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Kinetics of thermal and high-pressure inactivation of avocado polygalacturonase

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ABSTRACT

Despite high-pressure (HP) processed avocado products are nowadays commercialized, there is no information about the effect of pressure on avocado polygalacturonase (PG) yet. In the present study, PG inactivation kinetics in crude avocado extract was investigated under isobaric conditions at 20 °C. Moreover, PG inactivation kinetics under isothermal conditions at atmospheric pressure was also included to make comparisons with the processing technique usually employed in the industry. Both temperature and pressure inactivation of avocado PG, either at 60–70 °C/0.1 MPa or at 20 °C/350–500 MPa, could be mathematically described by first-order inactivation models. No thermal or pressure resistant fractions were detected. Thus, avocado PG appeared a rather labile enzyme which could be completely inactivated after relatively mild thermal or pressure treatments (10 min/70 °C/0.1 MPa or 15 min/20 °C/450 MPa). PG inactivation models developed for PG in crude avocado extract could predict PG inactivation in avocado purée relatively well.

Industrial relevance: High-pressure processed avocado products are, nowadays, commercially available, but no data exist about the effect of high-pressure on pectinases yet. This paper provides, for the first time in the literature, scientific data about kinetics of thermal and high-pressure inactivation of avocado polygalacturonase. Moreover, first-order inactivation models are issued to mathematically describe inactivation. All these data should be of high interest for the food industry since polygalacturonase can affect the texture of avocado halves and chunks and the rheological properties of guacamole, avocado purée and sauces during the storage.

1. Introduction

High-pressure (HP) processing has become, in the last decade, a successful technology in the avocado processing industry to obtain high quality products. Other food preservation techniques like freezing, conventional thermal treatments or the addition of chemical additives are effective to prolong the shelf-life of avocado products, but they produce substantial losses in the product quality, texture, color, smell and flavor (Bates, 1970; Daiuto, Vieites, Simon, De Carvalho, & Pegoretti, 2011; Degenhardt & Hofmann, 2010). In contrast, high-pressure processing reduces microbial counts and produces significant enzyme inactivation while retaining the fruit's fresh-like characteristics. These advantages have caused that, nowadays, there exist many avocado processors in United States, Mexico, Peru, Chile, Spain, Australia and New Zealand which have implemented this technique. Current users of HP processing are being able to market and export different high-value added, high quality, fresh and minimally processed avocado products such as avocado halves, chunks, purée, guacamole or avocado-based sauces (Purroy Balda, Tonello, Peregrina, & de Celis, 2011).

Despite that HP processed avocado products are commercially available, there is little information concerning the effects of high-pressure on their quality deteriorative enzymes. Enzymes are molecules with a complex structure maintained by a delicate balance of non-covalent forces (e.g., H bonds, ion pairs, and hydrophobic and Van der Waals interactions). Changes in external factors, such as pressure and temperature, can perturb this subtle balance and produce conformational changes that induce enzyme inactivation. From an engineering point of view, kinetic data about high-pressure inactivation of enzymes are indispensable for the design, the evaluation and the optimization of high-pressure processes (Hendrickx, Ludikhuyze, Van Den Broeck, & Weemaes, 1998). Most of the papers in the literature are focused on polyphenoloxidase (PPO) and lipoxygenase (LOX) pressure inactivation because these oxidative enzymes strongly affect the color, taste and nutritional value of avocado products. Results obtained showed that pressure processing of avocado paste at 600–700 MPa for 3–10 min is

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effective to reduce PPO and LOX activities by approximately half, although reactivations of both enzymes have been observed during the refrigerated storage of the product (Jacobo-Velázquez & Hernández-Brenes, 2010, 2012; López et al., 1998; Palou et al., 2000; Weemaes, Ludikhuyze, Van den Broeck, & Hendrickx, 1998a,b,c, 1999). Nevertheless, there is not yet any published study about the effect of high-pressure on avocado enzymes implied in the breakdown of cell-wall polymers, such as cellulose, hemicelluloses and pectin. They are particularly interesting because the textural and structural properties of vegetal foods largely depend on the integrity of the cell walls.

In dicotyledonous plants, the cell wall is approximately 30% cellulose, 30% hemicellulose, 35% pectin and 5% protein (Fry, 1988). Pectin is especially relevant because it is the common and major component of primary cell wall and middle lamella and constitutes, together with hemicelluloses, the matrix in which cellulose microfibrils are embedded. Thus, it has a strong impact on the structure-related quality characteristics of fruits, vegetables, and the related intermediate and end products (Duvetter et al., 2009; Jolie et al., 2012; Sila et al., 2008; Van Buggenhout, Sila, Duvetter, Van Loey, & Hendrickx, 2009). Pectin comprises a diverse group of heteropolysaccharides containing partially methylated D-galacturonic acid residues with side chain appendages of several neutral polysaccharides. Different pectinases (pectinmethylestease, polygalacturonase, β-galactosidase) have been identified in avocado fruit able to degrade this complex macromolecule (Awad & Young, 1979; Jeong & Huber, 2004; Wakabayashi & Huber, 2001; Zauberman & Schiffmann-Nadel, 1972). Among them, polygalacturonase (PG) has been pointed to induce loss of structural integrity of tissue systems and also may cause a viscosity or consistency loss in pastes and purées of plant sources (Jolie et al., 2012). Polygalacturonase cleaves the α-(1 → 4)-D linkages between two adjacent galacturonic acid residues in the homogalacturonan domain of pectin. The lower the degree of esterification of pectin, the better substrate it becomes for PG (Crelier, Robert, Claude, & Juillerat, 2001). Therefore, pectinmethylesterase, which catalyzes the de-esterification of pectine, enhances PG activity. Through depolymerization of cell wall pectins, PG has been probed to play a critical role in the softening of avocado fruit (Awad & Young, 1979; Zauberman & Schiffmann-Nadel, 1972) and, therefore, this paper is focused in this pectinase.

