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TESE

Processamento de *Orange Juice-Milk* por Micro-ondas

Carolina Pinto de Carvalho Martins

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PROCESSAMENTO DE *ORANGE JUICE-MILK* POR MICRO-ONDAS

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RESUMO

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Atualmente, o superaquecimento ainda é um grande problema no uso do aquecimento convencional para leite e vários produtos lácteos. O aquecimento por micro-ondas (AMO) tem o crédito de fornecer produtos lácteos de qualidade superior com vida útil estendida, representando uma boa alternativa ao tratamento térmico convencional. O presente estudo teve como objetivo avaliar o efeito do AMO (65 ◦C e 75 ◦C, por 15, 30 e 60 s) sobre os compostos bioativos, perfil de ácidos graxos, compostos orgânicos voláteis e aspectos físicos da bebida mista de suco de laranja e leite (BMSLL). E, além disso, avaliar o conhecimento sobre a tecnologia micro-ondas (MO), incluindo segurança do forno e a segurança dos alimentos para consumidores brasileiros e portugueses. O AMO apresentou menor índice de escurecimento e maiores níveis de ácido ascórbico, fenólicos totais e carotenoides, maior atividade antioxidante e maior atividade inibitória de α-amilase, α-glicosidase e enzima conversora de angiotensina (ECA) do que o produto pasteurizado, semelhante à bebida não tratada. Não foram observadas diferenças significativas nos níveis de compostos orgânicos voláteis e ácidos graxos. Temperaturas mais baixas (65 ◦C) e tempos de processo mais longos (60 s) resultaram em maior retenção de compostos bioativos. As condições operacionais ideais foram 915 MHz em comparação com 2.450 MHz devido aos maiores valores de fator de perda e profundidade de penetração, resultando em maior dissipação de calor e eficácia na distribuição de temperatura. As amostras tratadas por AMO apresentaram propriedades reológicas semelhantes às bebidas não tratadas com uma cor amarela ligeiramente mais intensa e um tamanho de partícula menor, especialmente em temperaturas e tempos de espera mais elevados. A migração dos compostos do recipiente para o alimento e as alterações da textura foram as principais preocupações relatadas. 3,6% dos brasileiros ainda usam vasilha de metal, 19,7% não leem as instruções de reaquecimento e 12,2% não leem as instruções de cozimento. Os consumidores portugueses têm um maior conhecimento dos níveis de potência e, em ambas as populações estudadas, o nível de escolaridade influenciou o conhecimento sobre a tecnologia. Brasileiros e portugueses eram indiferentes ou consideravam produtos tratados por micro-ondas como pouco seguros, respectivamente. O AMO pode ser considerado uma alternativa eficaz para o processamento de bebidas mistas de suco de laranja e leite, produzindo produtos com propriedades semelhantes ou aprimoradas em comparação com a pasteurização convencional. Entretanto, revela-se a necessidade de maior disseminação de informações que possam atingir a população de menor escolaridade, proporcionando melhor segurança operacional do forno micro-ondas e maior conhecimento dos riscos microbiológicos associados.

Palavras-chave: Aquecimento micro-ondas, tecnologias inovadoras, preservação de alimentos, produtos lácteos, segurança de alimentos.

ABSTRACT

MARTINS, Carolina Pinto de Carvalho. **Microwave Orange Juice-Milk Processing.** 2021. 110p. Thesis (PhD in Food Science and Technology). Institute of Technology, Department of Food Technology, Federal Rural University of Rio de Janeiro, Seropédica, RJ, 2021.

Currently, overheating is still a major problem in the use of conventional heating for milk and various dairy products. Microwave heating (MWH) has been credited with providing superior-quality dairy-based products with extended shelf-life, representing a good alternative to conventional heat treatment. The present study aimed to evaluate the effect of MWH (65 ◦C and 75 ◦C, for 15, 30, and 60 s) on the bioactive compounds, fatty acids profile, volatile organic compounds, and physical aspects of orange juice-milk beverage (OJMB). In addition, evaluate the knowledge of microwave technology, including microwave oven safety and microwaved food safety for Brazilians and Portuguese consumers. MWH presented a lower browning index and higher levels of ascorbic acid, total phenolics, and carotenoids, higher antioxidant activity, and greater α-amylase, α-glucosidase, and angiotensin-converting enzyme (ACE) inhibitory activity than the pasteurized product, similar to the untreated beverage. No significant differences were observed in the volatile organic compounds and fatty acids levels. Lower temperatures (65 ◦C) and longer process times (60 s) resulted in higher retention of bioactive compounds. MH can be an alternative to conventional pasteurization for OJMB processing. The ideal operating conditions were at 915 MHz compared with 2450 MHz because of the higher loss factor values and penetration depth, resulting in higher heat dissipation and temperature distribution effectiveness. The MWH samples had rheological properties similar to untreated beverages with a slightly more intense yellow color and a smaller particle size, especially at higher temperatures and holding times. The migration of compounds from the container to the food and textural changes were the main concerns reported. 3.6% of Brazilians still use metal containers, 19.7% do not read the instructions for reheating and 12.2% do not read the cooking instructions. Portuguese consumers had a higher understanding of the power levels, and in both populations studied, the level of education influenced knowledge about the technology. Brazilians and Portuguese were indifferent or considered microwaved-treated products as slightly safe, respectively. MWH can be considered an effective alternative for processing mixed beverages of orange juice and milk, yielding products with similar or enhanced physical properties compared with conventional pasteurization. However, there is a need for greater dissemination of information that can reach the population with the lowest level of education, providing better operational safety of the microwave oven, and more excellent knowledge of associated microbiological risks.

Keywords: Microwave heating, innovative technologies, food preservation, dairy products, food safety.

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INTRODUÇÃO

Os alimentos com alto teor de compostos bioativos e considerados funcionais tem ganhado cada vez mais espaço entre os consumidores preocupados com a saúde. As bebidas à base de suco de fruta, como a laranja, e de leite podem ser consideradas importantes fontes destes compostos.

A laranja é considerada fonte de importantes compostos reguladores da saúde humana, tais como a vitamina C, tiamina, riboflavina, ácido pantotênico, piridoxina, ácido fólico, vitamina A, vitamina D e vitamina E, além de compostos fenólicos e carotenoides (Giuffrida *et al*., 2019; Ruiz-De Anda, Ventura-Lara, Rodríguez-Hernández, & Ozuna, 2019; Zvaigzne & Kārkliņa, 2013), como os carotenos (α-, β-, e ζ-caroteno) e as xantofilas (β-criptoxantina, luteína e zeaxantina) (Escudero-López *et al*., 2016; Giuffrida *et al*., 2019; Hornero-Méndez *et al*., 2018).

O conteúdo de compostos bioativos, assim como a alta atividade antioxidante do suco de laranja tem sido associada a benefícios à saúde, como controlar os níveis de colesterol e pressão, e minimizar o risco de câncer (Hornero-Méndez *et al*., 2018; Zvaigzne *et al*., 2013). Além disso, a laranja pode servir como um agente doador de sabor e cor aos produtos lácteos melhorando características sensoriais e aceitação.

O leite e seus derivados são importantes para a dieta humana e fazem parte das recomendações nutricionais oficiais em muitos países (FAO, 2016; Rozenberg *et al*., 2016), pois contribuem com quantidades significativas de proteína, cálcio, magnésio, selênio, riboflavina, zinco, vitamina B12, ácido pantotênico e ácidos graxos essenciais (Bhat & Bhat, 2011; Hidayat, Du, & Shi, 2019; Raza & Kim, 2018).

Além disso, são fonte de peptídeos bioativos gerados pela proteólise da caseína (α-, β-, γ- e κ-caseína) e proteínas do soro do leite (β-lactoglobulina, α-lactalbumina, albumina sérica, imunoglobulinas, frações de lactoferrina e protease-peptona) (Tonolo *et al*., 2020), sendo a imunomodulação, atividade antimicrobiana, antitrombótica e anticâncer, regulação da pressão arterial e ligação a minerais ou vitaminas suas principais atividades biológicas (Bhat *et al*., 2011; Egger & Ménard, 2017; Sánchez & Vázquez, 2017; Tonolo *et al*., 2020), afetando beneficamente os sistemas: imunológico, digestivo, cardiovascular e nervoso (Korhonen & Pihlanto, 2006; Sánchez *et al*., 2017).

A pasteurização e esterilização térmicas convencionais são amplamente utilizadas na indústria láctea para promover a segurança microbiológica e aumentar a vida útil dos produtos (Munoz, Gou, Picouet, Barlabe, & Felipe, 2018). Embora o processamento térmico convencional tenha sido amplamente utilizado na indústria de laticínios, também se reconheceu que as altas temperaturas empregadas para eliminar as bactérias também levam à degradação térmica de compostos voláteis e termossensíveis, resultando na perda do valor sensorial e nutricional do leite e produtos lácteos (Bhushand, Vyawarea, Wasnik, Agrawal, & Sandey, 2017; Chandrasekaran, Ramanathan, & Basak, 2013).

Diferentemente dos mecanismos convencionais de convecção e condução, o aquecimento por micro-ondas se dá pela geração de ondas eletromagnéticas dentro de uma faixa de frequência de 300 MHz a 300 GHz (Chandrasekaran *et al*., 2013). As micro-ondas penetram no alimento, são absorvidas e convertidas rapidamente, proporcionando geração volumétrica de calor e, consequentemente, altas taxas de aquecimento (Salazar-González, Martín-González, López-Malo, & Sosa-Morales, 2012). Os mecanismos envolvidos neste processo são a condução iônica e rotação dipolar. A condução iônica ocorre quando um campo eletromagnético alternado é aplicado e as moléculas resistentes ao fluxo de íons e as colisões intermoleculares causadas pela mudança contínua na direção destes íons promovem atrito (Salazar-González *et al*., 2012), e a rotação dipolar está relacionada ao movimento de moléculas polares que tentam se alinhar com o campo elétrico alternado (Hossan, Byun, & Dutta, 2010).

É a capacidade de geração volumétrica de calor o principal atrativo do aquecimento micro-ondas, pois esta resulta em uma maior eficiência energética, tempos de aquecimento reduzidos e permite a obtenção de produtos com melhor qualidade sensorial e nutricional quando comparados aos métodos convencionais de aquecimento (Guo, Sun, Cheng, & Han, 2017; Marszałek, Mitek, & Skąpska, 2015; Saikia, Mahnot, & Mahanta, 2016; Salazar-González *et al*., 2012; Sattar *et al*., 2019).

JUSTIFICATIVA

Para minimizar os efeitos deletérios dos processos térmicos convencionais, foram estudadas tecnologias alternativas de processamento de laticínios, tais como campo elétrico pulsado (McAuley, Singh, Haro-Maza, Williams & Buckow, 2016), aquecimento ôhmico (Cappato *et al*. 2017 ; Cappato *et al*., 2018; Costa *et al*., 2018), tecnologia de dióxido de carbono (Amaral *et al*., 2017), luz ultravioleta (Kasahara, Carrasco, & Aguilar, 2015), ultrassom (Guimarães *et al*., 2018a, 2018b; Monteiro *et al*., 2018), tecnologia de luz pulsada (Miller, Sauer, & Moraru, 2012), plasma frio (Coutinho *et al*., 2018), entre outros.

Além destes, relatos sobre o uso de um sistema de micro-ondas para pasteurização em diversos produtos já foram realizados (Géczi, Horváth, Kaszab, & Alemany, 2013; Hamid, Boulanger, Tong, Gallop e Pereira, 1969; Laguerre, *et al*., 2011; Lin & Ramaswamy, 2011; Manjunatha, Prabha, Ramachandra, Krishna, Shankar, 2012; Pina-Pérez, Benlloch-Tinoco, Rodrigo e Martinez, 2014; Rasooly, Hernlem, He, & Friedman, 2014; Tremonte, *et al*., 2014).

No entanto, o que se vê atualmente é a escassez de estudos sobre os efeitos da pasteurização micro-ondas em produtos lácteos (Figura 1). O conhecimento dos fatores que influenciam o processamento e seus efeitos sobre os alimentos são importantes para a determinação dos parâmetros relativos ao processo, sendo estes os grandes desafios para a utilização do aquecimento micro-ondas e sua aplicação prática em produtos lácteos.

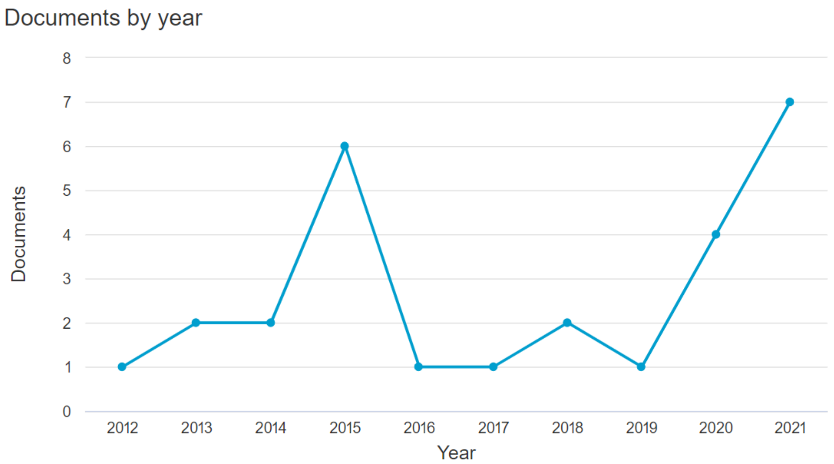

Figura 1. Número de estudos envolvendo aquecimento micro-ondas em produtos lácteos (2012 -2021).

Fonte: Scopus 2021, acessado em 10/08/2021.

Embora os estudos relacionados ao uso do aquecimento por micro-ondas em produtos lácteos sejam escassos na literatura, os benefícios de sua aplicação têm sido relatados em todo o mundo. Esse cenário faz com que esta seja uma tecnologia promissora para uso comercial que deve continuar a ser investigada em resposta às demandas dos consumidores por produtos com melhores características nutricionais e sensórias, sem comprometer a segurança. Desta forma, o presente trabalho apresenta grande importância para o desenvolvimento e avaliação dos efeitos do processamento micro-ondas em produtos lácteos.

OBJETIVOS

Objetivo Geral

Avaliar o efeito do aquecimento micro-ondas sobre os compostos bioativos, perfil de ácidos graxos, compostos orgânicos voláteis e aspectos físicos da bebida mista de suco de laranja e leite. Além de avaliar o conhecimento dos consumidores sobre a tecnologia micro-ondas.

Objetivos Específicos

Desenvolver uma bebida mista de suco de laranja e leite e processar por aquecimento micro-ondas em diferentes tempos e temperaturas (65 ºC e 75 ºC, por 15, 30 e 60 s);

Processar a bebida desenvolvida através da pasteurização convencional (75 ºC por 15 s) para fins de comparação;

Avaliar o efeito do aquecimento micro-ondas em relação aos compostos bioativos (compostos fenólicos totais, ácido ascórbico, carotenoides totais, capacidade antioxidante, índice de escurecimento, e inibição da enzima conversora de angiotensina I, α-amilase e α-glicosidade) durante o armazenamento (dias 1, 7, 14, 21 e 28);

Avaliar o efeito do aquecimento micro-ondas no perfil de ácidos graxos e índices de saúde (índice aterogênico, índice trombogênico, ácidos graxos desejados e ácidos graxos hipercolesterolêmicos saturados);

Avaliar o efeito do aquecimento micro-ondas nos compostos orgânicos voláteis;

Avaliar o efeito do aquecimento micro-ondas em relação às propriedades físicas através da análise de parâmetros de cor, reológica e de distribuição de tamanho de partícula;

Avaliar o conhecimento dos consumidores brasileiros e portugueses sobre a tecnologia micro-ondas, incluindo o uso e conhecimento sobre a segurança, as práticas de segurança ao reaquecer ou cozinhar alimentos, a atitude em relação à segurança de alimentos e as preocupações sobre alimentos preparados no equipamento.

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CAPÍTULO I

MICROWAVE PROCESSING: CURRENT BACKGROUND AND EFFECTS ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL ASPECTS OF DAIRY PRODUCTS

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ARTIGO PUBLICADO NA REVISTA

Comprehensive Reviews in Food Science and Food Safety

Qualis A1 - Ciência de Alimentos

Comprehensive REVIEWS in Food Science and Food Safety

Microwave Processing: Current Background and Effects on the Physicochemical and Microbiological Aspects of Dairy Products

Carolina P. C. Martins, Rodrigo N. Cavalcanti, Silvia M. Couto, Jeremias Moraes, Erick A. Esmerino, Marcia Cristina Silva, Renata S. L. Raices, Jorge A. W. Gut, Hosahalli S. Ramaswamy, Carmen C. Tadini, and Adriano G. Cruz

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ABSTRACT

Overheating is still a major problem in the use of conventional heating for milk and various dairy products, because it leads to the lowering of quality and sensory and nutritional values. Microwave (MW) heating has been credited with providing superior-quality dairy-based products with extended shelf-life, representing a good alternative to conventional heat treatment. The main drawback of MW heating refers to nonuniform temperature distribution, resulting in hot and cold spots mainly in solid and semisolid products; however, MW heating has been shown to be suitable for liquid foods, especially in a continuous fluid system. This review aims to describe the main factors and parameters necessary for the application of MW heating technology for dairy processing, considering the theoretical fundamentals and its effects on quality and safety aspects of milk and dairy products. MW heating has demonstrated great ability for the destruction of pathogenic/spoilage microorganisms and their spores, and also inactivation of enzymes, thereby preserving fresh characteristics of dairy products.

Keywords: dairy foods, dielectric properties, food preservation, innovative technologies, microwave processing, nutritional value

1 INTRODUCTION

Milk and dairy products are important for the human diet and are part of the official nutritional recommendations in many countries worldwide (FAO, 2016; Rozenberg *et al*., 2016). They contribute high-quality nutrition in the human diet through high concentrations of micro- and macronutrients with significant amounts of calcium, magnesium, selenium, riboflavin, zinc, vitamin B12, and pantothenic acid (Bhat & Bhat, 2011; Raza & Kim, 2018). Furthermore, several other components in milk and dairy products, such as whey proteins, different types of casein, bioactive peptides, antioxidants, vitamins, oligosaccharides, organic acids, highly absorbable calcium, lactoferrin, lactoperoxidase, glycomacropeptides, sphingolipids, and conjugated linoleic acid (CLA) have also been identified as bioactive substances responsible for benefits to human health, including protection against cardiovascular diseases and cancer, improved immunity, and control of the action of pathogenic microorganisms, especially those present in the gastrointestinal tract (Bhat & Bhat, 2011; Pardo, Altahona, & Pérez, 2013).

Safety and quality of milk and dairy products are mainly related to the elimination of contamination of raw milk by spoilage and/or pathogenic microorganisms, chemical residues, and risk factors (Hamann, 2010). Raw milk may be contaminated by microorganisms mainly during and after milking, which may occur through diverse sources and routes, including the interior of teats (mastitis, bovine tuberculosis, and other organism), vectors such as flies, human carriers, and others, soil, feces, and equipment (Muñoz, Gou, Picouet, Barlabé, & Felipe, 2018; Raza & Kim, 2018). Similarly, animal feed is also subject to contamination with chemical residues and/or contaminants, for example, from various pesticide sprays that are used on the same farm. Thus, ruminants are at danger from various types and sources of anthropogenic contaminants, and it is possible that these components are transferred to the milk and, subsequently, to humans (Raza & Kim, 2018).

Conventional thermal processing is the oldest treatment capable of destroying or inactivating microorganisms and enzymes that impair and/or modify the functionalities of dairy ingredients (Mishra & Ramchandran, 2015; Muñoz *et al*., 2018). Pasteurization and sterilization are the most frequent used treatments to ensure safety and to extend the shelf-life of milk for direct consumption or for later processing into various dairy products (Muñoz *et al*., 2018). To ensure that pathogenic microorganisms and/or endogenous enzymes are inactivated, the food material is maintained at a certain target temperature for a specific period of time. Normally, milk pasteurization, achieved through different time and temperature combinations, for example 72 °C for 15 s (FAO, 2004) or other suitable high-temperature, short-time treatment (HTST), whereas milk sterilization is carried out at a much higher temperature generally with an ultra-high temperature (UHT) process, which involves heating milk at 140 °C for 1.9 s (FAO, 2004).

Although conventional thermal processing has been widely used in the dairy industry, it has also been recognized that the high temperatures employed to kill bacteria also lead to the thermal degradation of volatile and thermosensitive compounds, resulting in the loss of sensory and nutritional values of milk and dairy products (Bhushand, Vyawarea, Wasnik, Agrawal, & Sandey, 2017; Chandrasekaran, Ramanathan, & Basak, 2013). In order to minimize the deleterious effects of conventional thermal processes, alternative dairy processing technologies have been studied, such as pulsed electric field (McAuley, Singh, Haro-Maza, Williams, & Buckow, 2016), ohmic heating (Cappato *et al*., 2017, 2018; Costa *et al*., 2018), carbon dioxide technology (Amaral *et al*., 2017), ultraviolet light (Kasahara, Carrasco, & Aguilar, 2015), ultrasound (Guimarães *et al*., 2018a,b; Monteiro *et al*., 2018), pulsed-light (Miller, Sauer, & Moraru, 2012), and cold plasma (Coutinho *et al*., 2018).

Novel thermal technologies such as dielectric heating by microwave (MW) and radio-frequency systems promote a volumetric heating, that is, the heat generated occurs inside the food, thereby obtaining higher quality products with greater thermal efficiency when compared to conventional thermal processes (Bhushand *et al*., 2017; Mishra & Ramchandran, 2015). Because the first study reported on the use of a MW system for milk pasteurization (Hamid, Boulanger, Tong, Gallop, & Pereira, 1969), many studies have been carried out on the pasteurization and sterilization of different food products (Géczi, Horváth, Kaszab, & Alemany, 2013; Laguerre *et al*., 2011; Lin & Ramaswamy, 2011; Manjunatha, Prabha, Ramachandra, Krishna, & Shankar, 2012; Pina-Pérez, Benlloch-Tinoco, Rodrigo, & Martinez, 2014; Rasooly, Hernlem, He, & Friedman, 2014; Tremonte *et al*., 2014).

