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Combined high hydrostatic pressure and carbon dioxide inactivation of pectin methylesterase, polyphenol oxidase and peroxidase in feijoa puree

Carmen Ortuño [a,*], Trang Duong [b], Murat Balaban [b], Jose Benedito [a]

[a] Department of Food Technology, Universitat Politècnica de València, Camí de Vera s/n, E46022 Valencia, Spain
[b] Department of Chemical and Materials Engineering, University of Auckland, Auckland 1142, New Zealand

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ABSTRACT

A combined treatment of high hydrostatic pressure (HHP) and dense phase carbon dioxide (DPCD) was investigated to inactivate pectin methylesterase (PME), peroxidase (POD) and polyphenol oxidase (PPO) in feijoa (*Acca sellowiana*) puree. The treatments were HHP (HHP); carbonation and HHP (HHPcarb); carbonation + addition of 8.5 mL CO_2/g puree into the headspace of the package and HHP (HHPcarb + CO_2). The different samples were treated at 300, 450 and 600 MPa, for 5 min.

The residual POD and PPO activity decreased in the order HHP > HHPcarb > HHPcarb + CO_2 at all pressures used. Treatments with HHP at 300 MPa increased POD activity to 140%. The residual PME activity of HHPcarb and HHPcarb + CO_2 samples at 600 MPa (45–50%) was significantly ($p < 0.05$) lower than for HHP treatment (65%).

The simultaneous application of HHP and DPCD seems to synergistically enhance the inactivation of the enzymes studied, the CO_2 concentration being a key process factor.

1. Introduction

Enzymes and microorganisms in foods cause quality deterioration and spoilage during storage and distribution. In the food industry, non-thermal processing alternatives have been developed in response to an increasing consumer demand for fresh-like and high quality food products. These technologies aim to economically produce safe, nutritious, and tasty foods using less severe processing conditions [1–3].

The application of high hydrostatic pressure (HHP) allows the inactivation of undesirable enzymes [4] in liquid and solid food systems, without altering their quality to the same extent as thermal treatments and with a comparable preservation effect. Park et al. [5] reported that by increasing the pressure in HHP treatments (25 °C – 5 min) from 200 to 600 MPa, the residual activity of polyphenol oxidase (PPO), lipoxygenase (LOX) and pectin methylesterase (PME) in carrot juice decreased from 83%, 78% and 80% to 10%, 30%, and 45%, respectively. Nevertheless, some undesirable enzymes, such as PPO and some isozymes of PME, are highly pressure resistant [6]. In this case, higher temperatures are needed to inactivate these enzymes, thereby negating the non-thermal advantages of HHP process.

Similarly, DPCD has been reported to inactivate different microorganisms in liquid foods [2,7–9] without exposing them to the adverse effects of heat which allows retain their fresh-like physical, nutritional, and sensory properties [10]. Similarly to HHP, DPCD has also been proven effective in inactivating many undesirable enzymes, including PPO [11,12], peroxidase (POD) [12], and PME [13,14]. However, in some cases the inactivation level was less than satisfactory [15,16].

Therefore, there is increasing interest in process intensification, with simultaneous application of different non-thermal technologies, seeking for synergistic effects. In this regard, DPCD could be a good candidate to enhance the effect of HHP processing. It is well known that the effect of HHP is enhanced at lower pH, moreover, it is assumed that CO_2 could dissolve in the hydration layer associated with the enzyme and could decrease the local pH [17], therefore the presence of CO_2 in sample medium might create an acid environment, and positively interact with pressure to destroy or damage the structure of enzymes. Few studies have shown synergistic effects of combining DPCD and HHP process on inactivation of PPO, LOX and PME enzymes in orange [18] and carrot [5] juice. Corwin and Shellhammer [18] first carbonated enzyme preparations at atmospheric pressure, then treated them with HHP. They showed that CO_2 had an additional inactivation effect on PME at 500 MPa. Park et al. [5] reported that a sequential application of

* Corresponding author at: ASPA Group, Food Technology Department, Universitat Politècnica de València, Camí de Vera s/n, E46022 Valencia, Spain.
Tel.: +34 96 3873683; fax: +34 96 3879839.
E-mail addresses: carminaocases85@gmail.com, carorca1@upvnet.upv.es (C. Ortuño).

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Table 1
Moisture content, °Brix, pH, firmness, and color of fresh feijoa.

	% Moisture	°Brix	pH	Firmness (N)	Color		
					L^*	a^*	b^*
Fresh Feijoa	83.33 ± 0.30	11.8 ± 0.8	3.30 ± 0.02	20.67 ± 3.87	54.56 ± 2.56	−8.32 ± 2.07	14.01 ± 3.26

All data shown are means ± SD.

DPCD at 4.9 MPa (5 °C – 5 min) and HHP at 200 MPa (25 °C – 5 min) improved the inactivation of the PPO, LOX and PME enzymes in carrot juice with a residual activity of 35%, 17% and 45%, respectively, compared with the residual activity of DPCD (40%, 20% and 50%, respectively) and HHP (83%, 78% and 80%, respectively) treatments.

