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

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ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 16 September 2014
Accepted 21 September 2014
Available online 28 September 2014

Keywords:
Browning
Polyphenoloxidase
Enzyme
High Pressure Processing
High hydrostatic pressure
Kinetics

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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http://dx.doi.org/10.1016/j.jfoodeng.2014.09.030
0260-8774/

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 ($p < 0.05$).

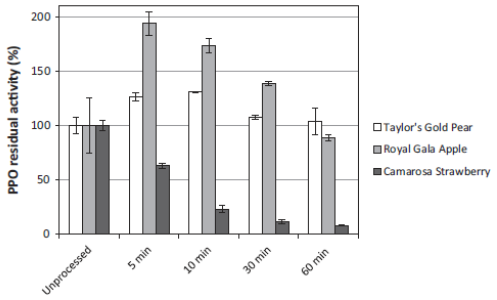

Fig. 1. The effect of 600 MPa HPP at 34 °C for processing times between 5 and 60 min on PPO residual activity (%) of Taylor's Gold pear, Royal Gala apple and Camarosa strawberry purees.

2.4. Polyphenoloxidase (PPO) residual activity in five fruit cultivars after 600 MPa HPP

The five fruit cultivars' purees mentioned in Section 2.1 were processed at 600 MPa-room temperature (34 °C) and 600 MPa–62 °C for 15 min to determine the PPO inactivation and residual activity. One-way analysis of variance (ANOVA) (Statistica 12, Statsoft®, USA) and separation of the ten means (Tukey's honest significant difference, HSD) were carried out. Significantly different treatments were marked with different letters ($p < 0.05$).

Then, the effect of high pressure (600 MPa) at room temperature was further investigated for 5, 10, 30 and 60 min in pear (cv. Taylor's Gold), apple (cv. Royal Gala) and strawberry (cv. Camarosa).

2.5. Modeling the kinetics of PPO inactivation by 600 MPa HPP-thermal processing in three fruits

In this experiment, Taylor's Gold pear, Royal Gala apple and Camarosa strawberry were chosen because they are common commercial cultivars, well known for their sweet tastes, which are suitable for industrial processing into purees and sauces. The effect of 600 MPa combined with mild temperature on fruit endogenous PPO activity was investigated for varying treatment times up to 60 min. Average processing temperatures during the holding constant pressure phase (600 MPa) of the HPP cycle were 57, 62 and 71 °C. The first order, fractional conversion and Weibull models were attempted to model PPO inactivation data and the performance was compared using R^2 (coefficient of determination), χ^2 (chi-square) and residuals distribution analysis (Sant'Anna et al., 2010), as these models had successfully described enzyme inactivation in foods (Dalmadi et al., 2006; Ludikhuyze et al., 2003; Shalini et al., 2008; Terefe et al., 2010). The temperature dependence of the estimated rate constants/coefficients was also investigated. TableCurve 2D software (version 5.01, Systat software Inc., USA) was used to fit different models and to determine the goodness of fit.

2.5.1. First order model for linear inactivation data

First the linearity in the inactivation of PPO was checked by plotting $\ln(A/A_0)$ vs time (Eq. (1)):

$$\frac{A}{A_0} = \exp(-k_T t) \quad \text{or} \quad \ln\frac{A}{A_0} = -k_T t \tag{1}$$

where A is enzyme activity in a sample processed for time t (min), A_0 is initial enzyme activity of the raw unprocessed sample, A/A_0 is the residual activity of the enzyme and is the first order inactivation rate constant at temperature T (min^{-1}).

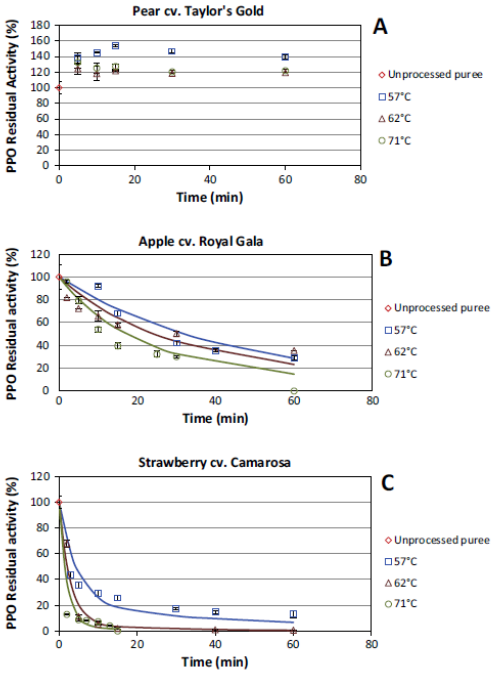

Fig. 2. The effect of combined 600 MPa High Pressure and thermal processing (57, 62, 71 °C) of the fruit puree on Taylor's Gold pear's polyphenoloxidase (PPO) (A), Royal Gala apple's PPO (B) and Camarosa strawberry's PPO (C) (solid lines represent the first order biphasic kinetic model).

