Thermal inactivation of polyphenoloxidase and peroxidase in Jubileu clingstone peach and yeast isolated from its spoiled puree

Andréa Menezes LOPES[1]*, Ricardo Peraça TORALLES[2], César Valmor ROMBALDI[3]

Abstract

The thermal inactivation of yeast isolated from spoiled Jubileu peach puree and that of polyphenoloxidase (PPO) and peroxidase (POD) in cv. Jubileu, which is widely cultivated in southern Rio Grande do Sul state, Brazil, were studied. PPO and POD were extracted using the protein powder method and submitted to partial purification by precipitation followed by dialysis. The enzymatic activity was determined measuring the increase in absorbance at 420 nm for PPO and 470 nm for POD. The yeast used in this investigation was isolated from spoiled Jubileu peach puree at 22 °Brix, with total initial microbial count of 22 × 10^2 UFCmL^{-1}. Stock cultures were maintained on potato dextrose agar (PDA) slants at 4 °C and pH 5 for later use for microbial growth. In all cases, kinetic analysis of the results suggests that the thermal inactivation was well described by a first-order kinetic model, and the temperature dependence was significantly represented by the Arrhenius law. Both enzymes were affected by heat denaturation, and PPO was more thermostable. PPO was also more thermostable than the yeast isolated from peach puree. The D60-values were 1.53 and 1.87 min for PPO and yeast isolated from spoiled Jubileu peach puree, respectively.

Keywords: peach puree; heat resistance; yeast; enzymatic browning, blanching.

1 Introduction

The natural color and taste of fruits are important attributes for the consumer's choice (Toralles et al., 2006). Preserving them in minimally processed fruits, purees, nectars, and juices is a big challenge (Guerrero-Beltrán et al., 2004; McLellan & Padilla-Zakour, 2005; Quevedo et al., 2011).

Polyphenoloxidase (PPO) and peroxidase (POD) are enzymes associated with oxidative deterioration reactions that can cause browning of these products when not properly controlled (Brito et al., 2005; Toralles et al., 2010). Besides the color change, enzymatic browning can induce severe changes in flavor and texture and cause nutritional losses (Valderrama & Clemente, 2004; Garcia & Barrett, 2005; Prohens et al., 2007; Bi et al., 2013).

Polyphenoloxidases catalyze the oxidation of phenolic substrates using oxygen as a hydrogen acceptor in two different types of reactions. PPO (EC 1.14.18.1, monophenol, L-dopa: oxygen oxidoreductase) is involved in the hydroxylation of monophenols in order to originate o-diphenols and PPO (EC 1.10.3.1, 1,2-benzenodiol: oxygen oxidoreductase), which catalyzes the removal of hydrogen from o-diphenols to produce an o-quinonone (Ramírez et al., 2003).

Peroxidase catalyzes four types of reactions: peroxidatic, oxidatic, catalatic, and hydroxylation. For phenolic substrates, only the peroxidatic reaction is important. In this case, POD (EC 1.11.1.7., H_2O_2 donor, oxidoreductase) catalyzes the reaction of hydrogen peroxide or other organic peroxide, which is reduced, whereas an electron donor (AH_2) is oxidized. The electron donor may be ascorbate, phenols, amines, or other organic compounds (Brito et al., 2005). In many cases, the oxidation product is colorful and serves as the basis for the colorimetric determination of peroxidase activity. On the other hand, the oxidative reaction can occur in the absence of hydrogen peroxide; however, it requires the presence of oxygen and cofactors (manganese and phenol). Hydroquinone, dihydroxyfumaric acid, and ascorbic acid are the possible substrates to be treated in this type of reaction (Pinto, 2008). Furthermore, the enzyme action aims primarily to control the level of peroxides generated in almost all cellular compartments and, in the absence of a hydrogen donor, peroxidase converts hydrogen peroxide into H_2O and O_2. This catalatic reaction is at least a thousand times slower than peroxidation (Pinto, 2008; Ranieri et al., 2001).

