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Physicochemical and nutritional characteristics of blueberry juice after high pressure processing

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ABSTRACT

This study was carried out to investigate the impact of high pressure processing (HPP) at different pressure (200, 400 and 600 MPa) and treatment times (5, 9 and 15 min) on ascorbic acid, total phenolics, anthocyanin stability and total antioxidant capacity, were also studied at different physicochemical parameters such as pH, °Brix and color. HPP treatments resulted in more than 92% vitamin C retention at all treatment intensities. On the other hand, total phenolic content in the juice was increased, mainly after HPP at 200 MPa for all treatment times. The total and monomeric anthocyanin were similar or higher than the value estimated for the fresh juice being maximum at 400 MPa/15 min (16% increase). Antioxidant capacity values were not statistically different for treatments at 200 MPa for 5–15 min in comparison with fresh juice, however for 400 MPa/15 min and 600 MPa for all times (8–16% reduction), the lowest values were observed for total antioxidant capacity determined with TEAC method. No significant changes were observed in pH and °Brix. Color changes (a*, b*, L* and ΔE) were not visually noticeable for pressurized beverage for all pressures and times.

1. Introduction

Blueberry juices are becoming more attractive to consumers due to their high levels of antioxidants, which promote human health. Blueberry juice with higher amounts of anthocyanins and other antioxidant phytochemicals such as vitamins C and E among other beneficial substances, is healthful to humans (Nindo, Tang, Powers, & Singh, 2005). Vitamin retention studies to assess the effects of food processing on the nutritional value of foods are of great importance to food technologists and consumers. Ascorbic acid has been used as quality indicator in fruits and vegetables (Esteve, Farré, & Frígola, 1996; Giannakourou & Taoukis, 2003) because this is a temperature-sensitive bioactive compound, which is also acting as a valid criterion for other organoleptic or nutritional components (Blasco, Esteve, Frigola, & Rodrigo, 2004; Esteve, Farre, & Frígola, 1995). Anthocyanins are the major polyphenolics present in blueberry juice, which are responsible for the red, violet, purple, and blue color of this fruit (Kong, Chia, Goh, Chia, & Brouillard, 2003; Prior, 2003), and exhibit a wide range of antioxidant protection and therapeutic benefits including the integrity of genomic DNA, potent cardioprotective, neuroprotective, antiinflammatory, and anticarcinogenic properties. Among berry fruits, blueberry has the highest antioxidant capacity, which is mainly related to the anthocyanin content (Juranic & Zizak, 2005; Kalt, Forney, Martin, & Prior, 1999).

Thermal blanching above 80 °C remains the most widely adopted technology for preservation of fruit juices, however at these process conditions, blueberry products typically lose their natural flavor and some of their nutritional value (Rossi, Giussani, Morelli, Lo Scalzo, Nani & Torregiani, 2003). Consumer demand for minimally processed foods, has led to interest in non thermal technologies such as HPP (Hendrickx & Knorr, 2002). The application of HPP allows microbial and enzyme inactivation in order to enhance safety and shelf-life of perishable foods, while reducing thermal effects on nutritional and quality parameters (Butz, Fernández García, Lindauer, Dieterich, Bognár & Tauscher, 2003; Knorr, 1993). A commercial application of HPP consists of subjecting the packaged or unpacked food in water at pressures of 100–900 MPa for 1–20 min at room temperature, which results in the inactivation of vegetative microorganisms and enzymes while maintaining physicochemical and nutritive properties, allowing the storage of the product at 4–6 °C (Cheftel, 1995).

Over the last 20 years, HPP has been investigated and several commercial products, including fruit juices, i.e. mandarin, grapefruit, apple, orange, carrot juices and broccoli–apple juice mixture treated by HPP are currently available on market (Buzrul, Alpas, Largeteau, & Demazeau, 2008; Hayakawa, Kanno, Yoshiyama, & Fujio, 1994). In addition, at this stage of development of HPP technology, evaluating the influence of process variables on the stability of bioactive compounds as well as antioxidant capacity and physicochemical parameters of blueberry juice is a key factor in defining treatment conditions to avoid the loss of these important properties of foods and to obtain a food beverage with high benefits for the health of the consumer. Thus, the operating pressure, and holding times at the pressure set point were

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changed over a wide range, with the aim of optimizing the processing condition, in order to obtain a beverage with high content in bioactive compounds and optimal physicochemical characteristics, and in any case with an assured microbiological stability of the processed juice.

2. Materials and methods

2.1. Samples

Blueberries (*Vaccinium myrtillus,* harvested in Poland) were purchased at Berlin's wholesale market (Berlin, Germany). The fruits were washed, drained and chopped. Then, the squeezed blueberry juice was centrifuged at 4000×*g* for 15 min and the supernatant was filtered using a steel sieve with a mesh of 2 mm.

2.2. Chemicals

Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2-azinobis(3-ethylbenzothiazoline 6-sulphonate)), potassium hydroxide, sodium and disodium phosphate, acetonitrile (special grade), magnesium hydroxide carbonate and tetrabutyl ammoniumhydrogen sulfate were obtained from Fluka (Steinheim, Germany) L(+)-ascorbic acid, ethanol, methanol metaphosphoric acid and sodium chloride (special grade) were obtained from Merck (Darmstadt, Germany).

