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# Inactivation of polyphenol oxidase by microwave and conventional heating: Investigation of thermal and non-thermal effects of focused microwaves

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ABSTRACT

Emerging technologies, such as focused microwave heating of liquid foods, have been studied to reduce quality losses due to the high temperatures of conventional processing. Besides faster heating, microwaves can also have non-thermal effects on inactivation; however, this is a controversial issue. The objective of this study was to compare conventional and focused microwave heating under similar conditions for the inactivation of two polyphenol oxidases (PPOs): mushroom tyrosinase in buffer and the PPO present in coconut water. Small samples under stirring were treated at temperatures between 50 and 90 °C and three kinetic models were adjusted considering the whole time-temperature history. The Weibull model could best describe inactivation in both heating processes, which was more effective with microwave heating for temperatures over 70 °C. Validation runs show that the model can satisfactorily describe the PPO inactivation. This study contributes for the design of liquid food pasteurization by focused microwave technology.

## 1. Introduction

Thermal processing of foods targets the most heat resistant microorganism that compromises safety or the most heat resistant enzyme that compromises shelf life. However, thermal processing may cause deleterious changes in the nutritional and sensory qualities of the food, such as color, flavor, vitamin content and texture. Therefore, novel food processing technologies aim to reduce nutritional and sensory losses by high temperatures (Awuah, Ramaswamy, & Economides, 2007; Cullen, Tiwari, & Valdramidis, 2012). In recent years, focused microwave heating has been studied for the continuous flow thermal processing of liquid foods. Advantages over conventional heating include rapid volumetric temperature increase without surface overheating, improved process control and higher energy efficiency (Cullen et al., 2012; Saxena & Chandra, 2011; Tang, 2015; Tewari & Juneja, 2008). Some studies report microwaves showing less undesired impact than conventional heating on food attributes, antioxidant activity and bioactive content (Guo, Sun, Cheng, & Han, 2017).

Studies have successfully used microwave heating to inactivate undesired enzymes in foods, such as polyphenol oxidase (PPO), responsible for off-colors in fruit-based products (Damodaran, Parkin, & Fennema, 2009). De Ancos et al. (1999) showed how the PPO activity decreased in strawberry, kiwi and papaya purées as microwave power was increased. Matsui et al. (2007; 2008) reduced PPO activity in green coconut water by 90% at 90 °C with a multimode microwave oven. Benlloch-Tinoco et al. (2013) observed greater PPO inactivation in kiwi purée by microwaves when compared with conventional heating. Marszałek et al. (2015) decreased PPO activity in strawberry purée by 80% with continuous flow microwave heating at 90 °C.

"Non-thermal" or "specific" effects from microwave heating, supposedly another advantage of this technology, have been a subject of debate for decades (Fleming, 1944; Kubo et al., 2020). On the one hand, some authors state that additional inactivation is caused by electric fields that can alter the conformation of enzymes, which are susceptible to structural modifications that influence their functionality (George, Bilek, & McKenzie, 2008; Porcelli et al., 1997; Zhang, Balasubramaniam, Dunne, Farkas, & Yuan, 2011). The mechanism of enzyme inactivation can be analyzed at molecular level through techniques such as circular dichroism and fluorescence spectroscopy, which can provide information about secondary and tertiary structural changes, respectively (Iqbal et al., 2019). On the other hand, other authors state that only thermal effects contribute to inactivating enzymes or microorganisms, since microwaves do not have sufficient energy to break covalent bonds (Awuah et al., 2007; Benlloch-Tinoco,

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Martinez-Navarrete, & Rodrigo, 2014; Shaman, Mizrahi, Cogan, & Shimoni, 2007; Xu et al., 2016). Evidence of such effects may have arisen from inadequate comparisons between microwave and conventional heating, since some assays were performed with different time–temperature profiles, with multimode microwave cavities without control of electric field distribution, with comparisons between batch treatments and continuous flow processing, or with inactivation kinetic data obtained under unmatching conditions (Awuah et al., 2007; Dogan Halkman, Yücel, & Halkman, 2014; Kozempel, Cook, Scullen, & Annous, 2000; Shaman et al., 2007).

In this context, there is potential for improving the pasteurization of liquid foods such as green coconut water. Coconut water is valued for its delicate flavor, unique composition and therapeutic properties, but widespread distribution of this increasingly demanded product is difficult due to changes caused by PPO (Prades, Dornier, Diop, & Pain, 2012; Tan, Cheng, Bhat, Rusul, & Easa, 2014). Process conditions necessary for its inactivation lead to loss of sensorial characteristics (Campos, Souza, Coelho, & Gloria, 1996).

Kinetic models allow understanding inactivation while aiding to achieve a safe and stable product. These models could be used to compare enzyme inactivation by different treatments, assessing the interesting potential of emerging technologies (Awuah et al., 2007; Cullen et al., 2012; van Boekel, 2008). Therefore, this study aims to compare conventional and focused microwave heating under similar conditions through kinetic models for inactivation of two PPOs: commercial mushroom tyrosinase in buffer solution and PPO present in coconut water. For that, a suitable comparison using similar time–temperature profiles and heating rates between technologies is needed, as well as adequate kinetic model fitting (Kubo et al., 2020). Results should prove useful for designing liquid food pasteurization by focused microwave technology.