In peaches, the activity of PPO and POD has long been known and has still been widely studied (Reyes & Luh, 1960; Flurkey & Jen, 1978; Alba et al., 1996; Girner et al., 2002). For Brazilian cultivars, although thermal inactivation of PPO and POD in Granada clingstone peaches (Toralles et al., 2005) and even the potential for enzymatic browning of some Brazilian peach cultivars (Toralles et al., 2004) are known, there is very little literature pertaining to the thermal inactivation of other peach cultivars. Knowing such inactivation kinetics is very important because, traditionally, the control of enzymatic browning in fruit juices and purees results from the combination of heat treatment and chemical inhibitors (McEvily et al., 1992; Funamoto et al., 2003; Toralles et al., 2010).

On the other hand, knowing the types of microorganisms present in purees, as well as their thermal resistance, is of

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[1] *Escola de Química e Alimentos, Universidade do Rio Grande – FURG, Rio Grande, RS, Brasil, e-mail: andrealopes@furg.br*
[2] *Laboratório de Bioquímica, Departamento de Química, Instituto Federal Sul Riograndense – IFSUL, Pelotas, RS, Brasil*
[3] *Departamento de Ciência e Tecnologia Agroindustrial, Faculdade de Agronomia Eliseu Maciel, Campus Universitário, Universidade Federal de Pelotas – UFPEL, Pelotas, RS, Brasil*
**Corresponding author*

fundamental importance to produce a microbiologically stable product. More than 160 types of microorganisms have already been identified at different stages of peach puree processing, particularly the species *Bacillus badius*, *Penicillium sp*., and *Saccharomyces cerevisiae* (Garza et al., 1994a). The last one, when not properly inactivated during pasteurization, may cause fermentation of the peach puree basically in two key steps: the transformation of sugar into pyruvate and of pyruvate into ethanol (Guerrero-Beltrán & Barbosa-Cánovas, 2006; Fazio, 2006). However, no report was found on the use of thermal treatment to inactivate yeasts isolated from peach puree obtained from a Brazilian cultivar.

The present research studied the thermal inactivation of PPO, POD, and yeasts in cv. Jubilee peach with the objective of defining an indicator for thermal inactivation. The parameters selected were: inactivation rate constant (k), activation energy (E_a), half-life time ($t_{1/2}$), reduction time (D), and value (Z). Cultivar Jubilee was chosen due to its low potential for browning and its sensory attributes that are favorable for the elaboration of puree, nectars, and juices (Toralles et al., 2006).

2 Materials and methods

2.1 Peach puree

Ripe and sound-appearing clingstone cv. Jubileu peaches were picked from a commercial orchard in Pelotas/RS, Brazil, and stored at 1-3 °C for two days at most during the experiment. Jubileu peach puree was obtained directly in plants of the Food Technology Department of IFSUL - Pelotas, using the methodology described by Toralles & Vendrusculo (2007). Following the process proposed by these authors for Granada peach puree, the peaches were initially peeled, pitted, and blanched at 75 °C for 4 minutes. Later, the blanched pulp was refined to a 1 mm particle size to form the peach puree. The puree was stored in a stainless steel tank for the addition of 0.08% (w/w) ascorbic acid and then immediately centrifuged in a continuous separator (Westfallen – Germany KO 2006) at 8,380 rpm. A conventional homogenizer (Kirchfeld – Germany HL) was used to homogenize the centrifuged peach puree under a high pressure of 100 bar. After that, those authors recommend concentrating the homogenized peach puree by vacuum evaporation using a falling-film evaporator (Wiegand - Germany) in order to reach 32 °Brix. In the current study, for technical reasons, the peach puree was concentrated at 22 °Brix. Finally, the thermal treatment of puree was carried out in completely filled and hermetically sealed glass flasks 9 cm in diameter and 10 cm tall, which were then submerged in a water bath at 100 °C for 15 minutes. Three sample flasks were placed in a circulator bath at 1.7 °C and stored at -20 °C for six years. The experiments were performed three times by analyzing the parameters, and also the physical and chemical analyses of the peach puree were determined by the second methodology of the Institute Adolfo Lutz (2008).

