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Effects of Microwave Electromagnetic Radiation on Milk Microbiology and Physicochemical Properties

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Abstrac

Conventional thermal treatments for milk processing in small and medium-sized enterprises (SMEs) often fail to fully meet Colombian food quality and safety regulations, particularly in microbial inactivation and nutrient preservation. In response, non-conventional thermal treatments, such as microwave heating, offer a promising alternative due to their ability to generate efficient volumetric heating and reduce processing times compared to traditional methods. This study evaluated the effects of microwave electromagnetic radiation on the microbiology and physicochemical properties of milk under different exposure times and power levels. In phase 1, a randomized experimental design was applied using a 1500 W microwave at 2450 MHz to treat 100 ml milk aliquots for different exposure times (0 to 50 seconds). In phase 2, a pilot-scale continuous flow system with an 18-liter capacity was developed, operating at 700 W and 1000 W with exposure times of 10, 15, and 20 seconds. Microbiological and physicochemical analyses demonstrated that treatment at 1000 W for 20 seconds met the standards of Decree 616 of 2006 and the Pasteurized Milk Ordinance (PMO), achieving significant microbial reduction without altering physicochemical properties such as fat, protein, and total solids content. In contrast, treatments at 700 W were ineffective for microbial inactivation, indicating the importance of using higher power levels. Additionally, the continuous flow system improved heat distribution, making the process more homogeneous and efficient. These findings suggest that the use of microwaves in continuous flow systems is a viable alternative for improving the microbiological quality of milk, optimizing its processing in SMEs through an efficient method with minimal impact on the final product composition while maintaining regulatory compliance.

Keywords: Continuous flow system, Electromagnetic radiation, Microbial inactivation, Microwave treatment, Milk, Pasteurization, Physicochemical properties, Power levels, Thermal processing, Microbiological quality.

Efectos de la Radiación Electromagnética de Microondas en la Microbiología y las Propiedades Fisicoquímicas de la Leche

Resumen

Los tratamientos térmicos convencionales para el procesamiento de leche en pequeñas y medianas empresas (PYMEs) a menudo no cumplen completamente con las normativas colombianas de calidad e inocuidad alimentaria, especialmente en la inactivación microbiana y la preservación de nutrientes. En respuesta, los tratamientos térmicos no convencionales, como la calefacción por microondas, ofrecen una alternativa prometedora debido a su capacidad de generar un calentamiento volumétrico eficiente y reducir los tiempos de procesamiento en comparación con los métodos tradicionales. Este estudio evaluó los efectos de la radiación electromagnética de microondas en la microbiología y las propiedades fisicoquímicas de la leche bajo diferentes tiempos de exposición y niveles de potencia. En la fase 1, se aplicó un diseño experimental aleatorizado utilizando un microondas de 1500 W a 2450 MHz para tratar alícuotas de leche de 100 ml durante distintos tiempos de exposición (0 a 50 segundos). En la fase 2, se desarrolló un sistema de flujo continuo a escala piloto con una capacidad de 18 litros, operando a 700 W y 1000 W con tiempos de exposición de 10, 15 y 20 segundos. Los análisis microbiológicos y fisicoquímicos demostraron que el tratamiento a 1000 W durante 20 segundos cumplió con los estándares del Decreto 616 de 2006 y la Ordenanza de Leche Pasteurizada (PMO), logrando una reducción microbiana significativa sin alterar propiedades fisicoquímicas como el contenido de grasa, proteínas y sólidos totales. En contraste, los tratamientos a 700 W fueron ineficaces para la inactivación microbiana, evidenciando la importancia de utilizar mayores niveles de potencia. Además, el sistema de flujo continuo mejoró la distribución del calor, haciendo el proceso más homogéneo y eficiente. Estos hallazgos sugieren que el uso de microondas en sistemas de flujo continuo es una alternativa viable para mejorar la calidad microbiológica de la leche, optimizando su procesamiento en PYMEs mediante un método eficiente, con mínimo impacto en la composición del producto final y garantizando el cumplimiento normativo.

Palabras clave: Calidad microbiológica, Inactivación microbiana, Leche, Niveles de potencia, Pasteurización, Procesamiento térmico, Propiedades fisicoquímicas, Radiación electromagnética, Sistema de flujo continuo, Tratamiento con microondas.

