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Inactivation kinetics of peroxidase and polyphenol oxidase in peach juice treated with gaseous ozone

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Abstract The effectiveness of gaseous ozone for inactivating peroxidase (POD) and polyphenoloxidase (PPO) in peach juice was investigated. The suitability of first-order and Weibull models to describe inactivation kinetics was also analysed. Peach juice was exposed to ozone (0.11 and 0.20 mg O_3 min^{-1} mL^{-1}) in a bubble column up to 12 min at 20 ± 1 °C. Enzyme activities were reduced due to treatments. The magnitude of the inactivation increased with ozone level and exposure time. Reductions in activity after 12 min of treatment ranged between 99.5% and 99.8% for POD and between 93.9% and 97.3% for PPO, depending on ozone concentration. Inactivation curves were successfully fitted with the first-order and Weibull models; although, based on the root-mean-square error, the corrected Akaike and the Bayesian Schwarz criterion, the Weibull model showed stronger capability in all cases.

Keywords Deteriorative enzymes, first-order and Weibull models, inactivation kinetics, Ozone, peach juice.

Introduction

Endogenous enzymatic activity may cause sensory and nutritional deterioration in raw, minimally or fully processed fruit and vegetables (McEvily *et al*., 1992; Espachs-Barroso *et al*., 2003). Thermal treatment has traditionally been the most applied method for inactivating enzymes and microorganisms in fruit and vegetable juices (Chen *et al*., 1993). Nevertheless, high processing temperatures significantly affect juice quality, provoking nutrients loss, colour modifications and off-flavour development (Mawele *et al*., 1996; Espachs-Barroso *et al*., 2003; Rivas *et al*., 2006). These limitations have evidenced the need of novel non-thermal technologies.

Ozonation is a relatively simple to operate and cost-effective non-thermal technology for processing of foods. Ozone is a triatomic allotropic state of oxygen that has a high oxidation potential and auto-decomposes rapidly to harmless oxygen, leaving no residues in food and environment (Khadre *et al*., 2001). Its half-life in the gaseous phase is of about 12 h, but in distilled water, it is reduced to only 20–30 min at 20 °C (Khadre *et al*., 2001). Ozone has a strong antimicrobial activity against bacteria, fungi, protozoa, spores and viruses, attributed to its oxidation reactions with unsaturated lipids of the cell envelope, intracellular enzymes and genetic materials (Patil *et al*., 2010a). This oxidation activity is not only associated with its molecular form but also with activated oxygen species resulting from ozone decomposition, such as the superoxide anion radical, the hydroperoxide radical, singlet oxygen and finally the hydroxyl radical (Korycka-Dahl & Richardson, 1978; Perry & Yousef, 2011).

Various environmental factors influence ozone effectiveness, among them temperature, medium pH, humidity, additives (surfactants, sugars, etc.), amount of organic matter in the matrix (Restaino *et al*., 1995) and solids content (Choi *et al*., 2012).

In 2001, the Food and Drug Administration (FDA) approved ozone as a direct additive to food, leading to research in the application of ozone for processing of various fruit juices, including apple cider (Steenstrup & Floros, 2004), apple juice (Patil *et al*., 2010a; Choi *et al*., 2012), orange juice (Tiwari *et al*., 2008a,b; Patil *et al*., 2010b), grape juice (Tiwari *et al*., 2009a) and tomato juice (Tiwari *et al*., 2009b). Ozonation of fruit

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juices has been mainly carried out in bubble columns, usually employed as multiphase contactors and reactors in various industries (Cullen *et al*., 2009).

The impact of ozone on quality of fruit juices depends on their composition (% soluble solids, pH and % matter organic), applied ozone dose and treatment conditions. Several authors have significant changes in colour due to ozone application in apple cider (Choi & Nielsen, 2005; Williams *et al*., 2005), orange juice (Angelino *et al*., 2003; Tiwari *et al*., 2008b), blackberry juice (Tiwari *et al*., 2009c) and strawberry juice (Tiwari *et al*., 2009d). However, ozone treatment has been reported to have no effect on non-enzymatic browning, cloud value, pH, °Brix and titratable acidity of orange and tomato juice (Tiwari *et al*., 2008a,b, 2009a) and apple cider (Choi & Nielsen, 2005).

