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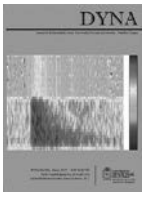

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# Inactivation of *Bacillus licheniformis* in liquid coffee extract by microwave heating in domestic oven and precise temperature measurements using an optical fiber sensor

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**Abstract**
This study evaluates the inactivation of *Bacillus licheniformis* in liquid coffee extract using heating microwaves. To achieve accurate measurements of temperatures, an optical fiber sensor was developed and calibrated for real-time and in situ measurements of the extract. Using a central composite design, the effects of the processing time, power levels and sample volume were evaluated together with the concentration of the soluble solids of the liquid coffee extract and the position of the sample in the microwave cavity. In addition, the physicochemical and sensory properties of the product were evaluated. The results indicate that the greatest reduction of the microorganism occurred when the processing time was 19 seconds at a power level of 6 using a sample volume of 11 mL and that the highest biological destruction occurs when the sample is placed in the center of the microwave cavity.

*Keywords*: microwave; coffee extract; *Bacillus licheniformis*; inactivation.

# Inactivación de *Bacillus licheniformis* en extracto líquido de café por calentamiento por microondas en hornos domésticos y sensor de fibra óptica para mediciones precisas de temperatura

**Resumen**
En este estudio se evalúa la inactivación de *Bacillus licheniformis* en extractos líquidos de café usando calentamiento por microondas. Para lograr medidas precisas de temperatura, se desarrolló y calibró un sensor de fibra óptica para medir en tiempo real e in situ la temperatura del extracto. Usando un diseño central compuesto, se evaluaron diferentes condiciones de tiempo de proceso, niveles de potencia y volumen de muestra; además, fue evaluado la concentración de solidos solubles del extracto de café y la posición de la muestra en la cavidad del microondas. Adicionalmente, se evalúan las propiedades fisicoquímicas y sensoriales del producto. Los resultados muestran que la mayor reducción del microorganismo ocurre con un tiempo de proceso de 19 segundos, nivel de potencia 6, volumen de muestra 11 mL y que la más alta destrucción biológica ocurre en el centro del horno.

*Palabras clave*: microondas; extracto de café*; Bacillus licheniformis*; inactivación.

## 1. Introduction

In recent years, food quality has become more important both commercially and in terms of policy; in particular, the safety of these products must be guaranteed during their commercialization phase and consumption. Thus, new technologies involving unconventional (non-thermal) treatment have emerged, with certain advantages in

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maintaining nutritional, organoleptic, sensory and physical properties relative to traditional methods of conservation based on the application of heat.

*Bacillus licheniformis* is one type of food contaminating microorganism that has been detected in canned vegetables, milk and baby food in previous studies [1]. However, because these microorganisms have the ability to grow at an acidic pH, they can also be present in liquid coffee extract.

It is noteworthy that liquid coffee extract, as a product of an aqueous extraction of roasted coffee that is then concentrated, has been used as a base ingredient for cocktails and liquors, beverages in dispensing machines, cookies, pastries and ice cream and, given its composition, presents a high risk of contamination by this organism [2].

A non-thermal food preservation option involves the application of the microwave technique, which uses electromagnetic waves between infrared and radio waves (300 MHz - 300 GHz), corresponding to the range of wavelengths between 1 m and 1 mm. In food processing with microwaves, the frequencies conventionally used are 2450 MHz for domestic ovens and 915 MHz for industrial processing [3,4].

The type of equipment and process conditions are critical in microwave heat treatment. The design of the microwave oven (size, geometry, etc.) can significantly affect the magnitude or the spatial variation of the energy absorption in the product, which results in process efficiency. Additionally, the presence or absence of devices contributing to improving the uniformity of heating, such as revolving plates, are crucial, as these devices affect the temperature distribution within the material [5,6].

The microwave and heat treatment have different bactericidal mechanisms, although it is not clear whether microbial inactivation is due to heating or to electromagnetic energy [7]. It has been observed that the exposure of bacterial suspensions to microwave radiation reduces the viable cell count and increases loss of DNA and proteins, which suggests that there is damage to the cell [8]. In studies with *Escherichia coli* and other coliform bacteria, it was observed that after irradiation with microwaves, structural changes occurred in the cell walls that do not occur with heat treatment. Therefore, it can be deduced that the bactericidal mechanisms of the microwave irradiation are involved in cell membrane and cell wall damage [9].

