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High hydrostatic pressure treatment and storage of soy-smoothies: Colour, bioactive compounds and antioxidant capacity

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Chemical compounds:
Lycopene (PubChem CID: 446925)
Beta-carotene (PubChem CID: 5280489)
Ascorbic acid (PubChem CID: 54670067)
Caffeic acid (PubChem CID: 689043)
p-Coumaric acid (PubChem CID: 637542)
Chlorogenic acid (PubChem CID: 1794427)
Hesperidin (PubChem CID: 10621)
Genistin (PubChem CID: 5281377)
(−)-Epicatechin (PubChem CID: 72276)
Daidzin (PubChem CID: 107971)

A B S T R A C T

Bioactive compounds: lycopene, α-, β- and ε-carotenes, ascorbic acid, chlorogenic, p-coumaric, caffeic, hesperidin, narirutin, genistein, daidzin, daidzein, catechin and epicatechin were quantified in order to provide new information on high pressure (HP) processing (550 and 650 MPa/3 min/20 °C) compared to pasteurization (80 °C/3 min) in a multifruit-soymilk smoothie. Antioxidant activity (FRAP and DPPH), colour differences and storage effects (45 days/4 °C) were also investigated. HP maintained better original colour ($\Delta E < 2.82$) than pasteurization ($\Delta E = 3.70$), and did not modify the content of bioactive components (α- and ε-carotenes, ascorbic acid, total polyphenols); it even increased the concentration of lycopene and β-carotene and had higher antioxidant capacity than in heat-treated samples. Most remained quite stable under cold storage. About 55% of the ascorbic acid, the main compound relating to antioxidant capacity ($r = 0.7399$ for FRAP and $r = 0.8944$ for DPPH), was retained at the end of the storage period.

1. Introduction

The beneficial effects of fruits, vegetables and their derivatives are associated with bioactive components such as carotenoids, phenolic compounds and vitamin C. The health-giving properties of these compounds have been strongly linked with a decreased risk of several chronic diseases, including cardiovascular disease, stroke, diabetes, Alzheimer's disease, cataracts, and certain types of cancer (Liu, 2003). Smoothies are an increasingly popular way of consuming fruits, and contribute to the intake of bioactive compounds (Keenan et al., 2010). The addition of soymilk to these beverages supplies proteins which increase their nutritional and functional values, and also make them suitable for people with lactose intolerance and bovine milk protein allergy.

Recent studies by Morales de la Peña, Salvia Trujillo, Rojas Graü, and Martín Belloso (2011) reported that a fruit juice-soymilk beverage was a good source of vitamin C and isoflavones, and displayed high antioxidant capacity. The potential of soy is currently being explored by the food industry as its consumption is assumed to have beneficial health effects in humans (Rostagno, Villares, Guillamón, Garcia-Lafuente, & Martinez, 2009). Because of their relatively high consumption, oranges and orange juices are an important source of polyphenolic compounds (caffeic acid,

Abbreviations: HPP, high pressure processing; TP, thermal processing; TPC, total phenolic compounds; FRAP, ferric reducing antioxidant power; DPPH, 2,2, diphenyl-1-picrylhydrazyl; TPTZ, 2,4,6, tripyridil-s-triazine.

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hesperidin and narirutin), ascorbic acid, and carotenoids (Barba, Cortés, Esteve, & Frígola, 2012; Klimczak, Małecka, Szlachta, & Gliszczyńska-Świgło, 2007; Plaza et al., 2006). Cantaloupe is one of the most widely consumed fruit crops in the world due to its pleasant flavour and nutritional value. It is a good source of pro-vitamin A, vitamin C and flavonoids and its pulp extract has high antioxidant and anti-inflammatory properties (Ismail, Chan, Mariod, & Ismail, 2010). Papaya is a good source of carotenoids, vitamin C and hydroxycinnamic acids (Gayosso-García Sancho, Yahia, & González-Aguilar, 2011). Vegetables such as carrots are often part of the composition of smoothies. Carrot juice is a popular beverage consumed throughout the world, and widely accepted as an important source of healthy components such as carotenoids, vitamins and hydroxycinnamic acid derivatives (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001; Martínez-Flores, Garnica-Romo, Bermúdez-Aguirre, Pokhrel, & Barbosa-Cánovas, 2015).

