heat-exchanger (Universitat de Lleida, Lleida, Spain). A gear pump was used to maintain the juice flow rate at 60 ml/min through a stainless steel heat-exchange coil, which was immersed in a hot water shaking bath. After thermal processing, the products were immediately cooled in a heat-exchange coil which was immersed in an ice-water bath.

2.5. Determination of vitamin C

Total vitamin C was determined by HPLC by the method of Soliva-Fortuny et al. (2004) with some modifications. A sample of 25 ml of orange juice or *gazpacho* was homogenized with 10 ml of extraction solution (10% metaphosphoric acid + 0.5% 2,3 dimercapto-1-propanol, BAL, a thiol reducing reagent). The resulting mixture was centrifuged at 15,300*g* for 15 min at 4 °C (Centrifuge Avanti™ J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vacuum-filtered through Whatman No. 1 paper and diluted to 50 ml with distilled water. Then, samples were passed through a 0.45 μm membrane filter. An aliquot of 20 μl was injected into the HPLC system using an NH_2-Spherisorb S5 Column (250 × 4.6 mm, 5 μm). The eluent was acetonitrile:5 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 (40:60). The flow was isocratic at a rate of 1 ml/min at room temperature. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA, USA) at 254 nm. Results were expressed as vitamin C retention related to the untreated sample.

2.6. Determination of antioxidant capacity

The antioxidant capacities of orange juice and *gazpacho* were studied through the evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) radical. From the methodological point of view, the widespread use of the stable $DPPH^{\bullet}$ radical-scavenging model is recommended as easy and accurate, with regard to measuring the antioxidant activity of fruit and vegetable juices or extracts (Da Porto, Calligaris, Celloti, & Nicoli, 2000; Espín, Soler-Rivas, Wichers, & García Viguera, 2000; Sánchez-Moreno, 2002).

The determination was based on the method proposed by De Ancos et al. (2002). This analysis was carried out employing a reaction mixture of aliquots (0.010 ml) of the supernatant of samples of orange or *gazpacho* centrifuged at 6000*g* for 15 min at 4 °C (Centrifuge Medigifer; Select, Barcelona, Spain), 3.9 ml of methanolic $DPPH^{\bullet}$ (0.025 g/l) and 0.090 ml of distilled water. The samples were shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured on a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm against a blank of methanol without $DPPH^{\bullet}$. Results were expressed as percentage of inhibition of the radical $DPPH^{\bullet}$, that is the decrease in absorbance with respect to the control value ($DPPH^{\bullet}$ initial absorption value).

Untreated samples of orange juice and *gazpacho* were pumped through the HIPEF system, as well as through the heat-exchanger without receiving any treatment to check that no differences in vitamin C content or antioxidant capacity existed before and after passing the orange juice or *gazpacho* through the systems.

2.7. Statistical analysis

Each processing condition was assayed in triplicate and determinations were also performed in triplicate.

Analysis of variance was used to determine the effect of processing factors on vitamin C and antioxidant capacity of orange juice and *gazpacho*. The least square difference (LSD) test was employed to determine differences among the treatments ($p < 0.05$). Statistical analysis was performed with the Statgraphics plus version 5.1 for Windows package (Statistical Graphics Co., Rockville, MD, USA).

3. Results and discussion

3.1. Effects of HIPEF treatment conditions on vitamin C retention of orange juice and gazpacho

Vitamin C contents of fresh orange juice and *gazpacho* were 56.3 mg/100 ml and 22.6 mg/100 ml, respectively. The content of vitamin C in fresh orange juices has been widely studied and the results obtained in the present work are in the range of those published in the literature, which varied from 25 mg/100 ml to 68 mg/100 ml (Farnworth, Lagacé, Couture, Yaylayan, & Stewart, 2001; Fernández-García, Butz, Bognàr, & Tauscher, 2001; Kabasakalis, Siopidou, & Moshatou, 2000; Lee & Coates, 1999; Rapisarda, Bellomo, & Intelisano, 2001; Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003). Commercial orange juices showed 31–56 mg/100 ml, 64 mg/100 ml and 49 mg/100 ml in slightly pasteurized, traditionally pasteurized and frozen orange juices, respectively (Sánchez-Moreno et al., 2003). Nevertheless, very little information was found about vitamin C in *gazpacho*. Slightly pasteurized *gazpacho* exhibited 6–11 mg/100 ml of vitamin C (Arnao et al., 2001; Pinilla, Plaza, Sánchez-Moreno, De Ancos, & Cano, 2005). Pinilla et al. (2005) reported 7–13 mg/100 ml and 8 mg/100 ml of vitamin C in traditionally pasteurized and frozen *gazpacho*, respectively. The lower values of vitamin C content in the above-mentioned commercial *gazpacho* compared to those obtained in the present work could be attributed to the different formulations, the origin and varieties of the raw material, and the processing equipment used for their production.