Some researchers attribute the lethal effect exerted by the microwave to heat generated while others propose a non-thermal effect due to the microwave energy itself [10]. However, determining whether the microwave radiation affects the chemistry of biological molecules and the setting of the cellular components of the microorganisms independent of the thermal effect generated by the waves [11].

Microwave heating primarily involves two mechanisms: ionic migration and dielectric heating. In food, water is often the main component responsible for dielectric heating because of its bipolar nature: the molecules attempt to follow the electric field associated with the electromagnetic radiation and oscillate, thus producing heat. The second main mechanism of microwave heating involves oscillatory ion migration in foods, which generates heat under the effect of the oscillating electric field. Therefore, a food that has a low thermal conductivity coefficient may be rapidly heated using microwaves, which does not occur in the conventional methods [4].

When microwaves are applied to food, the direction of the electromagnetic field changes millions of times per second, mainly causing the water molecules and minerals to oscillate when orienting with the polarity of the field, producing collisions and friction between the molecules, which leads to an increase of the temperature inside the food, a fact that differentiates this method from traditional thermal treatments [4].

Microwaves are typically produced by a magnetron, a device that converts electrical energy into an electromagnetic field. To avoid interfering with radar signals and the radio waves used in telecommunications, the radar waves can overlap with the bands of microwaves. In this non-thermal technology, dielectric, thermal and geometric properties play a fundamental role in determining the energetic interaction between the electric field and food [6].

The objective of this study was to find the optimum conditions for the application of microwave technology to a domestic oven in liquid coffee extract as a method of preservation of the product, ensuring its physicochemical and sensory quality.

## 2. Methodology

The samples that were subjected to the microwave processing were obtained from preparing a culture of *Bacillus licheniformis* for 24 hours, then centrifuging at 4000 rpm for 20 minutes to form a pellet that was then resuspended with coffee extract at 45 °Brix. The initial concentration of bacteria was on average 2 x $10^5$ CFU/mL.

Electromagnetic tests were performed in a domestic microwave oven HACEB (AREZZO HM-07 ME BL) with a capacity of 0.02 $m^3$ (0.7 $ft^3$) at a frequency of 2450 GHz, which allows the power level and processing time to be varied.

As a contribution to the state of the art of this technology to achieve accurate measurements that relate time and spatial variation, in this study, the temperature of the liquid extract was measured in real time using a fiber optic sensor developed at the Laboratory of Photonics of the National University of Colombia. The sensor consists of a Bragg network recorded in the core of a single optical mode Fiber Bragg Grating (FBG), for which the spectral response depends linearly on temperature. The FBG was appropriately encapsulated in a glass capillary closed end tube to avoid undesirable effects due to mechanical stresses that can alter the spectral response of the network. The advantage of this sensing platform is that the photons, and not the electrons, are the basic elements of the spread of the measurement signals, not being affected by the microwave signal. In addition, as the structure is completely made of glass, it meets the safety requirements that must be followed with this type of oven.

The detection system is complemented by a FBG interrogator (Micron Optics). Initially, the sensor was calibrated using a calorimeter to determine the relationship between the wavelength $\lambda$ of the probe FBG and the temperature of the sample (Fig. 1).

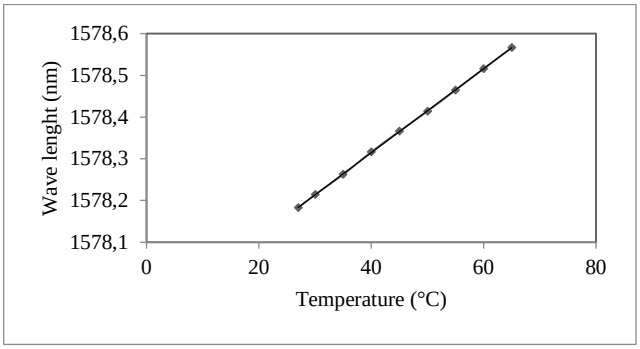


Figure 1. Calibration of the FBG sensor using a calorimeter.
Source: The authors.

