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Pressurization and cold storage of strawberry purée: Colour, anthocyanins, ascorbic acid and pectin methylesterase

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ABSTRACT

The effect of pressurization and cold storage of strawberry purée on colour, anthocyanins, ascorbic acid and pectin methylesterase was investigated. Samples were treated at pressures of 100, 200, 300 and 400 MPa, at two temperatures, 20 °C and 50 °C, for 15 min. Ascorbic acid content remained fairly constant after all the high pressure treatments in comparison to the control. However, the values of the samples pressurized at 50 °C were lower than the samples pressurized at 20 °C. Concentration of anthocyanins remained similar after all pressure treatments at both temperatures. Pectin methylesterase decreased in the samples pressurized at 50 °C, and it is remarkable that with this enzyme inactivation a gel-network formation in the strawberry purée was not found. However, a gel-network was observed in the control and in all the samples pressurized at 20 °C throughout cold storage. Immediately after pressurization, strawberry colour showed significant differences between the two temperatures, but no differences were observed between pressures. However, after cold storage no significant differences were observed, for hue and chroma parameters, between the samples pressurized at the two temperatures.

1. Introduction

The strawberry is a fruit with high consumer demand due to its attractive colour and flavour. It is grown in a wide range of climates and, among the berries, its worldwide production ranks only second after the grape.

Phenolic compounds are ubiquitous phytochemicals present in plant foods with numerous biological activities including antioxidant properties (Szajdek & Borowska, 2008). Strawberries are rich sources of micronutrients and phytochemical compounds including flavonoids such as anthocyanins, flavonols, hydrolysable tannins and phenolic acids (Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). However, anthocyanins remain quantitatively the most important phenolic constituent in ripe fruits. Anthocyanin pigments are responsible for the red colour of strawberry fruits. Lopes da Silva, Escribano-Bailón, Pérez-Alonso, Rivas-Gonzalo, and Santos-Buelga (2007) have found, using HPLC-DAD-MS, twenty five defined anthocyanins, most of which contained pelargonidin (Pg) as aglycone and some cyanidin (Cy) derivatives, although pelargonidin-3-glucoside (Pg-3-glu), pelargonidin-3-rutinoside (Pg-3-rut) and cyanidin-3-glucoside (Cy-3-glu) represent over 95% of the total anthocyanins, Pg-3-glu being the main anthocyanin present in most strawberry fruits (Lopes da Silva, De Pascual-Teresa, Rivas-Gonzalo, & Santos-Buelga, 2002).

Since colour is a critical quality parameter in food, due to its influence on consumer acceptance, colour measurement has gained attention from food scientists and the industry (Du & Sun, 2005; Quevedo, Aguilera, & Pedreschi, 2010). To investigate colour quality in a systematic way, it is necessary to measure colour as well as pigment concentration (Ngo, Wrolstad, & Zhao, 2007). Colour has always been a great challenge in foods after industrial processing, and many parameters are involved in its stability, such as pH, temperature, light, oxygen, enzymatic and non-enzymatic reactions and L-ascorbic acid (L-AA) (Bakowska, Kucharska, & Oszmianski, 2003). Colour stability is influenced by self association (condensation of anthocyanins) (Brouillard & Dangles, 1994) and copigmentation (interaction of anthocyanins with polyphenols) (Bishop & Nagel, 1984).

Abbreviations: HP, high pressure; PME, pectin methylesterase; L-AA, L-ascorbic acid; PPO, polyphenoloxidase; POD, peroxidase.

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There are many companies offering a broad variety of strawberry products (frozen strawberry, concentrates, jam, juices, nectar, syrup, and dairy products) on the market. These companies are interested in maintaining high quality parameters. High pressure (HP) processing has been described as an alternative treatment to conventional ones with specific impact over food colour (Hayashi, 1991).

Nowadays, HP treatment is considered as a promising technology for food preservation from the nutritional and sensorial quality point of view (Krebbers et al., 2003). However, HP can significantly alter vegetal tissues and induce changes in their structure and texture (Otero & Préstamo, 2009; Préstamo & Arroyo, 1998). For this reason, most of the commercialized HP processed fruit and vegetables come in the form of purée or juice (e.g., guacamole, fruit jams and juices).

Today, there is a demand from the strawberry industry to preserve strawberry purée for use as a base product for the preparation of strawberry juices and soft drinks, or for addition to ice creams and yoghurts. However, strawberry changes colour after heating and with storage in refrigerated conditions. It has also been observed that strawberry purée is capable of forming gels during cold storage, due to the action of some enzymes such as pectin methylesterase (PME) (Knorr, 1999; Rovere, 1995).

This study has been designed to offer an evaluation of strawberry purée characteristics after HP treatment and following cold storage, focussing on anthocyanin content, colour changes, L-AA and PME enzyme activity, as an alternative method to the conventional methods (vacuum filling and pasteurization) the strawberry industry is already using.

2. Materials and methods

2.1. Sample

Strawberries (*Fragaria x ananassa*, cv. Camarosa), grown in Huelva (Spain) and harvested at table ripeness were used, 2 kg of strawberries were blended (Moulinex-Blender 4 speeds, for 30 s) and sieved for homogenization. Amounts of 50 g of strawberry purée were distributed into plastic bags (Polyskin XL Doypack, Amcor Flexibles Hispania, Granollers, Spain), heat-sealed (without air space, to prevent plastic breaking during the HP treatment) and kept refrigerated at 5 °C. The samples were immediately HP treated.