The Arrhenius equation shows the temperature dependence of the PPO inactivation rates (Eq. (2)):

$$\ln(k_T) = \ln(C) - \frac{E_a}{R}\left(\frac{1}{T}\right) \tag{2}$$

where T is the temperature (K), E_a is the activation energy (kJ/mol), R is the universal gas constant (8.314 J/(mol.K)) and C is Arrhenius constant.

The first order kinetics can also be expressed by D- and z-values, the Bigelow model commonly used by microbiologists. The decimal reduction time D_T is the time needed for 90% reduction in activity at temperature T. It can be estimated from the negative reciprocal of the slope of the $\log(A/A_0)$ versus time plot (Eq. (3)):

$$\frac{A}{A_0} = 10^{-\frac{t}{D_T}} \quad \text{or} \quad \log\frac{A}{A_0} = -\frac{t}{D_T} \tag{3}$$

D_T-value can be calculated from k_T and vice versa using the following equation:

$$D_T = \frac{2.303}{k_T} \tag{4}$$

The z-value is defined as the temperature increase required for a 10-fold decrease in D-value and is calculated from the negative reciprocal of the slope of $\log D$ versus temperature line (Eq. (5)):

$$\log D_T = \log D_{T_{ref}} - \frac{T - T_{ref}}{z} \tag{5}$$

Table 2
Summary of the performance of selected models fitted to apple and strawberry PPOs inactivation data by combined 600 MPa High Pressure and thermal processing.*

Fruit	Model	R^2	χ^2	Residual plot	Remarks
Apple cv. Royal Gala	First order	0.524–0.936	0.0040–0.0160	Patterned	Low R^2 and patterned residual plot; rejected
	First order biphasic	0.931–0.981	0.0005–0.0120	Random	Good fit for effect of temperature on k; accepted
	Fractional conversion	0.894–0.933	0.0035–0.0073	Patterned	Patterned residual plot; rejected
	Weibull distribution	0.932–0.985	0.0005–0.0074	Random	Poor fit for dependence temperature parameters; rejected
Strawberry cv. Camarosa	First order	0.351–0.914	0.0043–0.0180	Patterned	Low R^2 and patterned residual plot; rejected
	First order biphasic	0.900–0.964	0.0002–0.0059	Random	Good fit for effect of temperature on k; accepted
	Fractional conversion	0.901–0.979	0.0007–0.0058	Patterned	Patterned residual plot; rejected
	Weibull distribution	0.983–0.989	0.0003–0.0006	Random	Poor fit for dependence temperature parameters; rejected

* Good models have higher R^2 (coefficient of determination), lower χ^2 (chi-square values) and random residuals. Estimated k values (rate constants) and Weibull distribution time coefficients (at 57, 62 and 71 °C) should increase with temperature.

where is the D-value at T_{ref}. T_{ref} can be any reference temperature.

2.5.2. Non-linear models

When non linearity was observed, the two fractions or biphasic (Eq. (6)), Weibull (Eq. (7)) and fractional conversion (Eq. (8)) models were attempted:

$$A = A_S \exp(-k_S t) + A_L \exp(-k_L t) \quad (6)$$

where A_S and A_L are activities of the stable and the labile fractions, respectively and k_S and k_L are the inactivation rate constant of stable and labile fractions, respectively.

$$\log \frac{A}{A_0} = -bt^n \quad (7)$$

where b and n are scale and shape factors, respectively (Peleg and Cole, 1998).

$$A = A_0 + (A_0 - A_\infty) \exp(-kt) \quad (8)$$

where A_∞ is residual activity after prolonged treatment time.

