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Influence of high-intensity pulsed electric field processing parameters on antioxidant compounds of broccoli juice

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ABSTRACT

The effect of high-intensity pulsed electric field (HIPEF) processing parameters (electric field strength, treatment time, and polarity) on broccoli juice carotenoids, vitamin C, total phenolic (TP) content and antioxidant capacity (AC) was evaluated. Results obtained from HIPEF-processed broccoli juice were compared with those of thermally treated (90 °C/60 s) and untreated juices. HIPEF processing parameters influenced the relative content (RC) of bioactive compounds, and the relative AC (RAC). Maximum RC of lutein (121.2%), β-carotene (130.5%), TP (96.1%), vitamin C (90.1%) and RAC (5.9%) was reached between 25 and 35 kV/cm and from 2000 μs to 500 μs. The highest RAC and RC of bioactive compounds were observed in HIPEF treatments applied in bipolar mode, except for vitamin C. HIPEF-treated broccoli juice exhibited greater RC of bioactive compounds and RAC than juice treated by heat. HIPEF technology could be considered a promising option for preserving the antioxidant quality of broccoli juice.

Industrial relevance: Vegetable juices are becoming more and more popular because of their wide range of health-related compounds. Particularly, broccoli juice is attracting the food industry attention because it contains high amounts of vitamins, carotenoids and phenolic compounds, among other bioactive compounds. Broccoli juice requires treatment conditions that protect its microbial, nutritional and sensorial quality. HIPEF is a non-thermal technology for liquid food preservation that inactivates microorganisms and enzymes without compromising the nutritional and sensorial features of foods. Consequently, this technology could be used in the food industry as an alternative for thermal treatment to preserve the bioactive compounds present in vegetable juices, offering to consumers a healthy product.

1. Introduction

Broccoli (*Brassica oleraceae* var. *italica*) is a green vegetable with a high content of chlorophylls, glucosinolates and other bioactive compounds, such as carotenoids (Singh, Upadhyay, Prasad, Bahadur, & Rai, 2007), vitamin C (Combs, 1998; Heinonen & Meyer, 2002), and phenolic compounds (Faller & Fialho, 2010; Mattila & Hellström, 2007). Epidemiological studies have demonstrated that these bioactive compounds stimulate the immune system (Combs, 1998; Harrison & May, 2009), prevent cardiovascular diseases (Vermerris & Nicholson, 2006), and exert chemoprotection against different types of cancer (Huang et al., 1992; Jeffery & Araya, 2009).

Recently, the consumption of broccoli has increased worldwide (Latté, Appel, & Lampen, 2011). As a result, new broccoli-based products, such as blends of broccoli and apple juices have been suggested to further increase its consumption (Houška et al., 2006). Overall, vegetable juices represent an interesting alternative for incorporating bioactive compounds in the diet and a special attention has been focused on broccoli juice due to its elevated content of bioactive compounds that provide important health benefits (Lee et al., 2013; Mandelová & Totusek, 2007).

Traditionally, juices have been processed by heat to avoid the harmful influence of enzymes and microorganisms. Nevertheless, thermal processing causes losses of nutritional and sensorial characteristics of foods. As alternatives to thermal processing, non-thermal technologies have been developed. High-intensity pulsed electric field (HIPEF) is a non-thermal preservation technology for liquid foods that has shown promising results for microbial reduction (Mosqueda-Melgar, Elez-Martínez, Raybaudi-Massilia, & Martín-Belloso, 2008) and enzymatic inactivation (Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2013). Besides, some studies have demonstrated that HIPEF processing is effective for maintaining bioactive compounds of liquid foods. In the same way, Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny, Gimeno-Añó, and Martín-Belloso (2007) reported that bioactive compounds such as, lycopene and vitamin C concentration, as well as increased antioxidant levels in HIPEF-treated tomato juice.

The effects of HIPEF processing on bioactive compounds in fruit juices have been widely reviewed (Odriozola-Serrano et al., 2013; Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009). Odriozola-

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Serrano et al. (2013) observed as general tendency that bioactive compounds, such as vitamin C, carotenoids and phenolics were better retained in HIPEF-processed juices than in those thermally-treated ones. However, literature related to the effect of HIPEF on bioactive compounds present in green vegetable juices is still scarce (Sánchez-Vega, Elez-Martínez, & Martín-Belloso, 2014). Therefore, the purpose of this study was to evaluate the effect of HIPEF processing parameters (electric field strength, treatment time, and polarity) on the content of carotenoids (lutein and β-carotene), vitamin C, total phenolic compounds (TP) and antioxidant capacity (AC) of broccoli juice. Furthermore, the effects of HIPEF and traditional thermal pasteurization on relative content (RC) of bioactive compounds and relative antioxidant capacity (RAC) of broccoli juice were also compared.

2. Materials and methods

2.1. Reagents

Magnesium carbonate, ethyl acetate, metaphosphoric acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), stock solutions of lutein and β-carotene were purchased from Aldrich Chemical Co. (St Louis MO, USA); sodium sulfate anhydrous, ascorbic acid, Folin–Ciocalteu reagent, gallic acid and diethyl ether were supplied by Scharlau Chemie, SA (Barcelona, Spain). Methanol and propanone were obtained in Teknokroma (Barcelona, Spain).