1. Introduction

Conventional heating remains a significant issue in the processing of foods such as milk and other dairy products due to overheating, which results in reduced quality, sensory attributes, and nutritional values. Microwave heating (MW) has been identified as a promising alternative to traditional thermal treatments, as dairy products of higher quality with extended shelf life are produced. However, the main drawback of microwave heating is the uneven temperature distribution, which leads to hot and cold spots, particularly in solid and semi-solid products; nevertheless, its effectiveness in liquid foods, especially in continuous flow systems, has been demonstrated (Martins et al., 2019).

Microwave heating is employed in various food processing applications, such as cooking, drying, pasteurization, and preservation (Chandrasekaran et al., 2013). This process is complex and is influenced by the propagation of microwaves and their interactions with food materials, which are primarily affected by their dielectric properties. Fundamental mechanisms of heat and mass transfer regulate heat dissipation (Salazar-González et al., 2012). Unlike conventional convection and conduction mechanisms, microwaves penetrate the food, are absorbed, and quickly converted into heat, generating volumetric heating that results in high temperatures (Martins, 2021).

The electromagnetic spectrum of commercially used microwaves operates in the ranges of 915 MHz and 2450 MHz, with lower frequencies allowing greater energy transfer. The temperature increase in food is determined by the power of the equipment and the physical and thermal properties of the sample, enabling foods with low thermal conductivity to heat rapidly with microwaves, something not achieved by traditional methods (Feng et al., 2012). Common frequency bands for microwave processing include 915 MHz and 2450 MHz. Microwave ovens operate at 2450 MHz, balancing efficiency and safety for domestic use. In industrial applications, frequencies such as 915 MHz are used, allowing greater penetration depth and suitability for processing larger volumes of material (Guo et al., 2017).

Microwave thermal treatment not only eliminates potential microorganisms in food to ensure safety, but also inactivates enzymes to maintain the nutritional value of food (Chen et al., 2017). Over the years, various continuous microwave flow systems have been developed. Microwave tunnels have been used to create continuous microwave-assisted thermal processes for non-fluid foods, mainly used for industrial continuous pasteurization/sterilization of ready-to-eat packaged foods (Stanley & Petersen, 2017).

At the molecular level, dipole rotation occurs when a dipole aligns in response to the strongest electromagnetic field affecting it. In food, where water is the main component along with proteins, sugars, and salts, this phenomenon causes molecular excitation. These molecules, interacting with each other, generate heat through friction due to the constant oscillation of the dipoles in the alternating electric field, resulting in efficient volumetric heating (Jiang et al., 2018). Ionizing radiation applied to food products (food irradiation) is considered one of the most important advances in the food industry since pasteurization, as it Improves food safety and extends shelf life (Käferstein & Moy, 1993; Gautam, 2024).

Irradiation involves the application of energy, such as light, heat, or electromagnetic rays, to food products without direct contact with the energy source. Sufficient application has a bactericidal effect without damaging the food with radioactivity. It has been shown that these processes do not significantly deteriorate the quality and nutritional properties of products (Bornhorst et al., 2017).

Microwave systems have been used in food pasteurization processes, where they have shown various benefits (Angoy et al., 2019). Significant improvements have been demonstrated in the food industry due to their ability to preserve food quality, achieve volumetric and selective heating, and attain high energy efficiency. Peng et al. (2017) demonstrated that microwave pasteurization (MW) of packaged carrots was much faster than with hot water. After 20 minutes of preheating at 60°C, microwave pasteurization reduced the total processing time needed to reach an F90°C value of 3 minutes, requiring only 3.22 minutes compared to 7.80 minutes with hot water. Similarly, for an F90°C value of 10 minutes, the microwave process took 4.96 minutes, whereas the hot water process required 13.96 minutes. These benefits enable faster and more uniform treatment of food, preserving its nutritional and organoleptic properties compared to conventional thermal methods (Joshi et al., 2023).

Although microwaves have been widely used worldwide since the 1960s, even at an industrial scale for continuous flow processes (Stanley & Petersen, 2017), their application in Colombia has been limited. The primary objective of this research is to determine the effect of microwave power and exposure time on the microbiological counts of aerobic mesophiles and the physicochemical properties of milk.