3. Results and discussion

3.1. Polyphenoloxidase (PPO) residual activity in five fruit cultivars after 600 MPa HPP

Table 1 presents the percentage residual activity (RA) of PPO in different fruit cultivars after 600 MPa HPP-thermal (62 °C) and HPP at room temperature (⩽34 °C) processes with a duration of 15 min. Regarding HPP-62 °C processing, with the exception of Taylor's Gold pear (RA = 122%), fruits' PPOs were inactivated after processing as follows: 88% RA for Nashi pear puree, 59% RA for Scirose and Royal Gala apples purees and 2% RA for Camarosa strawberry puree. Terefe et al. (2010) reported 95% RA for Aroma strawberry puree after similar processing, which demonstrates the high variability of PPO resistance within the same fruit and among different cultivars. The results demonstrate that 600 MPa–62 °C for 15 min can reduce the PPO activity in several fruits.

With respect to 600 MPa HPP at room temperature, Camarosa strawberry PPO showed the least RA of 19%. The same value was obtained by Guerrero-Beltran et al. (2004) with peach processed for 5 min at 517 MPa. Following that was Scirose apple puree with 40% RA. By contrast, Royal Gala apple, Taylor's Gold and Nashi pears' PPOs were activated after being submitted to the same processing conditions. Del Pozo-Insfran et al. (2007) and Terefe et al. (2010) also reported activation in muscadine grape (250% RA) and Aroma strawberry (113% RA), respectively. Fig. 1 presents more results of 600 MPa HPP-room temperature of Taylor's Gold pear, Royal Gala apple and Camarosa strawberry, for longer times (up to 60 min). Fruit types and processing time play an important role in PPO activation or inactivation. While strawberry PPO was inactivated from 63% RA (5 min) to 8% RA (60 min) without prior activation, pear and apple PPOs were activated by 26% and 94% after 5 min, respectively. This has also been observed with Boskop apple juice PPO, which showed activation after processing at mild temperature (45–55 °C) and/or pressure (200–500 MPa) followed by a decrease in activity after prolonged treatment times (>10 min) (Buckow et al., 2009). Other publications have reported enzyme activation after pressure treatments (Heremans, 1993; Johnson and Campbell, 1945; Mozhaev et al., 1996). HPP affects the protein conformation and leads to conformational changes, denaturation, aggregation or gelation (Hendrickx et al., 1998; Knorr et al., 2006; Terefe et al., 2014). Weemaes et al. (1997) reported that HPP (600–900 MPa) caused structural changes in mushroom PPO. The modifications in the enzyme's secondary and tertiary structure can either increase or decrease enzymatic activity by changing its substrate specificity and modifying functionality (Hendrickx et al., 1998). Our HPP-room temperature results demonstrated the importance of combining HPP with thermal processing for PPO inactivation, as temperature can destabilize the hydrogen bonding (Heremans, 1993; Johnson and Campbell, 1945; Mozhaev et al., 1996; Sulaiman and Silva, 2013). Additionally, the results from this work and from the literature show a high variability of PPO resistance to HPP and HPP combined with thermal processing. Fruit composition could also be the reason for different PPO inactivation/activation in different cultivars. For example, Rapeanu et al. (2006) concluded PPO resistance from Victoria grape was higher in the grape must than the buffer solution at the same pH.

3.2. Modeling the kinetics of PPO inactivation by 600 MPa HPP-thermal processing in Taylor's Gold pear, Royal Gala apple and Camarosa strawberry

HPP in combination of temperatures ranging between 57 and 71 °C were selected based on the results obtained in the previous section and maximum temperature supported by the HPP equipment. Experimental and predicted data for PPO RA versus time are shown in Fig. 2 for Taylor's Gold pear, Royal Gala apple and Camarosa strawberry PPOs. Pear's PPO was found to be resistant to 600 MPa-thermal processing for 60 min as shown in graph A of Fig. 2, without inactivation even at 71 °C, the maximum temperature tested. Therefore, the kinetics could not be modeled. Non-linearity was observed in PPO inactivation by HPP combined with thermal in apple and strawberry. Three non-linear models were attempted (Table 2). A good model presents higher R^2, lower χ^2 and random residuals; therefore Weibull and first order biphasic models better fitted the data. The k values obtained from the first order biphasic kinetics (Eq. (6)) showed good temperature dependence, as opposed to Weibull time coefficients (Eq. (7)). Therefore, the first order biphasic (Eqs. (2) and (6)) was the most appropriate model to describe the HPP-thermal inactivation of PPO in Royal Gala apple ($R^2 \geqslant 0.93$) and Camarosa strawberry ($R^2 \geqslant 0.90$) (Fig. 2, Table 2). The same model has been used previously with avocado, Victoria white grapes and Elsanta strawberry (Dalmadi