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Impact of high-pressure carbon dioxide on polyphenoloxidase activity and stability of fresh apple juice

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ABSTRACT

Freshly-extracted apple juice was exposed to high pressure carbon dioxide (HP-CO_2) treatment at 20, 35 and 45 °C at different pressure conditions (6.0, 12.0 and 18.0 MPa) for up to 30 min. Samples were analysed for residual enzymatic activity. The time needed for 90% enzyme inactivation (D_p) decreased when CO_2 pressure increased, while the CO_2 pressure sensitivity of the enzyme (z_p) showed no variation with temperature. The HP-CO_2 treatment at 12 MPa and 35 °C allowed the minimum residual enzyme activity (20%) to be reached in 10 min. Samples treated under these conditions showed lower polyphenoloxidase activity and higher microbial stability than untreated apple juice while presenting a sensory fresh-likelihood higher than thermally pasteurized apple juice.

1. Introduction

Consumption of unprocessed fruit juices has substantially risen over the last few years, mostly due to the increasing demand for good nutritional quality foods with fresh-like characteristics (Beuchat, 1996; Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martín-Belloso, 2009). As a consequence of inappropriate manipulation and storage, both spoilage and pathogenic microorganisms can grow, leading to hygienic and quality issues. Enzymatic activity can also contribute to quality depletion, along with physical and chemical changes during the storage (Raybaudi-Massilia et al., 2009). To guarantee product safety and provide an adequate shelf-life, unpasteurized juices are generally distributed under refrigerated conditions. They are traditionally obtained by a combination of formulation strategies such as water activity reduction, nutrient restriction, acidification as well as use of antimicrobial additives (Davidson, 2001). These preservation strategies hardly fit with the current demand for fresh-like juices that are free from additives, generating the need for developing novel non-thermal treatments for juice stabilization.

High pressure carbon dioxide (HP-CO_2) has been reported as a promising non-thermal technology for the stabilization of fresh products. During the treatment, food is in contact with pressurised CO_2 at temperature/pressure conditions that may be below or above the critical point (31.1 °C, 7.38 MPa). Typical CO_2 pressure is generally within 4 and 30 MPa, rarely exceeding 50 MPa. Temperature is generally between 20 and 50 °C, low enough to maintain the freshlikelihood of treated products (Manzocco et al., 2016).

Significant lethal effects of HP-CO_2 on different microorganisms have been demonstrated in fruit juices (Damar & Balaban, 2006; Ferrentino, Bruno, Ferrari, Poletto, & Balaban, 2009; Spilimbergo & Bertucco, 2003; Xu et al., 2011). In particular, the technology is known to promote up to 5 Log reductions in microbial counts, approaching those required for pasteurization (Ferrentino & Spilimbergo, 2011; Kincal et al., 2005). The germicidal activity of HP-CO_2 is due to the combination of temperature, pressure and specific effects of HP-CO_2. The treatment is associated with extracellular and intracellular acidification, destabilization of membranes and denaturation of microbial enzymes (Bothun, 2004; Bothun, Knutson, Strobel, & Nokes, 2005; Hutkins & Nannen, 1993; Jones & Greenfield, 1982). More controversial is the effect of HP-CO_2 in inactivating fruit enzymes leading to juice quality decay. For instance, inactivation of polyphenoloxidase responsible for browning of fruit juices, depends on the nature of the enzyme and is strongly affected by CO_2 pressure, temperature and treatment time (Gui et al., 2006; Liao, Zhang, Bei, Hu, & Wu, 2009; Spilimbergo, Komes, Vojvodic, Levaj, & Ferrentino, 2013; Zhou, Zhang, Hu, Liao, & He, 2009). The mechanisms involved in

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enzyme inactivation by HP-CO_2 include pH lowering (Balaban et al., 1991) and changes in the conformation of the secondary structure of the enzyme (Chen, Balaban, Wei, Marshall, & Hsu, 1992; Manzocco et al., 2016).

Based on these considerations, the present paper was addressed to investigate the impact of HP-CO_2 treatment on polyphenoloxidase activity and stability of fresh apple juice intended for refrigerated storage. To this aim, apple juice was exposed to HP-CO_2 treatments in a wide range of pressure, temperature and treatment time conditions. Apple juice was then submitted to the HP-CO_2 treatment leading to the minimum polyphenoloxidase activity at the mildest pressure/temperature combination and stored at 4 °C for up to 15 days. HP-CO_2 treated apple juice was monitored during storage for residual polyphenoloxidase activity, colour, microbial counts and sensory attributes. To verify the potential applicability of HP-CO_2 technology to produce fresh apple juice, data were compared to those relevant to an untreated apple juice. An apple juice submitted to conventional thermal pasteurization was also considered as additional control.

