

Proceeding paper

# Inactivation Kinetics of Peroxidase by Conventional and Microwave Processing of Mature Coconut Water <sup>†</sup>

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**Abstract:** Mature coconut water is considered a waste product in the process of obtaining copra. However, it is a material that still contains substances that can be beneficial to the human body. For the processing of this material, the application of emerging technologies such as microwaves could help to reduce quality losses. For this reason, the effect of conventional and microwave processing was assessed on the inactivation kinetics of peroxidase (POD) in mature coconut water. Samples in glass tubes were treated at three temperatures (60, 75, and 90 °C) and 5–130 s of holding time. First-order and Weibull kinetic models were adjusted considering the complete time-temperature profiles. Weibull model was the one that best described the inactivation of the POD enzyme, having the highest fit and lowest RMSE in both heating processes. Microwave heating generated an inactivation of the POD enzyme significantly faster than conventional processing in mature coconut water.

**Keywords:** emerging technology; mature coconut water; microwaves; enzymatic inactivation

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## 1. Introduction

Mature coconut is mainly used to obtain copra, a process in which coconut water is discarded or underutilized. Mature coconut water contains sugars and amino acids such as L-valine, L-threonine, L-isoleucine and L-leucine which are essential for humans [1]. The composition, physicochemical properties and enzyme inactivation of green and mature coconut water have been studied [2,3], where the presence and inactivation of polyphenol oxidase (PPO) and peroxidase (POD) enzymes was determined through the application of conventional heat treatments. In addition to conventional heat treatments, technologies such as pressure assisted thermal processing [4] and batch microwave treatment [5] have been used to study the inactivation of PPO and POD in green coconut water.

Microwave heating, an emerging technology, has been used to reduce quality losses occurring in conventional processing. Conventional and microwave processing have been compared for the inactivation of PPO in green coconut water [6]. The objective of this work is to compare the effect of conventional and microwave processing on the enzymatic activity of POD in mature coconut water, using similar time-temperature profiles, as well as kinetic models.

## 2. Materials and Methods

### 2.1. Sample Preparation

In this study mature coconuts that had reached 12 months of age were harvested from the farm of a producer from Benito Juárez, Guerrero, Mexico. Mature coconut was

washed with a dilute bleach solution, then water was extracted manually with a special knife by gravity, mixed, filtered and stored at -18 °C until use.

### 2.2. Conventional Processing

Samples with 5 mL of mature coconut water were placed in a glass tube. Thermal treatment was conducted by immersion of the glass tube in a stirred hot water bath with temperature control E100 (Lauda, Germany) followed by immersion in ice water bath manually agitated. Combination of three temperatures (60, 75 and 90 °C) and five immersion times (5, 10, 25, 60 and 130 s) were tested [6].

### 2.3. Microwave Processing

Microwave heating was conducted in a microwave system (CEM, mod. Discover system, USA, Matthews) at 2450 MHz. Coconut water samples of 5 mL were placed in a glass tube and inserted vertically into the microwave digester cavity under continuous microwave incidence. Temperature-time combinations were the same as conventional processing. Once temperature and holding time were achieved, the tube was quickly inserted into an ice water bath manually agitated.

### 2.4. Time–Temperature Profiles

Traceable data logging thermocouple probe thermometer (Digi-Sense WD-20250-02, Cole-Parmer, USA) was used to obtain time-temperature profiles. The probe tip was kept at the center of the sample. Data collection was every second.

### 2.5. Determination of Enzymatic Activity

POD activity assays were made in triplicate at 25 °C and pH 6.0, ensured using a buffer solution made of citric acid and Na<sub>2</sub>HPO<sub>4</sub> (Merck, Germany). POD activity was assayed as described in Augusto et al (2015) and adapted to microplates using a microplate reader (Synergy 2, BioTek Instruments, USA). Microplates from 96 wells were used, each containing 16 µL of mature coconut water, 225 µL of buffer solution at pH 6.0, 320 µL of 5% (m/v) pyrogallol solution, and 16 µL of 0.147 M H<sub>2</sub>O<sub>2</sub> solution (Merck, Germany) to start the reaction. The absorbance value was acquired every 11 s for 2.5 minutes. A unit of enzyme activity was defined as the amount of enzymatic extract capable of producing an increase of absorbance at 420nm at rates of 0.001 unit per minute [2,7].

