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The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage

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ABSTRACT

The effects of high-pressure processing – HPP – (450 and 600 MPa/3 min/20 °C) on the colour, carotenoids, ascorbic acid, polyphenols and antioxidant activity (FRAP and DPPH) of a smoothie were compared to thermal processing (80 °C/3 min). Stability during 45 days at 4 °C was also evaluated. HPP samples showed slight differences ($p < 0.05$) in colour compared to untreated smoothies. Both HPP significantly increased the extractability of lycopene, β-carotene and polyphenols compared to untreated samples. After HPP, ascorbic acid was retained by more than 92% of the initial content. The best results for antioxidant activity were obtained when HPP was applied at 600 MPa. FRAP and DPPH showed a high correlation with ascorbic acid ($R^2 = 0.7135$ and 0.8107, respectively) and polyphenolic compounds ($R^2 = 0.6819$ and 0.6935, respectively), but not with total carotenoids. Changes in bioactive compounds during the storage period were lower in the HPP smoothie than in the thermal-treated sample.

1. Introduction

Regular consumption of the bioactive compounds present in food has been strongly associated with a reduced risk of cancer, cardiovascular disease, stroke, diabetes, Alzheimer's disease, cataracts, and some of the functional declines associated with ageing (Dauchet, Amouyel, Hercberg, & Dallongeville, 2006). Polyphenols, vitamin C, carotenoids, tocopherols, casein phosphopeptides and certain minerals – among others – may protect human cell systems from oxidative damage through a variety of complementary and synergic mechanisms (Zhang et al., 2014).

Today consumers are increasingly demanding better-quality, healthier food, with good nutritional characteristics similar to their fresh equivalents, in order to ensure their food safety. The need to produce food following strict quality parameters has led to the implementation of new, less aggressive conservation technologies other than traditional heat treatments, including high-pressure processing, pulsed electric field, dense-phase carbon dioxide, ozone processing, ultrasound, ultraviolet light, irradiation, and ohmic heating (Oms-Oliu, Odriozola-Serrano, & Martín-Belloso, 2012).

High hydrostatic pressure technology, also known as high-pressure processing (HPP), involves applying very high pressures (100–1000 MPa) from 0 °C to 100 °C for a short time (a few seconds to over 20 min) to packaged food using water as a medium to transmit pressure. One major drawback in the fruit-juice industry is the loss of the sensory and functional quality that occurs during thermal pasteurisation. HPP technology is seen as an interesting treatment for the refrigerated storage of these products, as it can potentially stabilize bioactive compounds and antioxidant activity, thereby avoiding the loss of these important food

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properties and obtaining beverages with significant health benefits, while preserving the nutritional and sensorial characteristics and extending their shelf life (Barba, Cortés, Esteve, & Frígola, 2012; Cao et al., 2012; Patras, Brunton, Da Pieve, Butler, & Downey, 2009; Plaza et al., 2011; Zhou et al., 2014).

The aim of this study was to investigate the effect of high hydrostatic pressure and subsequent storage period (45 days) at 4 °C on colour, bioactive compounds (ascorbic acid, carotenoids, and polyphenols) and antioxidant activity (measured as FRAP and DPPH) in a smoothie, compared to the same untreated and thermally treated beverage.

2. Materials and methods

2.1. Chemicals

Lycopene, β-carotene, gallic acid, ferric chloride, TPTZ [2,4,6-tris(2-pyridyl)-*s*-triazine], Trolox [6-hydroxy-2,5,7,8-tetra methylchromane-2-carboxylic acid] and DPPH [2,2-diphenyl-1-picrylhydrazyl] were supplied by Fluka (Sigma–Aldrich, Buchs, Switzerland) with over 97% purity. Ascorbic acid, acetic acid, sodium chloride, magnesium carbonate, sodium carbonate and anhydrous sodium sulphate were obtained from Panreac (Barcelona, Spain). Metaphosphoric acid, HPLC grade hexane, dichloromethane, acetonitrile, acetone, methanol, petroleum ether and tetrahydrofuran were purchased from Scharlau (Barcelona, Spain). The water used was previously purified in a Milli-Q system (Millipore, Bedford, MA, USA). Sulphuric acid was acquired from Merck (Darmstadt, Germany). Stock solution of each standard was prepared in the adequate solvent and stored in glass bottles at refrigerated temperature (4 ± 1 °C) in the dark until use.