The extension of atmospheric carbonation could be to add gaseous CO_2 into the headspace of the packaged liquid food before HHP treatment. The CO_2 in the headspace could dissolve into the sample during the HHP treatment and the CO_2 concentration inside the sample could be higher than in carbonated samples. Therefore, the effect associated to CO_2, like the acidification of sample, could be increased, improving the CO_2 effects compared with only carbonated samples. No references have been found in the literature covering simultaneous application of HHP and DPCD techniques involving additional gases in the package for either enzymatic or microbial inactivation purposes.

Feijoa (*Acca sellowiana*), an exotic fruit in New Zealand, has many desirable nutritional characteristics such as good source of vitamin C, low in calories and high in minerals and fiber, and interesting bioactive components such as high antioxidant activity, high phenolics and phytochemicals content [19]. Therefore, the preservation of feijoa products by non-thermal technologies is advantageous to retain these desirable characteristics.

The objective of this study was to determine the effect of different levels of added carbon dioxide in a package on the efficiency of HHP treatment to inactivate POD, PPO and PME at different pressures in feijoa puree.

2. Materials and methods

2.1. Raw material

The feijoa (*A. sellowiana*) was supplied by Frans and Tineke de Jong grower, Southern Belle Orchards (Matamata, Waikato), New Zealand. 15 kg of feijoa were stored at room temperature until they started ripening and released a sweet aroma volatile, and then they were put into storage at 4 °C for 2–3 days, time necessary to perform the chemical–physical analyses. The fruit that was not used for the chemical–physical analysis was cleaned, peeled and chopped, put in Ziploc bags and stored at −20 °C until required for the preparation of samples for the inactivation treatments.

2.2. Chemical–physical analysis of feijoa

For the chemical–physical analysis, 30 feijoa pieces were randomly selected. Color, pH and firmness were determined directly on the fruit. Afterwards, a puree was made using the same feijoa fruits, and the moisture, °Brix and water activity, were determined.

2.2.1. Color determination

Color assessment was conducted at 25 °C using a CR400-Chroma Meter Colorimeter (Konica Minolta, USA) in CIE $L^*a^*b^*$ color space system after calibration with the reference tile.

The fruit color was measured in 9 different sites of the fruit (3 readings around each end of fruit and 3 at the equator) and averaged. 10 fruits from the 30 previously selected were measured and a total of 90 readings were done.

2.2.2. pH

The pH was measured directly inside the feijoa fruit at 25 °C using a digital pH meter (PerpHec LogR meter, model 320, Orion research Inc., USA) and pH was recorded after stabilization, for 30 selected fruit.

2.2.3. Texture analysis

The firmness of fresh feijoa (Table 1) was measured using a universal texture analyzer (TA.XT Plus Texture Analyser, Stable Micro Systems Ltd., UK) linked to a computer for data acquisition and processing (Exponent software, Stable Micro System Ltd., UK), using a small cylindrical probe (10 mm diameter). The maximum force (firmness, N) was measured and computed with a test speed of 0.03 mm/s and travel distance of 5 mm down on the fruit surface, at the center of its equator and at each side of the fruit (2 punctures per side). 30 pieces of fruit were measured.

2.2.4. Moisture content

The moisture content of fresh feijoa puree was determined using the official method [20] for a vacuum oven. 5 g of fresh feijoa puree were accurately weighed and placed on a ceramic crucible, dried at 70 °C and 10 mmHg vacuum for 24 h in a vacuum oven (VT 6205, Haraeus Vacutherm, Germany). The vacuum was released slowly and the dried samples were stored in desiccators at ambient temperature prior to weighing by an analytical balance (ED224S, Sartorius Ag, Germany). The moisture analysis was conducted in triplicate. The moisture content (Table 1) of the feijoa was calculated using the following equation:

$$\text{Moisture content}\,(\%) = \frac{\text{Total moisture loss after drying}\ (\text{g})}{\text{Inital weight}\,(\text{g})} \times 100 \quad (1)$$

2.2.5. °Brix

The °Brix of fresh feijoa puree (Table 1) was measured in triplicate at 25 °C using E-Line ATC range 0–18 °Brix refractometer (Bellingham + Stanley Ltd., UK).

2.2.6. Water activity

The water activity of fresh feijoa puree was measured in triplicate at 25 °C using a digital water activity meter (Aqua Lab 4TE, Decagon Devices, USA). The water activity of the fresh feijoa puree was 0.9901 ± 0.0018.

2.3. Sample preparation and storage

The frozen fruit was thawed at 4 °C for 12–14 h before processing. Thawed feijoa were blended (Laboratory blender, Model 38BL40, Waring Commercial, USA), until well mashed and mixed into a puree. 30 g portions of feijoa puree were poured into plastic bags (155 mm × 180 mm × 30 mm, SURT155180, Cas-Pak Products Ltd., New Zealand), vacuum sealed (Vacutherm, VT 6205, Germany) and stored at −20 °C until required.