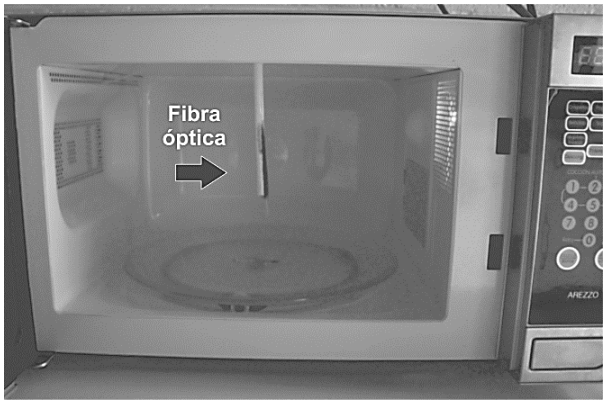


Figure 2. Location of the FBG sensor inside the home microwave oven.
Source: The authors.

The equation to fit the model was stablished as:

$$\lambda = 0.0101(T) + 1577.9 \text{ with } R^2 = 0.9999 \quad (1)$$

From Eq.(1), the following model for the temperature was obtained:

$$T = \frac{\lambda - 1577.9}{0.0101} \quad (2)$$

Fig. 2 shows the experimental assembly carried within the microwave cavity for the location of the optical fiber sensor. Its design allows the sensor to be placed in different regions of the oven cavity, making measurements that relate temperature with spatial variation and time.

### *2.1. Experimental analysis*

Three levels were assessed for each factor: power levels from 2 to 10 (dimensionless), processing times from 5 to 20 seconds and sample volumes from 10% to 65% of the total container volume (100 mL Schott beaker with external diameter of 50 mm and 70 mm height). The concentration of the sample expressed as degrees brix (45°Brix) and the position of the container within the cavity (center) were held constant. Using SAS software, a statistical design of the response surface (central composite) with three replicates at the center point was prepared, as shown in Table 1.

Table 1.
Statistical design of response surface using microwave heating in coffee liquid extracts.

| Run | Power level | Time (sec) | Volume (mL) |
|---|---|---|---|
| 1 | 2 | 5 | 45 |
| 2 | 2 | 19 | 45 |
| 3 | 10 | 5 | 45 |
| 4 | 10 | 19 | 45 |
| 5 | 6 | 5 | 11 |
| 6 | 6 | 5 | 79 |
| 7 | 6 | 19 | 11 |
| 8 | 6 | 19 | 79 |
| 9 | 2 | 12 | 11 |
| 10 | 10 | 12 | 11 |
| 11 | 2 | 12 | 79 |
| 12 | 10 | 12 | 79 |
| 13 | 6 | 12 | 45 |
| 14 | 6 | 12 | 45 |
| 15 | 6 | 12 | 45 |

Source: The authors.

Additionally, an optimization process was performed to determine which operating variables resulted in the greatest degree of biological destruction, i.e., a lower value of CFU/mL. During all the trials, the response variable was the colony forming units per volume, which was determined according to the standard NTC 4519 [12] and thermal kinetics of the process (extract temperature vs. process time). Quantification of the colony-forming units was performed in duplicate.

Once obtained the operating conditions at which the maximum biological destruction was achieved, the effects of the concentration of soluble solids (15 and 45 °Brix) and the position of the vessel in the cavity were evaluated: center, 6 cm from the center and 12 cm from the center (end of the rotary plate).

For the physicochemical and sensory testing of the sterilized product, it was assessed whether the drink was different from the conventional product, using the triangular sensory test according to the standards NTC 2681 and 4883 [13,14]. In addition, the physicochemical properties such as pH and acidity were determined according to standard NTC 4675 [15].

## 3. Results

### *3.1. Effect of process conditions*

Table 2 presents the microbial destruction results using the conditions defined in the experimental design and an initial concentration of bacterial inoculum of $2x10^5$ CFU/mL for all the samples analyzed. The minimum value was obtained when the process conditions were: power level of 6, time of 19 seconds and 11 mL volume. In terms of biological destruction, low volumes and high processing times are the most suitable. Analysis of variance demonstrated the significant effect of the operating conditions ($P < 0.05$) on the lethality. Table 3 lists the statistical results of experimental design.

