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Evaluation of the microwave-assisted enzymatic inactivation process of tomato pulp

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Abstract

In this study, the thermal and non-thermal effects of microwaves on the enzymatic inactivation of pectin methylesterase in the tomato pulp were validated. An amount of 30 kg of tomato pulp was subject to two different types of treatments: thermal effects and then non-thermal effects using microwaves, implementing a system that allowed simultaneous cooling of microwave heating. An experimental rotational central composite design was used to analyze the effect of the interactions of the variables involved in the process. The activity analysis of the PME enzyme, pH, titratable acidity, total soluble solid, and color was performed on both processed and non-processed tomato pulps, which showed that the heating process with thermal effect did not significantly change physical-chemical properties and were found to be more effective, reaching the enzymatic inactivation of 99% and the temperature of 80°C after 425 s of processing. However, the non-thermal analysis with temperatures at a maximum of 40°C attained 56.8% of enzymatic inactivation.

Keywords: microwaves; inactivation; enzymes; non-thermal effects.

Practical Application: Non-thermal effects of the pulp observed by microwaves.

1 INTRODUCTION

The tomato (*Lycopersicon esculentum*) is a vegetable highly consumed across the world due to its high level of fibers, mineral salts, and antioxidants such as vitamin C, carotenoids, and flavonoids (Pizarro-Oteíza & Salazar, 2022). It also contains two important enzymes: the pectin methylesterase (PME; EC 3.1.1.11) and the polygalacturonase (PG, EC 1.2.1.15) (Makroo et al., 2017), which are responsible for the degradation of the pectin and have a major role in the texture of the fruit.

The most important enzyme is the PME, as its thermal resistance is higher than most of the bacteria and fungi found in the tomato (Tajchakavit & Ramaswamy, 1997), therefore becoming an important index in the determination of the time-temperature variable during the pasteurization treatment.

Although conventional pasteurization is widely used to neutralize pathogenic microorganisms and enzyme inactivation, this form of treatment has a disadvantage, which is the loss of nutritional and sensorial quality in the substances, due to its exposition to high temperatures during long periods.

Several non-thermal and thermal emerging technologies are being developed to reduce enzymatic and microbial activities and to overcome the disadvantages of conventional methods concerning high temperatures.

Some of the non-conventional methods include ohmic heating, radio frequency heating, and microwave heating (Debbarma et al., 2021; Ferreira et al., 2021; Guo et al., 2017; Matsui et al., 2008; Salazar-González et al., 2014). Microwave utilization is an effective alternative to the pasteurization process as microwaves can transfer energy through the total volume of the material, due to its penetration and generation of heat ability through the non-electric material, reducing the temperature gradient in the material and the processing time (Ribeiro et al., 2022).

In addition to these features, there is a possibility of non-thermal effects in microwaves, which are largely discussed but not still distinguished between academics (Kubo et al., 2020; Shamis et al., 2012).

The objective of this study was to compare the efficiency of enzymatic inactivation by microwaves and to observe the possible thermal and non-thermal effects of microwaves, which have been the target of multiple studies. Therefore, the effect of microwaves on the pasteurization of tomato pulp is evaluated by analyzing the percentage of inactivation of the PME enzyme and the conservation of the organoleptic properties of the tomato pulp.

2 MATERIALS AND METHODS

The experiments were realized in the Microwave Laboratory owned by Innovatus Brasil—Microondas Desenvolvimento e Tecnologias LTDA. An amount of 30 kg of tomato of Deborah variety was purchased from Mercado Commercial de Campinas, washed, and then extracted for pulp, which was made with an industrial blender (Skymsen). Then, the seeds and the skin were removed, and 0.918 kg of tomato pulp/kg of tomato was obtained.