Available information on the effect of ozone on enzyme activity in fruit and vegetables is still insufficient; besides, to our knowledge, there are no studies reported in fruit juices. The reported investigations carried out on whole fruit and vegetables indicated that ozone treatment could reduce or increase the activity of different oxidative enzymes (Zhang *et al*., 2005; Rico *et al*., 2006; Yang & Rao, 2006; Chauhan *et al*., 2011; Kying Ong *et al*., 2014; Sachadyn-Król *et al*., 2016). Studies modelling enzyme inactivation kinetics in ozone-treated fruit juices are not available in the literature. Accurate model prediction of enzyme inactivation curves would be beneficial for selecting the optimum ozone dose in the preservation process design. Some authors applied the first-order model to describe enzyme inactivation kinetics using non-thermal technologies (Bendicho *et al*., 2002; Giner *et al*., 2002, 2005; Elez-Martínez *et al*., 2006; Soliva-Fortuny *et al*., 2006). This mechanistic model implicitly assumes all members in a population (in this case, the enzyme macromolecules) behave in the same way; the inactivation of a single macromolecule is due to one single event. The Weibull distribution is a flexible non-linear model that has been proved to describe well the inactivation of enzymes by several stress factors, such as pulsed electric fields (Giner *et al*., 2005; Elez-Martínez *et al*., 2006; Soliva-Fortuny *et al*., 2006; Quintão-Teixeira *et al*., 2013) and cold plasma (Pankaj *et al*., 2013). The Weibullian model does not assume the validity of any kinetic model. It takes biological variation into account and allows describing the spectrum of responses of a population to an adverse agent under different conditions (Peleg & Cole, 1998). The inactivation mechanism may vary from macromolecule to macromolecule, and the survival curve could be assumed as the cumulative form of the underlying distribution of the individual inactivation times.

This study aimed to evaluate (i) the effectiveness of ozone at two concentrations (0.11 and 0.20 mg O_3 min^{-1} mL^{-1}) for inactivating polyphenoloxidase (PPO) and peroxidase (POD) enzymes in peach juice at 20 °C using a bubble column and (ii) the suitability of first-order and Weibull models to describe the inactivation kinetics.

Materials and methods

Preparation of peach juice samples

Peaches (*Prunuspersica*, Pavíacv) were acquired in a local market and were kept at 4 ± 1 °C for 12 h. Juice samples were obtained using the methodology described by Garcia-Loredo *et al*. (2015). Juice samples were stored at −79 ± 1 °C (Panasonic ultra freezer, model MDF-U55V, Japan) until use. None of the samples were stored for periods greater than two months.

Ozone processing

The ozone processing equipment used for the treatment of the samples and the operating variables were reported by Garcia-Loredo *et al*. (2015). Two ozone levels at the inlet gas flow, 0.11 and 0.20 mg O_3 min^{-1} mL^{-1} (C1 and C2, respectively), were studied. Ozone gas was bubbled into 450 mL peach juice (20 ± 1 °C) for up to 12 min with regular sampling of 5 mL at 20 s, 1 min or 2 min intervals depending on ozone concentration. Treated and untreated juices (control) were stored at 4 ± 1 °C in sterile dark glass bottles to protect them from light. Analyses were performed immediately after processing.

Determination of POD and PPO activities

POD and PPO activities were determined using the methodology described by Montero-Prado *et al*. (2011) with slight modifications. The POD extract was prepared with the following extraction buffer (pH 6.4):0.04 M Na_2HPO_4 (MERCK, Germany), 0.06 M NaH_2PO_4 (Baker, Mexico), 1 M NaCl (MERCK, Germany) and 1% (w/v) polyvinylpolypyrrolidone (Sigma, Germany). Peach juice (5 mL) was homogenised with 20 mL of extraction buffer and left under stirring for 30 min at 4–5 °C. The mixture was centrifuged (15500 ***g***, 20 min, 4 °C) (Eppendorf, model 5804 R, Hamburg, Germany), and the pellet was discarded. The supernatant was used for assaying POD activity using the following reaction mixture in a total volume of 3 mL: 0.02 M Na_2HPO_4- 0.08 M NaH_2PO_4 buffer (pH 6.4), 8 mM guaiacol (Anedra, Argentina) and 0.5 mL of enzyme extract. The mixture was incubated at 30 °C during 2.5 min, and then, 1 mL H_2O_2 (12 mM) dissolved in sodium phosphate buffer (100 mM, pH = 6.4) was incorporated. The activity

was evaluated by measuring the increase in optical density (OD) at 460 nm during 120 s using a spectrophotometer (V-630, JASCO, Japan). The enzyme activity unit (U) was defined as the amount of enzyme required to increase 0.001 OD units per min at test conditions.