2.2. Sample preparation

Broccoli (*B. oleraceae* var. *italica*) was purchased at commercial maturity in a local supermarket (Lleida, Spain). Broccoli was cut discarding leaves and stalk, and then it was crushed. The resulting juice was filtered through cheesecloth and vacuum degassed for 10 min. The electrical conductivity of broccoli juice was analyzed (Testo 240 conductivimeter; Testo GmBh & Co, Lenzkirch, Germany), resulting in 0.9 S/m. Afterwards, broccoli juice was divided in three batches; one for HIPEF processing, the second for thermal treatment and the third was maintained unprocessed.

2.3. HIPEF treatments

HIPEF treatments were carried out using a continuous-flow bench-scale system (OSU-4F, Ohio State University, Columbus, OH) that held monopolar or bipolar square wave pulses. The treatment system consisted of eight colinear chambers serially connected. Each chamber consisted of two stainless steel electrodes separated by a gap of 2.92 mm, whose treatment volume was 0.012 cm^3. The treatment flow was controlled by a variable-speed pump (model 75210-25, Cole Parmer Instruments Company, Vernon Hills, IL, USA). The maximum temperature registered in the chamber outlet during HIPEF processing of broccoli juice never exceeded 35 °C. This temperature was maintained through a cooling coil connected between each pair of chambers and submerged in an ice-water shaking bath.

The input of electrical energy density (Q, J/m^3) supplied to the samples was calculated through Eq. (1) (Martín, Zhang, Castro, Barbosa-Cánovas, & Swanson, 1994):

$$Q = \frac{V_0 * I * t}{v} \tag{1}$$

where V_0 is the peak voltage (V), I the intensity of the current (A), t the treatment time (s), and v the volume of all treatment chambers (m^3).

2.4. Thermal treatments

Broccoli juice was heat-treated (90 °C for 60 s) in a tubular stainless steel heat exchange coil immersed in a hot water shaking bath using a gear pump (University of Lleida, Spain). Afterwards, the juice was immediately cooled in an ice water-bath through immersion of the heat exchange coil.

2.5. Bioactive compounds

2.5.1. Carotenoids

The extraction of carotenoids was based on the method described by Cano (1991) with some modifications. A portion of 20 mL of broccoli juice was mixed with 20 mL of chilled propanone and then magnesium carbonate (1 g) and sodium sulfate (10 g) was added. This mixture was vigorously homogenized for 1 min and filtered. The residues were washed with chilled propanone until they become colorless. The filtrate volume was reduced to 5 mL by rotoevaporation and transferred to a separatory funnel where diethyl ether (30 mL) and saturated sodium chloride solution (20 mL) were added. The washing procedure was repeated 3–5 times and then it was vigorously shaken. The organic phase was dehydrated with analytical grade sodium sulfate anhydride and the diethyl ether was eliminated by rotoevaporation. An aliquot of 4 mL of propanone was added to reconstitute the sample containing the carotenoids. Then this solution was filtered (0.45 μm, Millipore Iberica S.A., Spain) and analyzed by HPLC.

A portion of 25 μL of the carotenoid extract was injected into the HPLC equipment using a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 × 250 mm). A gradient elution was composed of methanol/water (75:25), as eluent A, methanol (100%), as eluent B (cleaning solution), and ethyl acetate, eluent C (Table 1). The flow rate was fixed at 1.0 mL/min and the column temperature was maintained at 30 °C. The 2996 Waters Photodiode Array Detector, (Milford, MA) was adjusted at 440 nm. Carotenoids were quantified by a comparison with external standards and expressed as mg of lutein or β-carotene per 100 mL of broccoli juice.

2.5.2. Vitamin C

Vitamin C content of broccoli juice was determined by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (2007). Briefly, broccoli juice was mixed with 4.5% metaphosphoric solution in a proportion 1:1. The mixture was homogenized and centrifuged at 2401 *g* for 10 min at 4 °C (Centrifuge AVANTI™ J-25, Beckman Instruments Inc., Fullerton, CA, USA). Then, the vacuum-filtered sample was passed through a Millipore 0.45-μm membrane to be injected in the HPLC system.

An aliquot of 20 μL of extract was injected into the HPLC system using a reverse-phase C18 Spherisorb ODS2 (5 μm) stainless-steel column (4.6 mm × 250 cm). The mobile phase was a 0.01% sulfuric acid solution adjusted to pH = 2.6. The flow rate was 1 mL/min at room temperature. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Identification of the ascorbic acid was carried out comparing the retention time and UV–visible absorption spectrum of the juice samples with those of the standards (ascorbic

Table 1
Mobil phase gradient for determination of carotenoids by HPLC.

Time (min)	Flow (mL/min)	A (%)	B (%)	C (%)
0	1	80	0	20
5	1	77.5	0	22.5
22.5	1	50	0	50
27.5	1	50	0	50
30	1	20	0	80
35	1	20	0	80
40	1	0	0	100
45	1	0	0	100
50	1	0	100	0
70	1	0	100	0
71	1	80	0	20
80	1	80	0	20

A: methanol/water (75:25). B: methanol (100%). C: ethyl acetate (100%).