2.2. Samples

The smoothies were obtained by mixing 500 ml of orange juice (*Citrus sinensis cv Valencia Late*), 135 ml of papaya juice (*Carica papaya cv Maradol*), 135 ml of melon juice (*Cucumis melo* L. *Cantaloup*), 130 ml of carrot puree (*Daucus carota L. cv Nantes*) and 100 ml of skimmed milk (Leche Pascual, Burgos, Spain). Melon samples were kindly supplied by Syngenta. The other ingredients used were purchased in several supermarkets in Madrid (Spain) and were chosen with an optimum degree of maturity. The oranges were squeezed in a Z14 blender (Zummo Mechanical Innovaciones SA, Valencia, Spain). The other fruits were pureed in a Vitamix VI-5086 blender (Ripex, Naucalpan de Juárez, Mexico). After mixing, ascorbic acid (20 mg/100 ml) was added and the samples were packed in polyethylene containers. Immediately after, the packed smoothies were randomly divided into four groups: untreated, which was kept as a control; thermally-treated; and HPP at different pressure conditions. Untreated and treated samples were stored at 4 °C for 30 and 45 days, respectively.

2.3. High-pressure and thermal processing

The smoothie was pressurised with a semi-industrial Hiperbaric 55 (Hiperbaric, Burgos, Spain). The containers were weighed before and after the treatments to ensure the goodness of the process. The smoothies underwent two high-pressure treatments: 450 and 600 MPa. In both cases the time and temperature were maintained constant (3 min, 20 °C). HP processing was compared to thermal treatment, as this is the most common option used in fruit juice production.

The thermal processing (TP) was performed at 80 °C for 3 min. After heating, the samples were immediately cooled in an ice water bath and then kept under refrigeration (4 °C). Equivalent thermal and HP processing conditions in terms of microbial safety were chosen for a clearer comparison of both treatments (Vervoort et al., 2011).

2.4. Colour

L^* (lightness), a^* (green to red), and b^* (yellow to blue) parameters were measured using a Minolta CR-200 Chroma Meter (Minolta, Osaka, Japan) in reflection mode. The values provided for each sample were the average of six replicates. The equipment was previously calibrated against a white colour standard. The total colour differences (ΔE^*) were calculated using the following formula:

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \quad (1)$$

where ΔL^*, Δa^* and Δb^* are the differences between the colour of the untreated and treated smoothies on day 0.

2.5. Carotenoids

Carotenoids (5 g) were extracted with tetrahydrofuran (THF) in sonication at room temperature during 15 min. Magnesium carbonate and ascorbic acid were added to prevent oxidation. After centrifugation (6500 rpm, 15 min), the extraction was repeated until the residue was colourless. The supernatants were then quantitatively transferred to a flask and THF was evaporated. The aqueous phase was washed four times in a separating funnel with petroleum ether and the organic phase was washed three times with sodium chloride solution. The aqueous phase was then discarded and the organic phases were pooled and dewatered with anhydrous sodium sulphate. The extract was evaporated to dryness under N_2 stream, and dissolved in 5 ml of hexane: methanol:acetonitrile:dichloromethane (40:20:20:20, v/v/v/v). Re-dissolution was ensured with 5 min of sonication. Before injection in the HPLC system, samples were filtered through a 0.45 μm nylon filter. Aliquots of 10 μL were injected, and chromatographic separation was performed at 30 °C with a Kinetex 5 μm C18 LC column 250 × 4.6 mm, coupled with a guard-pack C18 (5 μm) precolumn (Phenomenex, Torrance, California, USA) in an Agilent 1100 Series chromatograph (Agilent Technology, Palo Alto, CA, USA). The mobile phases were methanol (A), hexane:dichloromethane (1:1, v/v) (B), and acetonitrile (C). The gradient profile for the separation was set as follows: 0–10 min, linear, 15:10:75%; 10–36 min, gradient, 15:35:50%; and 36–41 min, gradient, 15:10:75 for return to initial conditions. The flow rate was set at 0.5 ml/min and the eluted compounds were monitored by a diode array detector (DAD) at 402, 440, and 475 nm. The spectrum was recorded between 200 and 600 nm. Identification was done by comparing the retention times with pure standards, coelution with a spike of standard and the specific spectrum of each compound analysed (Andrés, Villanueva, Mateos-Aparicio, & Tenorio, 2014). The α- and ε-carotenoids were quantified with the calibration curve of β-carotene. All operations were carried out in amber flasks, under yellow light and ensuring the samples did not heat to over 40 °C in order to prevent degradation of the analytes.

2.6. Ascorbic acid

The samples (5 g) were diluted with 4.5% metaphosphoric acid. Identification and quantification were done by HPLC with a Kinetex 5 μm C18 column (250 mm × 4.6 mm) at an oven temperature of 25 °C. The mobile phase was Milli-Q water (Merck Millipore, Billerica, Massachusetts, USA) acidified with H_2SO_4 (pH