2.1 Microwave process with a thermal effect

The microwave oven used to process the tomato pulp was built by Innovatus Brasil in a laboratory scale (Figure 1)

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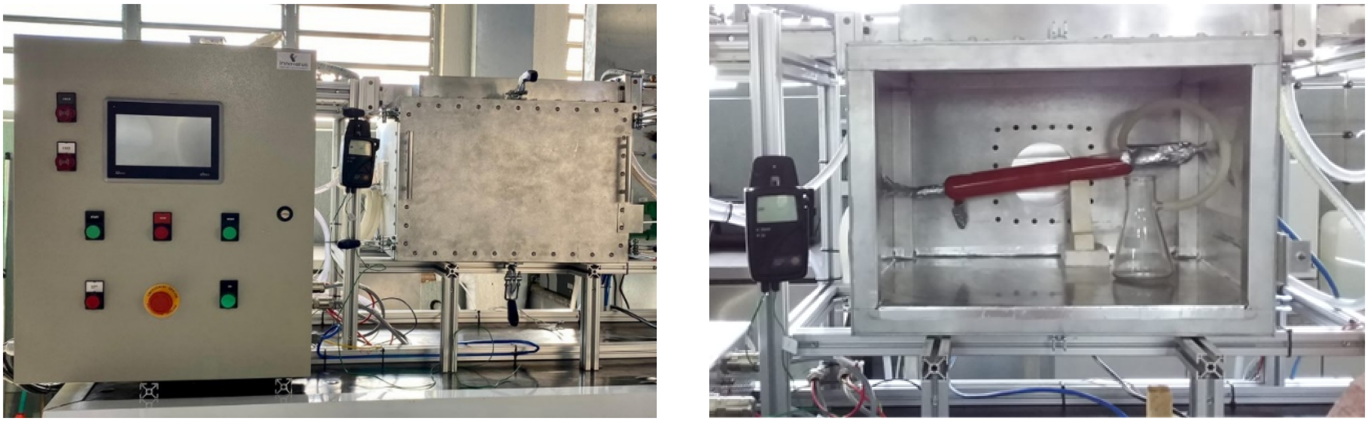

Figure 1. Prototype for batch process in the microwave oven.

containing a resonant cavity of 50 cm in length, 30 cm in height, and 36 cm in width, a power control panel, and an infrared sensor (Omega OSAT Series) coupled in its cavity. The equipment demands 220 AC to 13.3 A, and its nominal power is 2500 W.

To study the microwave power effect and the processing time to the inactivation of the PME, a minimal number of experimental essays were applied according to the rotational central composite design (RCCD). The power applied varied between 1250 and 2,500 W, and the processing time varied between 120 and 270 s. In total, 12 essays were defined by the RCCD.

For each essay, the condenser was filled with 200 mL of tomato pulp at 20°C and submitted to the microwave. During the process, the temperature was registered by the infrared sensor, and the samples were immersed in cold water immediately after each treatment to stop the thermal treatment. Afterward, the process was characterized by three repetitions.

2.2 Microwave process with non-thermal effects

To evaluate the non-thermal effects on the PME inactivation system by microwaves, the previously introduced equipment was utilized along with a simultaneous cooling process named the "non-thermal effect process." The cooling fluid (water) at 17°C was allowed through the condenser intern glass tube in a 40 mL/s flow rate to partially remove the tomato pulp absorbed energy and to keep the temperature ≤ 45°C. The temperatures were continuously registered and the sample was immersed in cold water after the treatment.

2.3 Physicochemical analysis

The pH was determined by a pH meter (OHAUS Starter 3100M) at room temperature, according to the Association of Official Agricultural Chemists (AOAC), and the titratable acidity was realized according to the AOAC 945.15. The content of soluble solids was measured in °Bx directly by a refractometer (HANNA HI96801) at room temperature. To measure the color of the pulp, the samples were disposed of in a Petri dish, under a white surfaced center, inside an illumination box, and a camera (Termovisor Profesional—FLIR series T450SC) was used to capture it.