The PPO extract was the same as that used for POD enzyme described above. The reaction mixture contained 2 mL 0.02 M Na_2HPO_4- 0.08 M NaH_2PO_4 buffer (pH 6.4) and 0.5 mL of enzyme extract. The mixture was incubated at 30 °C for 2.5 min, and then, 1 mL catechol (500 mM) (Sigma-Aldrich, Germany) dissolved in sodium phosphate buffer (100 mM, pH = 6.4) was incorporated. The changes in OD at 400 nm were recorded for 120 s. One unit of PPO activity (U) was defined as the amount of enzyme required to increase 0.001 OD units per min at test conditions.

Ozone treatments were performed in triplicate, and the enzyme activity in each replicate was measured twice. In all assays, the specific enzyme activity (U mg^{-1} of protein) was determined. Finally, the specific activity values were expressed as *Residual activity* (*RA*) according to Equation 1:

$$\mathrm{RA} = \left(\frac{A_t}{A_0}\right) \tag{1}$$

where A_t is the specific enzyme activity of peach juice after a given ozone exposure time t and A_0 is the initial specific enzyme activity of the untreated juice.

Protein determination

Protein concentration of the extracts was determined according to the modified Lowry method (Potty, 1969). The reaction mixture contained a mixture of reagent A (Na_2CO_3 (2% w/v) (Anedra, Argentine) dissolved in NaOH (1 N)) with $CuSO_4$ (Anedra, Argentine) (0.5% w/v) (ratio 50:1) (Reagent B), the Folin–Ciocalteu solution (Sigma-Aldrich, Germany)) (1 N) (Reagent C) and 1 mL of sample. Measurements were performed at 500 nm, using bovine albumin (Sigma-Aldrich, Germany) as standard (0.05–0.5 mg L^{-1}).

Mathematical modelling

Enzyme inactivation curves were fitted with the first-order (Equation 2) and Weibull (Equation 3) models.

The first-order models appropriate when the enzyme exhibits an exponential decreasing trend as a function of time (Van Loey *et al.*, 2003):

$$\mathrm{RA} = \exp(-kt) \tag{2}$$

where t (min) is the treatment time and k (min^{-1}) is the first-order inactivation rate constant.

The Weibullian distribution is a two-parameter function (van Boekel, 2002):

$$\mathrm{RA} = \exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right) \tag{3}$$

where t (min) is the treatment time, and α (min) and β (−) are the scale and the shape parameters, respectively. The shape parameter indicates concavity (tail-forming) or convexity (shoulder-forming) of the survival curve when it takes values below and above 1, respectively. The resistant frequency curves were generated from the values of α and β using the following equation:

$$f(t) = \frac{\alpha}{\beta}\left(\frac{t}{\alpha}\right)^{\beta-1}\exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right) \tag{4}$$

Other statistical parameters (distribution mode, t_{cm}; mean, $\bar{t}_c$; variance, σ^2_{tc} ; and coefficient of 'skewness', υ_1) were calculated from the following equations:

$$t_{cm} = \left[(\beta-1)/\beta\left(\alpha^{-\beta}\right)\right]^{1/\beta} \tag{5}$$

$$\bar{t}_c = \frac{\Gamma\left[\frac{(\beta-1)}{\beta}\right]}{\frac{1}{\alpha}} \tag{6}$$

$$\sigma^2_{tc} = \frac{\left\{\Gamma\left[\frac{(\beta+2)}{\beta}\right] - \left(\Gamma\left[\frac{(\beta+1)}{\beta}\right]\right)^2\right\}}{\left(\frac{1}{\alpha}\right)^2} \tag{7}$$