## 2. Materials and methods

### 2.1. Sample preparation

Commercial mushroom tyrosinase (EC 1.14.18.1, CAS 9002–10-2, Sigma-Aldrich Co. T3824, USA) was dissolved in 50 mL of potassium phosphate buffer (ionic strength of 50 mM and pH 6.5, PubChem CID 62657). This enzymatic stock solution was distributed in 1.0 mL microtubes and kept frozen at −30 °C. Prior to treatments, 1.0 mL of the stock solution was thawed and diluted in 10 mL of potassium phosphate buffer (Aguiar, Yamashita, & Gut, 2012; Campos et al., 1996; Meneses et al., 2013).

Green coconuts (*Cocos nucifera*, Dwarf variety) without signs of injury and purchased from a local market had their shells washed with a dilute bleach solution and perforated with extractor Coco Fácil (Brasil Iluminação, Brazil). Water from the coconuts was extracted by gravity, mixed, filtered (0.212 mm metal mesh) and stored at −30 °C until thawed for treatments (Matsui et al., 2008).

### 2.2. Conventional thermal treatment

Samples with 3 mL of buffer solution or coconut water were placed in a polyethylene pouch (3.5 cm × 14.5 cm, thickness 0.12 mm). Thermal treatment was conducted by immersion of the pouch in a stirred hot water bath with temperature control MA-184 (Marconi, Brazil) followed by immersion in a stirred ice water bath (under 10 °C) under manual agitation. Different combinations of temperature (between 50 and 96 °C) and immersion times (2 to 140 s) were tested to obtain values of residual activity between 3 and 95% (Aguiar et al., 2012).

Time-temperature profiles of the sample were obtained using a fiber optic thermometer Luxtron 812 (LumaSense Technologies, USA) connected to a fiber optic probe STF-1 M (LumaSense Technologies, USA). The tip of the probe was kept at the center of the sample, supported by a glass stick of height 30 cm and diameter 0.66 cm. The temperature was considered uniform due to the small sample volume and the stirring. Since the optical fiber response time was only 0.5 s, thermal inertia was ignored.

### 2.3. Microwave treatment

Microwave batch treatments at 2450 MHz were performed using the bench-scale microwave reactor Discover Reflux (CEM, USA) with maximum power of 300 W. A test tube (glass, length 200 mm and diameter 19.6 mm) containing 5 mL of buffer solution or coconut water and a magnetic bar stirrer with 7.2 mm in length was inserted in the application cavity (height and diameter 7 cm) where this reactor focuses microwaves. The sample height inside the tube was 26 mm. Time-temperature profiles were obtained as described for conventional thermal treatments. The temperature was admitted uniform due to constant stirring, small volume and power penetration depth of microwaves (frequency 2450 MHz), which was less than the tube radius, according to Franco et al. (2015) for coconut water and simulated solution.

In order to maintain similar time–temperature profiles and average heating rates between conventional and microwave paired treatments, a protocol was developed for the microwave treatments. Initially, a constant incident power was fixed so that the heating ramp was similar to the corresponding time–temperature profile obtained previously in the conventional heat treatment (come-up times between 20 and 30 s depending on the processing temperature). Shortly before the sample reached the intended processing temperature, the reactor configuration was changed to control the sample temperature by altering the incident power automatically. Using this second configuration at the beginning of the microwave treatments would yield come-up times longer than 60 s. After treatment, samples were immersed in a stirred ice water bath and agitated manually.

Commercial tyrosinase samples were initially heated at holding temperatures between 43 and 85 °C, and holding times to achieve 3% to 95% residual PPO activity. 25 time–temperature combinations were tested for both conventional and microwave heating. Coconut water samples were initially heated at holding temperatures between 60 and 90 °C, and holding times to achieve 3% to 95% residual PPO activity. 34 time–temperature combinations were tested for both conventional and microwave heating; running a larger number of experiments with coconut water addressed the increased uncertainty when modeling kinetic reactions in a complex food matrix containing several components.

### 2.4. Enzymatic assay

PPO activity was assayed with spectrophotometer Spectra Max Plus 384 (Molecular Devices, USA) at 420 nm and 25 °C with a 96 well flat bottom microplate (Greiner Bio-One, Austria). The absorbance was measured every 12 s for 5 min (commercial PPO) and every 20 s for 30 min (PPO in coconut water).

PPO activity in the mushroom tyrosinase buffer solution was determined according to Ndiaye et al. (2009). Each well contained 57 μL of pyrocatechol solution (0.175 M, PubChem CID 289, CAS 120–80-9, Sigma-Aldrich Co. C9510, Japan), 114 μL of potassium phosphate buffer (pH 6.5, 50 mM) and 29 μL of sample. The reference value was determined using 143 μL of potassium phosphate buffer and 57 μL of pyrocatechol solution. The average value of enzymatic activity was obtained from eight repetitions from each sample. PPO activity in coconut water was determined according to Campos et al. (1996). Each well contained 138 μL of potassium phosphate buffer (0.2 M, pH 6.0), 25 μL of coconut water and 37 μL of pyrocatechol solution (0.2 M, PubChem CID 289, CAS 120–80-9, Sigma-Aldrich Co. C9510, Japan). The reference value was determined using 163 μL of potassium phosphate buffer and 37 μL of pyrocatechol solution. The average value of enzymatic activity was obtained from four repetitions from each

sample.

