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Influence of high pressure processing on microbial shelf life, sensory profile, soluble sugars, organic acids, and mineral content of milk- and soy-smoothies

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ABSTRACT

Smoothies are an excellent way to intake nutrients and bioactive compounds from both the fruits and the milk or soymilk with which they are made. High pressure processing (HPP) is an efficient alternative to traditional thermal pasteurization (TP), obtaining microbiologically-safe smoothies with minimum impact on nutritional and sensorial properties. Untreated, pasteurized (80 °C/3 min) and HPP (450–650 MPa for 3 min at 20 °C) milk- and soy-smoothies were compared. Milk- and soy-smoothies showed a total reduction in microorganisms after pasteurization and HPP at the pressure conditions applied. HPP maintained microbial stability until the end of the storage period (45 days at 4 °C). Soluble sugars (glucose and fructose), organic acids (citric, malic, tartaric, oxalic and quinic) and minerals (sodium, potassium, calcium, magnesium, iron, copper, zinc and manganese) showed no significant changes after the treatments and storage. No significant differences were found in sensorial attributes between untreated and HPP samples, although the aroma and acceptability scores decreased significantly for pasteurized smoothies. Based on the data obtained, 450 MPa are sufficient to obtain safe smoothies whose organoleptic properties are equally acceptable to consumers as freshly-made smoothies.

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1. Introduction

Today's consumer is increasingly demanding new and healthier ready-to-eat products similar to their natural fresh equivalents, with minimal processing, good nutritional qualities and guaranteed food security (Hendrickx & Knorr, 2002). The food industry in the 21st century is responding to this demand by developing new functional products involving the application of novel processing technologies. Smoothies are an excellent way to intake the nutrients and bioactive compounds responsible for health effects. The addition of milk incorporates proteins and calcium and increases their nutritional value. Other variants such as the addition of soymilk facilitate consumption by the lactose-intolerant population. Their properties, appearance, taste and the fact that they are ready to drink are all decisive factors in their popularity (Keenan et al., 2010; Zulueta, Esteve, & Frígola, 2007).

One of the major drawbacks in the juice industry is the loss of sensory and nutritional quality that occurs with thermal pasteurization (Cardello, Schutz, & Lesher, 2007). HPP is an efficient alternative technique that involves applying very high pressures (100–1000 MPa) at below 0 °C to over 100 °C for a short time (a few seconds to 20 min) to the packaged food in order to eliminate harmful pathogens and the microorganisms responsible for vegetative spoilage, and to inactivate enzymes, with minimal modifications in nutritional and sensory quality (Oey, Lille, Van Loey, & Hendrickx, 2008). The effect of HPP on the quality characteristics of food has been mainly attributed to the stability of covalent bonds under high pressure (Knorr, 1993). This also avoids the use of additives in the formulation to give the consumer an increased sensation of a fresh product (Ferrari, Maresca, & Ciccarone, 2010; Keenan et al., 2010).

The aim of the present study is to investigate the effect of high-pressure treatment on the microbial shelf life, sensory profile, sugars, organic acids and minerals of a smoothie made with milk or soymilk, and determine whether these properties are maintained over 45 days under refrigerated storage at 4 °C, compared to the same untreated and thermal-treated beverages.

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2. Materials and methods

2.1. Preparation of smoothies

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 UHT, Burgos, Spain) or soymilk (ViveSoy Ligera, Pascual, Burgos, Spain). The fruits used were purchased in several supermarkets in Madrid (Spain) and were chosen with an optimum degree of maturity (intense colour, characteristic aroma and firmness). Melon samples were kindly supplied by Syngenta. The oranges were squeezed in a Z14 juicer (Zummo Mechanical Innovaciones SA, Valencia, Spain). The other fruits were pureed in a Vitamix VI-5086 blender (Ripex, 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; thermal-treated; and HPP at different pressure conditions. Untreated and treated samples were stored at 4 °C for 30 and 45 days respectively.

2.2. High pressure and thermal processing

The equipment used to pressurize the smoothies was a semi-industrial Hiperbaric 55 (Hiperbaric, Burgos, Spain). Smoothies underwent high pressure treatment at 450 and 600 MPa during 3 min at 20 °C for milk-smoothies, and 550 and 650 MPa for 3 min at 20 °C for soymilk-smoothies. HP processing was compared to thermal processing (TP) at 80 °C for 3 min, as this is the most common option used for producing fruit juices. Equivalent thermal and HP processing conditions in terms of microbial safety were chosen for a clearer comparison of both treatments (Vervoort et al., 2012).