2.2. High pressure treatment

Samples were treated in an HP machine (ACB GEC, Alshtom, France), with a 2.35 L vessel. The pressure chamber was thermoregulated, and water was the compressing fluid. The pressure applied was 15 MPa/s, for 15 min and rapidly released in approximately 9 s. Bags of strawberry purée were subjected to four different HP treatments (100, 200, 300, and 400 MPa) at two different temperatures (20 °C and 50 °C). Untreated HP samples were used as control. All treatments were done in duplicate, and after pressurization one set of the samples were lyophilized with a BenchTop 6K freeze dryer (VirTis, SP Scientific, New York, USA) and use for L-AA, PME and anthocyanin determinations. The other set was put in cold storage at 5 °C for six months and used for the colour measurements.

2.3. Anthocyanin extraction

Anthocyanins from lyophilized strawberry purée samples were extracted following the method described by Patras, Brunton, Da Pieve, and Butler (2009) with slight modifications. An equivalent of 1.25 g of raw strawberry purée (0.14 g of lyophilized purée) was extracted with 2.5 mL of methanol/acetic acid/water (25:1:24) from each sample. The mixture was homogenized for 2 min at 23,000 rpm using a SilentCrusher M homogeniser (Heidolph Instruments, Schwabach, Germany). The crude extract was mixed by an electric stirrer RZ-1 (Heidolph Instruments, Schwabach, Germany) for 20 min, rotating at 75 rpm at room temperature and centrifuged for 15 min at 2000 × *g*. Supernatant was evaporated under vacuum in a rotary evaporator (Rotavapor® R-200, Buchï, Flawil, Switzerland) and the residue was redissolved in 2.5 mL of solvent A for HPLC analysis (5 mL/100 mL aqueous formic acid). Samples after filtration through 0.45 μm nylon membrane were stored frozen (−20 °C) until further analysis.

2.4. Anthocyanin HPLC-DAD analysis

Analysis was carried out on a modular Waters Liquid Chromatograph (Milford, USA) equipped with two 515 HPLC pumps and InLine degasser connected to a photodiode array detector. The separation of anthocyanins was accomplished on a Nucleosil 120 − C_{18} column (250 × 46 mm, 5 μm) from Teknokroma, (San Cugat del Valles, Spain). The column temperature was maintained at 40 °C. The injection volume was 30 μL with an isocratic flow rate of 1 mL min^{-1} and the total run time was 93 min. Chromatographic separation was carried out following the method described by Sáenz-López, Fernández-Zurbano, and Tena (2003) with some gradient modifications. Solvents were (A) water/formic acid (95:5 v/v) and (B) acetonitrile establishing the following gradient: 0–2 min isocratic 100% A, at 5 min 8% B, at 60 min 20% B, at 70 min 30% B, at 71 min 50% B, at 73–80 min isocratic 100% B, at 83–93 min isocratic 100% A. The chromatograms (Fig. 1) were recorded at 520 nm and the UV spectra were collected from 200 to 650 nm. Identification of the component peaks was performed by the UV/Vis, MS and MS/MS spectra and retention times of the available standards. The HPLC system was coupled to a micrOTOF Q High-Resolution Mass Spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an Apollo II ESI/APCI multimode source. For quantification, calibration curves ranging from 0.04 mg L^{-1} to 25 mg L^{-1} were obtained from external standards of cyanidin-3-*O*-glucoside chloride ≥95% and pelargonidin 3-*O*-glucoside chloride ≥97% (Sigma–Aldrich, St Louis, MO, USA). All analyses were carried out in duplicate. The results are expressed in mean values.

2.5. Pectin methylesterase assay

PME (EC.3.1.1.11) activity was determined according to Hagerman and Austin (1986) with slight modifications. The activity measurement was carried out with a microplate (96 wells). Samples assays were prepared in a final volume of 200 μL using 100 μL of 5 g L^{-1} pectin solution (citrus pectin, Sigma, Madrid, Spain) in PBS (phosphate buffered saline), 5 μL of 1 g L^{-1} bromothymol blue in PBS buffer (pH 7.4), 35 μL of PBS buffer, and 60 μL (30 mg of lyophilized strawberry in 500 μL of PBS) of each sample. Colour change was monitored in a spectrophotometer (PowerWave XS microplate spectrophotometer, Biotek, Bad Friedrichshall, Germany) at 620 nm. The standard curve was built with pectinase (3000 U mL^{-1}, aqueous solution, from *Aspergillus niger*, Sigma, Madrid, Spain) and data were adjusted to a curve ($y = -111.24x + 203.07$, $r = 0.9873$). PME activity was defined as units of pectinase per mL. Each sample was replicated four times.

2.6. Colour measurement

Colour measurements were made externally on the surface of the plastic bag containing the strawberry purée on a CM-2600d spectrophotometer (Konica Minolta, Osaka, Japan) with specular component included (SCI), 10° standard observer angle, D65