Table 2.
Bacillus licheniformis counts after the microwave process in liquid coffee extract with 2 x105 CFU/mL initial populations for each test condition.

| Test | Power level | Time(s) | Volume (mL) | CFU/mL final |
|---|---|---|---|---|
| 1 | 2 | 5 | 45 | 1 x $10^4$ |
| 2 | 2 | 19 | 45 | 7 x $10^3$ |
| 3 | 10 | 5 | 45 | 1 x $10^4$ |
| 4 | 10 | 19 | 45 | 7 x $10^3$ |
| 5 | 6 | 5 | 11 | 9 x $10^3$ |
| 6 | 6 | 5 | 79 | 8 x $10^3$ |
| 7 | 6 | 19 | 11 | 4 x $10^3$ |
| 8 | 6 | 19 | 79 | 8 x $10^3$ |
| 9 | 2 | 12 | 11 | 1 x $10^4$ |
| 10 | 10 | 12 | 11 | 8 x $10^3$ |
| 11 | 2 | 12 | 79 | 1 x $10^4$ |
| 12 | 10 | 12 | 79 | 7 x $10^3$ |
| 13 | 6 | 12 | 45 | 9 x $10^3$ |
| 14 | 6 | 12 | 45 | 6 x $10^3$ |
| 15 | 6 | 12 | 45 | 7 x $10^3$ |

Source: The authors.

Table 3.
ANOVA model for the inactivation of *Bacillus licheniformis* using microwave heating.

| Effect | P value |
|---|---|
| Time | 0.1734 |
| Power | 0.0057 |
| Volume | 0.2997 |
| $(Time)^2$ | 0.0209 |
| Power*Volume | 0.0254 |

Source: The authors.

This model is presented in Fig. 3. The figure illustrates that there is a minimum CFU/mL when the volume is low and the power level is between 5 and 10.

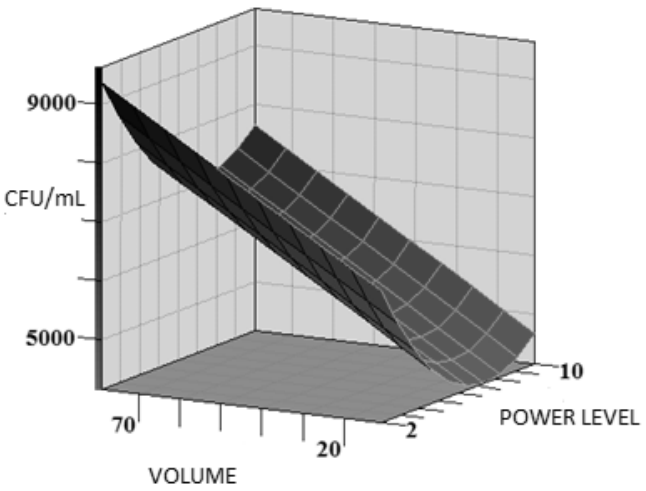


Figure 3. Response surface of CFU/mL with the power and volume factors for a processing time of 19 seconds.
Source: The authors.

Table 4.
Optimization of the inactivation model for *Bacillus licheniformis* by microwaves.

| Factor | Value |
|---|---|
| Power level (dimensionless) | 6 |
| Coffee extract volume (ml) | 11 |
| Time (seconds) | 19 |
| CFU/mL theoretical | 4233. 93 |

Source: The authors

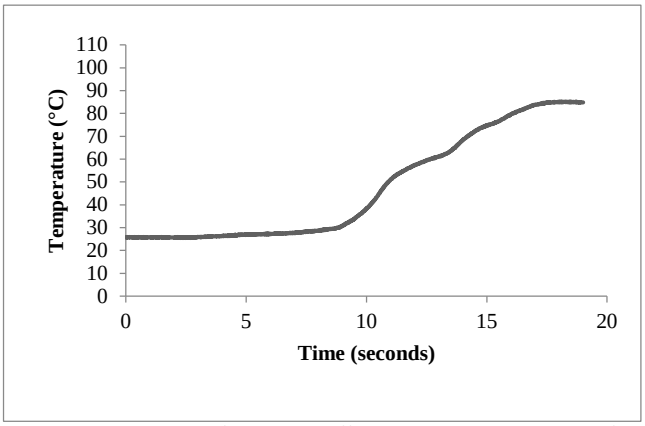


Figure 4. Heating curve of the liquid coffee extract obtained by optical fiber sensor.
Source: The authors

Table 4 shows the optimization of the model. These values match the values reported in Table 2 and Fig. 3, where the lowest value of CFU/mL was obtained when the process was performed under these conditions. According to the values observed, the model makes an over-prediction of the CFU/mL value by approximately 6%.