2. Materials and methods

This research was conducted in two phases. In the first phase, the process was carried out in "batches" using a Samsung microwave with a power of 1500 W at a frequency of 2450 MHz. The milk samples were collected during the reception stage at the Lácteos Corzo company. To ensure sampling quality, the instruments were pre-washed and disinfected. The milk in each container was vigorously mixed with a stainless-steel stirrer to ensure sample homogeneity. Aliquots were then taken and placed in clean containers with a capacity of one liter. The samples were shaken for one minute and transferred to sterile containers, which were sealed and labeled. Transportation to the laboratory was performed in a refrigerated portable cooler, maintaining temperatures between 0 and 4°C to preserve the milk's conditions. Transport time did not exceed 12 hours. This process was carried out in compliance with Colombian Technical Standard NTC 666 (Instituto Colombiano de Normas Técnicas y Certificación, 1996).

Sampling was performed in triplicate to ensure the representativeness of the results. Once at the laboratory, the milk samples were removed from the cooler and acclimatized to a temperature of 15°C. For thermal treatment, a 100 ml aliquot of milk was placed in 150 ml beakers, which were immediately covered with aluminum foil. The beakers were placed at the center of the microwave to ensure optimal radiation capture and uniform heating. The samples were exposed to microwave times of 0 seconds (control), 15, 20, 30, 40, 45, and 50 seconds. The sample corresponding to 0 seconds was not subjected to microwave exposure and was used as the untreated control. This control served as the reference baseline for microbiological and physicochemical comparisons. The microwave power was set to the maximum level, and the initial average temperature of the samples was 15°C. The maximum temperature reached in each treatment was measured with a Brixco digital thermometer immediately after the microwave exposure ended.

After microwave exposure, the surfaces of the beakers were covered again with aluminum foil and left to cool to 15°C. Subsequently, microbiological plating for aerobic mesophiles was performed according to Colombian Technical Standard NTC 399 (Instituto Colombiano de Normas Técnicas y Certificación, 2002). The treated milk samples were labeled, and serial dilutions were prepared up to 10-5. The dilutions were shaken, and the surviving microorganisms were destressed for 2 minutes near a flame, under aseptic conditions, following the indicated procedure. The determination of aerobic mesophiles was conducted using the deep plate count method. From the 10-5 dilution, 1 ml was transferred to sterile Petri dishes, to which 20 ml of Merck Standard Plate Count (SPC) medium was added and mixed with the inoculum. This procedure was performed in triplicate. The Petri dishes were

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incubated at 37°C for 24 to 48 hours. After this time, the colony count was performed using a CP-600/1 digital colony counter from Tecnal, and the counts were expressed as Log10 CFU/g.

In the second phase, a pilot continuous-flow system was designed and built (see appendix 1). This system was coupled to a concentric tube heat exchanger to cool the milk to 5°C. The system consisted of an LG microwave and two storage tanks. In addition, a Quad Input Logging Thermometer DT304 was installed to monitor the milk's temperature at both the entry and exit points of the pasteurization process. During this phase, power levels of 700 W and 1000 W were used, with exposure times of 10, 15, and 20 seconds. Unlike the first phase, no untreated control group was included in this stage, since the objective was to compare the effects of different power–time combinations in a continuous flow system. Physicochemical and microbiological analyses were carried out using the MilkoScan FT+ Foss Conveyor 5000 Basic equipment. These tests included the compositional analysis of fat, protein, and total solids (TS) through infrared spectroscopy (International Organization for Standardization (ISO) & International Dairy Federation (IDF), 2006). The aerobic mesophile count was also conducted in Colony Forming Units (CFU) using flow cytometry (statistically validated method, M-UD-0001L V3.1), as well as somatic cell count (SCC) following the standardized method ISO 13366-2, IDF 148-2:2006.

A completely randomized design was employed to structure the experiments in both phases. The factor of interest was the exposure time to radiation. To identify its effect, an Analysis of Variance (ANOVA) was performed. Subsequently, Tukey's test was applied to detect significant differences between means. Statistical analysis was performed using RStudio software (version 2024.04.0+73).