2.4 Activity determination of the PME enzyme

The PME activity was determined by titration according to the methodology described by Anthon et al. (2002), and 25 mL of citric pectin (Dinâmica, química contemporânea, Ltda.) was added to a concentration of 0.2 mL of NaCl in 1% balanced solution at 30°C and adjusted to a pH of 7.0. After the addition of 1 mL of the pulp sample, the pH of the solution was adjusted to 7.0 by 0.1 N NaOH addition and held at the same pH for 10 min with the addition of 0.02 N NaOH. The inactivation was given in percentage, according to the Equation 1:

$$\%\ \boldsymbol{Inactivation} = \left[1\frac{PEU\ activity\ after\ the\ treatment}{PEU_0\ activity\ before\ the\ treatment}\right].100 \qquad (1)$$

2.5 Statistical analysis

The experimental data on the enzymatic PME inactivation essays realized by microwaves with thermal and non-thermal effects were statistically analyzed using the Protimiza Experimental Design software, correlating the answer variables (% of inactivation) with the independent variables (power and time) using analysis of variance (ANOVA), and the results of process variables were obtained concerning the inactivation of the PME with a $p < 0.05$.

3 RESULTS AND DISCUSSION

In the physicochemical analysis, the pH value reported in Debora tomato pulp (4.79 ± 0.01) was larger than that reported in those cultivated in conventional systems (Nascimento et al., 2013; Oliveira et al., 2015). The normal pH range is 4.0–4.5. According to Monteiro et al. (2008), products with pH values beyond 4.5 require longer thermal processing time, resulting in higher use of energy and, therefore, a higher processing cost.

The acidity obtained was 0.37 g citric acid/100 g of tomato, an intermediary value than that previously presented by other authors (e.g., Monteiro et al., 2008). This parameter indicates the quality of organic acids and, along with the pH, the main factors involved in flavoring the fruits.

The value of soluble solids was 4.5°Brix as reported in the literature (Nascimento et al., 2013), with ranges between

4.0 and 6.0. The soluble solids average value of tomato fruits destined for industrial processes is 4.5°Brix. This parameter is important as it represents yield in industry, since the more the concentration, the more the yield.

The results obtained for the color parameters L*, a*, and b* in the tomato pulp were 39.83, 46.83, and 33.02, respectively, indicating a chromaticity C* of 57.3 and tonality °h of 35.18, which means that the sample has a major red tonality, once the angle of 180° represents pure green and an angle of 0° represents pure red.

3.1 Impact of microwaves on the physicochemical properties of tomato pulp

The results obtained by the physicochemical analysis of tomato pulp did not have significant differences in color, pH, °Brix, and titratable acidity parameters before and after the microwave treatment to both thermal and non-thermal effects (data not revealed).

The stability presented by the three physicochemical parameters obtained after the processing of the tomato pulp was similar to the research presented by Arjmandi et al. (2017) and Pérez et al. (2016), and in different food fluids treated with microwaves, such as mango puree (Rodríguez et al., 2011), none of the researchers related significant differences in samples that were heated and not heated after the microwave process.

3.2 Temperature profiles of microwave treatment

In comparison, the temperature profiles of the two microwave treatments showed different tendencies, due to variations in the configurations in the processes. In the microwave treatment with thermal effect, the maximum temperature achieved by the tomato pulp was 82°C during a 7-min treatment; however, in the process of non-thermal effect, the achieved temperature was 45°C under the same conditions of time and power.

Heating curves obtained from the microwave treatment on both processes presented a linear tendency with different inclinations, depending on the power applied to the processes.

3.3 PME Enzymatic inactivation by microwave treatments

The PME enzymatic activity value of the non-processed tomato pulp was 12 ± 0.4 PEU/mL on average, and this value is similar to that reported in the literature (Boas et al., 2000).

With the results obtained from the experiments, a comparison of the treatments was realized to observe the existence of non-thermal effects of the microwaves, using the temperature of 40°C as a reference point. The inactivation degrees were low, with values between 29.5 and 32.9%, in comparison with the other studies, which achieved a higher temperature and the inactivation between 60.5 and 98%, pointing to a lethality close to 100% in most cases.