Fig. 4 shows the variation of temperature of the sample using the optical fiber sensor with respect to processing time under the following conditions: power level of 6, time of 19 seconds and extract volume of 11 mL.

The results indicated that heating does not occur instantaneously, but a marked increase in temperature occurs for processing times higher than 50%. However, between 5-15 seconds, the temperature rapidly increases, coinciding with the results observed by Vadivambal and Jayas [16]. Another study conducted by Arimi and coworkers [17], reported similar behavior to that observed in this study. During the first 5 seconds, there is no change in the temperature of the sample, which can occur by the response time of the optical fiber

To analyze the thermal kinetics of the process, the values of the initial (Ti) and final temperatures (Tf) obtained for each sample are reported (Table 5). The conditions for which the minimum count of CFU/mL was obtained were a volume of 11 mL, a power level of 6 and a time of 19 seconds, for which the final temperature was 84.85°C, where this value is greater than the inactivation temperature (80°C) for bacterial vegetative cells [18].

Table 5.
Values of initial and final process temperature for the coffee extract in each test.

| Test | Power level | Time (seconds) | Volume (mL) | Ti (°C) | Tf (°C) |
|---|---|---|---|---|---|
| 1a | 2 | 5 | 45 | 20.2 | 22.08 |
| 2a | 2 | 19 | 45 | 21.78 | 30.79 |
| 3a | 10 | 5 | 45 | 21.49 | 22.77 |
| 4a | 10 | 19 | 45 | 22.77 | 79.8 |
| 5a | 6 | 5 | 11 | 23.17 | 24.36 |
| 6a | 6 | 5 | 79 | 21.98 | 22.77 |
| 7a | 6 | 19 | 11 | 25.74 | 84.85 |
| 8a | 6 | 19 | 79 | 24.75 | 35.25 |
| 9a | 2 | 12 | 11 | 26.34 | 30 |
| 10a | 10 | 12 | 11 | 25.94 | 100.9 |
| 11a | 2 | 12 | 79 | 20.79 | 23.86 |
| 12a | 10 | 12 | 79 | 26.53 | 32.67 |
| 13a | 6 | 12 | 45 | 27.82 | 95.64 |
| 14a | 6 | 12 | 45 | 27.62 | 93.66 |
| 15a | 6 | 12 | 45 | 27.03 | 97.62 |

Source: The authors

Table 6.
Effect of concentration of the liquid coffee extract on the inactivation of Bacillus licheniformis using microwaves.

| Brix degrees | CFU/mL |
|---|---|
| 15 | $1 \times 10^2 \pm 400$ |
| 45 | $6 \times 10^2 \pm 451$ |

Source: The authors

Table 7.
Values of power absorbed by the coffee extract at various ºBrix.

| | 15 °Brix | 45 °Brix |
|---|---|---|
| Ti (°C) | 24.95 ± 2.24 | 24.25 ± 0.62 |
| Tf (°C) | 98.74 ± 0.99 | 99.51 ± 1.02 |
| Power (W/m3) | 16651.32 ± 374.53 | 1697 9± 168.11 |

Source: The authors.

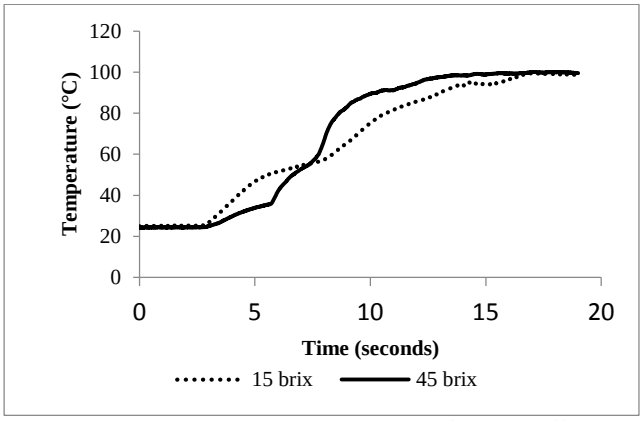


Figure 5. Microwave processing temperature curves for liquid coffee extract.
Source: The authors