$$\upsilon_1 = \frac{\left[\Gamma(\beta+3/\beta)/\left(\frac{1}{\alpha}\right)^3\right]}{\left[\Gamma(\beta+2/\beta)/\left(\frac{1}{\alpha}\right)^2\right]^{3/2}} \tag{8}$$

where Γ is the gamma function. The distribution mode, t_{cm}, represents the treatment time at which the majority of the enzyme population is inactivated. The mean, $\bar{t}_c$, corresponds to the inactivation time on average with its variance, σ^2_{tc}. The 'skewness' coefficient, υ_1, represents the skew of the distribution.

Statistical analysis

Model fit and the performance were evaluated using the adjusted determination coefficient (R^2_{adj}) and the root-mean-square error (RMSE), respectively (Alzamora *et al.*, 2005). The Bayesian Schwarz criterion (BIC, Quinn & Keough, 2002) and the corrected Akaike information criterion (AIC_c, Akaike, 1973) were used to detect model over fitting.

Both criteria can evaluate the efficiency of the parameterised model in terms of predicting the data, but the BIC criterion is a bit more conservative

because the penalty term is larger in BIC than in AIC_c. According to Akaike's and Bayesian's theories, the most accurate and parsimonious model yields the smallest AIC_c and BIC values (Quinn & Keough, 2002). Statistical analyses were carried out using InfoStat 2009 (InfoStat Group, FCA-UNC, Córdoba, Argentina).

Results and discussion

The effect of ozone dose on POD activity in peach juice is shown in Fig. 1. The initial POD activity (396.6 ± 3.53 U mg^{-1}) declined with increasing treatment time in a nonlinear tendency. The POD inactivation pattern indicated a strong dependence on ozone concentration. Peach juice treated for 3, 4 and 5 min at lower ozone concentration (C1) presented a significant decrease in the residual POD activity (52.1%, 33.0% and 19.0% of the initial value, respectively). From 6 to 12 min, POD activity reduction achieved in peach juice was slightly lower and not significant between time periods. Peach juice with less than 99.5% of the initial POD was obtained after 12 min of exposure. POD activity was dramatically reduced when processing at greater ozone level (C2). For example, a significant difference in POD activity was observed in peach juice exposed during 20 s, 40 s and 1 min: 73.3%, 40.1% and 20.4% of the initial POD, respectively. Non-significant differences were observed for POD activity in peach juice after 2–12 min of ozone treatment. When comparing the effect of both ozone concentrations after 1 min treatment, the residual activity was 3.6-fold lower in the peach juice exposed to C2.

Figure 2 shows the reduction in PPO activity in peach juice treated with ozone at both concentrations (C1 and C2). The initial value of specific PPO activity was 216.04 ± 3.51 U mg^{-1}. The reduction trend was similar to that of POD: the higher the ozone level, the lower the PPO activity observed, although ozone effectiveness was greater for inhibiting POD. There was a significant change in PPO activity when peach juice was treated with C1 during 1 min and less than 7% of residual PPO activity remained after 12 min exposure. PPO inactivation profile in peach juice treated with C2 was more abrupt showing a higher inactivation rate. Peach juice ozonised for 20 s and 40 s showed a significant decrease in the residual activity (68.2% and 51.5% of the initial PPO, respectively). Between exposure times: 5, 7, 10 and 12 min, the reduction in PPO activity was not significant. The remaining PPO level after 12 min of ozonation was about 2% of the initial PPO activity.

Yang & Rao (2006) reported a significant reduction of POD and PPO activities in peaches stored at 4 °C with an atmosphere of 8 ppm ozone. Rico *et al.* (2006) found that fresh-cut lettuce treated with ozone (1 ppm, 1 min) showed significantly lower PPO and POD activities than untreated samples. Zhang *et al.* (2005) reported that PPO activity and respiration rate of fresh-cut celery were inhibited by treatment with ozonated water (0.03, 0.08 and 0.18 ppm) and the effectiveness of inhibition increased as ozone concentration increased. According to Chauhan *et al.* (2011) findings, ozonation of fresh-cut carrots in water (1:2 w/v; 200 mg O_3 h^{-1}) for 10 min was found to reduce lignification and maintaining the quality of fresh-cut carrots during CA storage at 6 ± 1 °C. The authors