In this context, this review aims to describe the effects of the main process variables important for the application of MW technology for dairy processing, considering product quality and microbial inactivation.

2 MICROWAVE HEATING

2.1 General Aspects

MW heating of foods is a complex process that depends upon the propagation of MWs and their interactions with food materials, which are mostly determined by dielectric properties; and heat dissipation is governed by basic heat and mass transfer mechanisms (Salazar-González, Martín-González, López-Malo, & Sosa-Morales, 2012). In contrast to the conventional convection and conduction mechanisms, MWs can penetrate the food, are absorbed, and then quickly converted to heat, providing volumetric heat generation and, consequently, high heating rates. These result in a higher energy efficiency, reduced heating times, and allows the production of products with better sensory and nutritional qualities when compared to conventional heating methods (Salazar-González *et al*., 2012; Zhu, Kuznetsov, & Sandeep, 2007).

This ability of MW to generate heat within a material characterizes the main advantage of MW heating (Salazar-González *et al*., 2012). MW heating is a form of dielectric heating which is used industrially for the processing of food and also used domestically for cooking or thawing of food (Song & Kang, 2016). MWs are electromagnetic waves that are within a frequency band of 300 MHz to 300 GHz (Chandrasekaran *et al*., 2013); however, MW-heating applications have been limited to a few narrow frequency bands for industrial, scientific, and medical use to avoid interference with the radio frequencies used for telecommunication purposes. The typical bands are 915 ± 25 MHz and 2,450 ± 50 MHz with penetration depths ranging from 8 to 22 cm at 915 MHz and from 3 to 8 cm at 2,450 MHz depending on the moisture content. The domestic MW ovens operate at 2,450 MHz, whereas both frequencies are used for industrial purposes. It is worthwhile to note that outside of the United States, frequencies of 433.92, 896, and 2375 MHz are also used for MW heating (Ahmed & Ramaswamy, 2007; Guo, Sun, Cheng, & Han, 2017).

2.2 Operational Systems

MW equipment basically consists of a magnetron, responsible for converting electric energy into an oscillating electromagnetic field, and conductors of waves that reflect the electric field internally (wave guides), transferring it to the heating chamber (FDA, 2000). The heating chamber can be designed for batch or continuous flow operations and have appropriate mechanisms to prevent the leakage of MWs (FDA, 2000). Batch and continuous systems can be applied for solid or liquid foods depending on the equipment configuration. In batch equipment, a rotating antenna or a propeller is used to distribute the energy, or the food is placed on a turntable that rotates during the cooking operation (FDA, 2000). For liquid foods, a stirrer can be used to homogenize the MW absorption and heat distribution (Figure 1a). However, continuous systems have advantages over batch processing, with higher efficiency and easier cleaning and automation (Ahmed & Ramaswamy, 2007; Salazar-González *et al*., 2012). Continuous MW heating of solid foods normally occurs with the transportation of the solid material in trays or pouches inside the chamber where the food material receives MW radiation with the first patent published by Kenyon, Berkowitz, and Ayoub (1976). In the case of liquid foods, the fluid is pumped through helical coils into a MW oven (alternately, more MW ovens can be connected in series for heating, or several magnetrons can be arranged in an oven; Ahmed & Ramaswamy, 2007) (Figure 1b). After MW heating, the fluid passes through a retention section to allow a predefined holding time followed by cooling, and thermocouples are used to collect sample temperatures in the inlet and outlet sections, and fiber optic probes are used to monitor the temperature inside the MW cavity (Ahmed & Ramaswamy, 2007).

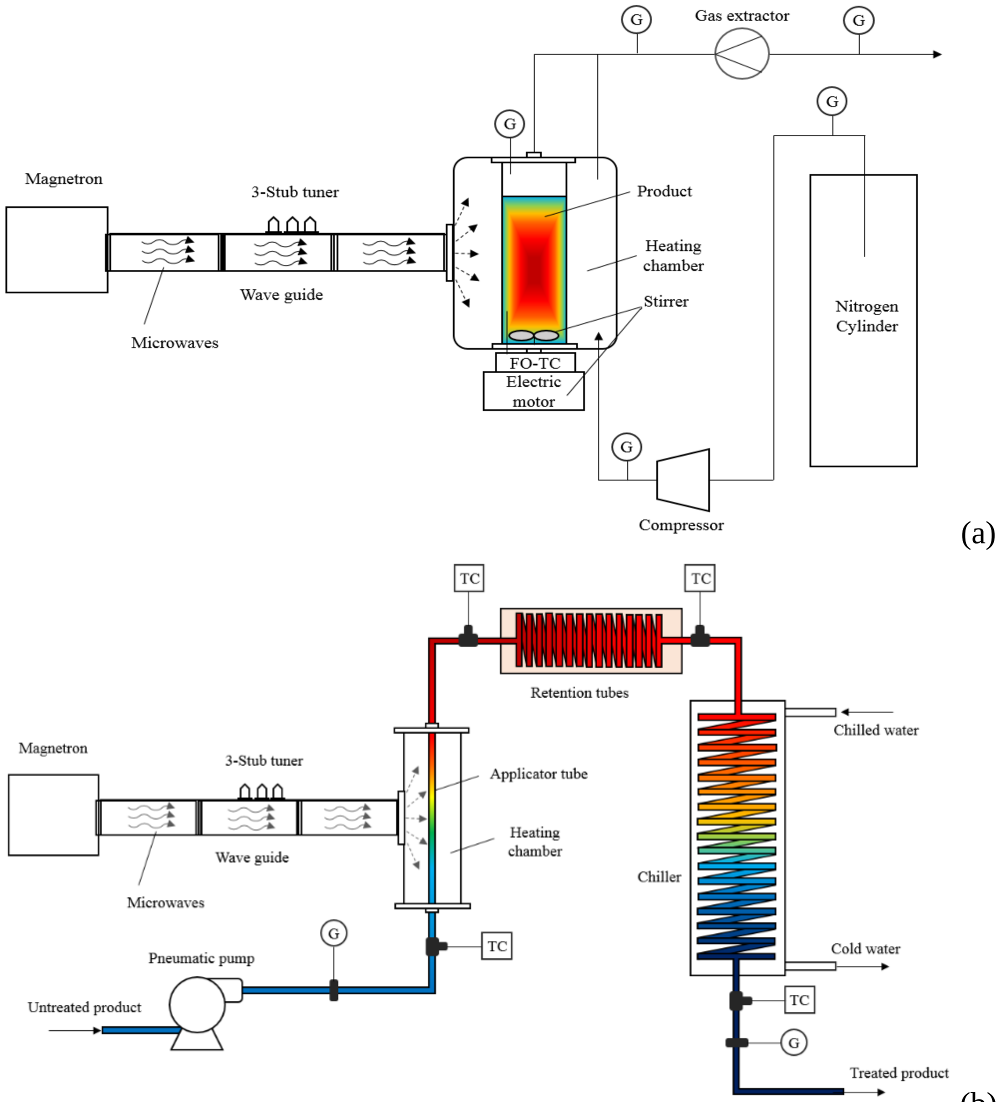

Figure 1. Microwave heating applied in batch (a) and continuous flow (b) systems. Thermocouple (TC), Pressure gauge (G), fiber optic thermocouple (FO-TC).

According to Stanley and Petersen (2017), several continuous flow MW systems have been developed along the years. For nonflowable foods, MW tunnels have been used to develop continuous MW-assisted thermal processes, mostly applied for industrial continuous in-pack pasteurization/sterilization of ready-to-eat meals, such as Sairem Labotrom 8000 in France (Sairem Company Website, 2015) and MicVac in Sweden (MicVac, 2015) both operating at 2,450 MHz. For flowable foods, MW systems such as UHT/HTST Microthermics (Microthermics, Raleigh, NC, USA) and EnbioJet (Enbio Technology, Kosakowo-k-Gdyni, Poland) have been developed and industrially applied. The Microthermics' pilot-scale unit can process pasteurization/sterilization of liquid foods at flow rates between 48 and 180 L/hr. The system uses a focused MW applicator operating at nominal power of 6,000 W and frequency of 2,450 MHz, and with maximum temperature of 150 °C. Another commercial MW system industrially used is the EnbioJet (Enbio Technology; Stanley & Petersen, 2017).

2.3 Heating Mechanisms

The heating of a food material by MWs is the result of two main mechanisms: ionic conduction and dipolar rotation (Franco, Yamamoto, Tadini, & Gut, 2015). In many applications these two mechanisms can occur simultaneously. Ionic conduction occurs due to the electrophoretic back-and-forth oscillation of ions when an alternating electromagnetic field is applied. When ionic molecules are exposed to MWs, the ions are forced to flow first in one direction and then in the opposite direction according to the rapidly directional changing of the electromagnetic field signal (Figure 2a). The resistance of the food molecules to this flow of ions, as well as the collisions between the molecules caused by the continuous change in the direction of the ions, results in friction by intermolecular collisions, promoting the volumetric heating of the food (Salazar-González *et al*., 2012). Therefore, a food with a high concentration of ions would have more frequent collisions and, therefore, would present a higher temperature rise than a food with a lower concentration of ions (Datta & Davidson, 2000). The dipolar rotation is related to the alternating motion of polar molecules that have a dipole moment (either permanent or induced by electric field), which try to align with the alternating electric field (Figure 2b). As the field decreases, the disorder is restored, which results in the release of thermal energy (Hossan, Byun, & Dutta, 2010). Because of the high frequency, this realignment occurs at million times per second, and molecules that try to oscillate at such frequencies generate intermolecular friction resulting in the heating of the material (Chandrasekaran *et al*., 2013). As heat is generated, it flows through the food components, spreading in all directions by conduction or convection mechanisms (Chandrasekaran *et al*., 2013; Hossan *et al*., 2010).

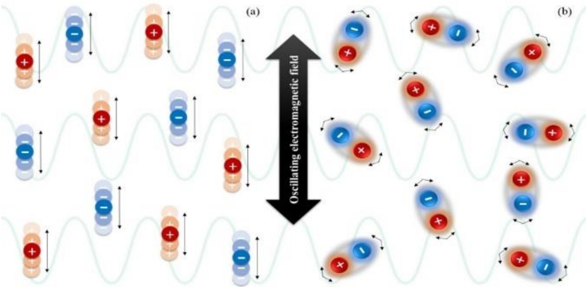

Figure 2. Ionic conduction (a) and dipole rotation (b) mechanisms associated with microwave heating.

2.4 Industrial Application

The industrial application of MW heating has been largely studied for many food systems and heat treatment operations in the food-processing industries with several review articles published in the literature (Chandrasekaran *et al*., 2013; Chizoba-Ekezie *et al*., 2017; Guo *et al*., 2017; Salazar-Gonzalez *et al*., 2012). According to these authors, the major applications of MW heating in the food industry are:

- tempering/thawing of frozen food products;
- precooking/cooking of various products for foodservice;
- drying of food materials;
- baking of various foods;
- extraction of food compounds;
- blanching of vegetables;
- heating and sterilizing of fast food, cooked meals, and cereals; and
- pasteurization and sterilization of various foods.

Historically, the first study on the use of a MW system for pasteurization of raw milk was reported by Hamid *et al*. (1969). The authors have assembled a glass tube fitted across a waveguide in which raw milk was passed through and exposed to MW energy during gravity falling. Since then, several other studies on MW heating of milk have been performed, the majority of them focused on the assessment of the potential enhancement of the milk shelf-life, the application of MW energy to inactivate milk pathogens and enzymes, the influence of MW treatment on nutritional and sensory aspects of milk, and the nonuniform temperature distribution during the MW treatment, in which many of these studies include a comparison with conventionally thermal treated milk samples (Chiu, Tateishi, Kosikowski, & Armbruster, 1984; Choi, Marth, & Vasavada, 1993a, 1993b; Clare *et al*., 2005; Dehghan, Jamalian, Farahnaky, Mesbah, & Moosavi-Nasab, 2012; Dumuta, Giurgiulescu, Mihaly-Cozmuta, & Vosgan, 2011; Géczi *et al*., 2013; Iuliana, Rodica, Sorina, & Oana, 2015; Jaynes, 1975; Kudra, de Voort, Raghavan, & Ramaswamy, 1991; Lin & Ramaswamy, 2011; Manjunatha, Prabha, Ramachandra, Krishna, & Shanka, 2012; Rasooly, Hernlem, He, & Friedman, 2014; Rodríguez-Alcalá, Alonso, & Fontecha, 2014; Sieber, Eberhard, & Gallmann, 1996; Tremont *et al*., 2014; Villamiel *et al*., 1996; Wang & Guohua, 2005).

Such studies have also promoted the development of new MW-assisted devices for processing liquid foods and their potential application in the dairy industry with the deposit of several patents (Bukreev, Eremin, & Chekrygina, 1996; Chekrygin, Eremin, Bukreev, Rakitin, & Pikul, 1998; Jinxia, 2016; Kindratovych, 2009; Pang, Guo, & He, 1987; Yasuhiro, Junichi, Yoshiki, & Keiji, 2017). Unfortunately, there is still a lack of industrial application of MW systems, especially for pasteurization and sterilization of milk. Since 2013 though, a European-funded project called MicroMilk has been developing a new continuous MW system with the purpose of promoting industrial application of MW heating on the pasteurization/sterilization of milk (MicroMilk Project Website, 2013). MicroMilk project aims to: (i) develop an electro-thermal model of MW cavity to enable controlled and uniform heating; (ii) design, simulate, optimize, and develop a system operating at 60 L/h; (iii) reduce the cleaning time and minimize the contamination sources of milk via equipment; (iv) decrease cost of milk treatment from €1,200/ton with state of the art systems to less than €1,000/ton; and (v) enhance the microbiological, nutritional, and organoleptic quality and the shelf life of milk (MicroMilk Project Website, 2013). The development made so far has focused primarily on the pasteurization of milk but the research right now is focused on the development of MW technology for industrial UHT processing and other thermal applications (MicroMilk Project Website, 2013). In spited all of those studies, there is still a long journey until the MW technology can be truly applied in the dairy industry.

3 DIELECTRIC PROPERTIES

Briefly, the dielectric properties of a food material can be described as the ability of this material to convert MW energy to heat (Curet, Rouaud, & Boillereaux, 2014). A good knowledge and understanding of effects of dielectric properties of foods is essential in designing dielectric heating equipment, developing successful pasteurization processes, and selecting optimal frequency ranges and bed thickness for uniform treatments based on radio-frequency or MW dielectric heating (Zhu, Guo, &Wu, 2012). Furthermore, the characteristics of the electromagnetic field, chamber geometry, and dielectric properties of the food material determine the absorption of MW energy, and an understanding of their influence is important for the prediction of the dielectric heating rates of food products (Muñoz *et al.*, 2018; Nelson & Datta, 2001). However, the thermophysical properties of foods, such as heat capacity, density, shape, and size, also play an important role in heating processes, especially those associated with the heat transfer mechanisms.

The fundamental electrical property through which the interactions between electromagnetic waves and dielectric materials are described as the complex permittivity of the material ($\varepsilon*$) (Ahmed & Ramaswamy, 2007; Sosa-Morales, Valerio-Junco, López-Malo, & García, 2010). The interaction of the electric field and a dielectric material basically depends on the frequency. For polar liquids, like water, this frequency dependence can be successfully computed by Debye's model (Álvarez *et al.*, 2017), which is mathematically expressed as follows:

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_S - \varepsilon_\infty}{1 + j2\pi f\tau} \quad (1)$$

wherein ε_S and ε_∞ represent real static (low-frequency) and real high-frequency permittivity, respectively; j corresponds to the complex constant ($j = \sqrt{-1}$); f is the frequency in Hz; and τ is the relaxation time in seconds, where $\tau = 2\pi f_R$. The frequency f_R is called the relaxation frequency and is the value at which the dipole displacement lags that of the driving field by $\pi/2$. At this frequency, the imaginary part of the complex permittivity (energy absorption) reaches its maximum (Nunes, Bohigas, & Tejada, 2006).

For nonpolar substances or complex molecules (e.g., polymers), some modifications are reported based on relaxation time distributions. The most extended ones are the Cole–Cole and the Cole–Davidson models (Álvarez *et al.*, 2017). Complex permittivity can also be expressed as the sum of the relative electrical permittivity or dielectric constant (ε') and the dielectric loss factor (ε"). These terms correspond, respectively, to the real and imaginary parts of the complex permittivity, as expressed in Equation (2). They can also be directly computed from relaxation time and static and high-frequency permittivities, as it is shown in Equation (3) and (4):

$$\varepsilon^* = \varepsilon^{'} - j\varepsilon^{"} \quad (2)$$

$$\varepsilon^{"} = \varepsilon_\infty + \frac{\varepsilon_S - \varepsilon_\infty}{1 + j(2\pi f)^2 \tau^2} \quad (3)$$

$$\varepsilon^{"} = 2\pi f\tau \times \left[\frac{\varepsilon_S - \varepsilon_\infty}{1 + j(2\pi f)^2 \tau^2}\right] \quad (4)$$

The relative electrical permittivity represents the ability of the material to polarize and to store electric energy in response to an applied electric field, whereas the dielectric loss factor is associated with energy dissipation as heat. These parameters are affected by composition, temperature, and electric field alternating frequency (Franco *et al*., 2015; Sosa-Morales *et al*., 2009).

One of the most important parameters used in electromagnetic theory is the loss tangent ($tan\delta = \varepsilon''/\varepsilon'$), which describes the ratio of dielectric loss factor to relative electrical permittivity (Chandrasekaran *et al*., 2013). The mechanisms that contribute to the dielectric loss factor in heterogeneous mixtures like milk include polar, electronic, atomic, and Maxwell–Wagner responses. The amount of energy converted into heat in the food is proportional to the value of the dielectric loss factor (Tang, 2005). In the frequencies 915 and 2,450 MHz, dipole rotation and ionic conduction are the predominant mechanisms for the dielectric loss factor (Ryynänen, 1995; Tang, 2005).

$$\varepsilon'' = \varepsilon_d'' + \varepsilon_\sigma'' = 2\pi f \tau \times \left[\frac{\varepsilon_S - \varepsilon_\infty}{1 + j(2\pi f)^2 \tau^2} \right] + \frac{\sigma}{2\pi f \varepsilon_0} \quad (5)$$

wherein subscripts d and σ represent the contributions of the dipole rotation and ionic conduction mechanisms, respectively, and ε_0 is the permittivity of free space (8.854 x 10^{-12} F.m^{-1}).

As previously mentioned, there is a relationship between the dielectric loss factor and the electrical conductivity, which is why the dielectric loss factor in the MW and radio frequencies is dominated by dipole rotation and ionic conduction mechanisms (Guo, Liu, Zhu, & Wang, 2011). The term ε_d'' of Equation (5) corresponds to the dielectric loss associated with the dipole rotation mechanism. However, liquids with dissolved salts have another mechanism that contributes to the loss factor which is the ionic conduction (ε_σ''). Free ions move with the electric field oscillations, but do not contribute to polarization. In addition, as they are bound to water molecules, they reduce the electrical permittivity (bound water). The loss factor due to ion conduction can be calculated as proposed by the Hasted–Debye model (Ryyanänen, 1995).

$$\varepsilon_\sigma'' = \frac{\sigma}{2\pi f \varepsilon_0} \quad (6)$$

wherein σ is the electrical conductivity (S m^{-1}) of the material, f is the frequency of the electromagnetic field (Hz), and ε_0 is the permittivity of the free space (8.854 x 10^{-12} F m^{-1}).

The dielectric properties are decisive when it comes to understanding the response of a given material to electromagnetic waves applied (Tang, 2005). When exposed to an electromagnetic field, the amount of radiant energy converted to thermal energy is proportional to the value of the loss factor (ε”) (Álvarez *et al*., 2017).

$$P = 2\pi f \varepsilon_0 E^2 \varepsilon'' \quad (7)$$

Therefore, the temperature increase in the material due to dielectric heating can be estimated as:

$$\rho C_p \frac{dT}{dt} = 2\pi f \varepsilon_0 E^2 \varepsilon'' = 55.63 \times 10^{-12} f E^2 \varepsilon'' \quad (8)$$

wherein P is the power dissipated per unit volume (W m^3), C_p is the specific heat of the material (J kg^{-1} ºC^{-1}), ρ is the density of the material (kg/m^3), E is the intensity of the electric field (V/m), f is the frequency of the electric field (Hz), dT/dt is rate of the temperature increase (°C/s), and ε_0 is the permittivity of free space (8.854 × 10^{-12} F/m). The equation shows that the increase of the temperature is proportional to the loss factor of the material, besides the intensity, frequency, and time of treatment of the electric field (Komarov, Wang, & Tang, 2005; Nelson, 1996).

The volumetric heating effect of the MW is mostly caused by the penetration of the MW into the food matrix. As the MWs reach the surface of the food, part is reflected and the other part penetrates into the material. Such MWs that penetrate the surface of the food have an initial amount of energy, which is continuously diminished as the MWs go deeper into the food. This phenomenon occurs due to the fact that part of the MWenergy is being absorbed progressively along their trajectory inside the food material (Yam & Lai, 2006; Yang & Gunasekaran, 2004).

The penetration depth (d_p) is defined as the distance below the surface of a material where the MW power level drops to a value of $1/e$ (e = 2.718) of its surface value, i.e., drops to 36.8% of its surface value (Sosa-Morales *et al*., 2010; Yam & Lai, 2006), and is expressed as (Metaxas & Meredith, 1983):

$$d_p = \frac{c}{2\pi f \sqrt{2\varepsilon' \left[\sqrt{1 + \left(\frac{\varepsilon''}{\varepsilon'} \right)^2} - 1 \right]}} \quad (9)$$

wherein c is the velocity of light in free space (2.9979×10^8 m/s) and f is the frequency (Hz) of the electromagnetic field. This equation is valid for a perpendicular wave over a semi-infinite body.