According to Table 5, the optimum conditions for the microwave inactivation of *Bacillus licheniformis* in liquid coffee extract were a power level of 6, a processing time of 19 seconds and 11 mL volume. This result agrees with the conditions reported by Chandrasekaran and coworkers [19], who observed that microwave-processed foods must have a low volume because the microwave technique in high moisture product has a low microwave penetration depth.

On the other hand, the treatments applied reduced 2 logarithm cycles in 19 seconds, which corresponds to D value of 12 seconds, which is due to gram-positive bacteria such as *Bacillus licheniformis* are more resistant to microwaves in compared to gram negative [20], this is because the treated bacteria were not completely lysed. Previous studies report process times longer than 19 seconds to inactivate bacteria in liquid products such as juices [6].

### *3.2. Effect of soluble solids concentration*

In this work, the effect of the concentration of sample measured as degree brix (°Brix) in the inactivation of the bacteria of interest was also assessed. The study to 15°Brix and 45°Brix was conducted under process conditions where the minimum recount CFU/mL was initially observed i.e., a power level of 6, a processing time of 19 seconds and an extract volume of 11 mL. The initial concentration of *Bacillus lichenformis* was $2x10^4$ CFU/mL.

Table 6 lists the values of CFU/mL of Bacillus licheniformis obtained by microwaves processing at various levels of concentration. .

The analysis of variance (ANOVA) indicates that the concentration of the coffee extract has no effect on the biological destruction process ($P>0.05$). This finding may be due to the electrical conductivity of the sample not changing significantly with the concentration of soluble solids, as indicated by Campañone and coworkers [21]. According to Simpson [22], if the heat generated by microwave heating is given by

$$q = E^2\sigma, \quad (3)$$

where $E$ is the electric field and $\sigma$ is the electrical conductivity of the sample, the heat generated under the evaluated conditions should not exhibit significant differences; thus, no effect of the coffee extract concentration on the microorganism inactivation is observed.

The values of temperature and absorbed power for each treatment as a function of the solids concentration are listed in Table 7. In addition, Fig. 5 illustrates the heating degree reached as a function of the processing time.

The results in Table 7 indicate that for the liquid coffee extract, the concentration of the samples expressed as ºBrix had no significant effect on the final product temperature and the level of energy absorbed by the sample.

This result occurs because the composition and thermal properties (specific heat) do not vary noticeably with ºBrix (less than 8%). According to Datta and Rakesh [23], these properties are critical in heating via microwave and could generate a substantial change in the process.

### *3.3. Effect of the position of the sample within the oven cavity*

According to Chandrasekaran and coworkers [5], one of the critical factors in the microwave process is the position of the container containing the sample in the cavity. Therefore, three positions were evaluated: center, 6 cm from the center

Table 8.
Effect of the position of the container in the microwave on the inactivation of Bacillus licheniformis using microwaves

| Position | CFU/mL |
|---|---|
| Center | 6 x $10^2$± 451 |
| 6 cm from the center | 2 x $10^3$± 462 |
| 12 cm from the center | 8 x $10^3$± 464 |

Source: The authors.

Table 9.
Values of power absorbed by the coffee extract located at various positions in the microwave.

| Position | Ti (°C) | Tf (°C) | Power (W/m$^3$) | Mean Power (W/m$^3$) |
|---|---|---|---|---|
| Center | 23.56 | 98.91 | 16739.48 | |
| Center | 24.46 | 98.91 | 16541.51 | 15903.60 |
| Center | 23.86 | 88.81 | 14429.83 | |
| 6 cm from the center | 26.34 | 102.77 | 16981.44 | |
| 6 cm from the center | 23.96 | 106.24 | 18279.25 | 18103.27 |
| 6 cm from the center | 23.17 | 108.91 | 19049.13 | |
| 12 cm from the center | 24.55 | 105.54 | 17993.29 | |
| 12 cm from the center | 24.65 | 106.83 | 18257.25 | 18543.21 |
| 12 cm from the center | 22.77 | 110.00 | 19379.08 | |

Source: The authors.