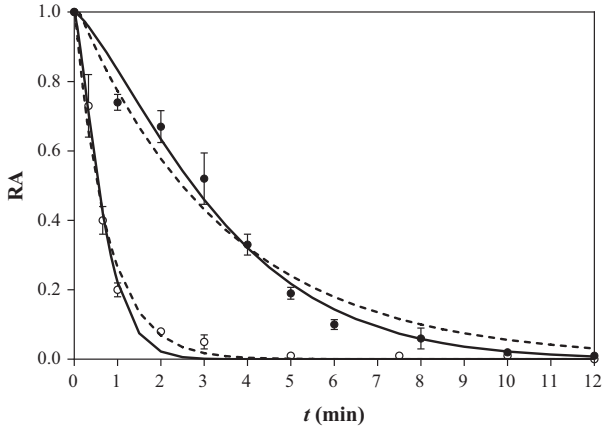

Figure 1 Experimental POD inactivation curves and fitted values derived from first-order (dashed line) and Weibull (solid line) models in peach juice treated with 0.11 mg O_3 min^{-1} mL^{-1} (●) and 0.20 mg O_3 min^{-1} mL^{-1} (○) at 20 °C.RA: residual activity. Error bars represent the standard deviation.

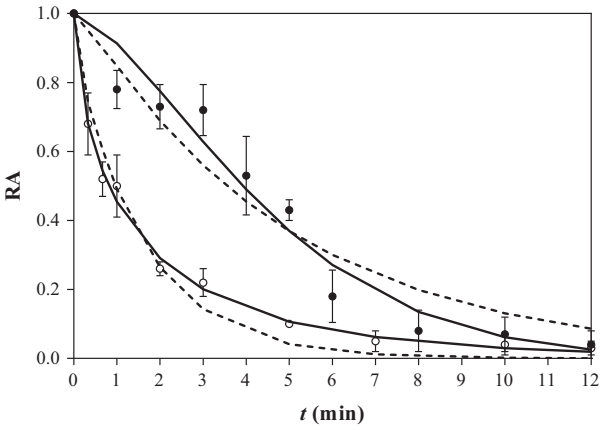

Figure 2 Experimental PPO inactivation curves and fitted values derived from first-order (dashed line) and Weibull (solid line) models in peach juice treated with 0.11 mg O_3 min^{-1} mL^{-1} (●) and 0.20 mgO_3 min^{-1} mL^{-1} (○) at 20 °C. RA: residual activity. Error bars represent the standard deviation.

observed a significant reduction in ascorbic acid, carotenoids and oxidative enzymes such as PPO and POD due to ozonation and CA storage. Similarly to our results, POD was more sensitive to the ozone exposure than PPO.

Several literature studies have suggested that the hyper-reactivity of ozone may contribute to the inhibition of several enzymes. Protein oxidation involves the covalent modification of the macromolecule induced either by direct reaction with ozone or by reactions with its decomposition by-products (Shacter, 2000; Zhang *et al*., 2013). These reactive oxygen species vary markedly in their reactivity, and the resulting damage is highly variable and complex. In some cases, damage is limited to specific residues, whereas in others, damage is widespread and nonspecific. Highly reactive oxygen species can interact with both amino acid side chains and protein backbones resulting in protein fragmentation or protein–protein cross-linkages. In general, less reactive species show greater selectivity with regard to the residues targeted and their spatial location. Physical and chemical properties of proteins, including conformation, structure and solubility, as well as enzyme activities, can change by oxidative modifications (Davies, 2016).

Figures 1 and 2 also showed the fitting of experimental data using the first-order (Equation 2) and the Weibull (Equation 3) models. The corresponding estimated parameters and the adjusted coefficient of determination (R^2_{adj}) values were listed in Table 1. Table 2 presented the specific statistics related to the Weibullian distribution calculated according equations 5-8. To compare the goodness of fit of the models, Table 3 displays RMSE, AIC_c and BIC values associated with the prediction of enzyme inactivation curves.