Hence, from dielectric properties, the penetration depth of electromagnetic energy in selected materials can be calculated for given temperatures. Food products are generally poor with respect of dielectric conduction of MWs. Table 1 presents the dielectric properties of several dairy products. Normally, food products have $\varepsilon_r'' < 25$, which implies a d_p ranging from 0.6 to 1.0 cm (Venkatesh & Raghavan, 2004).

Penetration depth is an important parameter, which serves as a guideline for the heating efficiency of a food. Given the fixed dielectric properties, the penetration depth of a material is inversely proportional to the frequency (f), so it is expected that deeper penetration will correspond to the lower frequencies, and that higher frequencies will result in higher surface heating (Sosa- Morales *et al*., 2010). The temperature dependence for penetration depth is a complex factor to be analyzed, because of the interactive effect of dielectric loss factors associated with dipole rotation and ionic conduction mechanisms, which must be taken into account (Ahmed, Ramaswamy, & Raghavan, 2007).

Table 1. Relative electrical permittivity (ε’) and dielectric loss factor (ε”) of dairy products, cited in the literature at usual frequencies (915 MHz and 2450 MHz) as a function of temperature (Continued on next page).

Dairy product	Composition (g/100 g)	*f* (MHz)	*T*(°C)	ε’	ε”	Reference
Raw milk	Water (88.20)	915	20	72.8	15.3	Munoz *et al*. (2018)
	Fat (3.55)		40	67.6	17.7	
	Protein (3.24)		60	63.4	20.7	
	Lactose (4.68)		80	59.7	25.0	
	Ash (0.32)		100	55.6	29.5	
			120	51.4	33.4	
			140	48.7	39.3	
			150	44.4	42.0	
		2450	20	70.3	15.9	
			40	66.2	13.1	
			60	62.7	12.9	
			80	59.7	13.9	
			100	55.0	15.1	
			120	51.2	15.9	
			140	48.3	18.0	
			150	43.0	18.2	
Skim milk	Water (90.44)	915	20	73.7	15.7	Munoz *et al*. (2018)
	Fat (0.99)		40	68.6	18.2	
	Protein (3.29)		60	63.5	21.7	
	Lactose (4.68)		80	58.7	25.4	
	Ash (0.60)		100	54.3	29.6	
			120	50.3	34.4	
			140	47.2	39.1	
			150	45.6	41.8	
		2450	20	71.2	15.1	
			40	67.0	12.6	
			60	62.3	12.1	
			80	57.5	12.7	
			100	53.3	13.6	
			120	49.2	14.9	
			140	46.0	16.4	
			150	44.4	17.3	
Concentrated milk (35 g/100 g)	Water (65.45)	915	20	57.7	26.3	Munoz *et al*. (2018)
	Fat (0.23)		40	56.2	33.1	
	Protein (11.99)		60	54.1	43.0	
	Lactose (19.39)		80	52.0	53.4	
	Ash (2.94)		100	51.3	63.1	
			120	49.6	69.2	
			140	46.4	80.0	
			150	45.5	92.9	
		2450	20	51.8	19.0	
			40	51.6	19.0	
			60	49.8	20.9	
			80	47.7	23.9	
			100	47.2	27.1	
			120	45.4	29.2	
			140	41.9	32.9	
			150	40.9	37.8	

Table 1. Continued.

Adulterated milk	30[a]	915	22	70.9	11.9	Murthy, Kiranmai, & Kumar (2017)
	25[a]			70.4	12.4	
	20[a]			69.9	12.8	
	15[a]			69.5	13.3	
	10[a]			69.0	13.7	
	5[a]			67.7	14.2	
Unadulterated milk	Fat (2.90)			68.1	14.3	
	Solids (8.42)					
	Protein (2.37)					
Adulterated milk	30[a]	2450	22	69.1	12.4	
	25[a]			68.5	12.6	
	20[a]			68.0	12.9	
	15[a]			67.5	13.2	
	10[a]			66.9	13.4	
	5[a]			65.6	13.5	
Unadulterated milk	Fat (2.90)			65.9	14.3	
	Solids (8.42)					
	Protein (2.37)					
Cow milk	Water (87.1)	915	25	57.7	12.4	Zhu, Guo, & Jia (2014)
	Solids (12.79)		35	47.3	11.1	
	Fat (4.38)		45	41.1	10.4	
	Protein (2.90)		55	36.5	10.1	
	Ash (0.9)		65	33.0	10.3	
			75	29.5	10.5	
		2450	25	55.9	11.7	
			35	46.2	9.1	
			45	40.2	7.6	
			55	35.8	6.4	
			65	32.4	6.1	
			75	29.3	5.7	
Goat milk	Water (88.4)	915	25	62.9	15.8	Zhu, Guo, & Jia (2014)
	Solids (8.64)		35	50.9	13.8	
	Fat (3.05)		45	39.7	11.9	
	Protein (3.06)		55	32.9	10.9	
	Ash (1.0)		65	27.5	10.3	
			75	24.1	9.8	
		2450	25	61.3	13.4	
			35	50.0	10.3	
			45	39.2	7.8	
			55	32.5	6.5	
			65	27.1	5.9	
			75	23.7	5.4	
Salted butter		915	30	12.5	36.4	Ahmed, Ramaswami & Raghavan (2007)
Unsalted butter				25.6	4.9	
Salted butter		2450		9.0	15.5	
Unsalted butter				24.5	4.3	
Cheese	Fat (0)	2450	20	43	43	Datta, Summu, & Raghavan (2005)
	Fat (12)			30	32	
	Fat (24)			20	22	
	Fat (36)			14	13	

Table 1. Continued.

Skim milk	Fat (0)	915	20	66.5	15.1	Coronel, Simunovic, & Sandeep (2003)
Milk	Fat (1.5)			68.7	15.8	
Milk	Fat (4)			68.9	15.5	
Chocolate milk	Fat (1.5)			66.5	16.9	
Milk	Fat (1)	2450	20	70.6	17.6	Kudra *et al*. (1992)
	Fat (3.25)			68	17.6	

All data are presented as mean values
*Added water content for milk adulteration

4 FACTORS AFFECTING THE DIELECTRIC PROPERTIES

Several factors can affect the dielectric properties of a material. These factors are associated with MW operating conditions (frequency, temperature) and food material properties (composition, structure, shape, size, and so on) (Datta, Summu, & Raghavan, 2005; Muñoz *et al*., 2018; Sosa-Morales *et al*., 2010; Tang, Hao, & Lau, 2002; Venkatesh & Raghavan, 2004; Zhu, Guo, & Jia, 2014).

4.1 Frequency

The dielectric properties of most materials vary considerably with the frequency of the applied electric field. At low frequencies (<200 MHz), ionic conductivity is considered the most important mechanism associated with dielectric heating, whereas both ionic conductivity and dipole rotation play an important role at practical MW and radio-frequency range (300 MHz to 300 GHz; Komarov *et al*., 2005).

Frequency is also important because it affects the penetration depth by the MW radiation. In general, the lower the frequency, the greater the depth of penetration (Ragni, Al-Shami, Berardinelli, Mikhaylenko, & Tang, 2007; Sosa-Morales *et al*., 2010). Frequencies of 2,450 MHz generally have penetration depths of 0.6 to 1.0 cm, commonly used in domestic applications, but frequencies of 915 MHz, which have deeper penetration power are used in industrial applications (Datta *et al*., 2005; Mishra & Ramchandran, 2015; Salazar-González *et al*., 2012; Sosa-Morales, Méndez-Obregón, & Lopez-Malo, 2010).

Zhu *et al*. (2014) observed in cow and goat raw milk that the relative electrical permittivity decreased with increasing frequency and the dielectric loss factor decreased with increasing frequency below 1,000 MHz and increased thereafter. According to the authors, the MW penetration depth of the studied milks also decreased with increasing frequency.

Muñoz *et al*. (2018) studied the dielectric properties of raw, skimmed and 35 g/100 g nonfat concentrate milk at temperatures ranging from 20 to 150 °C and frequencies between 10 and 2,450 MHz. They verified that, as the frequency increases, the penetration depth, loss factor, and relative electrical permittivity of the milk tend to decrease. According to the authors, with the frequency increase the dipoles are less able to follow the oscillations of the electric field and the energy storage capacity decreases, thus decreasing this property.

4.2 Temperature

Product temperature can also significantly affect the dielectric properties and influence the efficiency of the MW heating. The loss factor generally increases with increasing temperature at low frequencies due to ionic conductivity (Coronel, Simunovic, & Sandeep, 2003; Guan, Cheng, Wang, & Tang, 2004) and decreases with increasing temperature at high frequencies due to the free water dispersion (Wang, Wig, Tang, & Hallberg, 2003) and, as the temperature increases, the penetration depth decreases (Sosa-Morales *et al*., 2010; Vicente & Castro, 2008; Zhu *et al*., 2014).

Muñoz *et al*. (2018) noticed that at the higher frequencies (2,450 MHz) the relative electrical permittivity and the penetration depth tend to decrease and the loss factor tends to increase with temperature. Nonetheless, at the frequency of 2,450 MHz, the loss factor decreases slightly in the temperature range from 20 to 60 °C and it increases above 60 °C for raw and skimmed milk, whereas for concentrated milk at 35 g/100 g, the loss factor increased with the temperature.

4.3 Food Composition

Water is a strong absorber of MW energy and the major component in most foods and, consequently, the higher the moisture content the better the MW heating (Sosa-Morales *et al*., 2010; Vicente & Castro, 2008). The free or bound state of the water influences the dielectric properties of food materials in different ways. Free water molecules are obviously more freely oriented than bound water during the application of dielectric heating (Komarov *et al*., 2005). As the amount of free water increases, the relative electrical permittivity and the loss factor also increase (Mishra & Ramchandran, 2015).

Furthermore, the thermal conductivity of the frozen foods is higher due to the high thermal conductivity of ice, whereas the lyophilized foods have lower thermal conductivity (Ahmed & Ramaswamy, 2007). Thus, during the thawing of food the hotter part (surface that melts first) is rapidly heated and, at the same time, there may still be ice in the food material, representing a nonuniformity problem of MW heating (Ryynänen, 1995).

Organic materials and salts can also affect the dielectric properties of food materials, which mostly depend on how they interact and/or are bound between them and with water molecule (Chandrasekaran *et al*., 2013). Salt solutions act as conductors at MW frequencies; the salts dissolve and form ions that bind the water molecules and reduce their polarization, thereby decreasing the relative electrical permittivity and increasing the loss factor (Icier & Baysal, 2004).

The heat generated during dielectric heating depends on the contents of moisture, fat, protein, and minerals present in the product which are governed by various factors, such as milk type, lactation, season, breed and feeding practices, as well as compositional changes during the various processing steps. Therefore, different heating behaviors can be expected and need to be accounted for MW processing (Mishra & Ramchandran, 2015).

MW heating of milk occurs at a faster rate than in water for the same MW heating system due to the presence of ionic components in the milk (Kudra *et al*., 1991). The presence of salt in butter promotes a decrease in the relative electrical permittivity and penetration depth and increases the loss factor under constant conditions of temperature and moisture (Ahmed *et al*., 2007). Ahmed and Luciano (2009) have shown that the dielectric properties of the β-lactoglobulin dispersions were significantly influenced by concentration and temperature and that the relative electrical permittivity and the loss factor increased at denaturation temperature of 80 °C.

Muñoz *et al*. (2018) identified that relative electrical permittivity of raw milk was slightly higher than skimmed milk. This difference was explained by the different compositions (water, fat, and ash content) of both types of milk.

Murthy, Kiranmai, and Kumar (2017) studied the influence of water content (concentration) and milk freshness on the dielectric properties of the product simulating adulteration. They observed that the unadulterated/undiluted milk showed lower relative electrical permittivity and had the greatest loss factor when compared to the diluted milk, and that the depth of penetration increased with increasing water content. They concluded that changes in dielectric properties were sufficient to identify adulterations that could lead to economic losses, deterioration of the quality of the final products and a risk to consumer safety.

4.4 Physical Structure

The physical structure of foods, such as volume, density, and shape, affects the dielectric properties of foods and influences the absorption of MW energy (Mishra & Ramchandran, 2015). The capacity to absorb MW energy is proportional to the volume of the heated material (Mishra & Ramchandran, 2015), whereas the density is positively correlated with the relative electrical permittivity and loss factor, and therefore with the heating rate (Guo, Tiwari, Tang, & Wang, 2008; Vicente & Castro, 2008; Wang *et al*., 2003). Irregularly shaped foods exhibit more uneven heating (Salazar-González *et al*., 2012), a well-known problem in MW heating, particularly in solids with sharp edges.

5 MICROWAVE HEATING AND FOOD SAFETY

Raw milk may be contaminated by a vast variety of microorganisms, such as *Salmonella* sp., *Escherichia* O157:H7, *Campylobacter* sp., *Escherichia coli*, and *Listeria monocytogenes*, among others (Claeys *et al*., 2013; Gaulin *et al*., 2012; Hunt, *et al*., 2012; Serraino *et al*., 2013). One of the most important issues in the safety of milk and dairy products is the efficacy of the treatment applied for the purpose of removing all pathogenic microorganisms.

MW heating is a novel and alternative thermal technology that has been studied to ensure safety and increase shelf-life of milk and dairy products and to increase the nutritional and sensory quality of food products in comparison to conventional heating methods such as hot water, steam, hot gas, and so on (Mishra & Ramchandran, 2015; Salazar-González *et al*., 2012). The preservation effects of electromagnetic heating methods has been generally credited for their thermal effects, that is, resulting mainly from the increase of temperature, but other mechanisms may also be involved, such as (i) selective heating of the microbial cells to a higher temperature level than the surrounding fluid, leading to a more rapid/intense destruction; (ii) electroporation due to the electric potential that crosses the membrane which can generate pores in the cells leading to rupture of cell membranes and leakage of cellular material (a major influencing factor in the field of extraction and dehydration); and (iii) the magnetic field coupling the vital components of the cell, such as proteins or DNA, which can enhance the destruction effect (Guo *et al*., 2017).

MW thermal destruction of the microorganisms generally follows first-order kinetics, and the determination of the D-value (rate at a specific temperature necessary to reduce a population of organisms by 90%) and the z-value (temperature increase necessary to reduce the D-value of a microorganism in 90%) are of fundamental importance for the quantification of the microbial destruction delivered by the process (FDA, 2000). Factors such as pH, levels and types of preservatives, water activity, previous growth conditions of microorganisms of interest, food composition, and presence/absence of competitive microorganisms may also be able to influence the kinetics of thermal destruction of microorganisms within the food matrix (FDA, 2000).

Even though MW heating is based on volumetric heat generation and, therefore, presents a much more uniform temperature distribution than conventional methods, its nonuniform temperature distribution with the formation of cold spots is still one of the main problems associated with the use of this thermal technology, especially when reaching specific temperatures becomes a safety target (Chandrasekaran *et al*., 2013).

Thermal effect is the main mechanism considered to be effectively responsible for the inactivation of microorganisms and enzymes during MW heating. Thus, the determination of cold spots and the certainty that the product reaches the necessary temperature in all places to guarantee lethality, is the most important safety requirement that the dairy industry must achieve (Mishra & Ramchandran, 2015). The main problem is that in volumetric heating, the identification of the cold spot is not an easy task, because heat is continuously generated within the product, and is influenced by several factors, such as food composition, equipment design, frequency, and power level (Datta & Tomasula, 2015). This nonuniform temperature distribution results in the formation of overheated and under-heated spots; thus, microorganisms may not be completely and uniformly destroyed during MW treatment (Vadivambal & Jayas, 2010).

Coronel *et al*. (2003) studied the temperature profile of skimmed milk treated in a MW continuous system at 915 MHz with flow rates of 2 and 3 L/min at laminar flow conditions.

These authors identified that cold spots were mostly closer to the walls of the cylindrical polytetrafluoroethylene tube (0.039 mm of internal diameter and 0.124 m of length). According to Vicente and Castro (2008), nonuniform MW heating can result in undetected cold spots that can have serious impact on food safety, especially in solid and semisolid food products.

To reduce this nonuniformity in temperature distribution during MW heating several solutions have been proposed, such as combination of conventional and MW heating, food shape control, improvement of oven design, manipulation of heating cycle, and reduction of the power to achieve longer heating ramps, and so on (Vadivambal & Jayas, 2010).

Clare *et al*. (2005) demonstrated that MW processing can replace conventional sterilization (UHT) of the milk with guaranteed stability for a period of 1 year. Several later studies also highlighted the ability of MW heating to eliminate/inactivate microorganisms such as *E. coli*, *Salmonella* sp., *S. aureus*, *P. aeruginosa*, *B. subtilis*, *Cronobacter sakazakii*, to inactivate toxins such as Shiga (Stx2) toxin produced by toxigenic E. coli, and to inactivate enzymes, such as alkaline phosphatase in dairy products (Géczi *et al*., 2013; Laguerre *et al*., 2011; Lin & Ramaswamy, 2011; Manjunatha *et al*., 2012; Pina-Pérez *et al*., 2014; Rasooly *et al*., 2014; Tremonte *et al*., 2014; Table 2).

Table 2. Microwave heating (MWH) applied on milk and dairy products in order to inactivate microorganisms and enzymes, according to studies reported in the literature (Continued on next page).

Process target	Dairy product	Assay and results	Conclusions	Reference
- Mesophilic Bacteria - Coagulase-negative staphylococci (CoNS) - Lactic acid bacteria (LAB) - *Enterobactereaceae* - Fecal coliforms - Total coliforms - *Enterococcus* spp. - *Pseudomonas* spp.	Milk	Effect of domestic microwave heat treatment (750 W and 900 W/75 s) compared to boiling was studied to evaluate the safety of consumption of raw milk.	Microwave treatment at 750 W for 75 s resulted in partial inactivation of microorganisms in milk. The application of 900 W for 75 s yielded a milk with better microbiological quality whose effectiveness was proven with negative alkaline phosphatase test and positive peroxidase test.	Tremont *et al*. (2014)
- Shiga toxin (Stx2) produced by toxigenic *E. coli*	Solution of skim milk powder (5 g/100 g)	Effect of MW treatments (A = 25 min, 10% duty cycle for 165 kJ and 65 °C at the end, B = 20 min, 20% duty cycle for 198 kJ and 78 °C at the end) compared to treatment thermal (95 °C/5 min or 63 °C/30 min).	The low energy level in the MW treatment (165 kJ) and conventional heat treatment (63 °C/30 min) did not inactivate the Stx2 toxin, but the high energy level (198 kJ) and conventional heat treatment (95 °C/5 min) reduced its activity.	Rasooly *et al*. (2014)
- *Cronobacter sakazakii*	Reconstituted infant formula	Effect of MW treatments in the 400 to 900 W/0 to 120 s range and post-treatment storage study (5 °C/24 h) to determine recovery or death of damaged and surviving bacteria. Power levels of 800 W ($D_{79\ °C}$ = 14.33 s) and 900 W ($D_{88\ °C}$ = 13.66 s) reduced the initial population to undetectable levels (≥8 $\log_{10}$ cycles).	A high sensitivity of bacterial cells to MW was observed. Lower power intensities provide higher percentages of damaged cells that progressively die during refrigerated storage (up to 24 h) reaching reduction levels greater than or equal to 5 $\log_{10}$ cycles.	Pina-Pérez *et al*. (2014)
- Viable cell count	Milk	Effect of MWH (900 W) compared to conventional under identical conditions of final temperature and treatment time. The initial total viable cell count was 50,000 to 350,000 CFU/cm^3).	The treatments promoted a statistically significant decrease in the total viable cell count in comparison to the control, with no significant difference between the two heating methods.	Géczi *et al*. (2013)

Table 2. Continued.

- *E. coli* - *Salmonella* spp. - *S. aureus* - *P. aeruginosa* - *B. subtilis*	Whole milk Skim milk Milk concentrates Fresh cheese	The authors studied the effect of MWH (2,450 MHz/1,200 W) on the thermal resistance of bacterial cultures. In whole and skim milk, the time required for complete destruction of vegetative cells was 40 s, except for *E. coli* and *B. subtilis*, which were both effectively destroyed at 50 s. In concentrated milk, the complete destruction of the vegetative cells was achieved at 60 s. The same time was necessary in the fresh cheese; however, 50 s was sufficient for *Salmonella* spp.	Among the five bacterial cultures studied, S. aureus and *P. aeruginosa* were the most sensitive to MW radiation, while the *B. subtilis* culture was the most resistant.	Manjunatha, Prabha, Ramachandra, Krishna, & Shanka (2012)
- Alkaline phosphatase	Milk	Enzymatic inactivation by MWH (2,450 MHz) during continuous flow was compared to conventional pasteurization. The *D*-values of the conventional heating ranged from 1250 s at 65 °C to 1.7 s at 75 °C (z = 5.2 °C) in batch heating, 128 s at 65 °C to 13.5 s at 70 °C (z = 5.2 °C) in a continuous flow system. For MWH the *D*-value ranged from 17.6 s at 65 °C to 1.7 s at 70 °C (z = 4.9 °C) under continuous heating flow.	The *D*-values associated to the inactivation of alkaline phosphatase by MWH occurred much more rapidly than conventional process, emphasizing the possible existence of improved thermal effects of MW in the inactivation of enzymes.	Lin and Ramaswamy (2011)
- *G. stearothermophilus*	Infant formula	The authors studied the effect of microwave treatment (2,450 MHz) at 5, 7, 10, 12, 15, and 28 W/mL for 10 to 350 s on sterilization of non-pathogenic spore *G. stearothermophilus*. As a thermally resistant spore, a reduction of 5 log cycle was considered efficient rather than 12 log cycle reduction as required for *C. botulinum*.	The linear curves obtained with the destruction of the spores of *G. stearothermophilus* follow a first order kinetics. The minimum heating times that allowed an acceptable sterilization of the product were 14 s at 40 W/mL and 1.4 s at 60 W/mL.	Laguerre *et al*. (2011)

6 MW HEATING AND INTRINSIC QUALITY PARAMETERS IN DAIRY PROCESSING

The The major nutritional components of milk are proteins (casein and whey proteins), fats, carbohydrates (lactose), vitamins, and minerals (Pardo *et al*., 2013). This composition can be influenced by factors such as type and intensity of the heat treatment. The influence of such processes for food preservation should therefore be evaluated for quality retention in food products (Dumuta *et al*., 2011).