The absorbance was plotted against time and PPO activities were calculated from the slope of the initial linear part of the curve. One unit of enzyme activity was defined as an increase of 0.001 absorbance per second per mL of sample volume. The residual activity was determined as $A/A_0$, where $A$ is the average enzyme activity after treatment and $A_0$ is the average initial enzyme activity before treatment.

### 2.5. Kinetic models for enzyme inactivation

Food engineering literature traditionally describes inactivation of enzymes using the first-order kinetic model shown in Eq. (1), where $D$ (s) is the decimal reduction time at the processing temperature and $t$ (s) is the isothermal processing time. Parameter $D$ varies with temperature $T$ (°C) as described in Eq. (2), where $D_{ref}$ (s) is $D$ at a reference temperature $T_{ref}$ (°C) and $z$ (°C) is the temperature increase that decreases the decimal reduction time by 90%. This model has two parameters: $D_{ref}$ and $z$ (Awuah et al., 2007; Bigelow, 1921).

$$\log\left(\frac{A}{A_0}\right) = -\frac{t}{D} \tag{1}$$

$$\log\left(\frac{D}{D_{ref}}\right) = -\frac{T - T_{ref}}{z} \tag{2}$$

The first-order kinetic model of a two-component system considers two isoenzymes with different thermal resistances and independent inactivation. Eq. (3) shows the residual activity $A/A_0$ for this model, where $\alpha$ and $(1-\alpha)$ represent the initial activity fractions of the thermostable (subscript 1) and thermolabile (subscript 2) isoenzymes, respectively. This model has five parameters: $D_{ref,1}$, $z_1$, $D_{ref,2}$, $z_2$ (Eq. (4)) and $\alpha$ (Aguiar et al., 2012; Ling & Lund, 1978).

$$\frac{A}{A_0} = \alpha \bullet \text{alog}\left(-\frac{t}{D_1}\right) + (1-\alpha) \bullet \text{alog}\left(-\frac{t}{D_2}\right) \tag{3}$$

$$\log\left(\frac{D_j}{D_{ref,j}}\right) = -\frac{T - T_{ref}}{z_j},\ j = 1, 2 \tag{4}$$

Since $T$ varies with , the integrated lethality on enzyme activity should consider the whole time–temperature profile. In this case, Eqs. (1) and (3) become Eqs. (5) and (6), respectively (Aguiar et al., 2012; Awuah et al., 2007).

$$\log\left(\frac{A}{A_0}\right) = -\frac{1}{D_{ref}} \bullet \int_0^t \text{alog}\left(\frac{T(t) - T_{ref}}{z}\right) dt \tag{5}$$

$$\frac{A}{A_0} = \alpha \bullet \text{alog}\left(-\frac{1}{D_{ref,1}} \bullet \int_0^t \text{alog}\left(\frac{T(t) - T_{ref}}{z_1}\right) dt\right) + (1-\alpha) \bullet \text{alog}\left(-\frac{1}{D_{ref,2}} \bullet \int_0^t \text{alog}\left(\frac{T(t) - T_{ref}}{z_2}\right) dt\right) \tag{6}$$

A kinetic model based on the Weibull distribution function is sometimes used to describe microorganism or enzyme inactivation to take into account differences in thermal resistances among cells and molecules. Eq. (7) represents the Weibull model, where $\beta$ is the shape factor and $D_w$ (s) is the time for the first decimal reduction. $D_w$ dependency with $T$ may be described by Eq. (8), where $D_{w,ref}$ (s) is the time for the first decimal reduction at $T_{ref}$, and $z_w$ (°C) is the temperature increase that reduces $D_w$ by 90%. If $\beta = 1$, the Weibull model degenerates to the first-order model (Eq. (1)). This model has three parameters: $D_{w,ref}$, $z_w$ and $\beta$ (Serment-Moreno, Barbosa-Canovas, Torres, & Welti-Chanes, 2014; van Boekel, 2002).

$$\log\left(\frac{A}{A_0}\right) = -\left(\frac{t}{D_w}\right)^{\beta} \tag{7}$$

$$\log\left(\frac{D_w}{D_{w,ref}}\right) = -\frac{T - T_{ref}}{z_w} \tag{8}$$

The derivative of Eq. (7) with respect to time, for a constant $\beta$, yields Eq. (9), which shows that if $\beta \neq 1$, the momentary logarithmic inactivation rate depends on the momentary residual activity. For a non-isothermal treatment, the momentary logarithmic inactivation rate integrated along time (Eq. (10)) represents the accumulated reduction of enzyme activity (Mafart, Couvert, Gaillard, & Leguerinel, 2002). For a constant $\beta$, if $\beta = 1$ then Eq. (10) becomes Eq. (5).

