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Modeling the polyphenoloxidase inactivation kinetics in pear, apple and strawberry purees after High Pressure Processing

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ABSTRACT

High Pressure Processing of Royal Gala apple, Taylor's Gold pear and Camarosa strawberry purees was carried out to inactivate polyphenoloxidase (PPO). First, 600 MPa enzyme inactivation at room temperature was investigated. After 60 min, strawberry (8% RA, residual activity) and apple (89% RA) PPOs were partially inactivated and pear PPO was not inactivated, demonstrating the high variability in the resistance of different fruits' PPOs. Then, the fruit purees were submitted to 600 MPa combined with mild heat, and the PPO inactivation kinetics was modeled. The pear PPO was found to be resistant even after 60 min 600 MPa–71 °C process. Regarding apple and strawberry PPOs, 600 MPa-thermal inactivation of PPO followed a biphasic first order kinetics exhibiting stable and labile fractions. The k_S values (rate constant for stable fraction) at 57 °C and 71 °C were 0.0121 min^{-1} and 0.0184 min^{-1} for apple PPO, and 0.0182 min^{-1} and 0.0805 min^{-1} for strawberry PPO.

1. Introduction

Enzymatic browning is a concern in the fruit processing industry (Ferrar and Walker, 1996; Lambrecht, 1995; Martinez and Whitaker, 1995; Queiroz et al., 2008; Silva and Gibbs, 2004; Sulaiman and Silva, 2013; Vámos-Vigyázó, 1981). The activity of polyphenoloxidase (PPO, EC 1.10.3.1), an endogenous enzyme, causes browning when the fruit tissues are exposed to oxygen during processing and storage. Some other names for this enzyme are catechol oxidase, tyrosinase, phenolase, catecholase, o-diphenol oxidase. The optimal pH and temperatures for the activity of PPO are 5–8 and 20–40 °C, respectively (Dalmadi et al., 2006; Navarro et al., 2014; Siddiq et al., 1993; Wu et al., 2013; Yang et al., 2000). PPO catalyses the degradation of phenolic fruit constituents (o-diphenol oxidizes to o-quinones) in the presence of oxygen. The resulting o-quinone will subsequently polymerize with other o-quinone, protein or amino acids to produce browning compounds, the melanoidin pigments (Golangoldhirsh et al., 1984; Vámos-Vigyázó, 1981).

Currently, the most reliable method for controlling browning is thermal processing, often referred to in the industry as blanching. The thermal inactivation kinetics of PPO has been studied in several fruits and fruit cultivars, with the finding that temperatures in the range of 60–85 °C are required for PPO inactivation (Dimick et al., 1951; Goyeneche et al., 2013; Halim and Montgomery, 1978; Ludikhuyze et al., 2003; Silva and Gibbs, 2004; Soysal, 2008; Wakayama, 1995; Yemenicioglu et al., 1997; Zhou and Feng, 1991). The heat employed in thermal processing negatively affects the fruit flavour generating 'cooked-notes' (Silva et al., 2000). The heat can also destroy nutritive compounds in the fruits. Anti-browning agents such as ascorbic acid, sulphites, sodium chloride, cysteine, kojic acid and cinnamic acid are used for food preservation (Queiroz et al., 2008), although consumers have been choosing preservative-free foods, with a global trend to reduce the use of chemical food additives.

Due to the demand for fresh and minimally processed fruit products which are preservative-free, non-thermal food processing such as High Pressure Processing (HPP) has been researched and used commercially. HPP inactivates microorganisms responsible for food deterioration while retaining the original sensory properties and thermolabile nutrients of the raw fruits, as no heat or mild heat is used during processing (Butz et al., 2003; Dalmadi et al., 2008; Landl et al., 2010; Phunchaisri and Apichartsrangkoon, 2005; Terefe et al., 2014). HPP at room temperature can have limited effectiveness for the inactivation of enzymes associated with food spoilage, such as PPO (Weemaes et al., 1998a). Therefore, the combination of HPP with heat (<80 °C) has been studied, and the kinetics at 600 MPa reported

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for some fruits/cultivars. Although zero order was observed for Gala and Golden Delicious apple PPOs inactivation, the inactivation of Braeburn, Granny Smith and Red Delicious apple PPOs was modeled with the simple first order model (Falguera et al., 2013). Additionally, the first order biphasic model has been used to describe the inactivation kinetics for strawberry (cv. Elsanta), white grapes (cv. Victoria) and avocado (cv. not specified) PPOs (Dalmadi et al., 2006; Rapeanu et al., 2005; Weemaes et al., 1998b). Less commonly, second order kinetics has been registered for Fuji apple (Falguera et al., 2013) and 2.2 order model determined for HPP-thermal PPO inactivation in Boskop apple juice (Buckow et al., 2009).

Given the high variability of PPO resistance and inactivation kinetics among different fruits or fruit cultivars, the HPP-thermal inactivation of PPO in more fruits should be investigated. Additionally, enzyme studies are of crucial importance for emerging non-thermal food preservation technologies such as HPP. Several studies have processed the PPO extracts rather than the fruits containing the enzyme, thus not reproducing the industrial reality and providing misleading results. More kinetic studies will assist fruit processors in the selection of appropriate HPP conditions to avoid fruit browning during storage and distribution. The maximum pressure currently employed commercially is around 600 MPa. Therefore, in this work several fruit cultivars (purees) were processed by HPP at 600 MPa combined with thermal processing. The two objectives were as follows: (i) to investigate the effect of 600 MPa-room temperature and 600 MPa-thermal processing on five fruit cultivars' PPOs; (ii) to model the PPO inactivation kinetics for 600 MPa-thermal processed Taylor's Gold pear, Royal Gala apple and Camarosa strawberry purees.