The most important structural and chemical changes that occur during MW heating are protein denaturation and aggregation, Maillard reactions, and lactose isomerization, which are mostly related to thermal effects of MW heating (Iuliana *et al*., 2015). Hence, indicators traditionally employed in conventional thermal treatments can also be used to determine the severity of MW heating, such as appearance of lactulose, furosine, 5-hydroxymethylfurfural (HMF), fluorescence of advanced Maillard products, and soluble tryptophan (FAST) index, $_N{}^{\varepsilon}$-1-carboxymethyl-*L*-lysine (CML), glycoxidation products, denaturation, and aggregation of proteins (*β*-lactoglobulin), free fatty acids (FA), and so on (Dehghan *et al*., 2012; Laguerre *et al*., 2011; Rodríguez-Alcalá *et al*., 2014).

Dumuta *et al*. (2011) observed a reduction of protein bioavailability with the application of MW heating. This decrease was explained by the fact that the sulfhydryl groups of whey proteins, typically structured in the nucleus of the protein molecule, are exposed to heating. In addition, sulfhydryl groups may be formed because of hydrolysis or β-elimination of disulfide bonds during the heat treatment (Dumuta *et al*., 2011; Iuliana *et al*., 2015; Kamel, Ali, Farid, & Nadji, 2014). *β*-Lactoglobulin is often used as an indicator in the evaluation of heat damage to milk and dairy products because it is one of the most thermosensitive and major components of whey protein (Iuliana *et al*., 2015; Kamel *et al*., 2014).

The Maillard reaction is also one of the most important causes of sensory quality and nutrient loss in heat-treated milk and dairy products, which is mainly caused by a significant loss of the essential amino acid lysine (2% to 3% in pasteurized and 7% to 10% in UHT milk) due to its nonenzymatic reaction with lactose. Furthermore, it promotes the production of off-flavors and brown-colored pigments (melanoidins), as well the polymerization of milk protein (Dumuta *et al*., 2011; Iuliana *et al*., 2015; Tessier, Gadonna-Widehem, & Laguerre, 2006).

The Maillard reaction is a nonenzymatic browning chemical reaction between amino groups from peptides/proteins and carbonyl groups from reducing sugars, which is generally enhanced by heat. Initially, the reaction produces *n*-substituted glycosyl amine, which undergoes Amadori rearrangement, forming ketosamines, subsequently producing an extremely variable number of very reactive intermediate substances. These intermediates may react in several different ways, producing a wide range of volatile compounds, contributing to the flavor of the food, and nonvolatile brown-colored melanoidins, which are responsible for color (Mazzei, Botrè, Favero, Podestà, & Botrè, 2009).

In general, in the Maillard reaction in milk, lactose mainly reacts with the *ε*-amino group of lysine residue of milk proteins (mostly casein). The reaction then progresses with the formation of several intermediate compounds, such as HMF, formic acid, and methyl ketones, resulting in the formation of melanoidins and advanced glycation end-products (AGEs), which may be fluorescent cross-linking (e.g. pentosidine), nonfluorescent cross-linking (methylglyoxal-lysine dimers), and nonfluorescent non-cross-linking (CML and pyrraline; Deeth, 2010; Oh, Kim, Hoon Lee, Lee, & Park, 2017). AGEs have been related to several chronic and degenerative diseases, such as diabetes, renal failure (Šebeková & Somoza, 2007), atherosclerosis (Wang *et al*., 2012), and Alzheimer's and Parkinson's diseases (Li, Liu, Sun, Lu, & Zhang, 2012), as well as the formation of mutagenic compounds (Brands, Alink, van Boekel, & Jongen, 2000; Oh *et al*., 2017).

Furthermore, intermediate compounds can also be used to determine Maillard browning severity of the heating. For example, lactulosylamine, and subsequently N^{ε}-1-deoxylactulosyl-L-lysine, is one of the main Amadori compounds formed during the early Maillard reaction, which can be partially converted to stable N^{ε}-2-furoylmethyl-L-lysine (furosine) during acid hydrolysis, a stable compound widely used as a heating indicator. This modified lysine is not effective or available as a nutrient; hence, its decrease over time has become an indicator for the assessment of deleterious effects of heat treatments on the nutritional quality of milk and dairy products (Kamel *et al*., 2014). HMF is also used as a heat indicator, but it suffers from the disadvantage of being both formed and degraded during the progress of the Maillard reaction (Oral, Mortas, Dogan, Sarioglu, & Yazici, 2014; Sakkas, Moutafi, Moschopoulou, & Moatsou, 2014). Methyl ketones have been recently proposed as an indicator of heat treatment of milk; however, they can also be produced during storage from lipid oxidation (Deeth, 2010). Formic acid produced by the Maillard reaction contributes to the decrease in pH during heat treatment. The advanced Maillard products are only observed after very severe UHT heating and hence brown discoloration of milk during normal UHT heating is not usually observed. However, it may be observed in modified milks, such as lactose-hydrolyzed milks, or dairy products, such as *dulce de leche* and milk powders in which Maillard reactions are enhanced (Deeth, 2010). Maillard reaction also shows various effects on bioavailability, solubility, forming property, emulsifying property, and heating stability of milk proteins.

In addition, the decrease in lactose content also serves as an indicator of the severity of heat treatment, and during the first phase of this reaction lactose is isomerized into lactulose, another indicator (Dumuta *et al*., 2011). Lactulose is considered as a very useful indicator. It is not a substance of raw milk, is not detected in pasteurized milk and results from the conversion of glucose to fructose during heating. It is proposed as a heating indicator for UHT milks, considering that a laculose content higher than 600 mg/L corresponds to UHT milk, being rather stable during storage (Sakkas *et al*., 2014).

Laguerre *et al*. (2011) evaluated the impact of the heat treatment on the content of newly formed contaminants (NFC) through the FAST index, CML, and furosin, and observed that all indicators seemed to follow an exponential model as a function of time. The authors found that the best way to minimize NFC formation and nutrient degradation was to use high energy for a short time, similar to that obtained with the UHT process for conventional heating (Laguerre *et al*., 2011). CML is used to measure the overall contribution of various substrates, including lactose, vitamin C, and aldehydes derived from the Maillard reaction (Birlouez-Aragon, Moreaux, Nicolas, & Ducauze, 1997; Fu *et al*., 1996), and it has been reported to be well correlated with the extent of lysine blockage in milk (Leclère, Birlouez-Aragon, & Meli, 2002).

Dehghan *et al*. (2012) observed that MW heating, similar to HTST pasteurization, maintains the original level of protein in milk. However, lysine was considered the most sensitive amino acid and a decrease in color parameters *L* (lightness) and an increase in *b* (yellow-blue) was observed, suggesting the occurrence of the Maillard reaction. Similar results were also observed by Géczi *et al*. (2013) and Tremonte *et al*. (2014).

Tu *et al*. (2014) observed that MW heating at 400 W for 15 min changes the isoelectric point of proteins of conjugated protein-lactose compounds resulting in a more acidic environment. According to these authors, this can increase the application of milk protein, expanding its application to acidic foods. This phenomenon was achieved at a pH of 6.6, which apparently promoted an improvement in its antioxidant activity for *in vitro* digestion using 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azino-bis(3-ethylbenzothiazoline-6-

sulfonic acid) assays. Moreover, the microstructure of the compounds became irregular after MW heating, promoting greater protein solubility.

Dumuta *et al*. (2011) observed a reduction of the fat content in milk during MW heating. Milk lipids may undergo chemical and physical changes during processing and storage, such as auto-oxidation and formation of *trans* FAs (TFAs; Semma, 2002), and this fact may be an explanation for a decreasing fat content (Mishra & Ramchandran, 2015); however, such reduction in fat content was not observed in other similar studies (Dehghan *et al*., 2012; Géczi *et al*., 2013).

Sengar, Sharma, and Kumar (2015) indicated there was a decrease in the relative percentage of unsaturated FAs and an increase in the relative percentage of saturated FAs (SFAs) and free FAs as a function of MW energy and time. The content of the free FAs contributes to the development of off-flavor in oils and fats and the decrease in the C18:2/C16:0 ratio is indicative of the detrimental alteration in the lipid profile (Hassanein, El-Shami, & El-Mallah, 2003; Herzallah, Humeid, & Al-Ismail, 2005; Sengar *et al*., 2015).

However, Rodríguez-Alcalá *et al*. (2014) affirmed that MW heating at 650 W for 1.30 s did not change the concentration of total SFAs, monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), and CLA; and as FA composition was detailed, a better stability was achieved for most compounds in all samples.

Iuliana *et al*. (2015) and Dumuta *et al*. (2011) observed a reduction in the total solids content after the application of MW heating to cow milk and concluded that it resulted in a quantitative decrease in protein, lactose, and fat contents and also possible decrease in some vitamins, for example, A, B9, C, and E. The total solids content was reported to be an indicator of the nutritional value of milk and to have a direct effect on the use of raw milk for concentrated milk products. Furthermore, viscosity and electrical conductivity had positive correlations with protein and fat contents, respectively, also serving as indicators (Iuliana *et al*., 2015).

Bai, Saren, and Huo (2015) showed that the variables milk layer thickness (also known as milk skin or lactoderm, a sticky film of protein that forms on top of milk and milk-containing liquids), MW time, and MW power have the opposite effect on vitamin C concentration in milk treated by MW heating. Milk layer thickness was reported to be the most significant factor, and the effects of MW time and energy were dependent on this variable. One of the most serious threats to the nutritional value of milk is the destruction of heat-sensitive vitamins, like vitamin C, that may serve as an indicator of thermal degradation and nutritional quality (Bai, Saren, & Huo, 2015; Laguerre *et al*., 2011).

Table 3 describes the published studies about MW heating effects on quality parameters that are intrinsic to dairy products. There is a lack of studies on processed dairy products such as flavored milk, cheese, butter, fermented milk, and so on, which opens up a number of research opportunities in this area.

In the last decade, an alarming increase in the incidence of milk allergy has been observed. Cow milk is considered the most common cause of food allergy in young children (about 2.5%). This fact requires efforts for the development of technologies capable of reducing the immunoreactivity of allergens (Bøgh, Barkholt, & Madsen, 2015; El Mecherfi, *et al*., 2015).

Milk protein allergy begins with the generation of immunoglobulin E (IgE) by the immune system, which binds to the allergen at a specific site triggering subsequent immunological events (Sabra *et al*., 2003). Interruption of the IgE binding epitope in linear immunoglobulins can be reduced by fragmentation (Mondoulet *et al*., 2005). According to El Mecherfi *et al*. (2015), peptic hydrolysis of bovine *β*-lactoglobulin associated with MW heating reduced its in vivo allergen capacity. The authors applied MW heating combined with peptic hydrolysis at 50 W for 5 min, 100 W for 3 min, and 200 W for 3 min in whey protein or bovine *β*-lactoglobulin solution. Both conventional and MW heating treatments were performed under the same temperature and time conditions. Significant changes were reported in the tertiary and secondary structures of bovine *β*-lactoglobulin that may make this protein more accessible to protease attack, leading to more efficient hydrolysis of more resistant epitopes (El Mecherfi *et al*., 2015). Thus, MW treatment at 200 W might generate peptides with low IgE immunoreactivity in a short time (3 min of hydrolysis), and a potential use of these findings is to produce whey protein formulas with low IgE immunoreactivity and antigenicity (El Mecherfi *et al*., 2015).

Table 3. Assessment of the MWH effects on quality parameters in dairy products (Continued on next page).

Parameter	Dairy product	Assay and results	Conclusion	Reference
- Peroxide index - Colour - Free fatty acids (FFA) content - Iodine index - C18:2/C16:0 ratio - Differential Scanning Calorimetry (DSC)	Hydrogenated fat from palm and butter (3:2)	The MWH was applied for 5, 10, and 15 min in low and high-power configurations and temperature varied between 93 °C and 159 °C. FFA content, peroxide index and color parameters showed an increase of 16.6%, 19.27%, and 78.5%, respectively with MW power increase and time adjustment. On the other hand, iodine index and C18:2/C16:0 ratio presented a decline of 19.27% and 21.5%, respectively.	The DSC showed a positive correlation with FFA, and negative with iodine index and C18: 2/C16: 0 ratio at all process and power times. The physical-chemical and thermal behavior reveals the stability of the combined fat during MWH.	Sengar *et al*. (2015)
- Fat content - Protein content - Total solids content - Solids-not-fat content - Lactose content - Density - Dynamic viscosity - Surface tension - Specific gravity - Electrical conductivity	Milk	MWH (800 W) was applied in a range from 0 to 120 s. The contents of fat, protein, total solids and lactose decreased whereas solids-not-fat content increased during MWH. A negative correlation (R=−0.956) was observed between lactose content and electrical conductivity and positive correlation (R = 0.937) between protein content and surface tension.	The use of MWH can reduce the nutritional value of milk and it can also decrease its yield for concentrated products. Physical parameters were considered sensitive indicators of milk quality.	Iuliana *et al*. (2015)
- Vitamin C	Milk	The effect of the MW treatment varying the parameters: milk layer thickness (3, 4, and 5 cm); time (40, 60, and 80 s); and power (160, 320, and 480 W).	The variables milk layer thickness, microwave time and microwave power have the opposite effect on vitamin C concentration in milk treated by MWH. Milk layer thickness was the most significant factor, and the effects of MW time and energy were dependent on this variable.	Bai *et al*. (2015)

Table 3. Continued.

- Whey protein - Total protein content	Milk	The effect of the MW treatment (750 W and 900 W/75 s) compared to boiling was studied to evaluate the protein quality of raw buyer milk. In boiled milk the proportion of whey protein in the total protein content decreased from 19.3% to 4.3% in raw milk, whereas for MWH the values were 18.5% (750 W) and 16.5% (900 W)	MWH (750 W) did not lead to a significant reduction in soluble protein content compared to raw milk. However, boiling promoted significant reduction compared to raw milk and MWH (750 W and 950 W). The results suggest that boiling causes severe thermal damage to the nutritional quality of raw milk and MWH may be an alternative to preserve its nutritional characteristics.	Tremont *et al*. (2014)
- Fatty acids profile: (SFA, MUFA, PUFA CLA)	Milk Milk enriched with PUFA	Effect of MWH (650 W, 1.30 min) compared to pasteurization (72 °C, 30 s), HTST (85 °C, 30 s) and UHT (UHT1: 135 °C, 30 s; UHT2: 150 °C, 5 min).	The concentration of SFA, MUFA and PUFA remained stable for milk and milk enriched after pasteurization: HTST, UHT1, UHT2, and MWH. CLA concentration increased for HTST-treated milk and sterilization led to changes in the distribution of these fatty acids.	Rodríguez-Alcalá *et al*. (2014)
- Colour parameters - Protein content - Fat content	Milk	Effect of MWH pasteurization (900 W) compared to conventional heating under identical conditions of final temperature and treatment time. The initial fat and protein contents are 3.71 g/100 g and protein 3.32 g/100 g, respectively.	The milk protein and fat contents were not affected by any of the heating treatments. The color of the samples treated with MW differed not only from the untreated control, but also from the thermally treated traditional samples.	Géczi *et al*. (2013)
- Protein content - Aminoacids profile - Protein solubility (%) - Fat content - Fatty acids profile - Colour parameters	Milk	The effect of MWH (2,450 MHz and 540 W) with a heating ramp of 6 min until 85 °C, which was maintained for 15 s was compared to a HTST system under the same conditions. The content of protein, fat and fatty acids profile did not differ from raw milk in both pasteurization methods. The lysine content decreased significantly in relation to raw milk and HTST milk. The color parameter L∗ decreased and b∗ increased in the thermally treated milk.	The pasteurization of milk with MW does not have negative effects on the nutritional value of milk compared to the HTST method. Considering the shorter pasteurization time and the lower energy demand, the MW pasteurization of the milk has a higher potential for industrial application.	Dehghan, Jamalian, Farahnaky, Mesbah, & Moosavi-Nasab (2012)

Table 3. Continued.

- Protein content - Fat content - Total solids content - Lactose content - Density	Milk	MWH (800 W) was applied during times 0, 15, 30, 45, 60, and 120 s. The concentrations of fat, protein, total solids and lactose decreased with time while density increased. Physicochemical and composition parameters began to undergo pronounced changes at 31.71 s.	The increasing density values suggest sample concentration, especially during the second minute of the experiment, since the MWH occurred with open pots.	Dumuta *et al*. (2011)
- Vitamin C - Tryptophan - FAST index - carboxymethyllysine (CML)	Infant formula	MWH effect (2,450 MHz) at 5, 7, 10, 12, 15, and 28 W/mL and times 10 to 350 s on sterilization process of infant formula samples. A linear relationship was observed for the log of the vitamin C content. Moreover, FAST index and the CML content showed an increase as a function of time.	The maximum acceptable nutritional loss for sterilization was fixed at 50% for vitamin C and tryptophan, and the content of CML was set at 5.4 mg/100 g protein and the FAST index was set at 50% of the reference index. These values were reached at 14 s for 40 W/mL and 1.4 s for 60 W/mL.	Laguerre *et al*. (2011)

7 ADVANTAGES AND DISADVANTAGES

MW heating presents some advantages in comparison to the conventional techniques (Bhushand *et al*., 2017; Chandrasekaran *et al*., 2013; Guo *et al*., 2017; Mishra & Ramchandran, 2015; Salazar-González *et al*., 2012), which include:

(I) Faster heating: Volumetric heat generation with high heating efficiency (80% or more achieved);

(II) Shorter processing time: Uses about a quarter of the time used in conventional heating;

(III) Better quality retention in the processed product: because of the high heating rate the target temperature is reached quickly, thus reducing the thermal impact on the food and minimizes the deleterious effects on sensory and nutritional characteristics of the food;

(IV) Suitability of application for thermosensitive, high viscosity, and multiphase fluids;

(V) Possibility of safe handling, with pasteurization after packaging;

(VI) Ease of operation, low space requirement, and low energy consumption (high efficiency), reduction of noise levels, and low maintenance cost;

(VII) Heating systems can be switched on or off instantaneously;

(VIII) Minimal fouling due to the elimination of hot heat transfer surfaces, since the tubing used is transparent and remains relatively cooler than the product;

(IX) Environmentally clean processing, since the generation of MW does not produce any pollutants or toxic wastes.

The main drawback refers to nonuniform temperature distribution, resulting in hot and cold spots mainly in solid and semi-solid products; however, MW heating has been shown to be suitable for liquid foods in a continuous fluid system (Salazar-González *et al*., 2012). Nevertheless, in a heterogeneous system such as milk, fats, and colloidal dispersions are substances that obstruct the ions in their migration, reducing conductivity, which promotes nonuniformity in the generation of heating (Iuliana *et al*., 2015; Vadivambal & Jayas, 2010).

The thawing of food is a classic example of the possibility of non-uniformity of the MW process and one of its limitations, the surface is heated more rapidly and may be degraded and, at the same time, there may still be ice in some spots of the product (Ryynänen, 1995).

The lack of experimental data needed for MW heating modeling, analysis of energy, and manufacturing costs, the necessity of technical expertise to understand and minimize heat leakage, and high initial investment are also considered limitations of the process (Chandrasekaran *et al*., 2013; Khan *et al*., 2018). The only study about energy consumption during MW heating was performed by McConell (1974), who compared the energy consumption of a MW oven and the surface elements and oven of a conventional electric range. A wide variety of food types and menus were evaluated and the results indicated that MW oven consumed less energy in 100 of 127 samples tested. More robust studies need to be made in order to evaluate manufacturing costs of MW heating and compare with conventional thermal processes.

8 PERSPECTIVES

There is an increase in industrial interest in the application of MW heating. Research centers and industries are seeking to develop new technologies capable of minimizing energy costs and the destructive effects of conventional thermal processing on the sensory and nutritional aspects of food but must guarantee the microbiological safety and stability of the products.

The advances should be based on the lack of detailed information or validation procedures for the safety of dairy products treated by MW heating, and investigations should proceed towards:

(I) Understanding the effects and parameters associated with the physicochemical properties of food;

(II) Developing sophisticated methods of quality and process control to ensure a uniform heating rate;

(III) Developing more effective systems for identifying and measuring cold spots during food processing, especially solid, semisolid, and high-fat foods;

(IV) Determining the electrical effects on the inactivation of enzymes like the increase or reduction of their thermal resistance;

(V) the kinetics of inactivation of relevant microorganisms in various milk matrices;

(VI) Determining manufacturing cost as well as the capacity of milk or dairy products that can be processed by MW heating in comparison to conventional thermal treatments.

Although studies related to the use of MW heating with dairy products are scarce in the literature, the benefits of its MW use have been reported worldwide. This view makes it a promising technology for future commercial use that will continue to be investigated in response to consumer demands for fresh and healthy products without compromising safety, nutritional, and sensorial aspects.

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CAPÍTULO II

EFFECTS OF MICROWAVE HEATING ON THE CHEMICAL COMPOSITION AND BIOACTIVITY OF ORANGE JUICE-MILK BEVERAGES

EFFECTS OF MICROWAVE HEATING ON THE CHEMICAL COMPOSITION AND BIOACTIVITY OF ORANGE JUICE-MILK BEVERAGES

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Effects of microwave heating on the chemical composition and bioactivity of orange juice-milk beverages

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ABSTRACT

The effect of microwave heating (MH, 65 and 75 ◦C for 15, 30, and 60 s) on the bioactive compounds, fatty acid profile, and volatile compounds of orange juice-milk beverage (OJMB) was evaluated during 28 days of refrigerated (4 ◦C) storage. Conventionally pasteurized (75 ◦C/15 s) and untreated beverages were used as controls. MH-OJMB presented a lower browning index and higher levels of ascorbic acid, total phenolics, and carotenoids, higher antioxidant activity, and greater α-amylase, α-glucosidase, and ACE inhibitory activity than the pasteurized product, similar to the untreated beverage. No significant differences were observed in the volatile organic compounds and fatty acids levels. Lower temperatures (65 ◦C) and longer process times (60 s) resulted in higher retention of bioactive compounds. MH can be an alternative to conventional pasteurization for OJMB processing.