$$\left.\frac{d\log(A/A_0)}{dt}\right|_T = -\frac{\beta t^{\beta-1}}{D_w{}^{\beta}} = -\frac{\beta}{D_w} \bullet (-\log(A/A_0))^{\frac{\beta-1}{\beta}} \tag{9}$$

$$\log\left(\frac{A}{A_0}\right) = -\int_0^t \frac{\beta}{D_w} \bullet (-\log(A/A_0))^{\frac{\beta-1}{\beta}} dt \tag{10}$$

### 2.6. Parameter estimation

From the experimental temperature–time profiles and initial parameters guesses, the predicted residual activity for each run was calculated with software Excel 2013 (Microsoft, USA). Eqs. (5) and (6) were numerically evaluated by the trapezium method with a time step of $\Delta t$= 0.5 s (Aguiar et al., 2012). Eq. (10) was discretized as shown in Eq. (11), with a time step of $\Delta t$= 0.5 s, $\log(A/A_0)_0 = -10^{-4}$ and calculation from instant $t_{i-1}$ to avoid an explicit equation with $\log(A/A_0)_i$ at $t_i$ (Campanella & Peleg, 2001; Chen, Campanella, & Corvalan, 2007).

$$\log\left(\frac{A}{A_0}\right) = -\sum_{i=0}^{t/\Delta t} \frac{\beta}{D_{w,i}} \bullet (-\log(A/A_0)_{i-1})^{\frac{\beta-1}{\beta}} \Delta t \tag{11}$$

The parameters in Eqs. (5), (6) and (10) were iteratively estimated by minimizing the sum of squared errors $SSE$ between experimental and predicted final residual activities (Eq. (12)). A non-linear optimization problem is therefore defined, with model parameters as degrees of freedom and $SSE$ as an objective function. A generalized reduced gradient algorithm implemented in the Solver tool of software Excel 2013 (Microsoft, USA) was used. Trying different initial guesses avoided convergence to local optima.

$$SSE = \sum \left[ \left(\frac{A}{A_0}\right)_{experimental} - \left(\frac{A}{A_0}\right)_{calculated} \right]^2 \tag{12}$$

Adjusted models were evaluated by residual activity parity charts, minimized $SSE$ value, coefficient of determination ($R^2$) and root mean square error ($RMSE$).

### 2.7. Comparison of time–temperature profiles

Comparison of time–temperature profiles $T(t)$ was used to assure similar heating conditions between conventional and microwave treatments using the concept of equivalent processing time or integrated lethality, $F_0$ (s) in Eq. (13), based on parameters fitted for the Weibull kinetic model, as it proved to be the best model. $T_{ref}$ was fixed at 70 °C, and a time step of $\Delta t$=f 0.5 s was used. The proposal was to calculate the integrated lethality (proportional to the area under the $T(t)$ curve) for each pair of microwave and conventional heating runs, and then compare these values in a parity chart.

$$F_0 = D_w \left[ -\log\left(\frac{A}{A_0}\right) \right]^{\frac{1}{\beta}} \tag{13}$$

### 2.8. Validation of kinetic model

Tests with slow heating and cooling of buffer solution with tyrosinase and coconut water samples were used to validate the fitted

kinetic models. Experimental residual PPO activity after validation treatments was compared with the predicted activity from Eqs. (5), (6) and (10) using the adjusted parameters and the temperature–time profile. The samples were prepared according to item 2.1, and temperature was recorded as described previously.

The procedure for conventional heating of commercial tyrosinase in buffer solution was similar to item 2.2, with 5.0 mL samples placed in test tubes, which were agitated to obtain uniform temperature during treatment. The procedure for microwave heating was similar to item 2.3, with 15 mL samples placed in a test tube under magnetic stirring (the height sample inside the tube was 64 mm) and initially heated at a nominal incident power of 150 W. Experimental conditions for the validation runs were temperatures between 54 and 78 °C and holding times between 4 and 50 s.

The procedure for conventional heating of coconut water samples was similar to item 2.2, with 3.0 mL, but with slightly slower pouch agitation. The procedure for microwave heating was similar to item 2.3, with 5.0 mL, but no initial fixed power period. Experimental conditions for the validation runs were temperatures 65, 75 and 85 °C (maximum incident powers were 46, 60 and 88 W, respectively) and holding times between 1 and 160 s.

## 3. Results and discussion

### 3.1. Parameter estimation

The kinetic models presented had their respective parameters adjusted to data from 59 experimental runs (25 runs with buffer solution and 34 runs with coconut water) for each heating method. Table 1A presents adjusted parameters and evaluation criteria for the first-order kinetic model for PPO inactivation in buffer solution and green coconut water. The first-order model had been previously used to describe the PPO inactivation in green coconut water under conventional heating (Tan et al., 2014) and microwave heating in a multimode cavity (Matsui et al., 2007, 2008).