2.3. Microbiological analyses

Enterobacteriaceae were determined according to ISO 21528-2:2004 using the VRBG culture medium (crystal violet-neutral red-bile agar) and incubated at 37 °C, 24 h. Positive colonies were replated on nutrient agar to obtain pure cultures for confirmatory biochemical tests (oxidase and glucose fermentation). Mesophilic aerobic bacteria were determined in plate count agar (PCA) incubated at 30 °C, 72 h (ISO 4833-1: 2013). Yeasts and moulds were evaluated following the ISO 21527:2008 method in DRBC agar (dichloran-rose bengal-chloramphenicol) at 25 °C for 5 days. Beta-glucuronidase-positive *Escherichia coli* was enumerated in depth seeding in TBX medium (tryptone-bile-X glucuronide) and the characteristic colonies were subsequently counted in a chromogenic selective medium after incubation at 44 °C for 18–24 h (ISO 16649-1:2013). The method for detecting *Listeria monocytogenes* was on ALOA agar with aerobic incubation (24 h, 37 °C). Colonies suspected of being *L. monocytogenes* were isolated in pure culture in TSYEA agar (tryptone-soy yeast extract agar) and subjected to identification tests (hemolysis production, catalase test, CAMP test, Gram stain and utilization of carbohydrates) (ISO 11290-1:1996/A1:2004).

2.4. Physicochemical parameters

Total soluble solids (digital refractometer Atago RX-1000, Tokyo, Japan), pH (pH-meter (Microph 2000, Crison, Barcelona, Spain), titratable acidity (titration with 0.1 N NaOH up to pH 8.1), and density (ML204 analytical balance, Mettler Toledo, Barcelona, Spain) were determined.

2.5. Soluble sugars

Soluble sugars (glucose, fructose, sucrose) were analysed following the method described by Andrés, Tenorio, and Villanueva (2015). Analyses were performed on an HPLC (Agilent 1100, Santa Clara, CA, USA) coupled to a refractive index detector with a Rezex RPM-Monosaccharide Pb^{+2} column (300 × 7.8 mm, 8 μm; Phenomenex, Torrance, CA, USA) at 85 °C and Milli-Q water was used as eluent.

2.6. Organic acids

Organic acids were determined according to the method reported by Andrés et al. (2015) with slight modifications. The analysis was performed by the HPLC system equipped with a DAD at 215 nm with a Luna RP-C18 column (250 × 4.6 mm, 5 μm, Phenomenex, Torrance, CA, USA). Milli-Q water acidified with H_2SO_4 12 M (pH 2.55) was used as mobile phase on isocratic gradient with a flow rate of 0.4 ml/min.

2.7. Mineral elements

Freeze-dried samples were digested using a high-performance microwave digestion system (ETHOS One, Milestone, Sorisole, Italy) in a temperature ramp of 90 min, and the contents were then transferred to a 10 ml volumetric flask and made up to volume with water. Analyses were performed by ICP-OES (Varian 720-ES, Varian Inc., Walnut Creek, CA, USA) with a solution of yttrium as internal standard.

2.8. Sensory analysis

An untrained panel of 35 members collaborated in the sensory analysis. A descriptive test was used evaluating colour intensity, aroma (in terms of fruity smell), taste (in terms of sweetness, acidity and fruitiness), consistency and overall acceptability. The attributes were quantified using a 9-point scale, where 1 was the lowest value and 9 the highest according to Andrés et al. (2015). Samples were refrigerated, and 20 ml were served in transparent glasses immediately after opening in order to minimize organoleptic alteration. The beverages tested were numerically coded and served in a single session. Tap water and unsalted crackers were provided to the panellists to cleanse their palate between sampling.

2.9. Method validation

The methods were validated for this kind of matrix. All validation parameters are shown in Table 1. Linearity was assessed from the calibration curves obtained at five concentration levels of each compound. The correlation coefficient and linearity coefficient were also evaluated, indicating good linearity in the working range ($r \geq 0.990$ and $LC \geq 95\%$). The sensitivity of the method was evaluated by the limits of detection (LOD) and quantitation (LOQ). The methods and analytical techniques used were very sensitive for the compounds studied. Accuracy was measured by spiking a sample with each of the analytes of interest at three different concentration levels (low, medium and high in the range calibration) in triplicate. Reference materials were also used to assess accuracy in the analysis of mineral elements. The results are the average of all the measurements. The methods used showed good recoveries, from 89% (lactose) to 109% (L-malic acid). The standard deviation of these replicates was used to determine repeatability. Reproducibility was calculated by measuring the same standard sample on three

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