Keywords: microwave heating, Fruit juice, dairy beverages, food processing

1 INTRODUCTION

The consumption of orange juice has been associated with health benefits, such as the control of low-density cholesterol and high blood pressure (Hornero-Méndez *et al*., 2018). In addition, when used in technological applications, orange juice enhances the flavor and color attributes, thus improving the sensory characteristics of the products (Miranda *et al*., 2019). Milk and dairy products contain a significant level of proteins, minerals (calcium, magnesium, selenium), vitamins (riboflavin, zinc, B12, pantothenic acid), and essential fatty acids (Hidayat, Du, & Shi, 2019). They also contain bioactive peptides that display immunomodulatory, antimicrobial, antithrombotic, and anticarcinogenic activities (Tonolo *et al*., 2020). Thus, the production of orange juice-milk beverage (OJMB) can be an alternative to the dairy market.

Thermal pasteurization is the most commonly used method for the preservation of orange juices and milk, aimed at promoting microbiological safety and increasing the shelf life of the products (Munoz, Gou, Picouet, Barlabe, & Felipe, 2018: Paniagua-Martínez *et al*., 2018). However, high process temperatures can lead to the degradation of nutrients, bioactive compounds, and volatile organic compounds (VOCs), in addition to protein denaturation and non-enzymatic browning, resulting in great changes in the sensory and nutritional characteristics of the products (Martins *et al*., 2019). Therefore, there is a growing interest in developing alternative techniques that can minimize the changes in the nutritional and sensory characteristics of foods (Paniagua-Martínez *et al*., 2018).

Microwave heating provides greater energy efficiency and reduces heating times, which can provide products with better sensory and nutritional quality when compared to those subjected to conventional pasteurization (Martins *et al*., 2019). The process parameters (temperature, time, and power) play an important role in the process as they impact the product quality (Arjmandi *et al*., 2016). However, although the thermal effects and energy balance are well established, the industrial application of microwave heating is still limited by the lack of information on chemical reactions in each food matrix (Garnacho *et al*., 2019). Therefore, further studies on the effects of the application of microwave heating on food products are needed before industrial applications (Martins *et al*., 2019).

Studies on the application of microwave heating (MH) as an alternative to pasteurization of milk and/or dairy products are still scarce and are generally related to fluid milk (Iuliana, Rodica, Sorina, & Oana, 2015), infant formulas (Laguerre *et al*., 2011), and vegetable fat/butter blends (Sengar, Sharma & Kurma, 2015), with few studies on the application of MH in the processing of orange juice (Garnacho *et al*., 2019). To date, there are no studies on the use of MH in milk beverages and/or juice-milk beverages. Thus, the present study aimed to evaluate the effect of MH (65 ◦C and 75 ◦C, for 15, 30, and 60 s) on the bioactive compounds, fatty acids profile, and VOCs of OJMB.

2 MATERIALS AND METHODS

2.1 Processing of Orange Juice-Milk Beverage (OJMB)

OJMB was prepared according to Barba *et al*. (2012) and Zulueta *et al*. (2013). Initially, 50 mL of orange juice (*Citrus sinensis* L. Osbeck, 9°Brix), 20 mL of skimmed milk (0.3% fat, 8.3°Brix, Paulista, Danone, Amparo, SP, Brazil), and 30 mL of filtered water were mixed. Then, the dry ingredients (7.5 g sucrose, 0.1 g citric acid, and 0.3 g high methoxypectin) were added, totaling 107.9 mL. The beverage was then homogenized at 25 ºC in a blender (Philco®, 1200 W, 2 L) for 2 min until the ingredients were completely dissolved. According to temperature-holding time binomial, eight OJMB formulations were prepared as follows: T1 (without treatment), T2 (conventional pasteurization, 75 ºC/ 15 s), T3 (MH, 65 ºC/15 s), T4 (MH, 65 ºC/30 s), T5 (MH, 65 ºC/60 s), T6 (MH, 75 ºC/15 s), T7 (MH, 75 ºC/30 s), and T8 (MH, 75 ºC/60 s). The conventional pasteurization was carried out in a heating mantle (12 M, Fisatom Equipamentos Científicos Ltda., Perdizes, SP, Brazil). Batch microwave heating was performed by using a lab-scale microwave digester (Discover System 908005, CEM Corporation, Matthews, NC, USA) at 2450 MHz (Figure 1). For both heat treatments, approximately 100 mL of sample was heated in 250 mL round-bottom flasks, under magnetic stirring. The temperature was monitored using a fiber optic sensor (Fluoroptic STF-1 M, LummaSense Technologies, Santa Clara, USA) inserted at the center of the liquid. The fiber optic sensor was connected to a data acquisition system (Luxtron 812, LummaSense Technologies, Santa Clara, USA) that allowed temperature registration every 0.5 s to obtain the time–temperature profile. After the heating process, the flask was rapidly immersed in an ice-water bath until approximately 10ºC. The thermally processed samples were placed in sterile plastic bottles (polyethylene terephthalate, PET), and evaluated during the 28 days of refrigerated (4 ºC) storage.

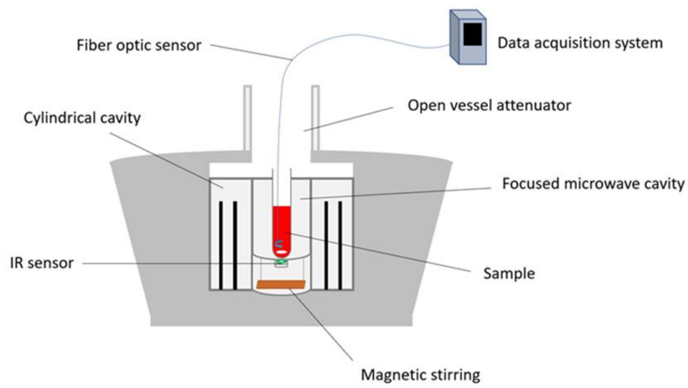

Figure 1. Schematic illustration of the lab-scale microwave digester (Discover System 908005, CEM Corporation, Matthews, NC, USA).

2.2 Bioactive Compounds

The total phenolics content was determined by the Folin-Ciocalteu method (Margraf *et al*., 2015). For that, 1 mL of Folin-Ciocalteu reagent was mixed with 1 mL of the sample in a flask for 5 min, then 1.5 mL of 10% Na2CO3 was added to react for 2 h at 25 ºC, and the absorbance readings were performed at 725 nm. The ascorbic acid content (mg/100 mL) was determined using a titrimetric method (Miranda *et al*., 2019). The total carotenoids content (μg/100 mL) was determined as proposed by Rodriguez-Amaya (2001) as follows: 5 g of the sample were homogenized in 10 mL hexane and 30 mL isopropyl alcohol, transferred to a separation funnel of 125 mL completed with distilled water, let to rest for 30 min, filtered, and the readings were performed at 450 nm. The antioxidant capacity (%) was determined according to the method developed by Brand-Williams *et al*. (1995) and adapted by Granato *et al*. (2015). For that, 150 μL of sample extract was mixed with 2.85 mL of a DPPH solution (0.006 μmol/Lin methanol) and the absorbance readings were carried out at 517 nm after 60 min. The antihypertensive activity was assessed by the angiotensin I converting enzyme (ACE) inhibition, according to the methodology proposed by Ferreira *et al*. (2019). For that, 20 μL of the ACE enzyme (0.1 unit/mL) was added to the flasks containing the samples (40 μL) and stored at 37 ºC for 30 min. Then, 250 μL HCl (1 mol/L) was added to inactivate the enzyme, the samples were dried and suspended in deionized water, and the absorbance readings were performed at 228 nm. The α-amylase and α-glucosidase inhibition were evaluated according to Ferreira *et al*. (2019). For α-amylase inhibition, 100 μL of the enzyme (in phosphate buffer at pH 6.8) were mixed with 100 μL of the sample extract and 250 μL of starch solution at 1% w/v and incubated for 5 min at 37 ºC. Then, 150 μL dinitro salicylic reagent was added and incubated at 100 ºC for 30 min. The absorbance readings were performed at 540 nm. For α-glucosidase inhibition, 100 μL of the enzyme solution (10 unit/mL) and 100 μL of the sample extract were placed in a microcentrifuge tube and incubated at 37 ºC for 10 min. Then, 50 μL of the substrate (4-nitrophenyl-D-glucopyranoside) was added and incubated for 20 min at 37 ºC. Finally, 1 mL of glycine solution (pH 11) was added and the readings were performed at 400 nm. The browning index was determined using the methodology proposed by Barba *et al*. (2012) as follows: the sample was centrifuged (824 × g, 20 min, 18 ºC), and the resulting supernatant was diluted with ethanol (1:1 v/v), filtered (Whatman 42) and the readings were performed at 420 nm.

2.3 Fatty Acids Profile

The lipids were extracted as reported by Batista *et al*. (2017). The identification and quantification of fatty acid esters were performed by gas chromatography-mass spectrometry (GC–MS, Agilent Technologies, 7890A-5975C, Santa Clara, California, USA) according to Silva *et al*. (2020). The atherogenic index (AI), thrombogenic index (TI), desired fatty acids index (DFA), and hypercholesterolemic saturated fatty acids index (HSFA) were determined according to Barlowska *et al*. (2018).

2.4 Volatile Organic Compounds

VOCs were extracted according to the methodology proposed by Cappato *et al*. (2018), and the identification was performed by GC–MS (Agilent Technologies, 7890A-5975C, Santa Clara, California, USA) using the Agilent Mass Hunter Qualitative Analysis software (Agilent Technologies) and the mass spectra library (linear retention indices, LRI) of the National Institute of Standards and Technology (NIST/EPA/ NIH, version 11, USA).

2.5 Statistical Analysis

The experiment was repeated three times and the analyses were performed in triplicate. The results were analyzed by one-way analysis of variances (ANOVA) followed by Fisher's test ($p < 0.05$). Principal component analysis (PCA) and cluster analysis (HCA) were also performed (Balthazar *et al*., 2018). All statistical analyses were carried out using the XLSTAT software for Windows Excel® version 2019.4.1 (Addinsoft, Paris, France).

3 RESULTS AND DISCUSSION

3.1 Bioactive Compounds

The results of the levels of the bioactive compounds are shown in Table 1. The MH-OJMB had a lower browning index and higher ($p < 0.05$) antioxidant activity, α-amylase, α-glucosidase, and ACE inhibition when compared to the pasteurized product. This result is directly associated with the higher ascorbic acid, TPC, and carotenoid levels found in MH-OJMB.

MH is a highly efficient volumetric heating system, resulting in greater thermal efficiency and less time to reach the desired temperature when compared to conventional pasteurization. Therefore, the preservation of thermosensitive compounds, such as ascorbic acid, carotenoids, and phenolic compounds is more pronounced (Martins *et al*., 2019). Phenolic compounds have been studied as possible natural inhibitors of α-amylase and α-glucosidase, which may contribute to the management of postprandial glycemia (Pradeep & Sreerama, 2015; Armstrong *et al*., 2020). MH does not seem to degrade bioactive peptides in dairy beverages, in addition to providing partial hydrolysis of proteins, resulting in the release of other bioactive peptides with ACE inhibitory activity (Ketnawa *et al*., 2019).

Browning of dairy products is associated with the Maillard reaction (MRP) and the consequent production of hydroxymethylfurfural (HMF). HMF levels are linearly correlated with the intensity of the heat treatment and the color changes (Francisquini *et al*., 2018). Our findings showed that MH-OJMB preserved its original color for not producing HMF (hypothesis) and not degrading carotenoids (Mapelli-Brahm, Stinco, Rodrigo, Zacarías, & Mel´endez-Martínez, 2018).

The MH process parameters (temperature and time) had a significant effect on the content of bioactive compounds ($p < 0.05$). The increase in temperature (65 ◦C to 75 ◦C) decreased the ascorbic acid content, while the processing time (15 to 60 s) led to a decrease in the carotenoids content ($p < 0.05$). Concerning the other parameters, there was an interaction between time and temperature ($p < 0.05$). Ascorbic acid is a highly labile component when exposed to high temperatures, and its degradation is commonly used as an indicator of the severity of thermal processing (Miranda *et al*., 2019). Carotenoids are susceptible to oxidative degradation, which is enhanced by the duration of processing at certain temperatures (Mapelli-Brahm *et al*., 2018).

MH-OJMB subjected to lower temperatures (65 ◦C) and higher processing times (60 s) showed higher TPC, antioxidant activity, and α-amylase and α-glucoside inhibition when compared to the other treatments, while the most drastic conditions (75 ◦C/60 s) resulted in greater ACE inhibitory activity ($p < 0.05$). The milder conditions (65 ◦C/ 15 s) resulted in higher retention of ascorbic acid ($p < 0.05$). In addition, lower temperatures retained higher contents of bioactive compounds, while higher processing times were required to promote the release of phenolic compounds from the plant matrix. Thus, the treatment at 65 ◦C/60 s was more suitable for maintaining the TPC levels, the antioxidant activity, and the α-amylase and α-glucoside inhibition. Finally, the most drastic condition (75 ◦C/60 s) was important to promote protein hydrolysis and the formation of bioactive peptides with ACE inhibitory activity. Further studies on the peptide formation and identification are required to investigate the peptides with ACE inhibitory activity.

In the present study, the conventional pasteurization resulted in a decrease in all bioactive compounds when compared to the untreated product ($p < 0.05$). When appropriate processing conditions (65 ◦C/60 s) were used, MH resulted in OJMB with higher ($p < 0.05$)

concentrations of total phenolics and higher α-amylase inhibition when compared to the untreated product. In contrast, a reduction of, ascorbic acid levels, carotenoids, ACE inhibition, and α-glucosidase inhibition was observed, despite being significantly lower ($p < 0.05$) when compared with the samples subjected to the conventional pasteurization.

During storage, the MH-OJMB presented a similar behavior to both the product subjected to conventional pasteurization and the untreated product. Overall, when comparing the results at days 1 and 28, there was a reduction of ascorbic acid, total phenolics, carotenoids, antioxidant activity, ACE-inhibitory activity, α-glucosidase inhibition, and α-amylase inhibition, as well as an increase in the browning index ($p < 0.05$). However, the MH-treated sample exhibited higher ascorbic acid, carotenoids, antioxidant activity (except for T7 and T8), ACE inhibitory activity, α-glucosidase inhibition, and α-amylase inhibition, and lower browning index when compared to the pasteurized samples ($p < 0.05$). The reduction of ascorbic acid, carotenoids, and total phenolics levels suggested the occurrence of oxidative reactions during the refrigerated storage, which impacts in a clear reduction of the antioxidant activity, and ACE, α-amylase, and α-glucosidase inhibition (Miranda *et al*., 2019). Besides the Maillard reaction, other factors may also be associated with the browning of the samples, including the oxidative and non-oxidative reactions of the polyphenols (Costa *et al*., 2017).

Table 1. Bioactive compounds, antioxidant activity, antihypertensive activity, enzymatic activity and browning index of the samples of orange juice-milk beverages during along 28 days of storage at 4°C (Continued on next page).

			Microwave/65ºC			Microwave/75ºC		
Parameters/ Storage time	T1	T2	T3	T4	T5	T6	T7	T8
Ascorbic acid								
1	15.99±0.17^{aA}	12.64±0.20^{fA}	15.17±0.17^{bA}	15.01±0.29^{bcA}	14.76±0.22^{cdA}	14.47±0.08^{deA}	14.37±0.08^{eA}	14.32±0.17^{eA}
7	15.10±0.09^{aB}	11.49±0.62^{dB}	14.41±0.15^{bB}	14.26±0.00^{bB}	14.26±0.15^{bB}	14.06±0.31^{bcB}	13.67±0.00^{cB}	13.72±0.09^{cB}
14	12.93±0.00^{bcdC}	10.63±0.46^{eC}	13.03±0.17^{bcC}	13.23±0.15^{abC}	13.48±0.09^{aD}	12.83±0.17^{cdC}	12.58±0.09^{deC}	12.23±0.17^{eC}
21	10.13±0.00^{aD}	7.85±0.16^{gD}	8.94±0.16^{cdD}	9.03±0.00^{cD}	9.76±0.16^{bD}	8.80±0.08^{deD}	8.62±0.14^{eD}	8.30±0.16^{fD}
28	7.51±0.18^{aE}	3.55±0.18^{eE}	4.26±0.00^{dE}	4.77±0.18^{cE}	5.28±0.18^{bE}	3.65±0.00^{eE}	3.45±0.18^{eE}	2.54±0.18^{fE}
Total phenolis count								
1	54.99±0.47^{bB}	44.00±0.36^{eD}	54.36±0.21^{cBC}	55.22±0.37^{bB}	56.12±0.21^{aB}	56.57±0.14^{aAB}	56.43±0.62^{aA}	53.73±0.14^{dC}
7	58.06±0.34^{aA}	56.35±0.14^{efA}	57.29±0.14^{bcdA}	57.83±0.47^{abA}	57.56±0.49^{abcA}	56.75±0.47^{defA}	56.21±36^{fA}	56.98±0.51^{cdeA}
14	55.90±0.41^{cB}	54.41±0.51^{dB}	54.86±0.14^{dB}	57.52±0.08^{aA}	55.63±0.08^{cB}	55.90±0.31^{cB}	56.75±0.00^{bA}	56.39±0.21^{bA}
21	50.90±2.34^{cC}	52.39±0.13^{bcC}	54.19±0.51^{aBC}	53.11±0.43^{abC}	53.96±0.28^{abC}	53.78±0.47^{abC}	53.74±0.71^{abB}	54.77±1.01^{aB}
28	51.98±0.43^{bcC}	52.84±1.91^{abcBC}	53.51±0.94^{abC}	53.15±0.16^{abC}	53.01±0.53^{abcD}	54.05±0.59^{aC}	52.61±1.15^{abcB}	51.44±0.41^{cD}
Carotenoids								
1	181.54±0.19^{aA}	141.98±0.67^{hA}	166.37±0.55^{cA}	153.70±0.58^{fA}	163.27±0.29^{dA}	155.15±0.60^{eA}	176.87±1.16^{bA}	148.13±0.58^{gA}
7	159.27±0.23^{aB}	109.28±0.38^{gB}	160.14±0.35^{aB}	153.37±0.45^{bA}	151.96±0.71^{cB}	150.49±0.82^{dB}	149.31±0.41^{eB}	148.19±0.65^{fA}
14	124.23±0.32^{fC}	86.81±0.15^{gC}	142.25±0.44^{dC}	133.49±0.55^{eB}	147.60±0.60^{bC}	148.48±0.12^{aC}	147.33±0.43^{bC}	146.51±0.53^{cB}
21	107.08±0.93^{dD}	79.74±0.50^{eD}	133.76±0.69^{bD}	121.79±0.26^{cC}	135.37±0.82^{aD}	134.54±0.45^{abD}	135.57±0.36^{aD}	133.65±0.49^{bC}
28	97.27±0.12^{gE}	75.55±0.76^{hE}	132.31±0.66^{aE}	119.77±0.14^{fD}	131.31±0.65^{bE}	130.22±0.41^{cE}	125.48±0.35^{dE}	123.50±0.38^{eD}
DPPH								
1	31.61±0.43^{bA}	27.35±0.08^{dA}	29.33±0.39^{cA}	28.71±0.52^{cA}	32.52±0.46^{aA}	29.13±0.71^{cA}	29.02±0.39^{cA}	28.59±0.36^{cA}
7	32.92±0.47^{aB}	30.76±0.85^{cB}	33.35±0.39^{aB}	33.66±0.28^{aB}	31.50±0.28^{bcA}	31.63±0.11^{bB}	31.69±0.21^{bB}	33.29±0.57^{aB}
14	27.20±0.42^{bC}	24.22±0.12^{dC}	26.86±0.12^{bcC}	26.59±0.51^{bcC}	29.23±1.43^{aAB}	25.91±0.41^{cC}	26.52±0.20^{bcC}	24.70±0.61^{dC}
21	19.93±0.56^{eD}	27.16±0.44^{aB}	23.76±0.34^{cD}	24.65±0.46^{bD}	26.64±0.34^{aD}	21.92±0.68^{dD}	24.87±0.34^{bD}	22.58±0.46^{dD}
28	16.40±0.65^{aE}	10.44±0.25^{dD}	13.37±0.19^{bE}	12.93±0.25^{bE}	13.64±0.71^{bD}	11.47±0.34^{cE}	10.17±0.34^{dE}	8.98±0.50^{eE}

Table 1. Continued.

