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Non-thermal pasteurization of apples in syrup with dense phase carbon dioxide

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ABSTRACT

The study investigated dense phase carbon dioxide (DPCD) applicability to a solid-liquid product consisted of apples in syrup as an alternative to the conventional thermal pasteurization. The effect of pressure (8, 10, and 12 MPa) and temperature (35, 45, and 55 °C) at 15 min was investigated to determine the inactivation of the natural microbiota (mesophilic microorganisms, total coliforms, yeasts and molds) and polyphenol oxidase enzyme. The optimal process conditions (12 MPa, 55 °C, 15 min) were determined. A storage study at 25 °C for 60 days was also performed to monitor the microbial, enzymatic and quality (pH, color, ascorbic acid content and total acidity) stability of the product.

DPCD induced inactivation to undetectable levels of mesophilic microorganisms, total coliforms, yeasts and molds. At the same conditions, polyphenol oxidase was totally inactivated while pH, color, and total acidity were slightly affected. The shelf life study of DPCD treated products revealed their microbial, enzymatic and quality stability.

1. Introduction

In the last years, the fruit and vegetable market has shifted from processed (i.e., canned) to fresh products due to an increasing trend in consumer markets.

Several studies demonstrated the deleterious effects of thermal technologies on the quality and nutritional attributes of the product thus drastically decreasing the consumers interests and forcing the food companies to the development of a new category of fruits and vegetables, the so called “minimally processed products” (Lozano, 2006).

The key to design practical minimal processing systems is to find innovative treatments able to uniformly preserve product quality parameters while maintaining overall fresh like attributes (Rocha et al., 1998). Dense phase carbon dioxide (DPCD) is receiving a high resonance as innovative non-thermal preservation technology: the treatment involves the contact between carbon dioxide (CO_2) at high pressure (up to 30 MPa) and the food, for a sufficient time (1–30 min) to achieve a certain inactivation degree, at mild temperature (up to 60 °C), thus overcoming some side effects induced on the quality of foods by thermal processes. Additionally, CO_2 presents several advantages compared to other solvents commonly use in food processing: it is relatively inert, inexpensive, nontoxic, nonflammable, recyclable and readily available in high purity leaving no residues when removed from the food after the process (Clifford and Williams, 2000). Furthermore, it is a Generally Recognized as Safe (GRAS) substance, which means it can be used for food processing.

The effect of DPCD on microorganisms in liquid products or on the surface of a solid sample has been already and largely investigated, but separately. Several research papers demonstrated the efficacy of the treatment to induce microbial and enzymatic inactivation of microorganisms in liquid foods such as fruit juices, beer, wine and milk (Damar and Balaban, 2006; Ferrentino and Spilimbergo, 2011). Most of DPCD inactivation studies currently available in the scientific literature have been performed on inoculated or spoiled foods (Arreola et al., 1991; Ferrentino et al., 2013a) or inoculated model systems such as physiological saline solutions or culture media (Ferrentino et al., 2013b). Experimental results are also available, showing the effects on the perceivable chemical–physical properties of the products immediately after CO_2 treatment and during storage, for example for orange (Arreola et al., 1991) and apple juice (Gui et al., 2006; Liao et al., 2010) but also for grape (Del Pozo – Insfran et al., 2006), grapefruit (Ferrentino et al.,

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2009), coconut (Cappelletti et al., 2015) and watermelon juices (Lecky and Balaban, 2005).

Regarding DPCD application to solid substrates, fewer papers in number have been published so far due to the complexity of the matrix, which can make CO_2 bactericidal action more difficult, and to the lack of information about the inactivation mechanism. Ferrentino et al. (2012) successfully applied the treatment for the inactivation of the natural microbiota of fresh cut coconut demonstrating DPCD pasteurization efficiency and quality attributes preservation of the products. Experimental results have been also published showing DPCD effects on other products as dry cured ham (Ferrentino et al., 2013b), fresh cut pear (Valverde et al., 2010), fresh cut carrot (Spilimbergo et al., 2013), spinach leaves (Zhong et al., 2008), alfalfa sprouts (Jung et al., 2009). In some of these studies, it has been reported that solid products undergo a softening caused by the decompression of the system from high to ambient pressure (Valverde et al., 2010; Spilimbergo et al., 2013). To avoid this drawback, the application of DPCD to solid products immersed in a liquid could represent a good alternative.

To our knowledge, no papers have been published so far investigating DPCD potentials for the pasteurization of a liquid-solid system. In this regard, the aim of the work was to test DCPD efficiency on the reduction of natural microbiota (mesophilic microorganisms, total coliforms, yeasts and molds) and polyphenol oxidase inactivation of apples in syrup. Measurements of color, pH, total acidity and ascorbic acid were performed on DPCD treated product and compared with the untreated. A shelf life study at 25 °C for 60 days was also addressed to determine the microbial, enzymatic and quality stability of the product during the storage.