2.2 Microorganisms and microbial growth

The microorganisms used throughout this investigation were isolated from spoiled Jubileu peach puree at 22 °Bix and counted as described by Siqueira (1995). The total initial microbial count, 22×10^2 UFC.mL^{-1}, was taken after 5 days of storage at 25 °C using potato dextrose agar plates. Stock cultures were maintained on potato dextrose agar (PDA) slants at 4 °C and pH 5. At this pH, the growth of yeasts in detriment of mold was observed. The yeasts were grown in shaken Erlenmeyer flasks (150 rpm) at 30 °C for 24 h in a culture medium (pH 5) with the following composition: yeast BDA (20 g.L^{-1}); glucose (20 g.L^{-1}); $(NH_4)_2S0_4$ (1 g.L^{-1}); KH_2P0_4 (2 g.L^{-1}); $MgS0_4.7H_20$ (0.5 g.L^{-1}); $FeS0_4$ (0.5 g.L^{-1}), and peptone (5 g.L^{-1}). Next, a new culture was carried out to purify the yeasts. The suspensions were counted to obtain a concentration of about at 10^8 yeast cells per milliliter and stored at about 4 °C until analysis.

2.3 Heat treatments and microbial count

Heat resistance was determined using a heated bath circulator (Marconi, Piracicaba, Brazil). Aliquots (4 mL) of the yeast suspension, which were isolated, using the procedure previously described in section 2.2, centrifuged at 3,500 rpm, and heated in sealed glass tubes (9 mm i.d., 1 mm wall) at different temperatures (55 to 65 °C) for different periods of time. After heating, the samples were cooled in an ice water bath. The menstruum (4 mL of glucose at 10% m/v) was then inoculated with 4 ml of the yeast suspension under constant agitation (1,500-2,000 rpm). The concentration of the final mixture for fermentation was 278 mM of glucose. This was conducted at pH 5 and appropriate temperature. After 1 hour of fermentation, 1 mL samples were then removed at intervals, transferred to tubes containing 9 mL of diluents, and the total microbial count was performed by the pour plate technique after storage for 5 days at 25 °C.

2.4 PPO and POD Assay

The polyphenoloxidase (PPO) and peroxidase (POD) enzymes were extracted from cv. Jubileu clingstone peaches using the acetone powder method, partially purified by $(NH_4)SO_4$ precipitation and dialysis as described by Toralles et al. (2005).

PPO activity was determined by measuring the increase in absorbance at 420 nm using a Varian Cary 100 UV-VIS spectrophotometer. The PPO reacting mixture contained 4.5 mL of catechol substrate in the homogenization buffer (100 mM citrate-200 mM phosphate buffer) and 0.5 mL of enzymatic extract. In the final reaction mixture, the concentration was 27.3 mM of catechol. The reaction was conducted at pH 6.2 and 30 °C.

The spectrophotometric method was also used to measure POD activity at 470 nm. Guaiacol, in the presence of hydrogen peroxide, was used as the substrate instead of catechol. The POD reaction mixture contained 4.5 mL of guaiacol:H_2O_2 in the homogenization buffer and 0.5 mL of the enzyme. In the final reaction mixture, the concentration was 25.8 mM of guaiacol:H_2O_2. The reaction was conducted at pH 5.0 and 30 °C. An aliquot of 0.5 mL of homogenization buffer was used as blank control for both enzymes. Enzyme activity was calculated based on the linear portion of the curve. One unit of enzymatic activity was defined as the amount that causes an increase of 0.01 unit of absorbance per minute and per milliliter of dialyzed enzyme extract. Protein was determined by the method of Lowry et al. (1951) using BSA as standard (Acros, New Jersey, USA); the protein content of the dialyzed enzyme extract was 400 μg.mL^{-1}.

2.5 Heat treatments of PPO and POD

Heat resistance was determined using a heated bath circulator (Marconi, Piracicaba, Brazil). Aliquots (0.5 mL) of the enzyme solution (400 μg/mL) in the homogenization buffer were heated in sealed glass tubes (9 mm i.d., 1 mm wall) at different temperatures (40 to 90 °C) for different periods of time. After heating, the samples were cooled in an ice water bath, and the remaining activity was measured using the procedure previously described.