3. Results and Discussion.

Figure 1 presents microbial counts in Colony Forming Units (CFU/mL) on a logarithmic scale, according to different microwave exposure times. The microbial load of the untreated control sample (0 s) was approximately 7.2 log CFU/mL, showing no statistical difference with samples exposed for up to 45 seconds ($p > 0.05$). Significant reduction was only observed at 50 seconds ($p < 0.05$). The 50-second treatment produced the highest microbial reduction (99.57%), in compliance with the standards of Decree 616 (MINISTERIO DE LA PROTECCION SOCIAL, 2006) and the Pasteurized Milk Ordinance (PMO) (U.S. Food & Drug Administration, 2023). This microbial inactivation is attributed to the temperature reached during this exposure time, which was 91.03 °C. The results obtained in this first experimental phase were fundamental for the design, construction, and implementation of the continuous flow system. Notably, during the second phase, shorter exposure times were applied since the milk flowed through a Teflon tube, which allowed a broader exposure surface, and a more homogeneous heating distribution compared to the 100 mL beakers initially used.

Figure 1. *Microbial count (Log10 CFU/mL) of milk samples exposed to microwave radiation at 1500 W for 15 to 50 seconds. The "Control" represents untreated milk (0 seconds). Bars indicate mean values ± standard deviation. Different superscript letters indicate significant differences between treatments (p < 0.05).*

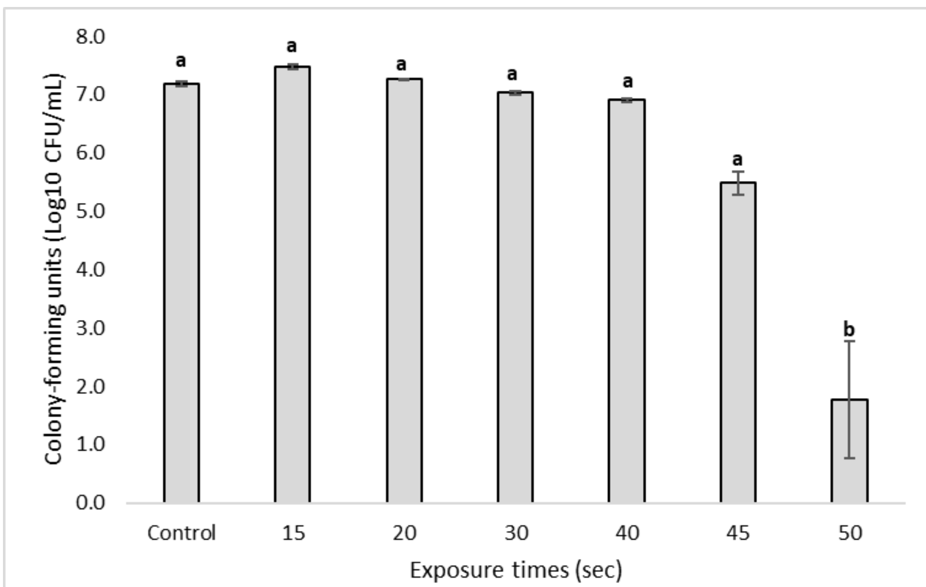

Table 1 shows the effect of thermal treatment of milk at different power levels and exposure times. The most significant results were observed with a 20-second exposure at 1000 W, where a considerable

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reduction in the aerobic mesophile count was achieved, complying with the current regulations in Colombia for human consumption. In contrast, at 1000 W for 10 and 15 seconds, a significant reduction in microbial load was not achieved. At 700 W, the exposure times of 10, 15, and 20 seconds were also insufficient to achieve the six-logarithmic-cycle reduction of pathogenic microorganisms or inactivation of peroxidase, with Escherichia coli predominating in the pathogenic microbiota. Significant reductions in aerobic mesophile counts were also reported by Tremonte et al. (2014) using power levels of 700 and 900 W during 75 seconds of exposure. Similarly, the most effective treatment was identified by Shahvandari et al. (2022) with 850 W for 90 seconds, achieving the complete elimination of microorganisms such as Staphylococcus aureus, coliforms, molds, yeasts, and total bacterial counts in some samples. Additionally, it was demonstrated that microwave irradiation can significantly reduce microbial and yeast counts, with the best power level being 600 W with an exposure time of 47 seconds (Kapcsándi et al., 2020).