### 2.6. Enzyme Inactivation Models

Inactivation of enzymes is usually described using the first-order kinetic model, shown in Equation (1), where b is the inactivation rate constant. Weibull distribution function has been employed as a kinetic model. Equation (2) represents the Weibull model, where b and c are scale and shape parameters.

$$A/A_0 = a \cdot \exp(-b \cdot x) \tag{1}$$

$$A/A_0 = a \cdot ((c-1)/c)^{(1-c)/c} \cdot (\text{abs}((x-x_0)/b + ((c-1)/c)^{(1/c))^{(c-1)}) \cdot \exp(-\text{abs}((x-x_0)/b + ((c-1)/c)^{(1/c))^{(c-1)}) \tag{2}$$

## 3. Results and Discussion

### 3.1. Time–Temperature Profiles

Comparison of time–temperature profiles during conventional heating and microwave processing are shown in Figure 1. The same conditions were programmed in both processes. It can be observed that microwave heating reaches the programmed tempera-

ture more quickly, however, as it is an accelerated temperature ramp, the maximum temperature reached is higher than that reached in conventional heating. In general, the heating and cooling process of the microwave treatment was faster than conventional heating.

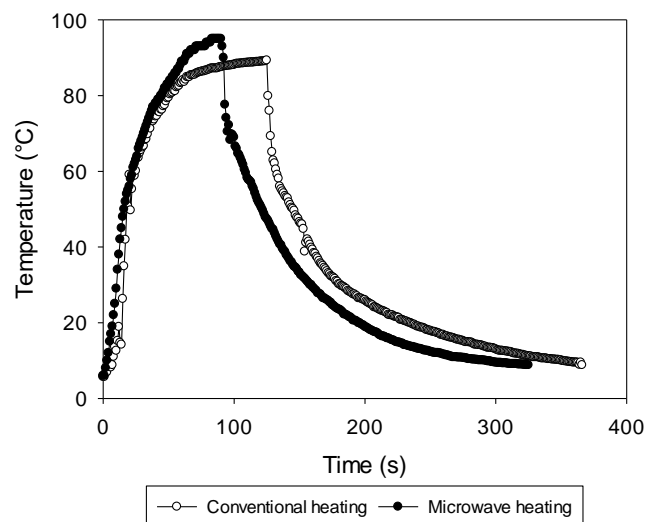


Figure 1. Sample temperature–time profiles for (○) conventional and (●) microwave heating.

### 3.2. Parameter Estimation and Evaluation of Model Fitting

Adjusted parameters for the first-order kinetic model and Weibull model are shown in Table 1. Data used to fit the models presented were obtained from 96 experimental runs (48 for each heating method). The first-order model has been employed to describe the inactivation of POD in green coconut water by conventional heating [2,3] and microwave heating has also been employed to inactivate POD in green coconut water [5] and modeled with first-order kinetics. The Weibull model has also been used to describe enzyme inactivation in coconut water, resulting in a better fit than the first-order model. In this study, for both models poor fits were obtained for the temperature of 60 °C, while at higher temperatures better fits were obtained by both models. It was also observed that the RMSE obtained from both models for microwave heating was lower than that obtained for conventional heating. In addition, the RMSE is generally lower in the Weibull model than the first-order model.

Table 1. Adjusted parameters and fitting performance of kinetic models for inactivation of peroxidase in mature coconut water.