2.6 Modeling

The first-order rate constants (k), microbial reduction, or enzyme denaturation were determined using the slopes of the denaturation time courses, according to Equation 1

$$\log\left(\frac{A_t}{A_o}\right) = -(k/2.303)t \quad \text{or} \quad \ln\left(\frac{A_t}{A_o}\right) = -kt \tag{1}$$

where A_o is the initial microbial load (N_o = UFC.mL^{-1}) or initial enzyme activity (in U.min^{-1}.mL^{-1}), and A is the residual microbial or activity after heating for the specified time. The slopes of these lines were determined by linear regression and the calculated rate constants were replotted in Arrhenius plots. Activation energies (Ea) were calculated from the slopes of the Arrhenius plots of ln(k) versus 1/T according to Equation 2

$$\ln(k) = -Ea/RT + \ln A \tag{2}$$

where R is the gas constant (8.314 J.mol .K), T is the temperature in K, and A is called the pre-exponential factor. Slopes were calculated by linear regression.

A useful indication of the rate of a first-order chemical reaction is the half-life $t_{½}$ of a substance, i.e., the time it takes for its concentration to fall to half of its initial value. The time for [A] to decrease from $[A]_o$ to ½$[A]_o$ in a first-order reaction is given by Equation 1 as

$$k.t_{\frac{1}{2}} = -\ln\left(\frac{\frac{1}{2}A_o}{A_o}\right) = -\ln(1/2) = \ln 2$$

Hence

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \tag{3}$$

The main point to observe about this result is that, for a first-order reaction, the half-life of a reactant does not depend on its initial concentration.

2.7 Decimal reduction time (D-value)

D-value is the time required at a certain temperature to inactivate 90% of microorganisms or enzyme activity and is given by Equation 4:

$$D = \frac{2.303}{k} \tag{4}$$

where D (minutes or seconds) is the decimal reduction time, and k is the inactivation constant rate (s^{-1}).

2.8 Z-value

The Z-value indicates the number of degrees the temperature must be increased to decrease the microbial population by one log cycle and measure the microbial heat resistance, calculated as follows:

$$LogD = -mT \tag{5}$$

where m is the slope (°C^{-1}), T is temperature (°C), and Z (°C) is computed from the negative inverse of the slope.

3 Results and discussion

3.1 Physical and chemical characteristics

Table 1 shows the physical and chemical characterization of the Jubileu peach puree after reaching concentration of 22 °Brix. In general, the physical and chemical values are not commonly reported for peach puree concentrate at 22 °Brix. Nevertheless, some associations are possible because peach puree is evaluated in single strength form or concentrated at 30-32 °Brix.

On the other hand, the value for single-strength peach puree was published by Garza et al. (1999). These authors reported total soluble solids content (°Brix) of 11.1, pH of 3.8, total acidity (citric acid g/100 mL) of 0.515, total nitrogen (mg nitrogen/100 mL) of 86.45 and fiber (% weight) of 1.5; the industrial puree was obtained from peaches of different varieties (Sudanell, Babygold, Carson, etc.). In general, the results for Jubileu peach puree at 22 °Brix were 2-to 3-fold higher than those reported by these authors for °Brix, pH, TA, and fiber.

On the other hand, Greenwood Associates (1974), a company that manufactures concentrated peach puree, reported total soluble solids content (°Brix) of 30-32, pH of 3.7-3.9, total acidity (citric acid g/100 mL) of 1.7, and maximum microbial values of yeast and mold < 100 CFU.g^{-1}. The total initial microbial count of 22 × 10^2 UFC.mL^{-1} was obtained in the present study after a long period of storage at –20 °C. This value is within the limit established by the Brazilian legislation in 2000 (Brasil, 2000).

Table 1. Physical and chemical characteristics of Jubileu peach puree.

Parameter	Value
Total nitrogen (mg nitrogen/100 mL)	340
Ash (%)	0.40
Moisture (%)	68
Total fibers (%)	4.0
Soluble solids content (° Brix) at 25 °C	22.0
pH	3.5
Titratable acidity [TA] (g citric acid/100g)	1.8
Reducing sugars (%)	11.0
Residual PPO activity	Not detected
Count plates (PDA)	22 × 10^2 UFC.mL^{-1}

3.2 Microbial reduction

Figure 1 shows the heat resistence of the yeast isolated from spoiled peach puree. The initial microbial yeast count was approximately 10^8 UFC.mL^{-1}. The yeast heat resistence (55-65 °C) was appropriately described using first-order kinetics. At 65 °C, 95% of the initial microbial yeast count was lost after 40 seconds. Above 58 °C, yeast reduction in the total count increased. Yeast isolated from a high-sugar strawberry product showed similar behavior (Truong-Meyer et al., 1997).