R^2_{adj} values for the first-order model varied from 0.92 to 0.98, indicating that in general the first-order model was suitable for describing the inactivation of POD and PPO by ozone within the experimental range of treatment times and ozone concentrations assayed. The k value increased $\approx$ from 0.29 to 1.35 min^{-1} and from 0.21 to 0.62 min^{-1} when ozone concentration increased from 0.11 to 0.20 mg O_3 min^{-1} mL^{-1} for POD and PPO, respectively. Thus, it was clearly evidenced that higher ozone concentration yielded a greater inactivation rate constant, this increase being larger for POD.

For the Weibull model, high R^2_{adj} values were obtained, showing that between 94% and 99% of the variation in the experimental data could be explained by the model (Table 1). For both enzymes, the scale factor α was strongly influenced by ozone level, varying for POD between 3.63 and 0.74 min and for PPO between 5.02 and 1.44 min for C1 and C2, respectively. POD inactivation curves exhibited β values >1 for both ozone levels, as expected according to the downward concavity. The shape factor did not depend on ozone concentration and took values of 1.33–1.34. On contrary, β parameter was greater than 1 (downward concavity) for PPO treated with 0.11 mg O_3 min^{-1} mL^{-1}, while it was lower than 1(upward concavity) for PPO treated with 0.20 mg O_3 min^{-1} mL^{-1}. β values <1 would indicate that the remaining macromolecules had the ability to resist the oxidative agent, whereas β values >1 could evidence that enzyme macromolecules became increasingly susceptible to treatment, in other words, there was a cumulative damage making it increasingly difficult for the enzymes to maintain their activity (van Boekel, 2002). Thus, the increase in O_3 level would imply a change in the mechanism of PPO inactivation.

Frequency distributions of resistances for POD and PPO are shown in Figs 3 and 4, respectively, and the associated statistics, mode, mean, variance and coefficient of skewness, can be observed in Table 2. Frequency distributions of resistances markedly changed with the ozone level and the enzyme assayed. For POD (treated with both ozone concentrations), frequency curves were asymmetric, skewed to the right (coefficient of skewness: 1.66–1.68), and characterised by a mode lower than the mean (Table 2).The increase in ozone dose led to a reduction of 80% in the mode and the mean. Frequency distribution of resistances corresponding to PPO exposure to C1 was also asymmetric, skewed to the right (coefficient of skewness: 1.55), with a mode (2.38 min) lower than the mean (4.54 min). On contrary, distribution of resistances for PPO exposure to C2 lacked of mode and was strongly skewed to the right (coefficient of skewness: 3.73), showing that the majority of the enzyme

Table 1 Kinetic parameter estimates of POD and PPO inactivation curves in peach juice treated with 0.11 and 0.2 mg O_3 min^{-1} mL^{-1}, modelled using first-order and Weibull models.

Enzyme	[O_3] (mg O_3 min^{-1} mL^{-1})	k (min^{-1})		R^2_{adj}	α (min)		β (−)		R^2_{adj}
POD	0.11	0.29	(−0.03)	0.97	3.63	(−0.16)	1.33	(−0.13)	0.98
	0.2	1.35	(−0.10)	0.98	0.74	(−0.03)	1.34	(−0.13)	0.99
PPO	0.11	0.21	(−0.03)	0.92	5.02	(−0.32)	1.50	(−0.22)	0.95
	0.2	0.62	(−0.08)	0.96	1.44	(−0.07)	0.65	(−0.04)	0.99

Table 2 Weibull model related statistics corresponding to POD and PPO inactivation curves in peach juice treated with 0.11 and 0.2 mg O_3 min^{-1} mL^{-1}.

Enzyme	$[O_3]$ (mg O_3 min^{-1} mL^{-1})	t_{cm} (min)	$\bar{t}_c$ (min)	σ^2_{tc}	υ_1
POD	0.11	1.24	3.34	6.53	1.68
	0.2	0.26	0.68	0.26	1.66
PPO	0.11	2.38	4.54	9.60	1.55
	0.2	–	1.98	10.01	3.73

macromolecules in the population were inactivated at exposure times lesser than 1 min (with a $\bar{t}_c$ = 1.98 min) (Fig. 4). When ozone concentration increased, the distribution of resistances for POD was narrower (lower variance value), meaning a more uniform sensitivity of the enzyme population to ozone treatment. However, for PPO, the heterogeneity of the response continued being high as it was reflected by the tails of the distribution and the great variance value.