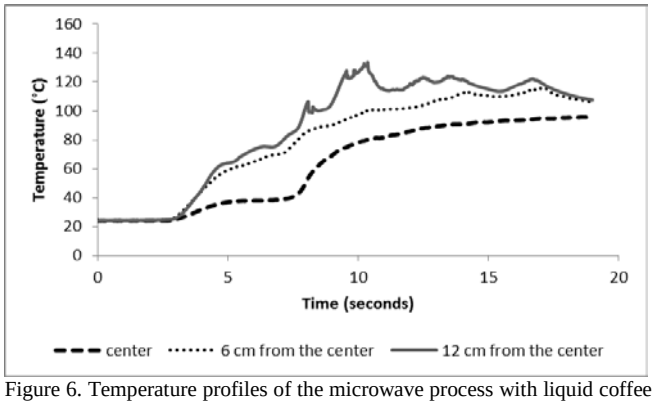


Figure 6. Temperature profiles of the microwave process with liquid coffee extract at various positions of the container in the microwave with a power level of 6, processing time of 19 seconds and 11 mL volume.
Source: The authors.

and 12 cm from the center (end of the rotary plate). The initial concentration of bacteria was 2 x $10^4$ CFU/mL in the liquid coffee extract at 45°Brix. Table 8 lists the biological destruction achieved as a function of position within the oven cavity.

Using a completely randomized unifactorial design, the positions were evaluated, and a statistically significant effect of the position on the final degree of biological destruction was observed ($P < 0.05$).

Table 9 lists the power absorbed by the sample using the temperatures measured at the beginning and end of the process. The final temperature reached during the process does not change significantly with the position inside the microwave. However, Fig. 6 illustrates how the temperature profile is highly variable. The results in Fig. 6 demonstrate that the thermal inertia of the process is higher at positions further from the center. Additionally, the temperature increases are higher for the positions close to the center.

Table 10.
Analysis of physicochemical properties of the liquid coffee extract subjected to microwave process.

| Treatment | pH | Acidity | °Brix |
|---|---|---|---|
| Treatment | 5.06± 0.01 | 42.10± 1.3 | 49.9± 0.1 |
| Control | 5.06± 0.01 | 40.67± 2.1 | 48.1± 0.1 |

Source: The authors.

Evaluating the position of the container within the microwave cavity revealed that the temperature increase occurs faster in the center than at the ends because the field is uniform to the extent that the product rotates [23]. Additionally, the increases in temperatures are higher at positions close to the center, which is due to the waves in these positions being reflected in the plate and losing energy that could be used to increase the temperature in the extract [24].

With respect to the power absorbed by the sample, Cañumir and coworkes [25], observed that the inactivation of *Escherichia coli* required a power between 900 and 720 W for processing times of 60 to 90 seconds in apple juice, while the results obtained from this study indicate that less time and a lower power for the inactivation of a more microwave-resistant bacteria are required. The possible differences may be due to variations in the dielectric properties of the products and to the distribution of the electromagnetic field within the oven cavity.

### *3.4. Process effect on the quality of the product*

Table 10 lists the physicochemical properties of the liquid coffee extract. No statistically significant differences ($P>0.05$) were observed between the control and treatment in terms of the values of pH, acidity and ºBrix.

In the sensory analysis, it was assessed whether there are significant differences between the coffee extract that has not been subjected to microwave treatment (control) and the treated coffee extract. No significant differences between the control and treatment ($P>0.05$) were observed; thus, the microwave treatment is considered a good alternative compared with conventional thermal processes as the microwave pasteurization preserves more flavor, color, nutritional value and quality [5,8].

## 4. Conclusions

Microwave technology can be used to help preserve the liquid coffee extract; a reduction of 2 logarithmical cycles was achieved in 19 seconds. The process conditions that minimize the recount CFU/mL of *Bacillus licheniformis* in liquid coffee extract of 15 or 45 °Brix without affecting the sensory properties of the product are a power level of 6, a processing time of 19 seconds and 11 ml sample volume. Additionally, the position of the sample inside the oven cavity should be considered to achieve adequate levels of biological destruction, with the central position meeting the highest standards of lethality.

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