A high retention of vitamin C content in orange juice and *gazpacho* was observed after HIPEF-processing (Figs. 1–4) with maximum values of 98.2% and 97.1% for HIPEF-treated orange juice and *gazpacho*, respectively. Retention of this vitamin after HIPEF treatment was always above 87.5% for orange juice and 84.3% for *gazpacho*, working with 35 kV/cm during 1000 µs at 200 Hz with monopolar pulses of 4 µs. These treatment conditions were reported by Elez-Martínez, Escolà-Hernández, Soliva-Fortuny, and Martín-Belloso (2004), Elez-Martínez, Escolà-Hernández, Soliva-Fortuny, and Martín-Belloso (2005), Elez-Martínez, Suárez-Recio, and Martín-Belloso (in press), Elez-Martínez, Aguiló-Aguayo, and Martín-Belloso (2006) Martín-Belloso, Díaz-Lázaro, and Elez-Martínez (2004) for pasteurization and high

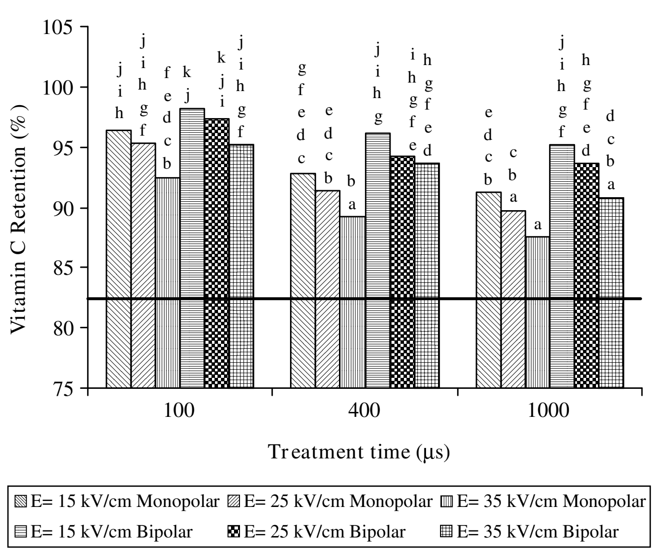

Fig. 1. Retention of vitamin C in orange juice after exposure to mono- and bipolar HIPEF at different electric field strengths (E) and treatment times (mean ± SD). Treatments were performed at 200 Hz and 4 µs square pulses. Horizontal line corresponds to a thermal treatment (90 °C, 1 min). Bars with different letters are significantly different ($p < 0.05$).

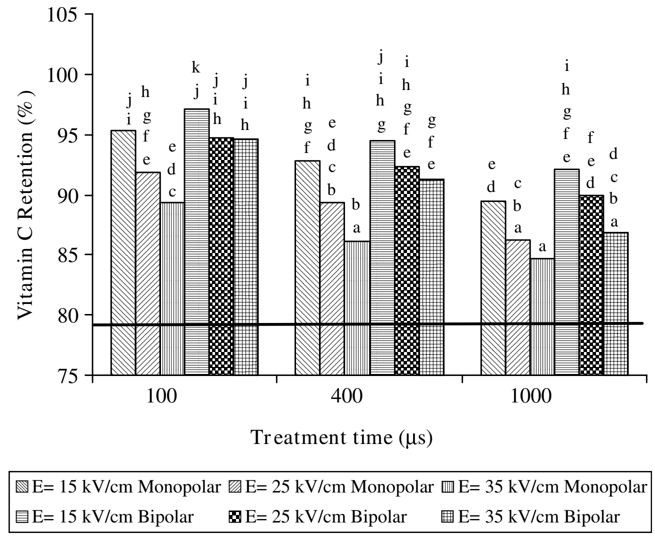

Fig. 2. Retention of vitamin C in *gazpacho* after exposure to mono- and bipolar HIPEF at different electric field strengths (E) and treatment times (mean ± SD). Treatments were performed at 200 Hz and 4 µs square pulses. Horizontal line corresponds to a thermal treatment (90 °C, 1 min). Bars with different letters are significantly different ($p < 0.05$).