ACE								
1	76.07±3.11^{aA}	51.83±0.50^{eA}	61.60±0.61^{dA}	66.88±0.36^{cA}	68.47±0.35^{bcA}	66.60±0.36^{cA}	67.57±0.31^{bcA}	69.27±0.21^{bA}
7	75.77±1.21^{aAB}	47.07±0.32^{gB}	59.77±0.45^{fB}	65.87±0.59^{deB}	67.40±0.44^{cB}	65.23±0.12^{eB}	66.53±0.35^{cdB}	68.77±0.23^{bB}
14	74.93±0.25^{aABC}	46.07±0.78^{gB}	59.40±0.56^{fB}	64.90±0.26^{dC}	66.70±0.35^{cB}	64.07±0.29^{eC}	66.03±0.21^{cB}	67.67±0.15^{bC}
21	73.13±0.67^{aBC}	44.93±0.75^{fC}	58.97±0.12^{eB}	64.03±0.31^{dD}	65.30±0.26^{cC}	63.83±0.25^{dC}	65.27±0.42^{cC}	66.70±0.26^{bD}
28	72.30±0.53^{aC}	44.43±0.49^{gC}	57.73±0.51^{fC}	62.97±0.21^{eE}	64.13±0.49^{dD}	63.03±0.21^{eD}	64.87±0.21^{cC}	65.90±0.10^{bE}
α-glucosidase								
1	75.33±0.30^{aA}	62.06±0.68^{eA}	66.56±0.20^{dA}	70.58±1.05^{cA}	72.13±0.68^{bA}	71.00±0.80^{cA}	66.67±0.13^{dA}	70.66±0.51^{cA}
7	73.51±0.34^{aB}	60.25±0.74^{eB}	64.35±1.08^{dB}	68.56±0.75^{cB}	71.11±0.50^{bA}	70.01±0.58^{bB}	64.53±0.30^{dB}	68.31±0.51^{cB}
14	73.14±1.36^{aB}	59.14±0.84^{fB}	63.86±0.74^{eB}	67.43±0.49^{cdB}	69.74±0.57^{bB}	68.49±0.52^{bcC}	63.35±0.44^{eC}	67.15±0.75^{dC}
21	71.34±0.47^{aC}	56.80±0.48^{fC}	61.61±0.39^{eC}	65.17±0.89^{dC}	68.45±0.57^{bC}	67.23±0.28^{cD}	62.09±0.38^{eD}	65.05±0.80^{dD}
28	69.12±0.73^{aD}	55.03±0.56^{gD}	59.89±0.71^{fD}	63.73±0.58^{dD}	66.20±0.88^{bD}	65.30±0.16^{bcE}	61.22±0.19^{eE}	64.42±0.21^{cdD}
α-amilase								
1	80.12±0.67^{dA}	64.94±0.43^{gA}	75.58±0.63^{fA}	83.60±0.24^{cA}	94.96±0.87^{aA}	88.60±0.89^{bA}	82.72±0.57^{cA}	78.17±0.35^{eA}
7	77.05±0.91^{eB}	62.81±0.31^{gB}	73.57±0.51^{fB}	81.22±0.36^{cB}	92.54±0.60^{aB}	85.24±0.28^{bB}	79.94±0.17^{dB}	76.19±0.56^{eB}
14	75.78±0.47^{dC}	59.26±0.25^{fC}	72.99±0.48^{eBC}	78.72±0.58^{cC}	91.80±0.31^{aB}	84.45±0.40^{bB}	78.06±0.88^{cC}	75.69±0.40^{dB}
21	73.16±0.43^{fD}	57.97±0.69^{gD}	72.53±0.27^{fC}	77.60±0.31^{cD}	90.59±0.30^{aC}	82.96±0.62^{bC}	76.21±0.60^{dD}	74.45±0.34^{eC}
28	72.30±0.66^{eD}	54.79±0.48^{gE}	70.87±0.56^{fD}	76.16±0.64^{cE}	89.22±0.68^{aD}	81.53±0.53^{bD}	74.66±0.26^{dE}	72.35±0.18^{eD}
Browing								
1	0.031±0.002^{bcD}	0.048±0.002^{aC}	0.033±0.002^{bC}	0.032±0.001^{bcC}	0.033±0.001^{bB}	0.030±0.001^{cB}	0.030±0.002^{cB}	0.032±0.002^{bcB}
7	0.039±0.001^{bD}	0.043±0.003^{aC}	0.032±0.001^{dC}	0.036±0.002^{bcB}	0.034±0.003^{cdB}	0.033±0.003^{cdB}	0.034±0.002^{cdAB}	0.035±0.004^{cdB}
14	0.061±0.010^{bC}	0.087±0.019^{aB}	0.038±0.002^{cB}	0.040±0.002^{cA}	0.040±0.001^{cA}	0.041±0.001^{cA}	0.036±0.001^{cAB}	0.043±0.001^{cA}
21	0.075±0.005^{bB}	0.097±0.011^{aB}	0.039±0.001^{cB}	0.039±0.001^{cA}	0.042±0.002^{cA}	0.039±0.001^{cA}	0.036±0.001^{cAB}	0.043±0.003^{cA}
28	0.104±0.006^{bA}	0.138±0.008^{aA}	0.041±0.002^{cA}	0.041±0.001^{cA}	0.042±0.003^{cA}	0.040±0.001^{cA}	0.037±0.001^{cA}	0.043±0.003^{cA}

* Data are expressed as the mean ± standard deviation of at least 3 replicates. $^{a-f}$Different letters in the same column indicate significant differences between samples ($p < 0.05$). Ascorbic acid, Total phenolic count, carotenoids, the DDPH, ACE, α - amylase, and α – glucosidase are expressed in mg 100 mL^{-}1, µg Gallic Acid /g, µg 100 Ml^{-}1, %, %, %, %, respectively. T1 (without treatment), T2 (conventional pasteurization, 75 ^{o}C/15 s), T3 (MH, 65 ^{o}C/15 s), T4 (MH, 65 ^{o}C/30 s), T5 (MH, 65 ^{o}C/60 s), T6 (MH, 75 ^{o}C/15 s), T7 (MH, 75 ^{o}C/30 s), and T8 (MH, 75 ^{o}C/60 s).

To gain insights from data, PCA was applied, and the results are presented in Figure 2. In a two-dimensional plot, about 83% of data variability was explained using two principal components. The MH-OJMB samples (T3-T8) were positioned close to the untreated product (T1), presenting higher concentrations of bioactive compounds (total phenolics, carotenoids, and ascorbic acid), antioxidant activity, and ACE, α-amylase, and α-glucosidase inhibition. Among the treatments, T5 (65 ◦C/60 s) was closer to T1, indicating that this condition provided characteristics closer to the untreated product, followed by T7 (75 ◦C/ 30 s) and T3 (65 ◦C/15 s). On the other hand, the sample OJMB which was subjected to conventional pasteurization (T2), did not present similar quality characteristics to the other formulations. This result is confirmed by the confidence ellipses, in which T2 was separated, the MH-OJMB samples were superimposed, and the formulations T3, T7, and T5 were more similar to the untreated product.

The dendrogram obtained using HCA (Figure 3) suggested three different groups: group I (T2), group II (T4, T5, T6, and T8), and group III (T1, T3, and T7). The samples T3 and T7 (65 ◦C/15 s and 75 ◦C/30 s, respectively) presented similar quality characteristics to the untreated sample (T1), which indicates the retention of bioactive compounds. However, the samples from group II showed more interesting results for beverage processing, once it presented higher retention of total phenolics contents, higher antioxidant activity, greater α-amylase inhibition, and lower browning index. The present results indicate that the MH-treated samples presented higher bioactive compounds levels when compared with the pasteurized sample. Therefore, the processing parameters of 65 ◦C and 60 s can be used for the maintenance and/or improvement in the concentration of bioactive compounds of the dairy beverage.

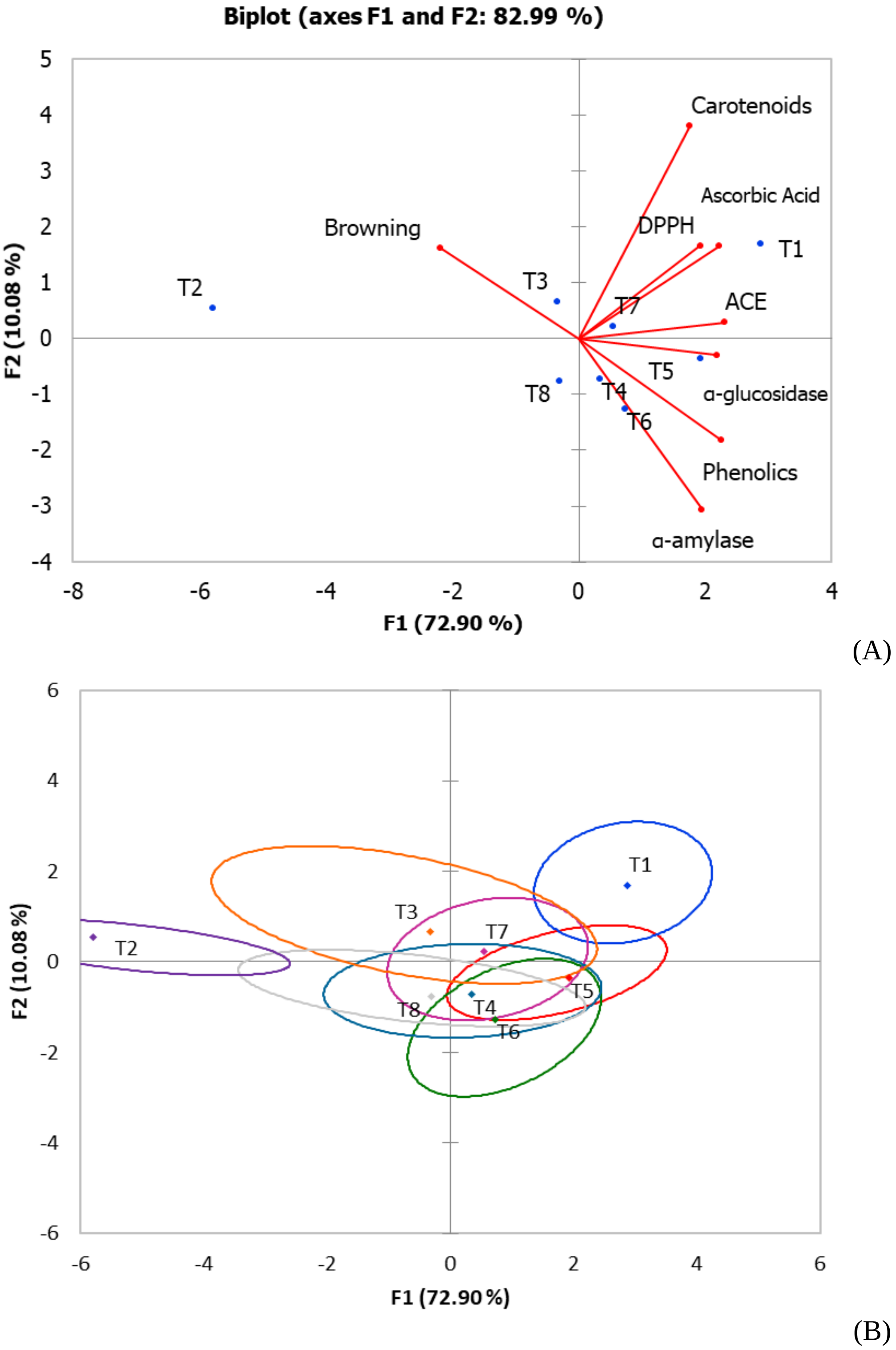

Figure 2. Principal component analysis (PCA) map for chemical analyses of orange juice-milk beverages. T1 —— (non-treated), T2 —— (pasteurization, 75 ◦C/15 s), T3 —— (microwave, 65 ◦C/15 s), T4 —— (microwave, 65 ◦C/30 s), T5 —— (microwave, 65 ◦C/ 60 s), T6 —— (microwave, 75 ◦C/15 s), T7 —— (microwave, 75 ◦C/30 s), and T8 —— (microwave, 75 ◦C/60 s).

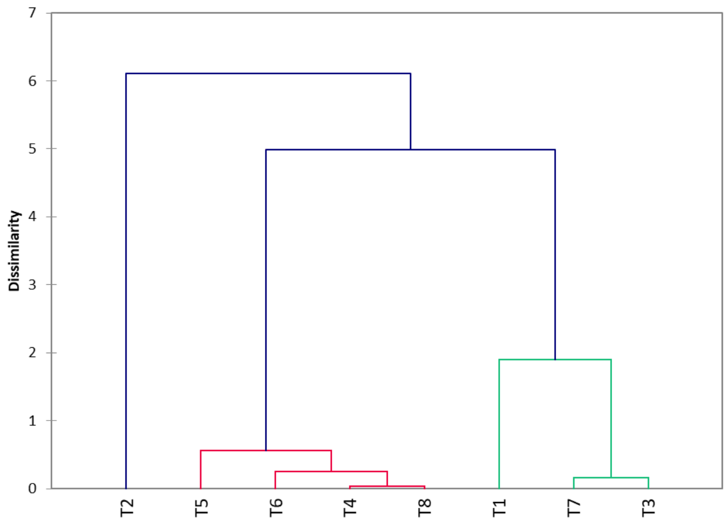

Figure 3. Dendrogram for chemical analyses of orange juice-milk beverages. T1 (non-treated), T2 (pasteurization, 75 ◦C/15 s), T3 (microwave, 65 ◦C/15 s), T4 (microwave, 65 ◦C/30 s), T5 (microwave, 65 ◦C/60 s), T6 (microwave, 75 ◦C/ 15 s), T7 (microwave, 75 ◦C/30 s), and T8 (microwave, 75 ◦C/60 s). group I —— (T2), group II —— (T4, T5, T6, and T8), and group III —— (T1, T3, and T7).

3.2. Fatty Acids Profile

Table 2 shows the fatty acids profile and the health indexes (AI, TI, DFA, and HSFA) of OJMB. The type of processing (pasteurization or microwave heating) and the process parameters (temperature and time) had a significant effect on the fatty acids profile and the health indexes ($p < 0.05$) of the samples. The MH-OJMB had a higher concentration of medium-chain fatty acids (MCFA) and lower concentrations of long-chain fatty acids (LCFA) and monounsaturated fatty acids (MUFA) when compared with the pasteurized product ($p < 0.05$), probably due to the lower stearic acid and oleic acid and higher octanoic acid levels ($p < 0.05$). Consequently, the MH-OJMB showed higher AI and lower DFA values than the pasteurized product ($p < 0.05$). It is worth- noting that the fatty acids profile of the MH-treated products was similar to the untreated product, indicating no effect of MH on the fatty acids profile.

Diets rich in MCFA favor the energy expenditure through thermogenesis, lipid catabolism, and body fat loss, in addition to controlling obesity and metabolic diseases and modifying the gut microbiota (Rial, Karelis, Bergeron, & Mounier, 2016). Lower SFA intake, such as stearic acid, is recommended, as a high intake of SFA can increase total cholesterol levels in the blood, which is a risk factor for cardiometabolic and cardiovascular diseases. High SFA diets can also increase adiposity and reduce insulin sensitivity (Gebreyowhans, Lu, Zhang, Pang, & Lv, 2019). In turn, the decrease in MUFA, especially oleic acid, is not recommended, as this fraction is associated with anticancer properties, reduced plasma cholesterol, improved autoimmune system, and reduced risk of inflammatory and cardiovascular diseases (Rodríguez-Alcalá, Castro-Gómez, Pimentel, & Fontecha, 2017).

Concerning the process parameters, changes in temperature (65 ◦C to 75 ◦C) resulted in a decrease in MCFA levels, while a higher process time (from 15 to 60 s) resulted in a decrease in LCFA and MUFA levels when compared to the pasteurized product ($p < 0.05$). The MH-OJMB samples subjected to the lower temperature (65 ◦C) and higher process time (60 s) and the most drastic conditions (75 ◦C/60 s) exhibited fatty acids profile and health indexes similar to those of the untreated product.

Table 2. Fatty acids profile (g 100 g^{-1} of fat) of the samples of orange juice-milk beverages.

Fatty acids	T1	T2	Microwave/65ºC			Microwave/75ºC		
			T3	T4	T5	T6	T7	T8
Short-Chain Fatty Acids (SCFA)	2.72±0.01^{bc}	2.32±0.10^{c}	3.55±0.42^{abc}	4.73±0.25^{a}	3.51±0.56^{abc}	2.69±0.23^{bc}	3.63±0.31^{ab}	3.44±0.27^{bc}
Butanoic (C4:0)	2.72±0.01^{bc}	2.32±0.10^{c}	3.55±0.42^{abc}	4.73±0.25^{a}	3.51±0.56^{abc}	2.69±0.23^{bc}	3.63±0.31^{ab}	3.44±0.27^{bc}
Medium-Chain Fatty Acids (MFCA)	12.04±0.20^{cd}	12.24±0.42^{cd}	14.43±0.26^{ab}	15.19±0.34^{a}	14.19±0.74^{ab}	12.42±0.69^{c}	13.11±0.27^{bc}	10.62±0.09^{d}
Hexanoic (C6:0)	2.51±0.08^{a}	1.57±0.09^{a}	1.54±0.31^{a}	2.26±0.14^{a}	1.56±0.03^{a}	1.86±0.86^{a}	1.34±0.43^{a}	1.38±0.11^{a}
Octanoic (C8:0)	2.79±0.01^{c}	2.20±0.09^{d}	5.75±0.22^{a}	5.35±0.17^{a}	5.68±0.20^{a}	4.43±0.02^{b}	4.82±0.08^{b}	2.46±0.03^{cd}
Docanic (C10:0)	3.10±0.13^{c}	4.69±0.02^{a}	3.67±0.17^{abc}	4.39±0.23^{ab}	3.62±0.42^{abc}	3.48±0.55^{abc}	3.55±0.43^{abc}	3.40±0.12^{bc}
Dodecanoic (C12:0)	3.64±0.26^{ab}	3.78±0.26^{a}	3.47±0.17^{ab}	3.18±0.20^{ab}	3.33±0.14^{ab}	2.66±0.40^{b}	3.40±0.34^{ab}	3.38±0.12^{ab}
Long-Chain Fatty Acids (LCFA)	23.97±0.43^{d}	33.79±0.58^{a}	29.48±0.38^{bc}	22.39±0.34^{d}	22.73±0.69^{d}	27.71±1.02^{c}	29.96±0.38^{b}	24.00±0.19^{d}
Myristic (C14:0)	7.52±0.56^{bc}	8.37±0.32^{ab}	9.59±0.48^{a}	6.54±0.28^{c}	7.33±0.07^{bc}	8.42±0.15^{ab}	8.42±0.45^{ab}	6.69±0.40^{c}
Myristoleic (14:1(ω-5))	0.14±0.03^{c}	0.35±0.02^{b}	0.56±0.01^{a}	0.17±0.02^{c}	0.14±0.02^{c}	0.11±0.01^{c}	0.67±0.07^{a}	0.14±0.01^{c}
Palmitic (C16:0)	3.33±0.15^{ab}	3.16±0.11^{b}	3.21±0.28^{ab}	4.25±0.17^{ab}	3.52±0.43^{ab}	4.47±0.55^{a}	3.83±0.09^{ab}	3.59±0.42^{ab}
Palmitoleic (C16:1(ω-7))	1.28±0.06^{d}	5.38±0.38^{a}	3.48±0.37^{b}	1.54±0.06^{d}	1.80±0.01^{cd}	2.42±0.11^{c}	4.12±0.03^{b}	2.43±0.11^{c}
Stearic (C18:0)	4.71±0.09^{a}	5.31±0.00^{a}	1.63±0.30^{cd}	1.74±0.06^{cd}	1.37±0.01^{d}	1.38±0.34^{d}	2.57±0.27^{b}	2.35±0.21^{bc}
Oleic (C18:1(ω-9))	3.12±0.10^{cd}	4.45±0.46^{ab}	3.68±0.21^{bc}	3.25±0.03^{cd}	3.21±0.02^{cd}	3.64±0.16^{bc}	4.82±0.19^{a}	2.56±0.09^{d}
Linoleic (C18:2(ω-6))	3.88±0.08^{d}	6.78±0.09^{a}	7.33±0.30^{a}	4.90±0.11^{cd}	5.36±0.23^{bc}	7.27±0.26^{a}	5.53±0.63^{bc}	6.24±0.02^{ab}
Linolenic (C18:3(ω-3))	nd*	nd*	nd*	nd*	nd*	nd*	nd*	nd*
Saturated Fatty Acid (SFA)	30.31±0.13^{ab}	31.40±0.11^{a}	32.42±0.05^{a}	32.45±1.11^{a}	29.92±1.79^{ab}	29.39±1.96^{ab}	31.56±0.68^{a}	26.69±0.56^{b}
Monounsaturated Fatty Acid (MUFA)	4.54±0.19^{d}	10.18±0.06^{a}	7.71±0.057^{b}	4.96±0.06^{d}	5.16±0.03^{d}	6.17±0.028^{c}	9.61±0.015^{a}	5.13±0.03^{d}
Polyunsaturated Fatty Acid (PUFA)	3.88±0.08^{d}	6.78±0.09^{a}	7.33±0.30^{a}	4.90±0.11^{cd}	5.36±0.23^{bc}	7.27±0.26^{a}	5.53±0.63^{bc}	6.24±0.02^{ab}
Atherogenic Index (AI)	4.40±0.16^{a}	2.38±0.05^{d}	3.00±0.15^{bc}	3.41±0.17^{b}	3.44±0.02^{b}	3.04±0.12^{bc}	2.70±0.22^{cd}	2.97±0.10^{bc}
Thrombogenic Index (TI)	3.69±0.02^{a}	1.99±0.03^{cd}	1.92±0.02^{d}	2.54±0.15^{b}	2.32±0.05^{bc}	2.12±0.16^{cd}	1.96±0.07^{d}	2.22±0.04^{bcd}
Desired fatty acids (DFA)	13.12±0.03^{de}	22.27±0.15^{a}	16.68±0.58^{b}	11.60±0.11^{f}	11.88±0.19^{ef}	14.82±0.32^{c}	17.71±0.74^{b}	13.72±0.21^{cd}
Hypercholesterolemic saturated fatty acids (HSFA)	14.48±0.14^{ab}	15.31±0.17^{ab}	16.28±0.02^{a}	13.98±0.25^{b}	14.18±0.64^{b}	15.54±1.10^{ab}	15.65±0.71^{ab}	13.66±0.13^{b}

* Data are expressed as the mean ± standard deviation of at least 3 replicates. a-f Different letters in the same column indicate significant differences between samples ($p < 0.05$). SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. AI = (C12:0 + 4 C14:0 + C16:0)/[ΣMUFA +ΣPUFA(n-6) and (n-3)]; TI = (C14:0 + C16:0 + C18:0)/[0.5 ×ΣMUFA + 0.5 ×ΣPUFA(n-6) + 3 ×ΣPUFA(n- 3) + (n-3)/(n-6)]; DFA = MUFA + PUFA + C18:0; HSFA = C12:0 + C14:0 + C16:0. T1 (without treatment), T2 (conventional pasteurization, 75 ◦C/15 s), T3 (MH, 65 ◦C/15 s), T4 (MH, 65 ◦C/30 s), T5 (MH, 65 ◦C/60 s), T6 (MH, 75 ◦C/ 15 s), T7 (MH, 75 ◦C/30 s), and T8 (MH, 75 ◦C/60 s).