Kinetic model	Conventional			Microwave			
	60 °C	75 °C	90°C	60 °C	75 °C	90°C	
First-order	a	1.5346	1.4508	0.9857	1.4488	1.33	0.9996
	b	1.1783E-13	0.0172	0.2227	1.23E-13	0.0304	0.479
	SSE	0.5912	0.9751	0.0572	0.4361	0.4565	0.0117
	R <sup>2</sup>	-3.967E-11	0.5063	0.908	-5.23E-11	0.7220	0.9841
	RMSE	0.384447656	0.49376108	0.11958261	0.33015148	0.33778692	0.05385165
Weibull	a	2.001	1.5894	16.5655	1.9256	1.4375	4.0289
	b	3746.1896	48.7804	4.4996	130	16.4036	2.0907
	c	1.1019	1.0308	1.001	1.5	1.818	1.001
	x0	431.3986	1.6163	-12.7192	130	6.2018	-2.9283
	SSE	0.0936	0.6665	0.0572	9.3229	0.0872	0.0117
	R <sup>2</sup>	0.8416	0.6626	0.9079	-20.3777	0.9469	0.9841

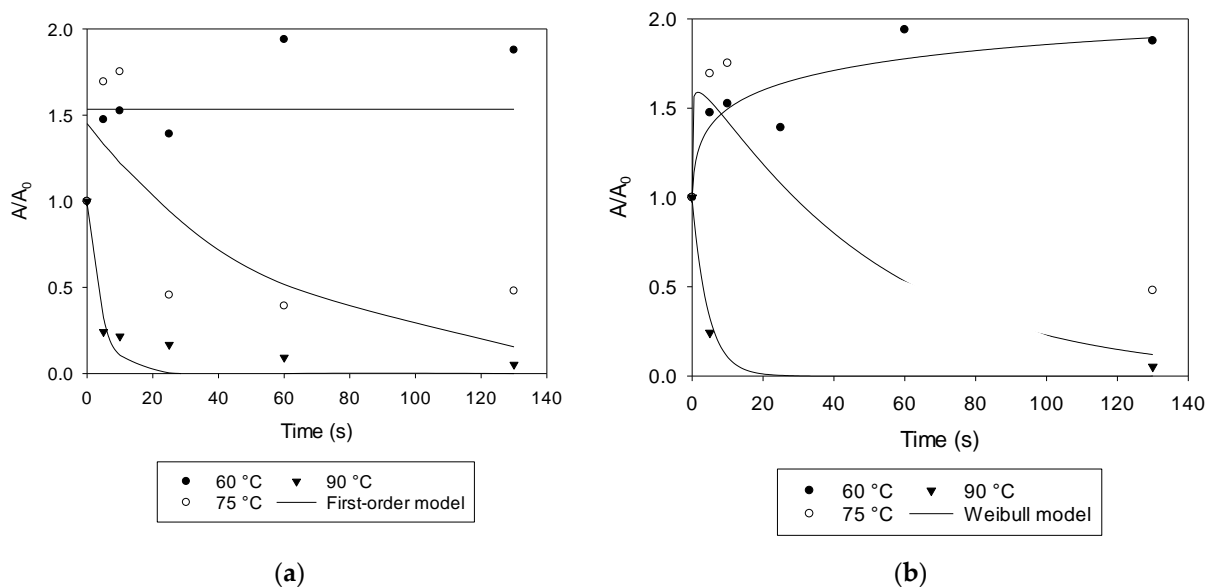
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RMSE	0.216333077	0.57723479	0.16911535	2.15905072	0.20880613	0.07681146
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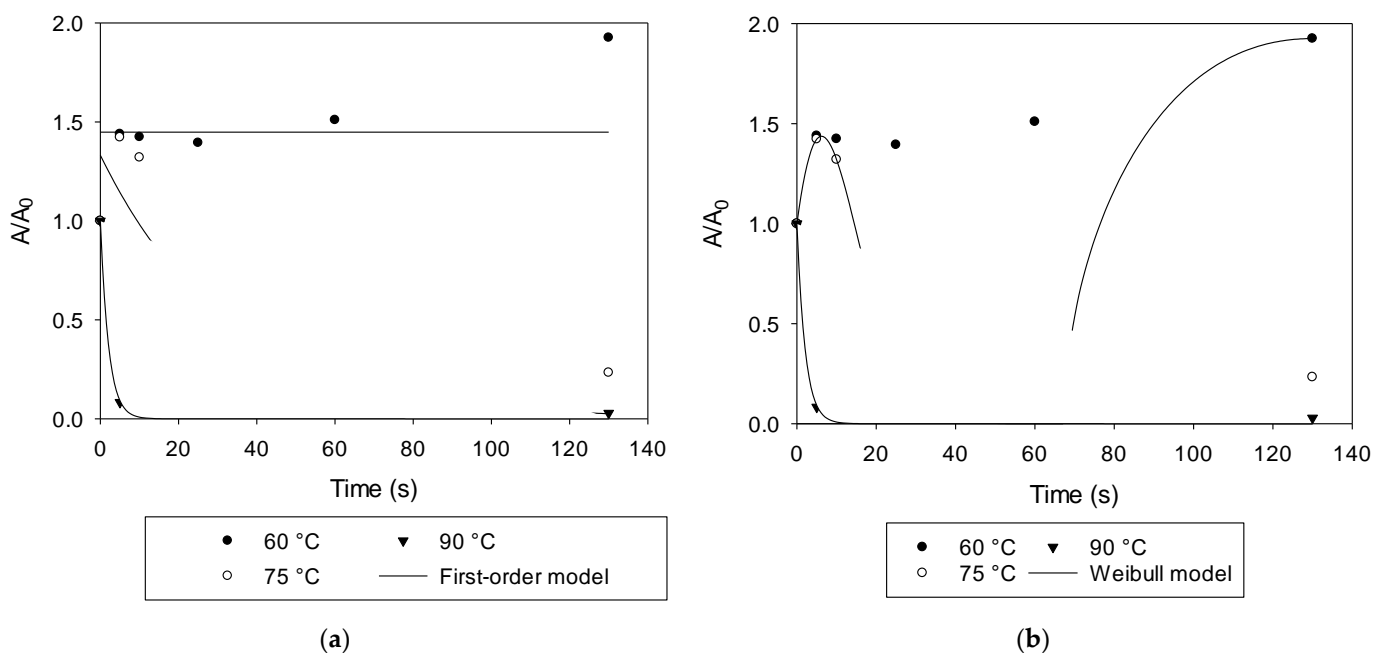
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### 3.3. Comparison between Conventional and Microwave Heating