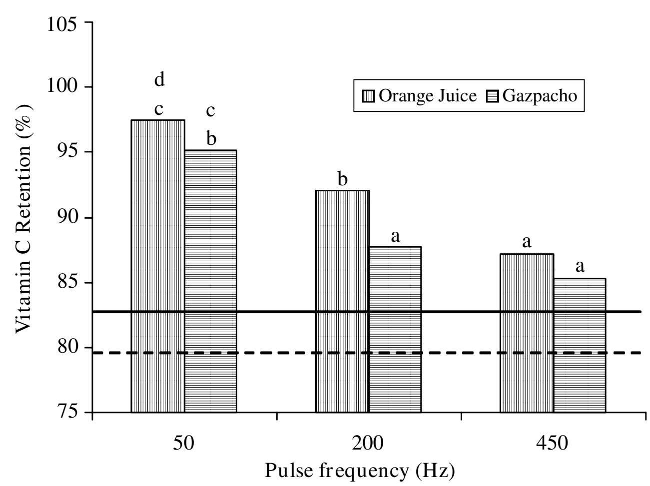

Fig. 3. Effects of pulse frequency on the retention of vitamin C in orange juice and *gazpacho* (mean ± SD). Treatments were performed at 25 kV/cm, 400 µs and square bipolar pulses of 4 µs. Horizontal and discontinuous horizontal lines correspond to a thermal treatment (90 °C, 1 min) for orange juice and *gazpacho*, respectively. Bars with different letters are significantly different ($p < 0.05$).

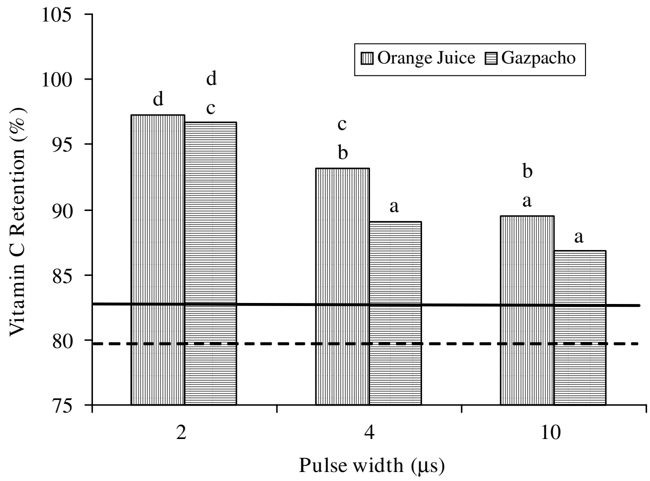

Fig. 4. Effect of pulse width on the retention of vitamin C in orange juice and *gazpacho* (mean ± SD). Treatments were performed at 25 kV/cm, 400 μs, 200 Hz and square bipolar pulses. Horizontal and discontinuous horizontal lines correspond to a thermal treatment (90 °C, 1 min) for orange juice and *gazpacho*, respectively. Bars with different letters are significantly different ($p < 0.05$).

enzyme-inactivation of orange juice and *gazpacho*. Min, Jin, Min, et al. (2003a) reported no differences between fresh orange juice and HIPEF-treated orange juice when they processed orange juice at 40 kV/cm for 97 μs at 2000 Hz with bipolar pulses of 2.6 μs and a maximum temperature of 45 °C. On the other hand, after processing orange juice by HIPEF with bipolar pulses of 4 μs at 800 Hz and 35 kV/cm during 750 μs (maximum temperature of 50 °C), a vitamin C retention of 93% was observed (Sánchez-Moreno et al., 2005). Evrendilek et al. (2000) reported that HIPEF-processing of apple juice did not alter the natural vitamin C of the juice (35 kV/cm, 94 μs, 952 Hz, monopolar pulses of 1.92 μs, maximum temperature 38 °C). Min et al. (2003b) did not observed differences in vitamin C retention between fresh and HIPEF-processed tomato juice at 40 kV/cm for 57 μs with bipolar pulses of 2 μs and a maximum temperature of 45 °C. All of these studies were performed by keeping constant the HIPEF treatment conditions. However, no information about the effect of HIPEF-processing parameters on vitamin C content in foods was found in the literature.