Fruit juices are usually preserved by pasteurization in order to inactivate microorganisms and enzymes. However, heat treatments may impair their sensorial and nutritional properties, including loss of vitamin C and carotenoids and changes in flavour and aroma at the temperatures required to inactivate the pectin methyl esterase: 90 °C for 1 min (Bull et al., 2004). In recent decades new technologies such as high pressure processing (HPP) have been developed to preserve foods. This technology applies very high pressures (100–1000 MPa) from 0 °C to less than 100 °C for a short time (a few seconds to around 20 min) to packaged food using water as a medium to transmit pressure (Barba, Cortés, et al., 2012; Barba, Esteve, et al., 2012). HPP has many advantages over heat processing. It can provide safe products with a reduced processing time, while maintaining maximum freshness and flavour in the product. It is also environmentally friendly, since it requires only electrical energy and it does not generate waste by-products (Toepfl, Mathys, Heinz, & Knorr, 2006).

Several studies related to the stability of bioactive compounds in pasteurized juices and HPP juices during refrigerated storage refer to the advantages of high pressure technology due to the stability of carotenoids and polyphenols, and the lower losses of vitamin C content with respect to the pasteurized juice, leading to an extension of the shelf life (Barba, Esteve, & Frígola, 2012; Bull et al., 2004; Esteve, Palop, Barba, & Frígola, 2009; Plaza et al., 2011; Polydera, Stoforos, & Taoukis, 2005).

The aims of the present paper were (1) to determine and compare the colour and bioactive compounds (carotenoids, ascorbic acid and polyphenols) in a soymilk smoothie treated by HPP with the equivalent untreated and heat-treated smoothie; (2) to measure the antioxidant activity (FRAP and DPPH) developed by these compounds; and (3) to evaluate the effect of refrigerated storage (4 °C) for 45 days on colour and bioactive content.

2. Material and methods

2.1. Chemicals

Lycopene, β-carotene, gallic acid, ferric chloride, epicathequin, catechin, TPTZ [2,4,6-Tris(2-pyridyl)-*s*-triazine], Trolox [6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] and DPPH [2,2-diphenyl-1-picrylhydrazyl] were obtained from Fluka (Sigma–Aldrich, Buchs, Switzerland) with over 97% purity. Ascorbic acid, acetic acid, sodium chloride, magnesium carbonate, sodium carbonate and anhydrous sodium sulphate were acquired from Panreac (Barcelona, Spain). Standards of chlorogenic acid, caffeic acid, *p*-coumaric acid, narirutin, hesperidin, daidzin, daidzein and genistein were obtained from Extrasynthèse (Genay Cedex, France) with over 90% purity. Metaphosphoric acid, hexane, dichloromethane, acetonitrile, acetone, methanol, petroleum ether and tetrahydrofuran were purchased from Scharlau (Barcelona, Spain). Ultra-pure water was supplied by a Milli-Q Advantage A10 water purifier system from Millipore Iberica (Madrid, Spain). Sulphuric acid was supplied by Merck (Darmstadt, Germany). All the reagents used were analytical grade and the solvents were HPLC grade. Stock solution of each standard was prepared in the adequate solvent and stored in glass bottles at −18 °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. cv. Cantalupensis), 130 mL of carrot puree (*Daucus carota* L. cv. Nantes) and 100 mL of soymilk (ViveSoy Ligera, 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 (intense colour, characteristic aroma and firmness). 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 (200 mg/L) 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 the two 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 pressurized 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: 550 and 650 MPa. In both cases the time and temperature were maintained constant (3 min, 20 °C). Time taken to reach the target pressures was approximately 120 s and depressurization was immediate (less than 3 s). The holding time in this study describes the time each sample was held at a given pressure and did not include the pressurization and depressurization times. The contribution of increased temperature during treatment, caused by adiabatic compression, was negligible. Water was used as the pressure-transmitting fluid. 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).

2.4. Colour

L^* (lightness), a^* (green to red), and b^* (blue to yellow) parameters were measured using a Minolta CR-200 Chroma Meter (Minolta, Osaka, Japan). 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:

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

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