Table 1B presents adjusted parameters and evaluation criteria of the two-component kinetic first-order model for PPO inactivation. Higher $R^2$ and lower *SSE* and *RMSE* than those in Table 1A were obtained, since the two-component model has three parameters more. The larger number of parameters and the higher non-linearity made the model fitting more time-consuming and local sub-optima were found in the process. The $\alpha$ values for PPO in buffer solution are closer to unity than in coconut water, showing that the thermal resistance of this commercial enzyme is more homogeneous than that of the native enzyme in coconut water. It is noteworthy that $\alpha$ did not seem to depend on the heating method, since the physical interpretation of this parameter is the initial activity of an isoenzyme before treatment.

Table 1C presents adjusted parameters and evaluation criteria of the Weibull kinetic model for PPO inactivation. All $\beta$ values were less than unity, which means that inactivation rates decrease with time – meaning that the least heat resistant isoenzymes were inactivated first in a *n*-component first-order model. $R^2$, *SSE* and *RMSE* values were similar to those on Table 1B, but the Weibull model has fewer parameters than the two-component first-order model and convergence to local sub-optima was not observed. PPO inactivation in green coconut water yielded higher *SSE* and *RMSE* and lower $R^2$ than mushroom PPO in buffer solution for the three models studied, due to the variability inherent to biological systems. Green coconut water PPO also showed to be more heat resistant than a commercial enzyme in buffer solution, in agreement with previous observations (Matsui et al., 2008).

### 3.2. Evaluation of model fitting

Fig. 1 presents parity charts comparing experimental residual PPO activity with predictions from adjusted models in Eqs. (5), (6) and (10) – for conventional and microwave heating and in buffer solution and green coconut water using the parameters in Table 1.

Modelling residual enzyme activity in a real food is prone to more uncertainty and variability than in a buffer solution, due to the interaction among food components. For the three models, in both buffer solution and in coconut water, as well as for both conventional and focused microwave heating, there is no observable trend in residuals and points are randomly scattered along the 45° line in the parity charts. The dispersion is larger for the first-order model, in accordance with the evaluation criteria values in Table 1A, whereas the two-component and Weibull models were able to better predict residual PPO activity within a margin of ± 10%.

Considering parity chart dispersion, evaluation criteria (*SSE*, *RMSE* and $R^2$) and number of parameters, the Weibull model was the most adequate among the three models studied to describe inactivation of

**Table 1**
Adjusted parameters and evaluation criteria for the three kinetic models adjusted for the inactivation of PPO in buffer solution and in green coconut water.

| Kinetic model | Commercial PPO in buffer | | | PPO in coconut water | | |
|---|---|---|---|---|---|---|
| | | Microwave | Conventional | | Microwave | Conventional |
| A – First-order | $z$ (°C) | 8.29 | 13.7 | $z$ (°C) | 12.6 | 23.3 |
| | $D_{70\ °C}$ (s) | 11.7 | 26 | $D_{70\ °C}$ (s) | 236 | 191 |
| | *SSE* | 0.158 | 0.143 | *SSE* | 0.297 | 0.436 |
| | $R^2$ | 0.961 | 0.944 | $R^2$ | 0.936 | 0.822 |
| | *RMSE* | 7.96% | 7.56% | *RMSE* | 9.34% | 11.3% |
| B – Two-component first-order | | | | | | |
| | α | 0.892 | 0.808 | α | 0.695 | 0.684 |
| | $z_1$ (°C) | 7.21 | 10.7 | $z_1$ (°C) | 10.1 | 17.1 |
| | $z_2$ (°C) | 45.3 | 40.0 | $z_2$ (°C) | 6.00 | 43.6 |
| | $D_{70\ °C,\ 1}$ (s) | 10.0 | 23.3 | $D_{70\ °C,\ 1}$ (s) | 501 | 387 |
| | $D_{70\ °C,\ 2}$ (s) | 0.830 | 1.20 | $D_{70\ °C,\ 2}$ (s) | 10.7 | 29.9 |
| | *SSE* | 0.114 | 0.087 | *SSE* | 0.153 | 0.207 |
| | $R^2$ | 0.966 | 0.961 | $R^2$ | 0.961 | 0.875 |
| | *RMSE* | 6.76% | 5.90% | *RMSE* | 6.70% | 7.80% |
| C – Weibull | | | | | | |
| | β | 0.266 | 0.543 | β | 0.456 | 0.499 |
| | $z_w$ (°C) | 3.56 | 9.79 | $z_w$ (°C) | 7.89 | 16.3 |
| | $D_{w\ 70\ °C}$ (s) | 10.2 | 26.7 | $D_{w\ 70\ °C}$ (s) | 1028 | 509 |
| | *SSE* | 0.102 | 0.090 | *SSE* | 0.139 | 0.229 |
| | $R^2$ | 0.969 | 0.959 | $R^2$ | 0.959 | 0.860 |
| | *RMSE* | 6.38% | 6.03% | *RMSE* | 6.39% | 8.21% |