Figure 2 shows the experimental data and the fit of the kinetic models to the enzyme inactivation produced by conventional processing. It is observed that, for 60 °C, both models lack fit, since they do not accurately describe the behavior of the data, however, the Weibull model is the best fit. It is noteworthy that at 60 °C the behavior of the enzyme activity is growth rather than decay and this may be the reason for the poor fit of the kinetic models. For the temperature of 75 °C, a better fit of the Weibull model to the experimental data is observed, since at this temperature an increase in enzyme activity occurs in the first seconds of heating and then the decay of activity begins. Both models correctly fit the behavior of the data for the 90 °C temperature.



**Figure 2.** Effect of conventional processing at (●) 60 °C, (○) 75 °C and (▼) 90 °C on the residual activity, modeled by (a) first-order and (b) Weibull models.



**Figure 3.** Effect of microwave processing at (●) 60 °C, (○) 75 °C and (▼) 90 °C on the residual activity, modeled by (a) first-order and (b) Weibull models.

The effect of microwave heating shows greater decay of residual POD activity than that presented by conventional heating (Figure 3). However, in the case of 60 °C, a greater increase in activity caused by microwaves is observed than that caused by conventional heating. Processing at 75 °C again shows an increase at the onset of heating and consecutively the residual POD activity starts to decrease. This behavior is best described by the Weibull model. Microwave heating at 90 °C generates a rapid decrease in residual POD activity, whose behavior can be accurately described by both the first order model and the Weibull model.

#### 4. Conclusions

The processing of mature coconut water by conventional and microwave heating showed a behavior not reported by the literature at temperatures below 75 °C. An increase in enzyme activity was observed at 60 °C, which could not be modeled acceptably by any of the models used. Further kinetic models that better describe the enzyme behavior at 60 °C and 75 °C should be explored. For temperatures of 75 °C and 90 °C, Weibull model was the one that best described the inactivation of the POD enzyme, having the highest fit and lowest RMSE in both heating processes. Microwave heating generated an inactivation of the POD enzyme significantly faster than conventional processing in mature coconut water.

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