In microwave treatments with non-thermal effect, the maximum lethality was achieved at 56.8% inactivation at a temperature of 40°C, which indicates the possibility of non-thermal effects, as the inactivation was possible only by temperature. According to the data obtained in the thermal effect treatment, an inactivation of 32.9% would be achieved at a temperature near 40°C.

In this context, there is a possible non-thermal interference in the microwave heating, although these effects cannot be easily separated from the heating effects, as observed in the non-thermal treatment previously exposed, concluding that there was no reduction until ambient temperature, and the temperature range reached during the experiments was inferior to the references in the literature (> 70°C).

In the inactivation of the PME enzyme on the tomato pulp in the studied conditions, it was observed that the power on the microwaves is an important parameter in the process to be controlled, in addition to the temperature, due to higher inactivation percentages, which can be related to the non-thermal effects.

In batch processing of tomato pulp, the microwave process with thermal effect can be considered adequate, as about 100% of the PME activity was reduced. Considering non-thermal effects and combining with a faster volumetric heating rate, there is a potential to reduce processing times and thus to improve tomato pulp quality.

Similar behavior was obtained by Cavalcante et al. (2021) in the study of non-thermal effects of the microwaves on the enzymatic inactivation of polyphenol oxidase (PPO) and peroxidase (POD), declaring that the PPO enzyme in temperatures beyond 70°C was observed for non-thermal effects, as a higher enzymatic inactivation was identified in the microwave treatment compared to the thermal conventional process.

Several studies have been released on the effects of microwaves in food processing, concerning the thermal effects compared with the thermal conventional process on the inactivation of microorganisms and enzymes. Most studies agree with the possible existence of non-thermal effects in different types of treatments (Cavalcante et al., 2021; Tajchakavit & Ramaswamy, 1995).

Furthermore, microwave non-thermal effects, such as changes in the structural conformation of an enzyme by microwave heating at temperatures between 70 and 90°C were observed in more studies (e.g., George et al., 2008).

However, distinguishing with precision the non-thermal effects of the electrical fields is complex, and it was observed that most experiments were performed through a comparison of the thermal treatment by microwave and the thermal conventional process. In this study, an alternative and comparative study proposal was explored, due to the difficulty in adjusting the temperature curves obtained in the thermal conventional processes by microwave heating, to obtain a precise comparison.

3.4 Statistics analysis

The analysis of variance (ANOVA) corresponds to the main effects and its interactions, and pattern error to the process in function of enzymatic inactivation percentage of the PME to an $\alpha = 5\%$ (significance level) was applied to the enzymatic inactivation results to both microwave treatments, and it was numerically analyzed on the quality of the adjust of the empirical model through a variance analysis presented in Table 1.

Table 1. Predictive empirical models of the enzymatic inactivation of the PME, with thermal and non-thermal effects.

Dependent variable	Equation	R^2
PME inactivation with thermal effect	$\%Inactivation = 64.54 + 13.16 * P + 22.19 * t - 8.36 * P * t$	0.9444
PME inactivation non-thermal effect	$\%Inactivation = 36.75 + 11.23 * P + 8.40 * t$	0.8858

P: microwave power (W); t: process time (s).