When a thermal pasteurization (90 °C, 1 min) was applied to orange juice and *gazpacho*, the retention levels of vitamin C were 82.4% and 79.2%, respectively. HIPEF-treated products retained a significantly higher content of vitamin C than did heat-pasteurized foods ($p < 0.05$) (Figs. 1–4). Min, Jin, Min, et al. (2003a, 2003b), Yeom et al. (2000) and Sánchez-Moreno et al. (2005) also reported higher levels of vitamin C retention in orange and tomato juices treated by HIPEF compared with those processed by thermal treatment. The maximum temperature reached during HIPEF-processing of samples was 35 °C, whereas 90 °C was maintained for 1 min for heat-pasteurized products. High temperatures led to a loss of vitamin C because heat is known to speed the oxidation process of ascorbic acid (Gahler, Otto, & Böhm, 2003). Moreover, the depletion of vitamin C in fresh juices is also attributed to oxidative enzyme reactions promoted by ascorbate oxidase and peroxidase (Davey et al., 2000; Lee & Coates, 1999). Similar levels of peroxidase inactivation were achieved when processing orange juice by heat or HIPEF treatment (Lee & Coates, 1999; Elez-Martínez et al., 2006). Thus, the higher retention of vitamin C of HIPEF-processed orange juice and *gazpacho* compared to thermally processed products might be due to the much lower processing temperature of HIPEF treatment.

Vitamin C retention depended significantly ($p < 0.05$) on the electric field strength, the treatment time, the pulse frequency, the pulse width and the pulse polarity used during HIPEF-processing of the samples ($p < 0.05$). The lower the electric field strength and the treatment time, the higher was the vitamin C retention in both orange juice and *gazpacho* (Figs. 1, 2). Figs. 1 and 2 also show that vitamin C retention was greater when HIPEF treatment of orange juice and *gazpacho* was conducted in bipolar rather than in monopolar mode. The retention of vitamin C in orange juice and *gazpacho* increased with a decrease of pulse frequency (Fig. 3). In the same way, the shorter the pulse width, the greater was the vitamin C retention (Fig. 4). To sum up, when the HIPEF-processing parameters increased in severity, vitamin C retention was lower. Therefore, minimal processing of foods to assure the microbial stability, as well as the original sensorial and nutritional characteristics of the food, making it more convenient and healthy, is gaining importance.

Orange juice showed a higher vitamin C content than *gazpacho* in both cases, HIPEF-treated and untreated. In addition, orange juice also exhibited greater vitamin C retention than did *gazpacho* ($p < 0.05$) (Figs. 1–4). These differences may be due to the lower pH of orange juice (3.58) than *gazpacho* (4.36), as more acidic conditions are known to stabilize ascorbic acid (Tannebaum, Archer, & Young, 1985). In addition, Gregory (1996) reported that the degradation of ascorbic acid seems to be influenced by many compounds which are involved in the oxidation reaction of vitamin C. Therefore, the influence of HIPEF on other orange juice and *gazpacho* compounds could affect the degradation of vitamin C and, thus, the levels of vitamin C retention.

3.2. Effects of HIPEF treatment conditions on antioxidant capacities of orange juice and gazpacho

Antioxidant capacities of orange juice and *gazpacho* were measured as free radical-scavenging capacity in a DPPH$^{\cdot}$ model. Fresh untreated orange juice and *gazpacho* exhibited 39.3% and 45.7% inhibition, respectively (Table 1). De Ancos et al. (2002) reported an inhibition of 37.5% DPPH$^{\cdot}$ in orange juice. Orange juice and *gazpacho* showed differences in their compositions of bioactive compounds. In this study, it has been observed that vitamin C content was higher in orange juice than in *gazpacho*. Moreover, some authors reported that orange juice had higher levels of carotenoids and phenols than *gazpacho* (Sánchez-Moreno et al., 2003; Pinilla et al., 2005). Thus, the higher free radical-scavenging capacity of *gazpacho* could be attributed to the differences between the bioactive compounds of the products.

Antioxidant capacity of HIPEF-treated orange juice and *gazpacho* were 36.1–41.2% and 42.1–47.4% inhibition of DPPH·, respectively, with non-significant differences between HIPEF-treated and untreated products ($p < 0.05$) (Tables 2–4). The only study about the effect of HIPEF treatment on the antioxidant capacity of food was that reported by Sánchez-Moreno et al. (2005) who treated orange juice with 35 kV/cm for 750 µs at 800 Hz with 4 µs bipolar pulses at less than 50 °C, and they did not observe any effect of this treatment on the radical-scavenging capacity of orange juice.