Table 2 shows values of k, D, and Z for the yeast isolated from spoiled peach puree and its heat resistance using Mcllvaine buffer at pH 5. The kinetic constants of the yeast varied from 2.53×10^{-3} to 8.61×10^{-2} s^{-1} in the temperature range of 55-65 °C and from 15.15 to 0.45 minutes for D values.

In contrast with the D values of the peach puree yeast, the results reported by Reveron et al. (2003) in a study on thermal resistance of *Saccharomyces cerevisiae* in Pilsen beer at pH 4.8, are lower than those obtained in the present study. They reported D_{60} value of 0.01 minute and Z value of 4.6 °C. The D_{60} value obtained in the present study, estimated by the equation of the TDT curve, was of 2.6 minutes, and the Z-value was 6.54 °C (Table 2). The Z-value is computed from negative inverse of the slope (m=–0.153); see Equation 5. It was also higher than the D_{60} values and Z-values of the three yeasts isolated from spoiled peach puree using Mcllvaine buffer at pH 4 and buffer at pH 7 reported by Garza et al. (1994b). No significant differences in the Z-value using buffer at pH 7 and pH 4 were observed by these authors probably because the yeasts isolated came from different sources of peach puree and not because of the influence of pH on the thermal resistance of yeast.

On the other hand, although there is no effect of pH differences on the thermal resistance of yeast, it was observed that during the counting of yeast and mould in the peach puree at pH 3, fungus colonies prevailed (data not shown). This is the reason why the cultivation and isolation of the peach yeast and fermentation were carried out at pH 5. According to Beuchat (2005), the optimum pH for S. cerevisiae growth is between 4 and 5.

In the thermal processing of fruit puree, yeast cannot be the most important spoilage agent. Carvalho-Filho & Massaguer (1997) stated that *Clostridium butyricum* was initially used as target organism for the thermal processing of banana puree. However, its pH limit of 4.6 favors *Clostridium botulinum* growth. They found similar D values at 115 °C for *C. butyricum* and *C. botulinum* (0.183 min and 0.236 min, respectively), which are higher than those obtained in the present study.

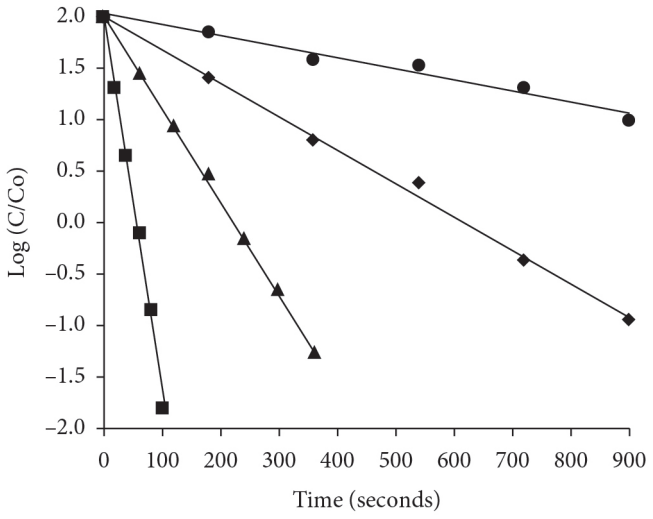

Figure 1. Thermal inactivation of yeast isolated from spoiled peach puree. Yeast extract was heated for the indicated times to 55(•), 58(♦), 61(▲), and 65 °C (■).