2.3. Microbiological assay

The number of surviving cells, N, after each test was determined, after a proper dilution of the treated samples in distilled water, by plate count method. The count of microbial colonies, grown on PCA (Plate Count Agar, Oxoid) slants at 32 °C for 72 h, was expressed in cfu/mL (colony forming units per milliliter of sample). The survival fraction, $S = N/N_0$ and the level of inactivation, log (S) were evaluated for each test.

2.4. HPP system

The samples, inserted in PE-LD bottles, were placed in polyethylene bags filled with water and were heat-sealed (MULTIVAC Thermosealer) before being placed in the HPP unit (U 4000 Unipress, Poland). The pressurization liquid was a mixture of distilled water and 1,2-propanediol (50:50 v/v). The pressure level, pressurization time, and temperature were controlled automatically. The treatment time stated in this study does not include come-up and come-down times. The samples were pressurized at 200, 400, and 600 MPa for specific times in a range from 5 to 15 min at a maximum temperature of 42 °C (initial temperature was 25 °C). All the treatments were applied in triplicate, with three bottles per replicate. Immediately after pressurization the samples were transferred to an ice/water bath, packed, and then stored under refrigeration (4 ± 1 °C) until needed for analysis.

2.5. Ascorbic acid

Ascorbic acid was determined according to Rückemann (Rückemann, 1980). All reagents were of analytical grade. The eluent contained 2.5 g of tetrabutyl ammoniumhydrogen sulfate in 945 mL distilled water and 55 mL methanol. A standard ascorbic acid solution of 50 mg ascorbic acid in 6% metaphosphoric acid was prepared with appropriate dilutions. 400 μL of blueberry juice was diluted with 1600 μL of 6% metaphosphoric acid. The samples prepared in this way were filtered through filters with a pore size of 0.22 μm and injected in the chromatograph (HPLC column, pump, variable wavelength monitor, Knauer GmbH, Berlin, Germany). Elution is obtained at a flow rate of 1 mL/min and eluate absorbance is measured at 251 nm. Retention was expressed as mg/100 g.

2.6. Total phenolic compounds

Total phenolic content was measured using the Folin–Ciocalteu method (Singleton & Rossi, 1965). Results were expressed as milligrams of gallic acid equivalents per gram of fresh weight.

2.7. Total monomeric, polymeric anthocyanins, monomeric index

Total anthocyanins, monomeric and polymeric anthocyanins, and monomeric index were determined using a modified method of Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999). A 10-fold diluted blueberry juice of 100 μL was mixed with 1700 μL of distilled water and 200 μL of 5% (v/v) HCl. The sample was held at room temperature for 20 min before measuring the absorbance at 520 nm in a 10 mm cuvette. This reading corresponds to the total anthocyanins content after considering the relevant dilution (A^{ta}). Separately, 100 μL of juice aliquot, 800 μL of 5% (w/v) SO_2, 1700 μL of distilled water and 200 μL of 5% (v/v) HCl were mixed, and absorbance was measured at 520 nm ($A^{SO}{}_2$) after 20 min at room temperature. From this reading, polymeric anthocyanins were obtained, and monomeric anthocyanins were obtained from the difference between total anthocyanin (A^{ta}) and polymeric anthocyanins ($A^{SO}{}_2$). From these measurements monomeric index absorbance units were calculated as follows:

$$\text{monomeric index} = \left(A^{ta} - A_2^{SO}\right) / A_2^{SO}.$$

Calculations of total anthocyanins were based on malvidin-3-glucosid (molar absorptivity 28,000 for blueberry). All spectrophotometric analyses were performed using a UV–visible spectrophotometer Lambda 20 (Perkin-Elmer, Überlingen, Germany).

2.8. Antioxidant capacity

The Trolox Equivalent Antioxidant Capacity (TEAC) test was adapted from Re, Pellegrini, Proteggente, Pannala, Yang and Rice-Evans (1999). This method is based on the capacity of antioxidants to quench the radical cation 2,2-azino-bis(3-ethylbenzothiazoline 6-sulphonate) (ABTS), which has a characteristic long-wavelength absorption spectrum showing a maximal peak at 734 nm. The ABTS radical cation is formed by the interaction of ABTS (7 mM) with $K_2S_2O_8$ (2.45 mM).

2.9. Physicochemical parameters

pH and °Brix (total soluble solid content (g/100 g)) were measured in accord to IFU methods (2001). Conductivity was measured at 20 °C using the Conductivity Meter WTW LF 323 (WTW, Germany). Color measurements were done in a Hunter Lab Labscan spectrophotometer (CR-200, Minolta, Japan) and the Hunter color parameters L*: lightness (0 = black, 100 = white), a* (−a* = greenness, +a* = redness) and b* (−b* = blueness, +b* = yellowness) were used. Tests for each sample were conducted in triplicate and the values were averaged. These values were then used to calculate hue degree (h^0 = arctangent [b*/a*]), chroma [$C = (a^{*2} + b^{*2})^{1/2}$], which is the intensity or color saturation, and ΔE, total differences of color, [$\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$] Calvo (2004).

2.10. Statistical analysis

Significant differences between the results were calculated by analyses of variance (ANOVA) and the possible interactions between the parameters. An LSD test was applied to indicate the samples between which there were differences. A multiple regression analysis was performed for each parameter to study the influence of pressure and time of treatment. Also the correlations between a pair of variables were studied. All statistical analyses were performed using