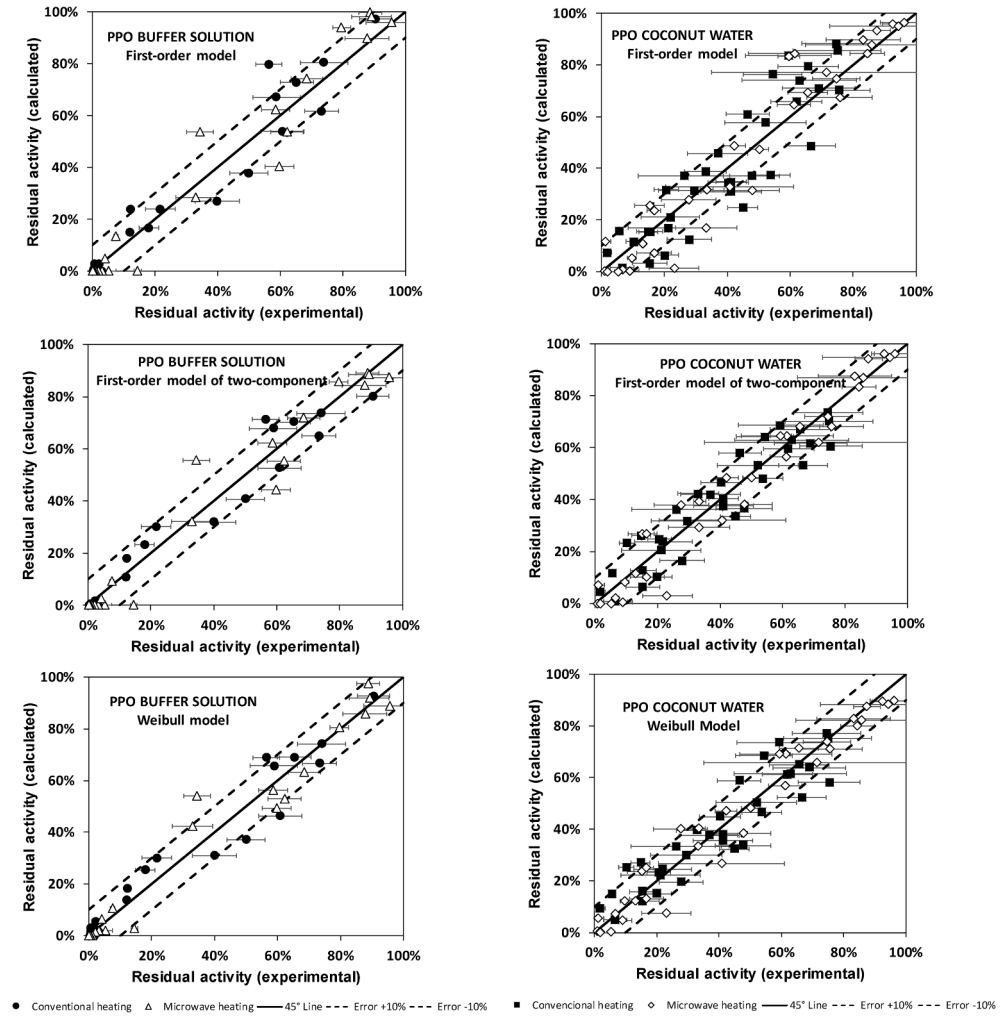


**Fig. 1.** Parity charts of experimental and calculated residual PPO activity for first-order, two-component first-order and Weibull kinetic models. Buffer solution, conventional heating: ●; Buffer solution, microwave heating: △; Coconut water, conventional heating: ◇; Coconut water, microwave heating: ■.

PPO by conventional or focused microwave heating in buffer solution and in green coconut water. Therefore, this model was selected to simulate PPO inactivation and to compare conventional and microwave heating. However, the discussion that follows does not depend on any given mathematical model, since comparing traditional and novel thermal processes relies actually on matching temperature profiles (Peleg, Corradini, & Normand, 2012). This means that selecting another kinetic model to describe residual PPO activity as a function of time and temperature with these data would still yield the same conclusions.

### 3.3. Validation of the Weibull kinetic model

The parity charts in Fig. 2 compare the residual PPO activity calculated by the Weibull model with experimental values obtained from validation runs in buffer solution and coconut water under different heating conditions than those used for model adjustment. As in Fig. 1, points are randomly scattered along the 45° line with residuals generally within a ± 10% margin. The good agreement between model predictions and experimental values, even with different heating rates, indicates that the integrated lethality as calculated with the Weibull model with a constant shape factor is effective in considering PPO inactivation on a non-isothermal process.

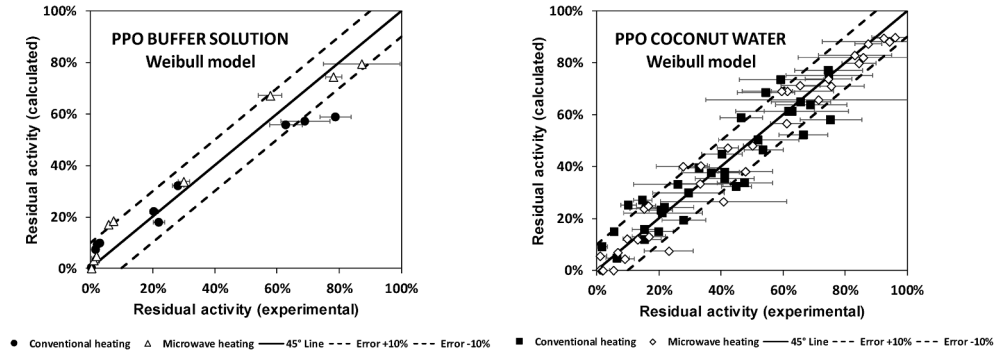


**Fig. 2.** Parity charts of experimental and calculated residual PPO activity for Weibull model validation. Buffer solution, conventional heating: ●; Buffer solution, microwave heating: △; Coconut water, conventional heating: ◇; Coconut water, microwave heating: ■.