3.3 Thermal inactivation of PPO and POD

Figure 2 shows the remaining Jubileu PPO activity in catechol. The initial PPO activity was 17.8 U.mg^{-1} at pH 6.2, equivalent to 100% of relative activity (RA). In Figure 2, that value is the same at residual activity (log %) = 2 for zero seconds. From that point on, thermal inactivation of Jubileu PPO showed apparent first-order kinetics, and its residual activity was typically linear between 50-60 °C. In the longest heating times, there is an apparent deviation from linearity. This deviation could indicate the presence of a second enzyme with greater thermal resistance. PPO and POD in Granada clingstone peaches showed this behavior (Toralles et al., 2004). Anthon et al. (2002) also observed such behavior in pectin methyl esterase in tomato juice. At 70 °C, 95% of the PPO activity was lost after 540 seconds, and similar loss was observed after incubation at 90 °C for about 60 seconds, suggesting that PPO catalytic structure was significantly changed when temperature increased.

Thermal inactivation of POD in guaiacol substrate also showed apparent first-order kinetics (Figure not shown), and the initial POD activity was 15.88 U/mg at pH 5 (RA of 100%). The activity rapidly decreased above 50 °C. At 65 °C, 95% of the activity was lost after 150 seconds. For PPO, similar loss was observed after incubation at 70 °C for about 540 seconds, suggesting that PPO has higher thermal stability than POD. These results suggest that PPO and POD catalytical structures are significantly changed when temperature increases. This

Table 2. Heat resistance data of the yeast isolated from spoiled Jubileu peach puree.

Heating temperature (°C)	k value (s^{-1})	D value (min)	Equation of the TDT curve	R^2	Z value (°C)
55	2.53×10^{-3}	15.15	Log D= –0.153T+9.58	0.999	6.54
58	7.37×10^{-3}	5.21			
61	2.05×10^{-2}	1.87			
65	8.61×10^{-2}	0.45			

Table 3. Inactivation parameters of Jubileu peach PPO and POD at 60 °C.

Enzyme	Parameters						
	T_{ref} (°C)	[a]k_{ref} (s^{-1})	[b]k_{obs} (s^{-1})	D-value (min)	$t_{1/2}$ (s)	[c]Ea(kJ.mol^{-1})	R^2
PPO	60	2.29×10^{-3}	2.22×10^{-3}	17.3	312.2	111.1±13.3	0.996
POD	60	1.6×10^{-2}	1.36×10^{-2}	2.4	43.1	97.2±20.9	0.976

[a]k_{ref} = estimated value for the rate constant for inactivation at reference temperature (T_{ref}). [b]k_{obs} = observed value for the rate constant for inactivation at reference temperature (T_{ref}). [c] value ± confidence interval at p = 0.05.

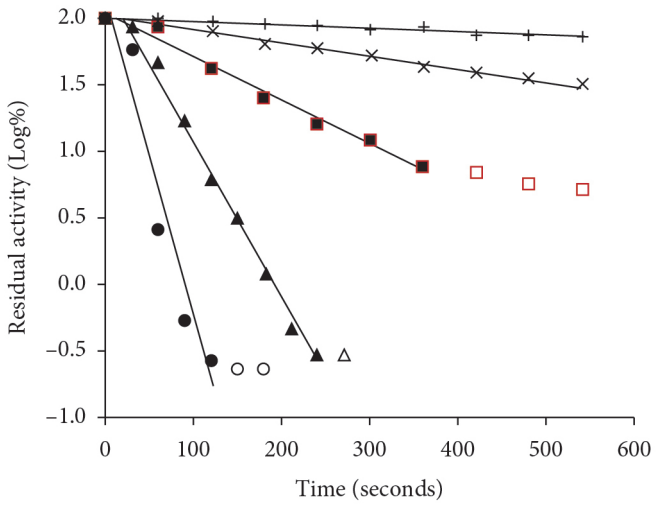

Figure 2. Thermal inactivation of polyphenoloxidase in catechol substrate. PPO extract from Jubileu clingstone peach was heated for the indicated times at 50 (+), 60 (×), 70 (■), 80 (▲), and 90 °C (●).

probably happens because of the significance of the noncovalent bond in maintaining the structure of enzymes. These bonds involve van der Waals forces, electrostatic interactions, hydrogen bonds, and hydrophobic interactions. When high temperatures disrupt noncovalent interactions, proteins unfold (Vieille & Zeikus, 2001; Kristjánsson & Ásgeirsson, 2003). Recent studies indicate that the active site tends to be more flexible than the enzyme as a whole, and that activity losses precede denaturation (Daniel et al., 2010).