2. Material and methods

2.1. Apple juice extract

A 10 kg batch of fresh apples "Golden delicious" were purchased at the local market and stored at 4 °C overnight. When the experiments were performed, apples had a dry matter content of 164.7 ± 1.6 g/kg, a soluble solid content of 13.3 ± 0.2 °Brix, a pH of 4.2 ± 0.2 and a titratable acidity of 4.6 ± 0.3 g/kg. Apple juice was prepared fresh for every trial from the same batch of fruits, to minimize sample variability. The juice was obtained by using a domestic juicer (Moulinex, mod. Vitae JU2000, Milan, Italy), filtered through two layers of cloth filter and centrifuged at 5000 g for 5 min at 4 °C (Beckman, Avanti™ J-25, High performance centrifuge, Brea, USA). The supernatant was filtered again through two layers of cloth filter and the resulting clear juice was immediately treated.

2.2. High-pressure CO_2 treatments

HP-CO_2 inactivation process was carried out in a double-batch apparatus. The system consists of two identical stainless steel cylinders with a screwed cap and an internal volume of 150 mL, connected in parallel. Each reactor was connected to an on-off valve that can be used to depressurise it independently from the other. The two reactors were submerged in a thermostatic water bath (CB 8-30e, Heto, Allerød, Denmark). For more details, please refer to Manzocco et al. (2016). Before starting the pressurisation, the temperature of the sample was allowed to reach equilibrium. The time needed to reach the desired temperature (20, 35 or 45 °C) and the pressurisation time were lower than 3 min. After reaching the desired pressure (6, 12 and 18 MPa), the pump was switched off and valves connected to each vessel were tightly closed. After increasing treatment time up to 30 min, vessels were depressurised. In all experiments, depressurisation was completed within 10 min and the outlet flow was controlled using a digital flowmeter (PFM 750, SMC Italia S.p.A., Milan, Italy). Control samples were prepared by treating the apple juice in the vessels at atmospheric pressure (0.1 MPa) and thus at CO_2 partial pressure equal to 0.0039 MPa.

2.3. Thermal treatment

Aliquots of 100 mL of apple juice were placed in plastic pouches (PA/PE, 20 × 28 cm, Savonitti, Codroipo, Italy). A thin layer of sample was obtained, being the maximum thickness of the filled pouches lower than 0.5 cm. Pouches were heated in a water bath (IKA-Werke, Staufen, Germany) at 71.1 °C for 6 s (FDA, 2004). After thermal treatment, samples were quickly cooled under running water at room temperature.

2.4. Apple juice storage

Aliquots of 10 mL of apple juice were introduced in Eppendorf® vials of 10 mL capacity and stored for up to 15 days at 4 °C in a refrigerated cell. At increasing time during storage, samples were removed from the refrigerator, equilibrated at 22 °C and submitted to the analysis.

2.5. Apple physical-chemical parameters

Soluble solid content (SSC) was measured using a table refractometer (Unirefrax, Bertuzzi, Milan, Italy) calibrated with distilled water.

Dry matter content of apple samples was determined gravimetrically by recording difference in weight before and after drying at 70 °C, until a constant weight was achieved (M.U.A.C.V, 1989).

Titratable acidity was determined by titration with NaOH 0.1 mol/L and phenolphthalein as indicator (Sigma-Aldrich, Milan, Italy), accordingly to the official M.U.A.C.V.method (1989) and expressed as g of acids/kg of fresh product.

Analyses of SSC and TA were carried out on the solution obtained after homogenization (Polyton, Kinematica, Luzern, Switzerland) and filtration of apple cubes through filter paper (Whatman #1, Whatman International Ltd, Maidstone, UK).

2.6. Temperature, pH

During HP-CO_2 treatments and thermal pasteurization, temperature was measured by a thermocouple probe (Hanna Instruments, Tersid s.r.l., Milan, Italy); pH was assessed using a pH-meter (Mettler Toledo 355, Lou Analyzer, Halstead, England).

2.7. Polyphenoloxidase activity

The polyphenoloxidase activity was assayed spectrophotometrically (Shimadzu UV-2501PC, UV-Vis recording spectrophotometer, Shimadzu Corporation, Kyoto, Japan) at 25 °C according to the methodology of Kahn (1995). The reaction was started by the addition of 500 μL of apple juice to 2 mL of 0.1 mol/L potassium phosphate buffer pH 7 and 1.5 10^{-3} mol/L L-Dopa (Carlo Erba, Milan, Italy). The absorbance at 420 nm was monitored each minute for 10 min. The changes in absorbance per min were calculated by linear regression, applying the pseudo zero order kinetic model. The eventual final stationary phase was excluded from regression data. The slope of the very first linear part of the reaction curve was used to determine polyphenoloxidase specific activity. The latter was defined as the amount of enzyme that produced 1 μmol of quinone per second (μkatal) (Lee, Tweed, Cookson, & Sullivan, 2010). The average polyphenoloxidase activity in untreated juice was found to be 0.047 μkatals. Polyphenoloxidase residual activity (*RA%*) upon treatments was calculated as the percentage ratio between enzymatic activity of the treated sample and that of the untreated one (Niu et al., 2010; de la Rosa et al., 2011; Xu et al., 2011).

2.8. Browning

Browning was assessed spectrophotometrically (Shimadzu UV-2501PC, UV-Vis recording spectrophotometer, Shimadzu