A 35.4% inhibition of DPPH· in orange juice and 39.6% inhibition of DPPH· in *gazpacho* were observed when products were thermally processed (90°C, 1 min), whereas untreated samples exhibited 39.3% inhibition and 45.7% inhibition, respectively. Orange juice, as well as *gazpacho*, processed by heat, showed a lower level of antioxidant capacity than did untreated orange juice and *gazpacho* ($p < 0.05$). Some HIPEF treatments, such as 1000 µs, 200 Hz, 4 µs pulse width, bipolar or monopolar mode and 15 or 35 kV/cm, led to similar levels of antioxidant capacity in orange juice and *gazpacho* with respect to thermal treatment (Table 2). When orange juice and *gazpacho* were processed at 30 kV/cm for 400 µs at 50 Hz with bipo-

Table 3
Effects of HIPEF pulse frequency and thermal treatment on antioxidant capacity (% inhibition DPPH·) of orange juice and *gazpacho*[a]

Pulse frequency (Hz)	Orange juice	*Gazpacho*
50	40.6 ± 2.3c	47.4 ± 2.8b
200	38.8 ± 2.4bc	43.1 ± 3.1ab
450	36.1 ± 3.0b	44.2 ± 2.8ab
Thermal treatment (90 °C/1 min)	35.4 ± 2.0a	39.6 ± 2.9a
Untreated	39.3 ± 1.5bc	45.7 ± 1.6b

Different letters in the same column indicate significant differences ($p < 0.05$).
HIPEF treatments were performed at 25 kV/cm, 400 µs and bipolar pulses of 4 µs.
[a] Values are means ± SD from three measurements of triplicate treatments.

Table 4
Effects of HIPEF pulse width and thermal treatment on antioxidant capacity (% inhibition DPPH·) of orange juice and *gazpacho*[a]

Pulse width (µs)	Orange juice	*Gazpacho*
2	40.8 ± 2.6c	44.6 ± 3.0b
4	39.1 ± 2.9bc	46.9 ± 2.5b
10	38.9 ± 2.1bc	43.4 ± 2.9ab
Thermal treatment (90 °C / 1 min)	35.4 ± 2.0a	39.6 ± 2.9a
Untreated	39.3 ± 1.5bc	45.7 ± 1.6b

Different letters in the same column indicate significant differences ($p < 0.05$).
HIPEF treatments were performed at 25 kV/cm, 400 µs, 200 Hz and bipolar pulses.
[a] Values are means ± SD from three measurements of triplicate treatments.

Table 2
HIPEF and thermal treatment effects on antioxidant capacity (% inhibition DPPH·) of orange juice and *gazpacho*[a]

Polarity mode	Electric field strength (kV/cm)	Treatment time (µs)	Orange juice	*Gazpacho*
Monopolar	15	100	41.2 ± 2.1de	46.6 ± 2.4bc
		400	37.6 ± 2.7abcd	44.6 ± 2.7bc
		1000	38.1 ± 2.2abcd	43.1 ± 2.9abc
	25	100	37.9 ± 2.6abcd	43.5 ± 2.8abc
		400	38.4 ± 1.7abcde	42.6 ± 3.0abc
		1000	36.3 ± 2.1ab	44.3 ± 2.7bc
	35	100	38.4 ± 2.2abcde	45.2 ± 2.6bc
		400	37.4 ± 2.0abcd	42.1 ± 2.7ab
		1000	37.1 ± 1.9abc	43.5 ± 3.1abc
Bipolar	15	100	38.5 ± 2.2abcde	47.1 ± 2.6c
		400	37.9 ± 2.4abcd	46.2 ± 2.9bc
		1000	37.8 ± 2.9abcd	43.9 ± 2.4abc
	25	100	40.2 ± 2.1cde	46.5 ± 3.0bc
		400	42.1 ± 3.5e	43.4 ± 2.6abc
		1000	39.6 ± 2.4bcde	44.3 ± 3.1bc
	35	100	37.4 ± 2.6abcd	43.2 ± 2.9abc
		400	39.8 ± 2.5bcde	45.9 ± 2.7bc
		1000	37.6 ± 2.2abcd	44.1 ± 3.1abc
Thermal treatment (90 °C/1 min)			35.4 ± 2.0a	39.6 ± 2.9a
Untreated			39.3 ± 1.5bcde	45.7 ± 1.6bc

Different letters in the same column indicate significant differences ($p < 0.05$).
HIPEF treatments were performed at 200 Hz and pulses of 4 µs.
[a] Values are means ± SD from three measurements of triplicate treatments.

lar pulses of 4 μs, the antioxidant activity was greater than that observed after heat-treatment (Table 3). After HIPEF treatments of 400 μs at 30 kV/cm, 200 Hz and bipolar pulses of 2 or 4 μs, orange juice and *gazpacho* showed higher levels of free radical-scavenging activity than did heat-treated products (Table 4). The antioxidant capacities obtained in heat- and HIPEF-treated foods suggested that HIPEF treatment may induce changes in antioxidant compounds of orange juice and *gazpacho*.