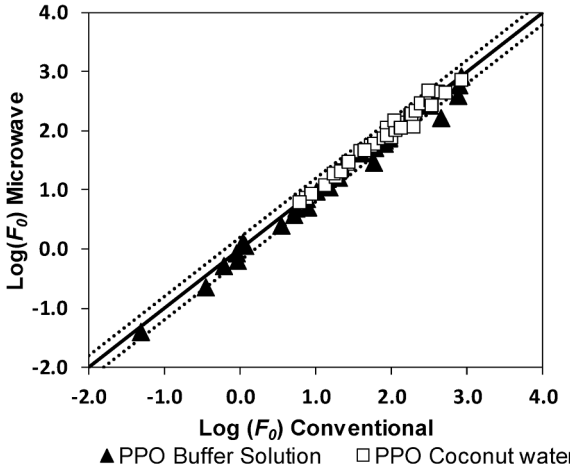


**Fig. 3.** Parity chart of log time process lethality ($\log(F_0)$) between conventional and microwave treatment at samples of PPO in buffer solution (▲) and PPO in coconut water (□).

### 3.4. Comparison between conventional and microwave heating

Fig. 3 shows a parity chart comparing the integrated lethality calculated from time-temperature profiles of each pair of conventional and microwave runs from process lethality from Eq. (13). Each of the 59 data points in Fig. 3 corresponds to a pair of a conventional heating run and a microwave heating run. If the $T(t)$ curves for microwave and conventional treatments are similar, one can expect the $F_0$ values for both treatments to be similar, and that would cause the data points to spread along the 45° line, as observed for most of the samples. This comparison is an alternative to overlapping 59 pairs of microwave and conventional treatments time–temperature profiles, to prove that heating conditions were indeed similar at temperatures close to $T_{ref}$. Thus, differences in PPO inactivation between both treatments may be caused by specific microwave effects, since the thermal effects were similar.

Comparing the adjusted kinetic parameters in Table 1C, one might infer that such specific effects exist, since different thermal treatments yielded different values for $D_w$, $z_w$ and $\beta$. However, to better compare both treatments, isothermal processes for PPO inactivation have been simulated with Eq. (7) with the estimated parameters in Table 1C at different process temperatures (Fig. 4). The higher resistance of native PPO in coconut water when compared to commercial PPO in buffer solution is evident, due to interactions between food components and the enzymes (Matsui et al., 2008).

Another interesting point in Fig. 4 is the shift in relative efficacy between conventional and microwave heating depending on the process temperature; inactivation by microwaves was higher than inactivation by conventional heating at 75 and 80 °C in buffer solution, and at 80 and 90 °C in coconut water. However, conventional heating was more effective in reducing PPO activity at lower pasteurization temperatures of 50, 60 and 70 °C. Microwave heating, therefore, seems to have an enhanced effect on PPO inactivation at higher pasteurization temperatures; combining this effect with a faster volumetric heating rate, there is potential for reducing processing times and improving quality.

Some authors have reported similar results for PPO inactivation in different fruit products processed when using microwave heating. Matsui et al. (2007; 2008) found that microwave heating promoted higher inactivation than conventional heating as reported in Campos et al. (1996). In another study with fixed incident microwave power (between 300 and 900 W), microwave heating was more effective in inactivating PPO in kiwi purée than conventional heating (Benlloch-Tinoco et al., 2013).

According to the PPO inactivation curves described by the adjusted Weibull model, non-thermal effects were observed at temperatures above 80 °C. Regarding protein denaturation by microwave heating, Shaman et al. (2007) did not observe non-thermal effects, and Xu et al. (2016) did not identify such effects on the inactivation of enzymes lipase and lipoxygenase. However, both teams studied processing temperatures lower than 70 °C. Additionally, non-thermal effects were detected in other studies as changes in the structural conformation of enzymes by microwave heating at temperatures between 70 and 90 °C (George et al., 2008; Porcelli et al., 1997). Samaranayake and Sastry (2016) observed that enzyme activity in tomato homogenate under ohmic heating could be either enhanced or inhibited depending on electric field strength, frequency and temperature. If there are electric field effects on enzyme inactivation, it would be expected that field strength is an important process parameter to control, in addition to temperature. In the present study, this correlation was confirmed: non-thermal effects were observed at higher pasteurization temperatures, which were reached with stronger electric fields.