Table 3 shows the Arrhenius parameters at 60 °C, half-lives ($t_{1/2}$), and activation energies (Ea). The values of the Arrhenius parameters were determined using Equation 2 by linear regression and Equation 3 for half-lives. High coefficients of determination (R^2) justify the use of the Arrhenius law for both enzymes. PPO half-life ($t_{1/2}$ = 312.2 s) was higher than that of POD ($t_{1/2}$ = 43.1 s). On the other hand, inactivation rate (k) of POD (1.36×10^{-2}) was higher than that of PPO (2.22×10^{-3}). Based on the half-life and inactivation rate, it is possible to prove that PPO is kinetically more stable than POD.

In general, k_{60}-values and half-life values obtained for Jubileu peach PPO and POD are higher than those of Granada peach PPO and POD found by Toralles et al. (2005). These authors reported k_{60} = 3.4×10^{-3} s^{-1} and $t_{1/2}$ = 214.6 s^{-1} for PPO, and k_{60} = 10.9×10^{-3} s^{-1} and $t_{1/2}$ = 67.2 s^{-1} for POD. They also revealed that PPO in Granada (Ea = 127.3 kJ.mol^{-1}) was the least heat stable and POD in Granada (Ea = 147.9 kJ.mol^{-1}) was the most heat stable. In the present study, considering the confidence interval, the activation energies were of equivalent magnitude.

Fortea et al. (2009), evaluating the activity of PPO and POD in table grape (Crimson Seedless), obtained approximately 2-fold higher values than those found in the current study for Ea, and similar thermostability for both enzymes, losing >90% of relative activity after only 5 min of incubation at 78 °C and 75 °C, respectively. In general, higher activation energy implies that a lower temperature change is needed to inactivate an enzyme.

In contrast with the D_{60} value (2.6 min) for peach puree yeast, the D_{60} value (17.3 min) obtained for Jubileu peach PPO was approximately 7.5-fold higher than that of yeast. Nonetheless, the D values of POD (2.4 min) and peach puree yeast were similar (Tables 2 and 3). Therefore, Jubileu peach PPO was more thermostable than the POD and yeast. Guerrero-Beltrán & Barbosa-Cánovas (2006), investigating *Saccharomyces cerevisiae* and PPO in mango nectar treated with UV light inactivation, also reported that PPO was the most UV-light stable.

A comparative study between Jubileu PPO and Granada PPO inactivation was carried out by a computer simulation using kinetic data. The analysis was performed at 75 °C and 80 °C; both are typical temperatures used during peach pure processing. At 75 °C, temperature used to blanch the Jubileu peach puree, 3 additional minutes should be added to the treatment proposed by Toralles et al. (2008) for the Granada peach puree at 75 °C for 4 minutes.

At 80 °C, it is possible to keep the thermal treatment for 4 minutes to blanch the Jubileu peach puree. Since blanching at 75 °C for 4 minutes is not sufficient to inactivate Jubileu PPO, 0.08% ascorbic acid had to be added to the puree after blanching. This must have inhibited the PPO activity until its concentration. This operation followed by pasteurization also guaranteed no residual activity in the final product, Jubileu peach puree at 22 °Brix (see Table 1).

4 Conclusion

PPO and POD of Jubileu clingstone peaches and yeast isolated from spoiled peach puree showed different heat resistances at real blanching and pasteurization temperature. Heat treatments significantly reduced peach PPO and POD activity. Heat denaturation was highly effective above 80 °C for PPO and above 60 °C for POD. The thermal stability of PPO was higher than that of POD. In addition, PPO was more thermally stable than the yeast isolated from Jubileu peach puree. The D_{60} values were 17.3 and 1.87 min for PPO and yeast isolated from spoiled Jubileu peach puree, respectively. In all cases,

heat inactivation was described using exponential decay (first order reaction), while the Arrhenius law described temperature dependence of all reactions. Finally, in the continuous blanching, increasing thermal treatment to 80 °C for 4 minutes is advisable to inactivate Jubileu PPO, and thus it would be an indicator for browning enzyme inactivation in peaches.

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