2. Materials and methods

2.1. Preparation of the five fruit cultivars samples

Ripe pear (*Pyrus communis* cv. Taylor's Gold and cv. Nashi, pH: 4.6 ± 0.07, Soluble solids: 16.5 ± 0.7 °Brix) and apple (*Malus domestica* cv. Royal Gala and cv. Scirose, pH: 4.1 ± 0.01, Soluble solids: 11.2 ± 0.4 °Brix) were sourced from a local fruit supplier. Ripe strawberries (*Fragaria ananassa,* cv. Camarosa, pH: 3.3 ± 0.05, Soluble solids: 9.3 ± 0.1 °Brix) were bought from a local farm (Phil Greig Strawberry Farm, Kumeu, New Zealand). The fruits from these five cultivars were peeled, cored, cut into smaller pieces and blended using a commercial blender. Each fruit cultivar's puree was packed in food grade retort pouches (Cas-Pak, New Zealand) composed of polyester coated with silicon oxide, laminated to nylon and laminated to cast polypropylene (PET-SIOX(12)//ON(15)//RCPP(70)). This bag was 1 mm thick, with low oxygen transmission rate (<2 cc/m^2/day) and could withstand temperatures up to 130 °C, being suitable for thermal and High Pressure Processing. Twenty grams of puree were vacuum packed in 150 mm × 105 mm pouches. Fruit thermal conduction was minimized by packing a small size fruit sample in a large surface area pouch, so that no temperature distribution occurred and fruit temperature could be considered uniform inside the bag. The packed samples were stored at −70 °C and thawed in a commercial refrigerator overnight before treatment. At least two replicates of packed samples were processed for each processing condition. The enzyme activity was determined for a raw unprocessed sample (A_0) and a processed sample (A) as described in the following section. The average enzyme residual activity ± standard deviation (RA = residual activity = A/A_0) was calculated and plotted.

2.2. Polyphenoloxidase (PPO) extraction and assay

Analytical grade chemicals, namely, catechol, polyvinylpolypyrrolidone (PVPP) (Sigma Aldrich, Germany) and Triton X-100 (Ajax FineChemical, Australia) were used for PPO extraction and assay.

For each processed and non-processed fruit sample, the PPO enzyme was extracted from the purees, and the enzyme activity was measured as follows. Enzyme extraction was carried out as described previously by Sulaiman and Silva (2013). Unprocessed and processed fruits (10 g) were mixed using a commercial blender for 3 min with 20 mL of 0.2 M sodium phosphate buffer (pH 7.0) and 4% (w/v) insoluble PVPP with the addition of 1% (v/v) triton X-100 and 50 μL of 1 M NaCl. The homogenates were then centrifuged in 1.5 mL centrifuge tubes at 14,000*g* for 30 min. The supernatant containing PPO was taken out and PPO activity was assessed spectrophotometrically at 420 nm, by recording the absorbance increase for 15 min (Perkin Elmer Lambda 35 UV–visible). The sample cuvette contained 3 mL of catechol substrate in a 0.07 M (pH 5.8) phosphate buffer and 100 μL of undiluted PPO extract from fruit. The pH of phosphate buffer used in the analysis was 5.8, since that is within the range for optimal enzyme activity. The blank was prepared by mixing 100 μL of distilled water with 3 mL catechol solution in phosphate buffer (pH 5.8). Enzyme activity was calculated from the linear portion of the plot of absorbance (mAbs) against time (min) and was expressed as mAbs/min. The sample was stored in the refrigerator prior to enzyme extraction, which was carried out within 24 h after each treatment. All activity analyses were carried out in replicates and the average result was registered for each sample of fruit enzyme extract.

2.3. High Pressure Processing (HPP)

Packed fruit puree samples were processed using the Avure 2L-700 HPP Laboratory Food Processing System (Serial No. 101130, USA) containing distilled water as the pressure medium in the treatment chamber. The HPP chamber was equipped with a thermocouple to register the temperature during the HPP cycle. This unit can operate at up to 600 MPa pressure and a temperature of around 70 °C. At the end of the constant pressure phase, the release of the pressure caused an instantaneous decompression. The samples were immediately cooled in an ice-water bath before the enzyme extraction. The pressure selected for this study (600 MPa) was based on previous results, which indicated effectiveness of PPO inactivation at HPP ⩾ 600 MPa (Buckow et al., 2009; Garcia-Palazon et al., 2004; Sulaiman and Silva, 2013; Weemaes et al., 1998a). The pressure–temperature-time processing conditions refer to the constant pressure phase of the HPP cycle. The total pressure increase took less than 2 min.

Table 1
Polyphenoloxidase (PPO) residual activity of different fruit cultivars purees submitted to High Pressure Processing (HPP) at room temperature (600 MPa, 15 min) and HPP combined with thermal processing (600 MPa, 62 °C, 15 min).*

Fruit cultivar	HPP + Thermal	HPP at room temperature
Pear cv. Taylor's Gold	122 ± 0.2*b*	120 ± 1.8*b*
Pear cv. Nashi	88 ± 1.5*c*	121 ± 6.9*b*
Apple cv. Scirose	59 ± 5.8*d*	40 ± 5.8*e*
Apple cv. Royal Gala	59 ± 4.7*d*	174 ± 6.4*a*
Strawberry cv. Camarosa	2 ± 0.7*g*	19 ± 4.3*f*

* Two replicates of the same processing conditions were carried out and PPO enzyme residual activity is expressed as the average ± standard deviation. The PPO residual activities with different letters are significantly different ($p < 0.05$).