2. Materials and methods

2.1. Preparation of canned apples

Red delicious apples, uniform in shape and not injured, were obtained from a commercial orchard in Trento (Italy). They were stored at 5–10 °C and used within 3 months. The day of the experiments, apples were washed with water, peeled, cored and cut in pieces (size of 30 × 20 × 20 mm). To inhibit the enzymatic browning, they were dipped for 5 min in an ascorbic acid solution (0.5% w/w). In the meanwhile, a syrup solution (14% w/w sucrose, 0.15% w/w citric acid and 0.0 5% w/w ascorbic acid) was prepared and added to the apples (1/2, ratio of mass of apples/mass of syrup).

2.2. DPCD apparatus

DPCD treatments were performed in a batch apparatus where liquid CO_2 (99.990% purity, Messer Group GmbH, Germany) was fed into a high pressure vessel by a volumetric pump (mod. LCD1/M910s, LEWA GmbH, Germany) with a maximum flow rate of 11 L/h.

The vessel consisted of a 310 mL stainless steel cylinder (height: 110 mm, inner diameter: 60 mm) equipped with a resistance temperature probe (Pt 100 Ω, Endress + Hauser, Milano, Italy), and a pressure gauge (Gefran, Brescia, Italy). The samples were loaded into the vessel, heated up to the set temperature with a heating rate of 1 °C/min. CO_2 was pumped until the desired pressure was reached (in about 1 min). The system was kept at the set-up pressure and temperature conditions for 15 min. Afterwards, the pressure was released slowly over a period of 2 min opening two micrometric valves (model 2S-4LN-SS, Rotarex, Brescia, Italy) placed on the output line of the system, both heated by an electrical resistance (80 W, model CSC2, Backer Fer, Ferrara, Italy) to prevent freezing during CO_2 expansion to ambient pressure. DPCD experiments were performed keeping constant the treatment time at 15 min to make the process competitive from an industrial perspective. The effect of pressure was investigated changing it from 8 to 12 MPa at 35, 45 and 55 °C. The effect of pressure lower than 8 MPa was not investigated in order to reach supercritical conditions. Moreover, pressures higher than 12 MPa were not applied as previous studies observed a not significant increase of CO_2 solubility in the liquid phase positively affecting the inactivation efficacy (Dodds et al., 1956; Spilimbergo et al., 2013). As concern the effect of temperature, previous published studies demonstrated that temperatures higher than 30 °C stimulate CO_2 diffusivity, cell membranes diffusivity thus enhancing the inactivation efficacy (Garcia-Gonzalez et al., 2007). However, higher temperatures were not applied to limit their deleterious effects on the quality attributes of the samples.

For each experimental run, 20 apple pieces of 2 g in 80 mL of syrup were loaded in the high pressure vessel and pressurized with CO_2.

After the treatment, the vessel was disconnected from the pressurization line and opened in a laminar flow hood to avoid any microbial contamination. The processed samples were collected in sterile tubes and cooled down immediately at 4 °C to be analyzed.

2.3. Shelf life study

Samples, treated at 10 MPa, 55 °C for 15 min, were stored at 25 °C for 60 days together with the untreated sample. During the storage, microbial, enzymatic and quality attributes were analyzed based on the methods subsequently described.

2.4. Microbial analyses

Samples were homogenized in a Stomacher 400 (International P.B.I., Milano, Italy) at 230 rpm for 2 min. The homogenate was serially diluted in phosphate buffer solution (PBS, Liofichem, Teramo, Italy) for the enumeration of the natural microbial flora (mesophilic microorganisms, total coliform bacteria, yeasts and molds) by plate count technique. Depending on the expected counts the adequate decimal dilution was plated on Petri dishes containing Plate Count agar (Liofilchem, Teramo, Italy), Chromatic Coli/Coliform agar (Liofilchem, Teramo, Italy), and Yeast Glucose Chloramphenicol agar (Liofilchem, Teramo, Italy). The incubation temperatures and times were: 30 °C for 48 h for mesophilic microorganisms, 30 °C for 24 h for total coliform bacteria, and 25 °C for 4 days for yeasts and molds count. At the end of the incubation periods, the number of colonies was counted. Microbial concentration was reported as Log (N), where N (CFU/mL) was the number of colony forming unit per mL of homogenized sample.

2.5. Polyphenol oxidase activity

Polyphenoloxidase (PPO) activity was determined according to the method of Soliva-Fortuny et al. (2002). The samples were homogenized and the enzyme activity was assayed spectrophotometrically by adding 3 mL of 0.05 M catechol and 75 μL of the sample to 1 cm path length quartz cuvette. Catechol solution was prepared with sodium phosphate monobasic monohydrate buffer solution (0.1 M and pH 6.5). Changes in absorbance at 420 nm were recorded every 5 s up to 3 min from the time the sample was added; the enzyme activity was expressed as changes in absorbance per min calculated by linear regression. Polyphenoloxidase residual activity was calculated as the percentage ratio between enzymatic activity measured for the treated sample and that detected in the untreated one. All determinations were performed in triplicate.

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