3.3. Volatile Organic Compounds

Table 3 shows the VOCs profile of OJMB. Hydrocarbons (14 compounds), followed by terpenes (13 compounds), alcohols (11 compounds), aldehydes (10 compounds), esters (9 compounds), acids (2 compounds), ketones (2 compounds), and phenols (1 compound) were identified. Terpenes qualitatively and quantitatively represent the largest group of VOCs in orange juices and are important for the characterization of the orange aroma (Miranda *et al*., 2019).

The beverages subjected to the conventional pasteurization or milder MH condition (65 ◦C/15 s) had the highest number of VOCs (n = 47), while the beverages subjected to the most drastic MH condition (75 ◦C/ 60 s) exhibited the lowest number of VOCs (n = 34). The samples subjected to 65 ◦C/60 s and 75 ◦C/15 s showed a comparable number of compounds (n = 39) to the untreated product (n = 40). Thus, the type of processing (pasteurization or microwave heating) and the process parameters (temperature and time) had a significant effect on the VOCs.

Twenty-five compounds were observed in all OJMB samples, indicating that they are inherent to milk and/or orange juice. These compounds are responsible for the green aroma (beta-pinene, 1-octanol), sweet and citrus (D-limonene, decanal, gamma-terpinene, 2-hexenal), fruity (octanal, o-cymene, hexanoic acid ethyl ester), pungent (alpha-terpineol), oily (1-nonanol), and notes of coriander (decanal) from orange juices (Miranda *et al*., 2019). In addition, these VOCs are also associated with fermented milk aroma (2-hexenal) (Ranadheera *et al*., 2019).

Some compounds that were not detected in the untreated beverage were observed in the OJMB subjected to the conventional pasteurization, which contributed to the fruity (1-hexanol), sweet (hexanoic acid, ethyl ester), and menthol (alpha-phellandrene) aroma of the beverages (Miranda *et al*., 2019). However, compounds that contribute negatively to the flavor, including bitter and saponaceous (*n*-decanoic), resin (3- carene), and unripe fruit (acetic acid decyl ester, geranyl acetate) were detected in those samples (Ranadheera *et al*., 2019). It is known that the heat treatment can lead to the degradation of VOCs, with changes in the sensory and functional characteristics of the product, in addition to leading to the formation of compounds that are responsible for the development of off-flavors (Amaral *et al*., 2018).

The MH-OJMB subjected to the lower temperature (65 ◦C) and higher process time (60 s) presented important compounds that are responsible for the sweet and fruity aroma (2-pentanone, 1-hexanol, acetic acid octyl ester), which were not present in the untreated product. Other compounds with negative impacts on the aroma, including the aroma of grass or saponaceous (hexanal) were not identified (Ranadheera *et al*., 2019). MH-OJMB subjected to the most drastic condition (75 ◦C/60 s) did not present 1-decanol (fatty aroma), citral (orange aroma), nootkatone (grapefruit aroma), octanoic acid, and ethyl ester (fruity) (Wei *et al*., 2018). In addition, this treatment presented 3-carene, which can negatively affect the sensory characteristics of the product (Wei *et al*., 2018). Citral is a compound derived from carotenoids and can be produced through the oxidative cleavage of the double bonds between carbons 7 and 8 of the β-cryptoxanthin molecule (Ranadheera *et al*., 2019). Thus, more drastic MH conditions had a negative impact on the VOCs profile of OJMB.

The sample subjected to the conventional pasteurization presented a greater number of VOCs, with the formation of compounds that can contribute negatively to the aroma of the product. In general, the MH process conducted at 65 ◦C/60 s is recommended, as this treatment presented VOCs that contribute positively to the aroma of the beverages.

Table 3. Volatile organic compounds (VOCs) of the samples of orange juice-milk beverages (Continued on next page).

					MW/65 ∘C			MW/75 ∘C		
Groups	Compounds	*LRI	T1	T2	T3	T4	T5	T6	T7	T8
	Total of VOCs Identified		**40**	**47**	**47**	**38**	**39**	**39**	**42**	**34**
Acids	Octanoic acid	2064	X	X	X	X	X	X	X	X
	n-Decanoic acid	2276		X						
	Total of acids		**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**
Alcohols	1-Hexanol	1386		X	X		X			
	1,6-Octadien-3-ol, 3,7-dimethyl-	1562	X	X	X	X	X	X	X	X
	1-Octanol	1572	X	X	X	X	X	X	X	X
	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	1619		X	X	X	X	X	X	X
	dl-Menthol	1654			X		X			
	1-Nonanol	1670	X	X	X	X	X	X	X	X
	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	1746			X	X				X
	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate	1767	X							
	1-Decanol	1771	X	X	X	X				
	6-Octen-1-ol, 3,7-dimethyl-, formate	1773	X							X
	3-Cyclohexene-1-ethanol, .beta.,4-dimethyl-	1833	X	X	X	X				
	Total of alcohols		**7**	**7**	**9**	**7**	**6**	**4**	**4**	**6**
Aldehydes	Hexanal	1139	X	X	X	X		X	X	X
	2-Hexenal, (E)-	1271	X	X	X	X	X	X	X	X
	Octanal	1329	X	X	X	X	X	X	X	X
	Pentanal, 2-methyl-	1420				X		X	X	
	Pentanal, 2-methyl-	1420	X	X			X			X
	Nonanal	1420			X					
	Decanal	1522	X	X	X	X	X	X	X	X
	Citral	1698	X	X	X	X	X	X	X	
	2,6-Octadienal, 3,7-dimethyl-, (E)-	1765			X					
	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-	1803	X	X	X	X	X	X	X	X
	Total of aldehydes		**7**	**7**	**8**	**7**	**6**	**7**	**7**	**6**
Esther	Dimethyl ether	1070	X	X	X	X	X	X	X	X
	Butanoic acid, ethyl ester	1099	X	X	X	X		X	X	X
	Hexanoic acid, ethyl ester	1278	X	X	X	X	X	X	X	X
	Octanoic acid, ethyl ester	1459	X	X	X	X	X	X	X	
	Acetic acid, octyl ester	1497	X	X	X	X	X	X	X	X
	Citronellyl butyrate	1675	X		X		X		X	
	Hexanoic acid, 3-hydroxy-, ethyl ester	1691		X	X		X	X	X	
	Acetic acid, decyl ester	1695		X		X	X		X	X
	Geranyl acetate	1767		X			X		X	
	Total of esther		**6**	**8**	**7**	**6**	**8**	**6**	**9**	**5**
Hydrocarbons	Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	1095	X	X	X	X		X	X	
	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	1169	X	X	X	X	X	X	X	X
	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1R)-	1285			X				X	
	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	1325	X	X	X	X	X	X	X	X
	Benzene, 1,3-bis(1,1-dimethylethyl)-	1453		X						
	Benzene, 4-ethenyl-1,2-dimethyl-	1460			X	X	X	X	X	
	Benzene, (2-methyl-1-propenyl)-	1464	X	X						
	2,4,6-Octatriene, 2,6-dimethyl-	1469		X						
	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z,E)]-	1610	X	X	X	X	X	X	X	X
	Cyclohexane, 1-methyl-4-(1-methylethenyl)-, trans-	1675						X	X	
	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	1741	X	X	X		X	X	X	X
	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	1744			X					
	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	1748		X					X	

Table 1. Continued.

	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	1776	X	X	X	X	X	X	X	X
	Total of hydrocarbons		**7**	**10**	**9**	**6**	**6**	**8**	**10**	**5**
Ketones	2-Pentanone	1082					X	X		
	Nootkatone	2538	X	X						
	Total of ketones		**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**
Phenols	Phenol, 2-nitro-	1828	X	X	X	X	X	X	X	X
	Total of phenols		**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Terpenes	3-Carene	1192		X	X	X	X		X	X
	.beta.-Pinene	1207	X	X	X	X	X	X	X	X
	D-Limonene	1263	X	X	X	X	X	X	X	X
	.alpha.-Phellandrene	1287		X	X			X		
	.gamma.-Terpinene	1287	X	X	X	X	X	X	X	X
	o-Cymene	1309	X	X	X	X	X	X	X	X
	(+)-4-Carene	1314	X	X	X	X	X	X	X	X
	.alpha.-Cubebene	1484	X	X	X		X	X	X	X
	.alfa.-Copaene	1519	X	X		X		X		
	cis-muurola-4(14),5-diene	1615	X		X	X	X		X	X
	Caryophyllene	1616			X			X		
	alpha-terpineol	1710	X	X	X	X	X	X	X	X
	7-epi-.alpha.-selinene	1785	X	X	X	X	X	X	X	X
	Total of terpenes		**10**	**11**	**12**	**10**	**10**	**11**	**10**	**10**

*LRI – Linear Retention Index; X = presence.

4 CONCLUSION

This study is the first investigation about the use of microwave heating to replace the conventional pasteurization of orange juice-milk beverages. The findings showed that the microwave heat-treated beverages had lower browning and higher concentrations of ascorbic acid, total phenolics, and carotenoids, higher antioxidant activity, and higher α-amylase, α-glucosidase, and ACE inhibition when compared with the pasteurized product. The process condition at 65 ◦C/60 s is recommended, once it provided the retention of the VOCs and higher bioactive compounds and bioactivity in the final beverage. Therefore, the MH proved to be an effective alternative for the processing of a mixed beverage of orange juice and milk, providing products with improved nutritional value when compared to the product subjected to conventional pasteurization, thus being considered an outstanding technological innovation.

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CAPÍTULO III

MICROWAVE HEATING IMPACTS POSITIVELY ON THE PHYSICAL PROPERTIES OF ORANGE JUICE-MILK BEVERAGE

MICROWAVE HEATING IMPACTS POSITIVELY ON THE PHYSICAL PROPERTIES OF ORANGE JUICE-MILK BEVERAGE

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RESEARCH ARTICLE

Microwave heating impacts positively on the physical properties of orange juice-milk beverage

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ABSTRACT

The impact of microwave heating (MWH, 65, and 75°C: 15, 30, 60 s) on the physical properties of orange juice-milk beverages (OJMBs) compared with those subjected to conventional heating (75°C/15 s) and untreated was evaluated. The ideal operating conditions were at 915 MHz compared with 2450 MHz because of the higher loss factor values and penetration depth, resulting in higher heat dissipation and temperature distribution effectiveness. The MWH samples had rheological properties similar to untreated beverages with a slightly more intense yellow colour and a smaller particle size, especially at higher temperatures and holding times.

Keywords: Microwave heating, Milk juice beverages, Physical parameters, Pasteurisation.

1 INTRODUCTION

The growing consciousness of consumers about the association between health and diet and the increasing demand for sustainable processes have stimulated new food product development aligned with the use of natural ingredients and the application of innovative and sustainable technologies (Saraiva *et al*. 2020; Weber *et al*. 2020). Vegetables and fruit present an important role in the human diet (Jideani *et al*. 2021). Previous studies have already reported a lower chronic disease risk in populations with high consumption of vegetables and fruits (Tresserra- Rimbau *et al*. 2019; de la Fuente *et al*. 2021). The FAO/WHO report recommends consuming a minimum of 400 g of fruit and vegetables per day to decrease the risk of nontransmissible chronic diseases, such as heart diseases, cancer and diabetes (WHO-FAO 2021).

In the past years, the demand for milk beverages mixed with fruit juices has increased due to consumers' demand for nutritious and healthy foods (Martins *et al*. 2021). These beverages represent a balanced source of minerals and bioactive peptides (de La Fuente *et al*. 2021; Martins *et al*. 2021). Orange juice-milk beverage (OJMB) has great potential on the market of functional food products. Orange juice is an accepted fruit juice due to its very appreciated sensory characteristics. Furthermore, it shows an essential nutritional profile, which contributes to daily fruit consumption (Paravisini and Peterson 2019). Orange has a wide range of vitamins (A, B_1, B_5, B_6, B_9, C, D and E). Its daily consumption provides several health benefits: bone and skin health promotion, cognitive functions improvement, cardiovascular system control and anticarcinogenic action (Alhabeeb *et al*. 2020; Hussain *et al*. 2021; Miles and Calder 2021). Milk plays an essential role in the human diet contributing to significant concentrations of minerals (zinc, calcium, phosphorus, magnesium and potassium), vitamins (A, B_1, B_2, B_5, B_{12} and D), high-quality proteins (casein, whey protein and bioactive peptides), carbohydrates (lactose) and fatty acids (Martins *et al*. 2019).

Furthermore, milk has been considered one o the most reliable health-promoting foods to reduce the risk of sarcopenia, osteoporosis, cardiovascular disease, metabolic syndrome, digestive illnesses and cognitive decline (Hidayat and Du 2019; Yun *et al*. 2020; Kumar *et al*. 2021).

Conventional heating (CVH) or pasteurisation is the thermal processing commonly used to provide microbiological safety and prolong the shelf life of beverages and foods (Gonz´alez-Monroy *et al*. 2018). Pasteurisation consists of a high-temperature short-time (HTST) processing used to inactivate enzymes and microorganisms (e.g. 72°C for 15 s). However, high temperatures can negatively impact foods' nutritional, sensory and functional quality (Martins *et al*. 2019; Funcia and Gut 2020).

Microwave heating (MWH) is based on the capacity of dielectric materials to absorb microwave energy and produce heat (Chaouki *et al*. 2020). Microwave heating is considered more efficient than CVH, and it shows lower energetic costs, shorter processing times and faster heating rates. These advantages result in better sensorial, nutritional and functional products (Cavalcanti *et al*. 2019; Devi *et al*. 2021; Kutlu *et al*. 2021). Our previous study indicated that MWH could improve the bioactive peptides content without changing the volatile compounds and fatty acid profile of OJMB (Martins *et al*. 2021). However, other aspects necessary for consumer acceptance must be evaluated. In this sense, this study aimed to assess the effect of MWH processing (65°C and 75°C, for 15, 30 and 60 s) on the physical aspects (colour attributes, rheological properties, and particle size) of OJMB.

2 MATERIALS AND METHODS

2.1. OJMB Beverage Processing

Orange (*Citrus sinensis* (L.) Osbeck), ‘Pera’ variety, and ultra-high temperature (UHT)-treated skim milk (0.1% fat, Paulista, Danone, Amparo, SP, Brazil) were obtained in supermarkets. The OJMB was prepared, as detailed by Barba *et al*. (2012), by mixing 50% (v/v) of orange juice (9 °Brix), 30% (v/v) of filtered water and 20% (v/v) of skim milk. The dry ingredients – citric acid (0.1% w/v), sucrose (7.5%, w/v) and 0.3% high methoxyl citrus pectin – were added to the content. Then, the OJMB was homogenised in a blender (Philco®, 1200 W, 2 L, Rio de Janeiro, Brazil) for 2 min and at 25°C. The beverage was prepared immediately before the thermal treatments.

2.2 Dielectric Property Measurement and Power Penetration Depth

The dielectric properties were determined by a vector network analyzer (E5061B; Agilent Technologies, Malaysia) with a coaxial cable (N6314A; Agilent Technologies, Bayan Lepas) and connected to an open-ended coaxial-line probe (85093C; Agilent Technologies), as described by Cavalcanti *et al*. (2019). We evaluated 915 and 2450 MHz, which are the most used frequencies in industrial and domestic applications of microwave processing. The network analyzer determined the reflection coefficient at the sample–probe interface. The dielectric constant (ε’) and dielectric loss factor (ε’) were calculated using the dielectric probe kit software (85070E; Agilent Technologies). The interferences were minimised using an electronic calibration module (85093C; Agilent Technologies). Calibration was executed using three standards: short circuit, open circuit (air) and deionised water (25°C). For that, 150 mL of the sample (in an Erlenmeyer flask) was immersed in a thermostatic oil bath TC-550 (Brookfield). For each isotherm (5–90°C), five frequency sweep readings (500–3000 MHz) were made. The temperature was controlled using a thermocouple (±1°C tolerance) (Mileto, São Paulo, Brazil). The samples’ electrical conductivity (σ_{cond}) was measured using a conductivity meter YSI3200 and YSI3252 cell (YSI, Yellow Springs, OH, USA).

Power penetration depth (DP) is related to the depth in which the power is reduced to 36.8% (1/e) (e = Euler number) of the incident power at the surface of the material. Penetration depth considers the dielectric properties and wavelength of the material and is calculated using Eq. 1 (Risman 1991):

$$d_p = \frac{c}{2\pi f\sqrt{2\varepsilon'\left[\sqrt{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2}-1\right]}} \quad (1)$$

where c is the speed of light in free space (2.9979 × 10^8 m/s), and f is the electromagnetic wave frequency.

2.3 Thermal Processing

The OJMB samples were divided in eight treatments: T1 (untreated raw beverage), T2 (CVH, 75°C/15 s), T3 (MWH, 65°C/15 s), T4 (MWH, 65°C/30 s), T5 (MWH, 65°C/60 s), T6 (MWH, 75°C/15 s), T7 (MWH, 75°C/30 s) and T8 (MWH, 75°C/60 s). Microwave heating was conducted in a microwave digester (Discover System 908005; CEM Corporation, Matthews, NC, USA, 300 W) at 915 MHz (based on the previous section results). Conventional heating was performed in a heating mantle (12 M; Fisatom Equipamentos

Científicos Ltda., Perdizes, SP, Brazil). For this, 100 mL of OJMB was heated in round bottom flasks (250 mL) under magnetic stirring, regardless of the heat treatments. A fibre optic sensor (Fluoroptic STF-1 M; LumaSense Technologies, Santa Clara, USA) was included at the liquid centre for temperature control. The temperature was registered every 0.5 s using a data acquisition system (Luxtron 812; LumaSense Technologies, Santa Clara, CA, USA). After the heating treatment, the samples were cooled in an ice-water bath (10 ± 1°C), transferred to sterile polyethylene terephthalate bottles and stored under refrigeration at 4°C.

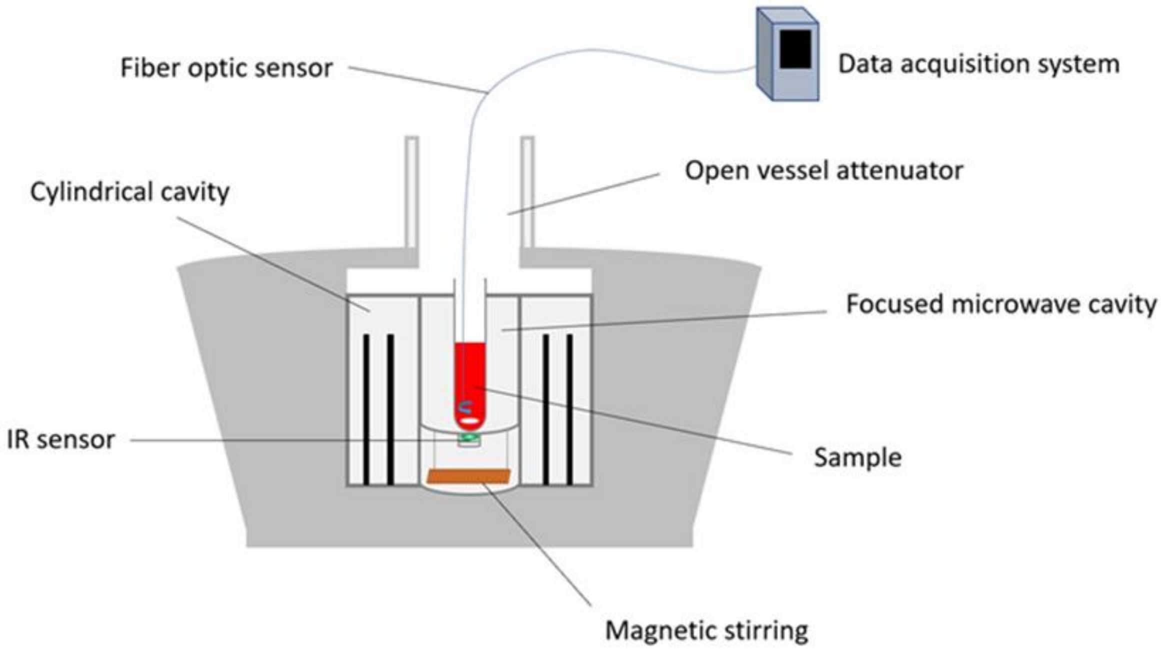

Figure 1. Microwave heating scheme

Figure 1 shows the MWH apparatus. During the heating ramp, the equipment was configured to operate at fixed power mode, while during the holding time, the equipment was configured to operate at fixed temperature mode.

2.4 Colour Analysis

Colour parameters (L^*, a^*, b^*) were determined using a colorimeter (Color Quest XE Hunter Lab, USA). Chroma (C^*), hue angle (h_{ab}) and total colour difference (ΔE^*) were also calculated (Pathare and Opara 2013).

2.5 Rheological Analysis

The rheological properties were evaluated using a controlled stress rheometer (AR1500ex, TA Instruments, England). The equipment had a plate–plate geometry of 40 mm in diameter and a 53-µm gap, and the samples were left to rest for 10 min on the plate to recover the structure. The temperature was 25 ± 0.1°C, and it was kept constant using a Peltier system. Three shear rate sweeps were performed: the first with increasing shear rate (0–300 s^{-1}), the second with decreasing shear rate (300–0 s^{-1}) and the third with increasing shear rate (0–300 s^{-1}). In addition, three shear rate sweeps (up–down–up programme) were performed to eliminate thixotropy and calculate the steady-state rheological properties of the samples. Finally, the third curve data were fitted to the power law model (Eq. 2) through nonlinear regression using STATISTICA 8.0 software (StatSoft, Tulsa, OK, USA).

$$\sigma = K\dot{\gamma}^n \quad (2)$$

where σ is the shear stress (Pa), K is the consistency index (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the flow behaviour index (dimensionless).