Tabla 1. *Microbiological results at different exposure times and power levels for milk samples.*

Time (s)	Temp. (°C)	A.M (LogCFU/mL)	Coliforms CFU/mL	Listeria monocytogenes	Salmonella spp.	E. coli (CFU/mL)
			700 W			
10	57.0 ± 1.0a	2.9 ± 0.0a	+	-	-	+
15	56.0 ± 1.0a	2.8 ± 0.0a	+	-	-	+
20	54.0 ± 0.6a	2.9 ± 0.0a	+	-	-	-
			1000 W			
10	67.0 ± 2.0a	5.7 ± 0.3a	+	-	-	+
15	69.0 ± 2.0a	5.1 ± 0.1a	+	-	-	-
20	77.3 ± 4.7b	1.9 ± 0.4b	-	-	-	-

Note: data are reported as mean ± SD, n = 3. Different letters indicate significant differences at $p < 0.05$ (Tukey's test). The symbols (+) and (-) denote the presence and absence of the indicated microorganisms, respectively.

Table 2 presents the results of the physicochemical characterization of milk after microwave thermal treatment, showing that the physicochemical properties remained stable with exposure times of 10, 15, and 20 seconds at 700 W and 1000 W. Zhang et al. (2023) reported that up to 300 W, the physicochemical properties of milk were unaffected. However, other studies have found that higher power levels can induce changes in protein structure, which was not observed in this study due to shorter exposure times.

On the other hand, it was demonstrated by Thum et al. (2020) that temperatures of 75 °C applied for 20 seconds inactivated over 95% of the bioactive proteins in milk. In contrast, it was observed in our study that short times at 1000 W achieved a significant reduction in microbial load without affecting physicochemical properties. Martins et al. (2021) concluded that microwave use at low temperatures and longer times favors the preservation of bioactive compounds.

Tabla 2. *Physicochemical properties of milk at different exposure times and power levels for milk samples.*

Time (s)	Density (g/mL)	Fat (%)	Total solids	Protein (g/Kg)	Cryoscopic index	MNU
			700 W			
10	1.029	4.53 ± 0.37a	12.9 ± 0.85a	33.5 ± 2.7a	465 ± 46a	11.8 ± 0.81a
15	1.029	4.55 ± 0.14a	13.2 ± 0.38a	34.5 ± 1.2a	494 ± 20a	11.7 ± 0.53a
20	1.030	4.49 ± 0.15a	13.3 ± 0.15a	36.3 ± 0.7a	510 ± 10a	10.9 ± 0.36a
			1000 W			
10	1.030	4.66 ± 0.18a	13.4 ± 0.37a	35.5 ± 1.3a	497 ± 21a	11.9 ± 0.90a
15	1.029	4.25 ± 0.53a	12.7 ± 1.12a	33.5 ± 3.0a	474 ± 51a	11.7 ± 2.15a
20	1.030	4.39 ± 0.29a	13.2 ± 0.51a	36.1 ± 1.7a	506 ± 27a	11.2 ± 0.91a

Note: data are reported as mean ± SD, n = 3. Different letters indicate significant differences at $p < 0.05$ (Tukey's test).

4. Conclusions

Microwave thermal treatment is presented as an effective alternative to conventional methods for milk processing, ensuring microbial inactivation without compromising the physicochemical properties of the product. The use of higher power levels, such

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as 1000 W, is essential to guarantee microbiological safety, while lower power levels are insufficient to meet the required standards. The pilot continuous-flow system improved heat distribution, making the process more efficient and homogeneous, suggesting that this approach could optimize milk pasteurization processes. Additionally, this method offers benefits both in preserving the product's nutrients and in energy efficiency. Finally, continuous-flow microwave treatment is considered a viable alternative for improving microbiological safety and maintaining milk quality, positioning itself as a competitive option compared to traditional thermal methods.

5. Competing Interests

The authors have no conflicts of interest to declare that are relevant to the content of this chapter.

6. Acknowledgement

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***Appendix 1**. Continuous-flow microwave equipment.*

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