Statistic criteria to evaluate the fitting ability demonstrated that both models constituted good alternatives to quantify the enzymes response to ozone (Table 3). However, based on the RMSE, AIC_c and BIC values, Weibull model showed the best performance in all cases. Weibull model had the strongest predictive ability according to AIC_c and BIC criteria, which take both, fit and parsimony, into account (Coroller *et al.*, 2006).

Estimated parameters of both tested models explained, from a different point of view, the influence of ozone on POD and PPO response. First-order model describes the inactivation from a kinetic point of view, determining an apparent reaction rate constant. This constant would represent the rate of an irreversible reaction that predominates over the others involved in the complex inactivation process. Weibull parameters allow knowing the distribution of resistances to ozone in the enzyme macromolecules population. No references on the Weibull equation as a model to describe oxidative inactivation of enzymes under ozone treatments have been published up to now. However, it is expected that different macromolecules in the enzyme population would present different inactivation times during ozone treatment, that is, their sensitivity would be distributed. In first place, enzyme inactivation by oxidant agents is a complex process involving several events (such as formation and/or disruption of different interactions and/or bonds, decomposition of amino acids, aggregation and/or dissociation) that can vary according to the reactivity of the reactive oxygen species (Davies, 2016). In second place, enzymes characterised by several isoenzymes can often be subdivided into two (or more) fractions with different processing stability (Van Loey *et al.*, 2003).

In previous studies, Garcia-Loredo *et al.* (2015) demonstrated that ozone treatment of peach juice under the same conditions was effective for reducing *Escherichia coli* ATCC 11229 and *Listeria innocua* ATCC 33090 populations inoculated into the juice. Reductions after 12 min exposure ranged from at least 3.9 up to 4.9 log units according to ozone concentration and microorganism, being *L. innocua* ATCC 33090 more sensitive than *E. coli* ATCC 11229 at both ozone levels. The effectiveness of ozone processing will depend on its ability to inactivate microorganisms and enzymes. Thus, for designing and optimising preservation process of peach juice using ozone, it is useful to compare the relative resistance of enzymes and microorganisms. Weibull parameters for PPO and POD inactivation were contrasted with those previously reported by Garcia-Loredo *et al.* (2015) for the inactivation *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 in peach juice undergone ozone treatment in the same process conditions.

Similarly to POD and PPO, for the microorganisms studied by Garcia-Loredo *et al.* (2015), α and $\bar{t}_c$ decreased when ozone concentration increased. β values for *E. coli* were greater than unity at both concentrations, following a similar inactivation pattern than POD. *L. innocua* population decrease showed the same trend regarding β *v*alues compared to PPO, that is $\beta > 1$ for 0.11 mg O_3 min^{-1} mL^{-1} and $\beta < 1$ for 0.20 mg O_3 min^{-1} mL^{-1}. Besides this, $\bar{t}_c$ values were in a range of 6.4–3.3 min and 3.5–1.8 min for *E. coli* and *L. innocua*, respectively. Therefore, PPO appeared to be more resistant to ozone than *L. innocua* while POD showed lesser resistance to ozone than both

Table 3 Minimum RSME, AIC_c and BIC values for POD and PPO inactivation curves in ozone-treated peach juice.

		RMSE		AIC_c		BIC	
Enzyme	$[O_3]$ (mg O_3 min^{-1} mL^{-1})	First order	Weibull	First order	Weibull	First order	Weibull
POD	0.11	0.004	0.002	−22.85	−28.39	−22.33	−28.81
	0.2	0.002	0.001	−32.38	−36.06	−31.87	−37.04
PPO	0.11	0.010	0.006	−13.8	−18.08	−13.51	−18.51
	0.2	0.004	0.001	−22.5	−41.26	−22.00	−41.68