The main objective of this work was to describe high-pressure inactivation kinetics of avocado polygalacturonase. The effect of high-pressure processing on the stability of PG should be a subject of major interest for avocado processors because this endogenous enzyme might strongly affect the texture of pressure-treated avocado halves and chunks and the rheological properties of guacamole, avocado purée and sauces during their storage. To make comparisons with the conventional processing technique usually employed in the industry, thermal inactivation kinetics was also included in the study. This is particularly interesting because the published researches on thermal stability of avocado PG are very limited (Barash & Khazzam, 1970; Wakabayashi & Huber, 2001). For the experiments, a batch of crude avocado PG extract was prepared and its thermal and pressure stabilities were tested in wide temperature and pressure ranges, respectively. From the results obtained in these preliminary experiments, appropriate temperature and pressure levels were selected to perform a detailed kinetic study. In this study, inactivation kinetics was investigated under isothermal conditions at atmospheric pressure and under isobaric conditions at 20 °C. Finally, the transferability of the results obtained in crude avocado PG extract to avocado purée was evaluated.

2. Materials and methods

2.1. Raw material and purée preparation

Avocados (*Persea americana* Mill, cv. Hass), imported from Chile, were purchased, at commercial maturity, from a local shop (Leuven, Belgium). Then, they were peeled, cut into small pieces and blended using a kitchen blender (Waring blender 7010G, Torrington, CT, USA) to obtain a homogenous batch of avocado purée. This purée was vacuum-packed in plastic bags, frozen in liquid nitrogen and stored at −40 °C, for one month maximum, until use.

Avocado purée was, on the one hand, used to prepare crude PG extract (Section 2.2) for which the thermal and high-pressure stability was studied. On the other hand, the purée itself was either thermally or high-pressure treated to investigate the in situ PG inactivation. In this case, crude PG extract was prepared after the thermal and high-pressure treatments.

2.2. Preparation of crude avocado PG extract

A batch of crude avocado PG extract was prepared according to the method described by Wakabayashi and Huber (2001) with some modifications. Avocado purée, previously thawed in a water bath at 20 °C, was mixed with 95% ethanol (1:2, w/v) and stirred for 20 min. The homogenate obtained was subsequently filtered through a cheesecloth and the residue was re-suspended in 95% ethanol (1:4, w/v), stirred for 1 h and filtered. These washing steps with ethanol were necessary due to the high oil content of avocado mesocarp which makes the sample processing difficult. Wakabayashi and Huber (2001) proved that the levels of PG activity recovered are not adversely affected by these ethanol pre-washes. The residue obtained was suspended in 50 mM Na-acetate buffer, pH 3.0, (1:4, w/v), stirred for 1 h and centrifuged at 20,000 *g* for 30 min. Since avocado PG remains wall-bound at low pH (Wakabayashi & Huber, 2001), the purpose of this washing step was to remove soluble pectins, weakly associated proteins and low molar mass cell constituents to reduce the amount of reducing groups in the final extract. Then, the supernatant was discarded and the pellet was dissolved in 50 mM Na-acetate buffer, pH 6.0, containing 1.8 M NaCl (1:3, w/v) and stirred overnight to extract ionically bound cell wall proteins. Finally, the suspension was centrifuged for 30 min at 20,000 *g* and the supernatant, that is the crude avocado PG extract, was filtered through a cheesecloth. This crude extract was divided into aliquots, frozen in liquid nitrogen and stored at −40 °C until use. All the previous steps were performed at 4 °C.

2.3. Thermal treatments

Isothermal treatments were performed, at atmospheric pressure, in a thermostated water bath in a temperature range from 47.5 to 95 °C. Crude PG extract samples, previously thawed, were filled in capillary tubes (Hirschmann, Germany; 1.15 mm i.d. × 150 mm length) while thawed purée samples (2 g) were vacuum-packed in polyethylene bags to minimize heating and cooling lags.

In experiments to test the thermal stability of avocado PG, samples were maintained in the water bath for 10 min. Then, they were immediately cooled in ice water. These experiments, carried out to select the most appropriate temperature range to be employed in the subsequent kinetic study, were performed at least in duplicate. In the kinetic study, crude extract samples were removed from the bath after preset time intervals, between 1.5 and 140 min, and cooled in ice water.

The residual PG activity was measured in all the samples as described below (Section 2.5). In purée samples, PG extraction was performed just after the thermal treatment.

2.4. High-pressure treatments

High-pressure experiments were carried out in a laboratory scale multivessel HP equipment (Resato, Roden, The Netherlands) at 20 °C and pressures between 0.1 and 750 MPa. The equipment consists of 8 individual vessels surrounded by thermostatic mantles which were connected to a cryostat for temperature control during the experiments. The pressure medium was a commercial glycol–oil mixture (TR15, Resato). The samples, either crude extract or purée, were filled in flexible micro tubes (0.25 and 0.4 mL, Carl Roth, Karlsruhe, Germany)