The oscillating electric field in microwave heating possibly promotes changes in protein structure and consequently affects its functionality (Zhang et al., 2011). In an extensive review, Kubo et al. (2020) discuss possible mechanisms for non-thermal effects of oscillating electric fields on enzyme activity: since proteins have polar groups in their structure and present a dipole moment, microwaves could induce translational and rotational molecular motion without directly breaking covalent bonds. The authors observe that energy levels in microwaves may not be high enough to affect primary structure of enzymes; however, they also cite a number of works that showed interactions between microwaves and polar groups altering an enzyme secondary or tertiary structure and, therefore, its functionality. Other mechanisms proposed include removal of the prosthetic metallic group or weakening of chemical bonds by microwave excitation to higher energy levels. When comparing heating technologies, it is imperative to match time–temperature conditions (as shown in Fig. 3) and to measure temperature with an adequate sensor inserted in the sample without interferences from the electric field (as the fast-response fiber optic thermometer utilized in this study).

## 4. Conclusions

It was possible to fit the three kinetic models to represent the inactivation of PPO by conventional or focused microwave heating in buffer solution and green coconut water, taking into account the whole time–temperature profile. The Weibull model was the most adequate to describe PPO inactivation. This model was selected to simulate PPO inactivation curves and compare conventional and microwave heating. Nevertheless, this does not mean that the other kinetic models cannot describe PPO inactivation. The predicted PPO inactivation by focused microwave heating was higher than conventional heating at temperatures above 70 °C, suggesting noon-thermal effects at higher microwave power levels. However, conventional heating was more effective at temperatures below 70 °C.

The validation demonstrated that the model accounts for effects of the whole time–temperature history, and it is able to satisfactorily predict the residual enzyme activity achieved after treatment by conventional or focused microwaves. This will contribute to future studies, providing important subsidies for scale-up and implementing focused microwaves in continuous industrial processes for heat treatment of liquid foods.

## CRediT authorship contribution statement

**Tiago Augusto Bulhões Bezerra Cavalcante:** Conceptualization, Methodology, Formal analysis, Resources, Visualization, Writing - original draft. **Eduardo dos Santos Funcia:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing - review

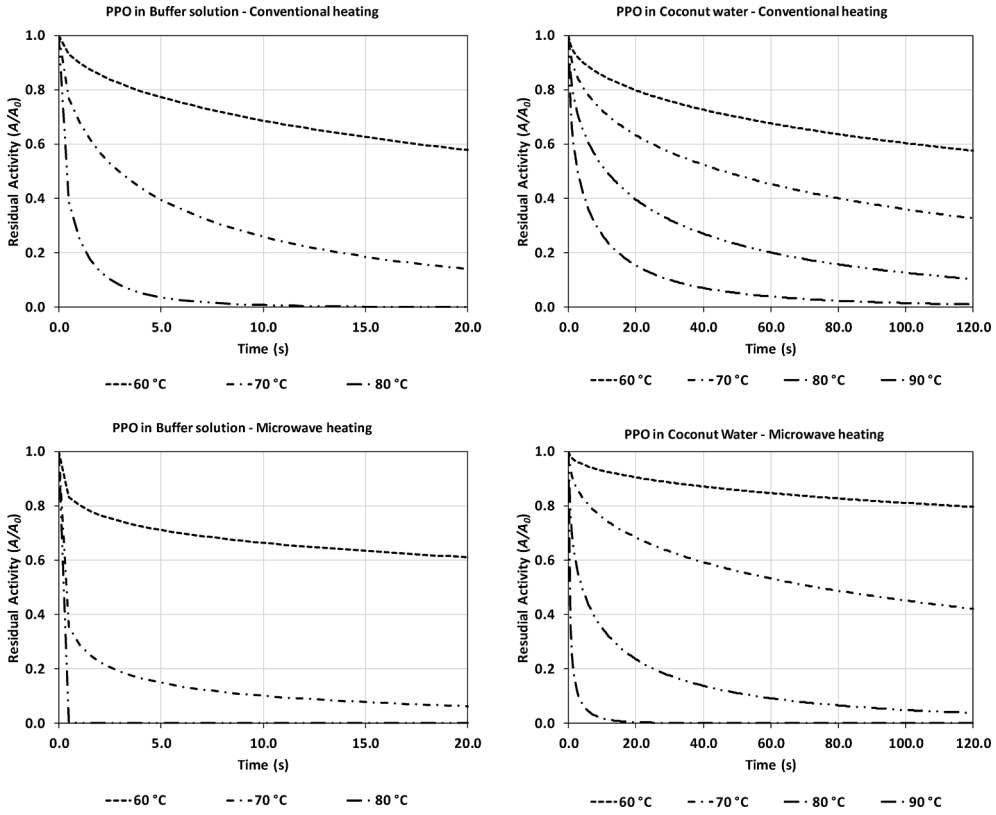


**Fig. 4.** Simulation of PPO inactivation in buffer solution and coconut water by conventional and microwave heating as predicted by the adjusted Weibull kinetic model.

& editing. **Jorge Andrey Wilhelms Gut:** Funding acquisition, Conceptualization, Supervision, Writing - review & editing, Project administration.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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