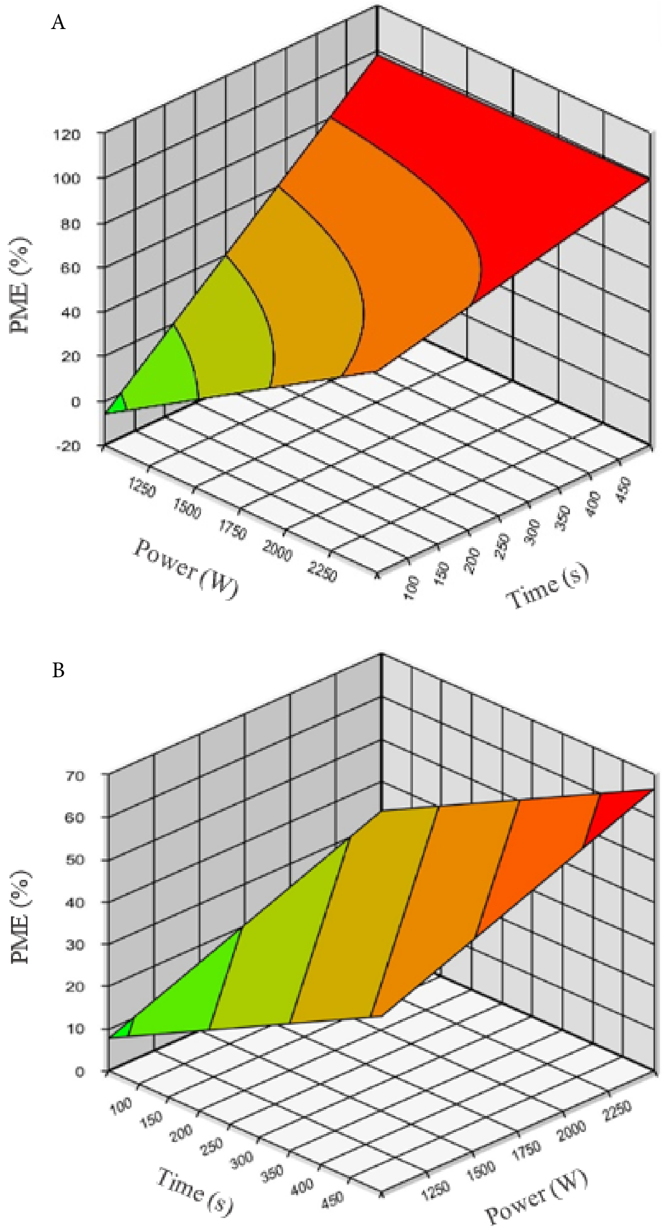

Figure 2. Response surface plots for the percentage change of pectin methylesterase (PME) inactivation in tomato pulp as a function of microwave power and process time, with (A) thermal effects and (B) non-thermal effects.

The model adjustment was expressed by determination coefficients (R^2), which were 0.9444 and 0.8858, to the processes with thermal and non-thermal effects, respectively, and indicated the variation percentage in the enzymatic inactivation, the result of the microwave power, and processing time applied to each treatment.

After the model obtained (Table 1), surface answers were generated which describe the dependency of enzymatic inactivation in each process (Figure 2). A significant growth in the PME inactivation was observed, as the microwave power intensity increased and the processing time was longer.

To both processes, only one linear effect had more relevance on the PME inactivation of tomato pulp, which was processed by microwave irradiation with thermal and non-thermal effects. Benlloch-Tinoco et al. (2013) described a linear effect between time and PME inactivation in kiwi puree by microwave, and Tajchakavit and Ramaswamy (1997) also described the same effect on the PME inactivation in orange juice by microwave.

From this multiple response evaluation, the highest percentage in the PME inactivation (100%) was achieved by microwave power with thermal effect of up to 2,500 W for 250 s. However, in the non-thermal effect by microwave power, 65% of the PME inactivation was achieved, by applying 2,500 W for 500 s.

The thermal effect process by microwave can be considered adequate for the batch production of tomato pulp because 100% of the PME activity was reduced.

Considering the non-thermal effect and matching it with a faster volumetric heating rate, there is a potential to reduce processing time and improve pulp quality, related to color parameters.

However, the microwave process with non-thermal effect did not achieve the minimum inactivation of 80% requirement of the PME enzyme in the tested conditions, so other time and power conditions must be studied for the requirement to be achieved.

Although the physicochemical properties of the tomato pulp, especially the color, were influenced by thermal treatments, the microwave treatment was able to preserve the redness in the tomato pulp, which is one of the main quality indicators.

4 CONCLUSION

Observing the non-thermal effects of the microwaves in the enzymatic inactivation of the PME in tomato pulp, this proposal could become a practicable process for juices and pulps pasteurization, due to the achievement of smaller temperatures without alteration in the physicochemical and organoleptic properties such as color and flavor.

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