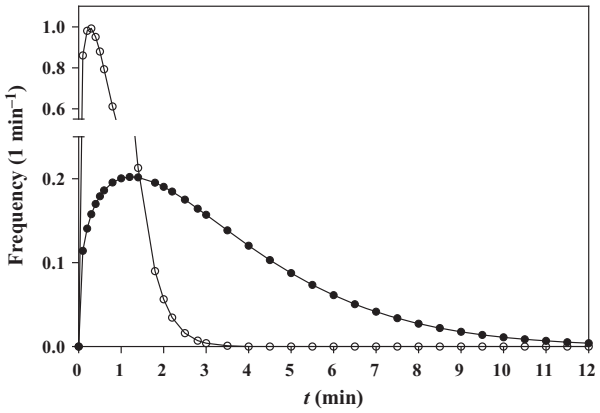

Figure 3 Weibull frequency distribution of resistances corresponding to POD inactivation in peach juice treated with 0.11 mg O_3 min^{-1} mL^{-1} (●) and 0.20 mg O_3 min^{-1} mL^{-1} (○) at 20 °C.

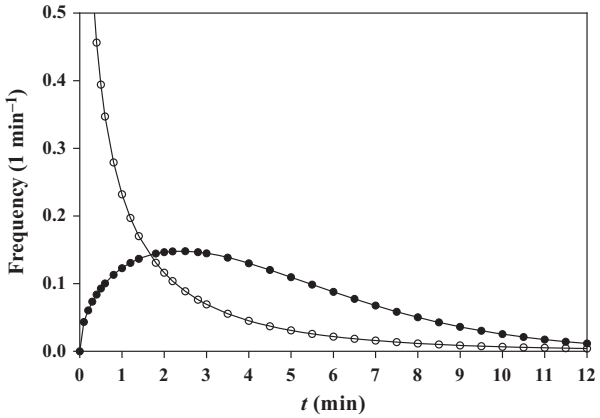

Figure 4 Weibull frequency distribution of resistances corresponding to PPO inactivation in peach juice treated with 0.11 mg O_3 min^{-1} mL^{-1} (●) and 0.20 mg O_3 min^{-1} mL^{-1} (○) at 20 °C.

microorganisms. *E. coli* was the most refractory to the oxidant agent.

From the point of view of PPO and POD inactivation at the highest dose (C2), it seemed that ozone treatments not longer than 5 min are required, as the activity of both enzymes is not reduced further with increasing exposure time. However, taking into account microorganisms inactivation (i.e. *E. coli*), the ozone exposure up to 12 min would be beneficial to increase the inactivation level.

Previous literature research indicated that ozonation of fruit juices could result in significant changes in colour, depending on the dose and the food matrix. The impact of ozone treatments (in the same operative conditions as in this work) on peach juice colour was evaluated by Jaramillo-Sánchez (2014). Slight but significant variations in colorimetric parameters and functions in juices exposed to the highest ozone concentration (0.20 mg O_3 min^{-1} mL^{-1}) were found as compared with control juice. L* parameter decreased (from 29.8 to 26.7) and a* parameter and browning index function increased (from 9.01 to 10.8 and from 96.2 to 105.8, respectively) during the first minute of ozonation and remained rather constant for higher treatment times. These changes were reflected in a slight increase in browning. Browning development could be associated not only with the enzymes action at the beginning of ozone exposure, but also with non-enzymatic browning. Ozone could induce non-enzymatic browning development by the oxidation of phenolic compounds (Chubey & Nylund, 1969; McEvily *et al*., 1992; Cullen *et al*., 2010). Nevertheless, colour changes induced by ozone treatments at the doses used in this work, although significant, were too slight to greatly impair peach juice quality.

Conclusions

Ozone reduced POD and PPO activities in peach juice. Both enzymes activities decreased as ozone concentration and treatment time increased. PPO appeared to be more resistant to the oxidant agent than POD. Maximum activity reductions after 12 min of treatment ranged between 99.5% and 99.8% for POD and between 93.9% and 97.3% for PPO, depending on ozone concentration. Inactivation curves were successfully described with the first-order and Weibull models, although, based on the RMSE, AIC_c and BIC values, the Weibull model showed the best performance in all cases. Weibull model parameters and statistics showed that when ozone level increased, both the treatment time at which the majority of the enzymes population was inactivated and the inactivation time on average decreased. Also POD response to ozone was more homogenous (lower variance) at greater ozone concentration.

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