In this study, a slight reduction of vitamin C content in orange juice and *gazpacho* was observed when those foods were treated with HIPEF. The variation in vitamin C retention due to HIPEF was not reflected in a depletion in free radical-scavenging capacity of the products. It has been reported that vitamin C and flavonoid compounds make a significant contribution to the antioxidant capacity of orange juice, whereas the contribution of carotenoids is negligible (Arena, Fallico, & Maccarone, 2001; Gardner et al., 2000; Miller & Rice-Evans, 1997; Rapisarda et al., 1999). *Gazpacho* soup contains polyphenols (quercetin, kaempherol and chlorogenic acid) and vitamin C from tomatoes, green pepper, and onions, which may supply antioxidative-related health benefits (Pinilla et al., 2005). Besides vitamin C, there are other compounds which also contribute to *gazpacho* antioxidant capacity (Arnao et al., 2001). In consequence, the small depletion of vitamin C retention, followed by the permanence of the antioxidant capacity of HIPEF-treated products, could be due to the stability of other antioxidant compounds, such as carotenoids and phenols, or to their variation during HIPEF-processing which results in the formation of new compounds with higher antioxidant capacity. Nevertheless, further investigations are still needed to explain this phenomenon.

The strongest HIPEF conditions selected for this study were similar to those used for high enzyme-inactivation (Elez-Martínez et al., in press, 2006), that are more severe than those needed for the destruction of microorganisms in orange juice and *gazpacho* (Elez-Martínez et al., 2004, 2005; Martín-Belloso et al., 2004). *Lactobacillus brevis* and *Saccharomyces cerevisiae* suspended in orange juice were inactivated up to 5.8 log reductions and 5.1 log reductions, respectively, when orange juice was processed at 35 kV/cm for 1000 μs using a 4 μs pulse width in bipolar mode and 200 Hz (Elez-Martínez et al., 2004, 2005). Using the same HIPEF-treatment conditions, a 93% peroxidase inactivation and a 75% pectin methyl esterase inactivation were achieved in orange juice (Elez-Martínez et al., in press, 2006). An HIPEF treatment of 35 kV/cm, 1000 μs, 200 Hz and bipolar pulses of 4 μs led to 5.1 log reductions in *L. brevis* inoculated in *gazpacho* (Martín-Belloso et al., 2004). Even under these severe conditions, HIPEF-processed foods retained more vitamin C and the antioxidant capacity was higher than did the heat-treated products. Therefore, stable orange juice and *gazpacho*, with minimal changes in their health-promoting properties, could be obtained by processing orange juice and *gazpacho* by HIPEF.

4. Conclusions

HIPEF-treated orange juice and *gazpacho* better retained vitamin C than did thermally-treated products. Vitamin C retention depended on the food and the process parameters, such as electric field strength, treatment time, pulse frequency, width and polarity. Bipolar pulses led to higher contents of vitamin C and the lower the electric field strength, the treatment time, the pulse frequency and the pulse width, the higher was the vitamin C retention. Greater levels of retention of vitamin C were achieved in HIPEF-treated orange juice than in HIPEF-treated *gazpacho*. HIPEF treatment did not affect the antioxidant capacity of orange juice and *gazpacho*. A pasteurization HIPEF treatment of 35 kV/cm for 1000 μs at 200 Hz and bipolar pulses of 4 μs led to 90.8% and 86.8% vitamin C retentions, and 37.6% and 44.1% inhibition of DPPH$^{\cdot}$ in orange juice and *gazpacho*, respectively. Higher contents of vitamin C were not related to higher levels of antioxidant capacity. The presence of other bioactive compounds may contribute to antioxidant capacity of orange juice and *gazpacho*. Further research is required to elucidate the effects of HIPEF-process parameters on the bioactive compounds of foods. HIPEF-processing has good prospects for use in the food industry as an alternative to thermal pasteurization, in order to achieve healthy foods.

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