2.6 Particle Size Distribution

The particle size analysis was performed using a Malvern Mastersizer integrated into a laser light scattering (Worcestershire, UK), according to Costa *et al*. (2018). The samples were included in the sample unit containing deionised water and pumped through the optical cell. Stirring at 500 g with 20–30% obscuration was used. The following parameters were determined: $d_{0.1}$ is the particle size diameter below which 10% of the material is contained; $d_{0.5}$ is the particle size diameter below which 50% of the material is included; $d_{0.9}$ is the particle size diameter below which 90% of the material is contained; span is an indicator of how far the $d_{0.1}$ and $d_{0.9}$ are apart from each other, normalised with the midpoint $d_{0.5}$; $D_{4,3}$ = particle volume mean diameter, corresponding to the sphere diameter that has the same average volume of the constituent particles; $D_{3,2}$ = particle surface area mean diameter, which corresponds to the mean diameter proportional to the volume and surface area ratio.

2.7 Statistical Analysis

The experiment followed a completely randomised design and was repeated three times. Each analysis was performed in triplicate. Data were submitted to one-way analysis of

variance (ANOVA), and Fisher's post hoc test determined significant differences between mean values (P < 0.05). In addition, principal component analysis (PCA) was performed with the physical analysis results to obtain a twodimensional map and confidence ellipses. The data set consisted of 24 × 16 matrices, with the lines representing the samples and the columns representing the studied parameters (Balthazar *et al*. 2018). The statistical analyses were conducted using the XLSTAT software for Windows Excel® version 2019.4.1 (Addinsoft, Paris, France).

3 RESULTS AND DISCUSSION

3.1 Dielectric Properties

The dielectric properties provide information about the interaction between electromagnetic fields and food. This knowledge is fundamental for the modelling, simulation and optimisation of MWH processes (Tao *et al*. 2020). For example, the dielectric constant is associated with a material's ability to store electromagnetic radiation, while the dielectric loss factor indicates the material's ability to convert electromagnetic energy into heat (Zheng and Sun 2021). Additionally, the penetration depth of microwave power is related to the heating uniformity and is a critical parameter for microwave process development. Overall, temperature and frequency significantly impact the dielectric properties (Hernandez-Gomez *et al*. 2021).

In Figure 2a, an increase in temperature and frequency resulted in a decrease in the dielectric constant. These results are associated with reducing the polarisation under thermal agitation and the reduced dipole response under higher frequencies, respectively (Franco and Tadini 2017). This pattern is typical of water-rich foods and has been massively reported in the literature for several liquid foods, such as grape juice (García *et al*. 2001) and tamarind and green beverages (Gonzàlez-Monroy *et al*. 2018). Hernandez-Gomez *et al*. (2021) also reported that the dielectric constant was inversely proportional to temperature and frequency when evaluating Mexican sauces subjected to MWH.

Figure 2b shows that a temperature rise may cause a loss factor increase or decrease, depending on the frequency range. To understand this behaviour, it is essential to point out that MWH is mainly governed by the combined effect of two mechanisms, namely dipolar rotation and ionic conduction. Thereby, the loss factor can be mathematically expressed as $\varepsilon'' = \varepsilon''_{\sigma} + \varepsilon''_{d}$, where ε''_{d} is the dipole loss and ε''_{σ} is ionic loss (Iris *et al*. 2020). Generally, ionic conductivity shows a significant role at low frequencies, whereas, at high frequencies, the primary mechanism is the free water dipole rotation (Kubo *et al*. 2018). Thus, the loss factor increased with temperature at low frequencies because of the ionic conductance and decreased at high frequencies because of free water dispersion (Sosa-Morales *et al*. 2010). Furthermore, higher temperatures favour the mobility of charged ions due to the decrease in viscosity when the temperature increases. At the same time, lower frequencies tend to increase the length of ion motion. On the contrary, dipole loss is associated with the water molecule dipolar relaxation in which the loss factor increase with frequency is visualized at low temperatures. In other words, the loss factor plot can be considered the transition from an ionic-governed loss to a dipolar-governed loss (Franco *et al*. 2015).

Figure 2c shows that penetration depth declines with the frequency increase, but a remarkable shift can be noticed in temperature dependence with frequency. At lower frequencies, the penetration depth decreases with temperature increase, which means the penetration depth decreases with heating favouring the cold spot appearance. On the other hand, at higher frequencies, the penetration depth increases with temperature, which means as the food is heated, the radiation penetrates more deeply, promoting more homogeneous heating (Cavalcanti *et al*. 2019). Additionally, it is worth mentioning that the heating pattern of the material will also depend on the dimensions and geometry of the material and the microwave cavity.

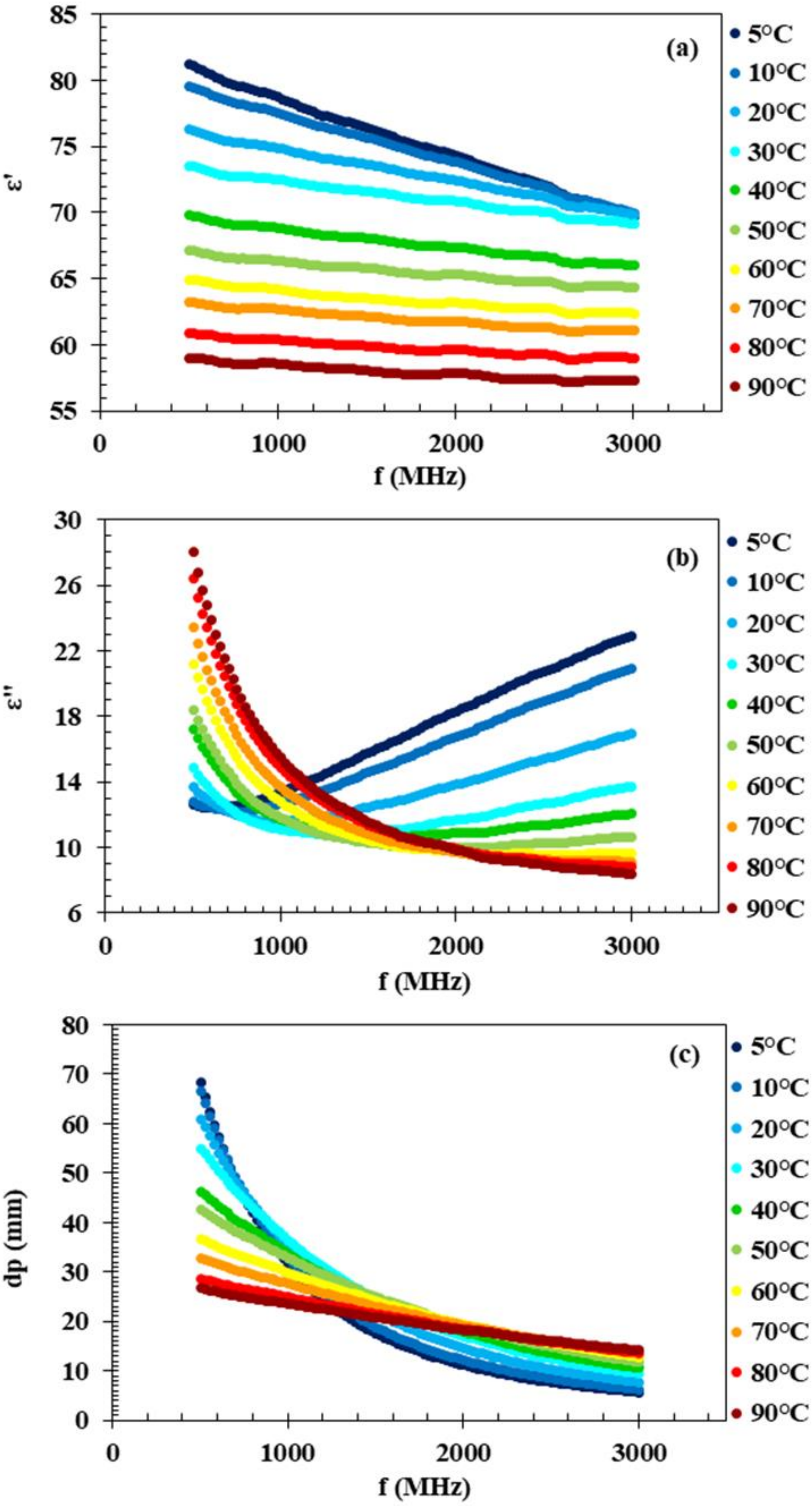

Figure 2. Dielectric constant (a), dielectric loss factor (b) and penetration depth (c) of orange juice-milk beverage at different temperatures (5–90°C) and electric field frequencies (500–3000 MHz).

Figure 3a–c exhibit the dielectric constant, loss factor and penetration depth. The dielectric constant of the OJMB decreased with temperature for both frequencies, with an almost linear behaviour (Figure 3a). Despite that, the dielectric constant showed a better fit to the quadratic equation ($0.988 \leq R^2 \leq 0.998$). For example, at 915 MHz, the mean dielectric constant dropped from 79.1 to 58.7. However, lower values were visualised at 2450 MHz, where the dielectric constant decreased from 72.4 to 57.5. This result indicates a decrease in the material's ability to store electromagnetic energy with temperature. On the contrary, the loss factor exhibited a somewhat contrasting behaviour in which the loss factor fell with the increase in temperature at 2450 MHz but declined at 915 MHz. Considering the heating temperatures like 65 and 75°C, this suggests that better efficiency in converting electromagnetic energy to heat can be achieved at 915 MHz. Similar results were observed by Hernandez-Gomez *et al*. (2021) when evaluating Mexican sauces subjected to MWH. Furthermore, an opposite behaviour profile was observed for the penetration depth, where an increase with temperature was observed at 2450 MHz, whereas a decrease was observed at 915 MHz. This behaviour may be associated with the thermal runaway phenomenon. An increase in temperature leads to a high loss factor and, simultaneously, a penetration depth decrease that results in a heterogeneous distribution of temperature (Cavalcanti *et al*. 2019). In this way, a greater uniform microwave dielectric heating may be achieved at 915 MHz (Hernandez-Gomez *et al*. 2021).

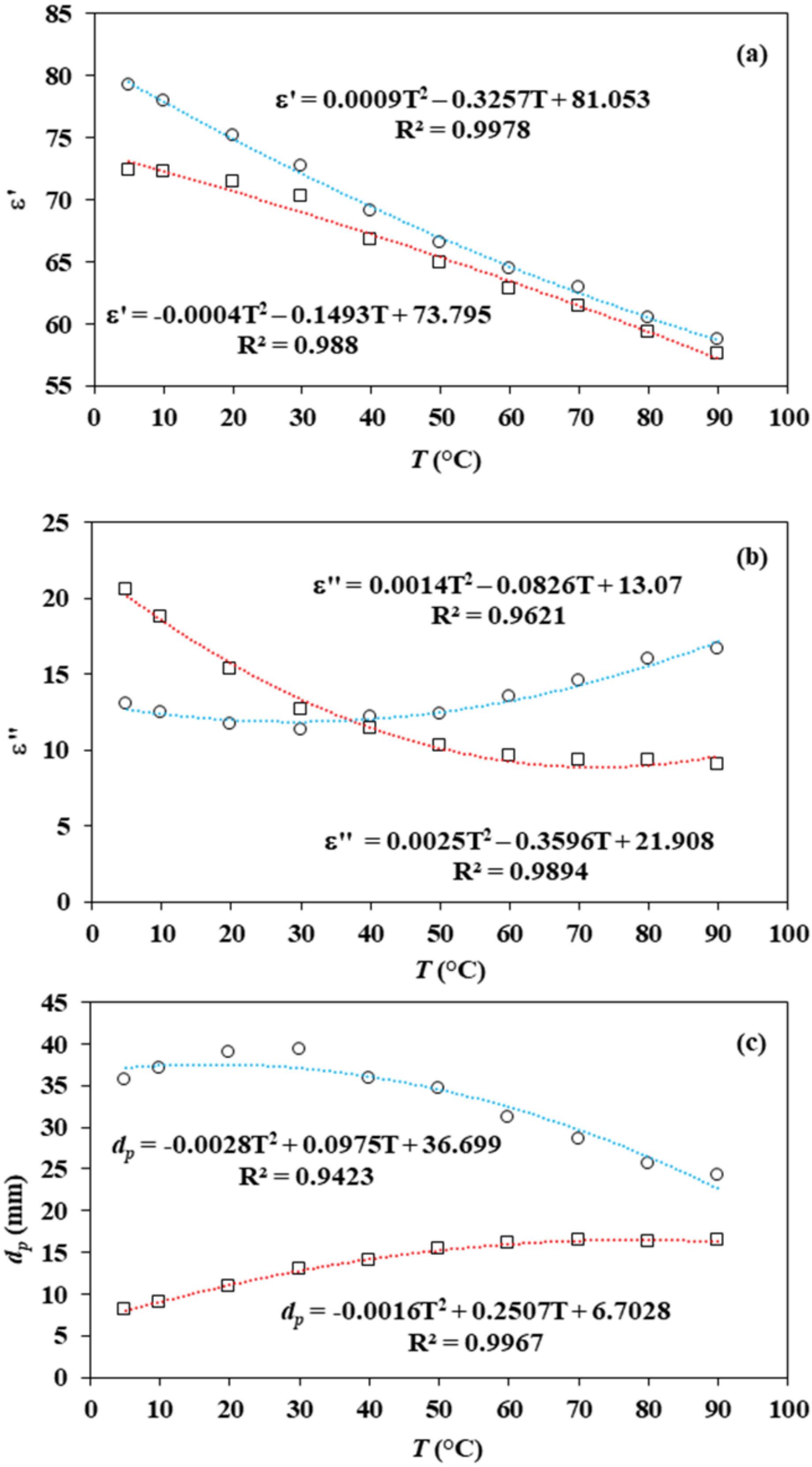

Figure 3. Dielectric constant (a), dielectric loss factor (b) and penetration depth (c) of orange juice-milk beverage at different temperatures (5–90°C) at 915 MHz (○) and 2450 MHz (□).

3.2 Physical Properties

Figure 4a–c show the physical characteristics of the OJMB concerning their colour attributes (L^*, a^* and b^*), rheological properties and particle size distribution (PSD) respectively. In Figure 4a, a slight variation in the CIE $L^*a^*b^*$ colour space was observed. Overall, all samples have shown a light-yellow colour with L^* ranging from 65.5 to 66.0 and yellowness ranging from 28.6 to 29.5. The heated microwave samples at 65°C for 60 s (T5) and 75°C (T6–T8) showed slightly higher values of lightness (L^* = 66.0) and yellowness (b^* = 29.3–29.5) in comparison with the other samples (Table 1). Since the colour parameter a^* was approximately zero, its impact on Eq. 2 was neglectable, resulting in C^* values equal to b^*, in terms of significant numbers. Nevertheless, a slight shift was observed from a positive a^* value, at the untreated raw beverage (0.083), to negative values, at the heat-treated samples (−0.143 to −0.037). This phenomenon can also be observed through the hue angle (hab) change from 89.8 degrees (T1) to 90.3–90.1 degrees (T2–T8). All samples presented minimal differences in comparison with the untreated raw beverage ($\Delta E^* < 1.0$); however, the highest differences were observed for the MWH-treated samples at 75°C (ΔE^* = 0.716–0.876). Barba *et al*. (2012) found a shift from positive a^* values in the unprocessed OJMB (1.35) to negative values in the heat-treated samples at 90°C (−0.37) and 98°C (−0.47). The same authors have reported higher L^* (71.1) and b^* (37.0) for the unprocessed OJMB compared with our study. Furthermore, they have also observed an increase in yellowness in the thermally treated samples at 90°C (42.6) and 98°C (42.8).

In Figure 4b, the flow curves of the OJMB have shown a similar behaviour for all samples; however, the one treated by microwave at 75°C for 15 s (T6) exhibited the most intense shear stress. The experimental data were fitted to different mathematical models (data not shown), and the most suitable model was chosen based on the highest determination coefficient (R^2). Based on these criteria, the power law model was the most appropriate one in which its rheological parameters apparent viscosity (η_{ap}), consistency index (K) and flow behaviour index (n) are presented in Table 2. Significant differences ($P < 0.05$) can be observed between the treatments with respect to apparent viscosity (η_{ap} = 11.4–16.15 mPa s), consistency index (K = 106.3–135.9 mPa s^n) and flow behaviour index (n = 0.438–0.562) ($P <$ 0.05). It is noteworthy that no trend regarding temperature and holding time was noticed between the treatments, though.

Regarding the flow index, it is clear that all samples have shown a typical pseudoplastic behaviour ($n < 1$), which can be corroborated by the shape of the flow curves observed in Figure 4b. Abbasi and Mohammadi (2013) also reported a pseudoplastic behaviour for the milk-orange juice mixture added with Persian gum fitted to the power law model; however, the control had a better adjustment to the Bingham model. In our study, the sample T2 had the smallest n value, suggesting that CVH promoted the most significant deviation from the Newtonian flow. In addition, this same sample has shown the highest consistency index (135.9 ± 0.1 mPa s^n). Overall, the MWH-treated samples (T3–T8) presented flow behaviour and consistency indexes closer to the untreated beverage (T1). This result indicates that MWH preserved the rheological properties of the OJMBs.

In Figure 4c, it can be seen that the particles in the size range of 100–1000 μm were relatively numerous in the OJMB. Still, the untreated sample (T1) and the sample treated by CVH (T2) had the narrowest distribution, which is corroborated by the fact that these samples gave the smallest spans among the treatments (T1: span = 1.77; T2: span = 1.90) (Table 3). In contrast, the MWH-treated beverages have shown a slightly wider distribution with spans ranging from 2.18 to 2.80, indicating a greater heterogeneity in the PSDs. Generally, the particle volume mean diameter ($D_{4,3}$) and surface area mean diameter ($D_{3,2}$) were quite higher for the untreated sample ($D_{4,3}$ = 361 μm; $D_{3,2}$ = 162 μm) and the sample treated conventionally ($D_{4,3}$ = 301 µm; $D_{3,2}$ = 109 µm) when compared with the MWH-treated beverages (215 µm ≤ $D_{4,3}$ ≤ 243 µm; 60.2 µm ≤ $D_{3,2}$ ≤ 80.6 µm). $D_{4,3}$ is highly associated with large particles, whereas D3,2 is associated with smaller particles (Maniglia *et al*. 2020). The same pattern can be found in $d_{0.1}$, $d_{0.5}$ and $d_{0.90}$, where a decrease is also observed from T1 to T2 and from T2 to the MWH-treated samples (T3–T8). The magnetic stirring might cause this reduction in the particles size for the thermal-treated samples during heating. Although a more pronounced decline in the particles size was observed for the MWH-treated samples, no significant effect of the temperatures and holding times was observed herein. Sucheta and Misra (2020) also observed a smaller particle size in pectin from black carrot pomace subjected to MWH than CVH.

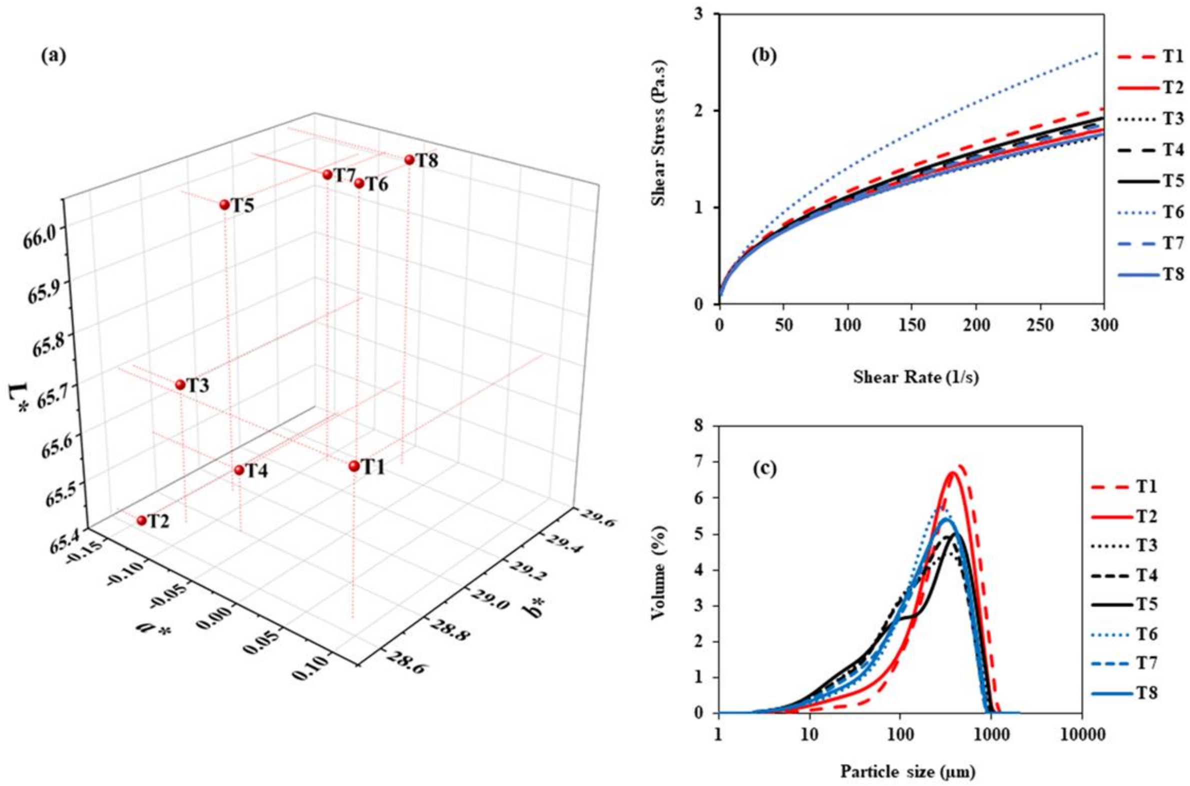

Figure 4. 3D plot of the colour attributes *L*a*b** (a), flow curves (b) and particle size distribution (c) of orange juice-milk beverage. T1 (untreated beverage), T2 (CVH, 75°C/15 s), T3 (MWH, 65°C/15 s), T4 (MWH, 65°C/30 s), T5 (MWH, 65°C/60 s), T6 (MWH, 75°C/15 s), T7 (MWH, 75°C/ 30 s) and T8 (MWH, 75°C/60 s).

Table 1. Colour parameters of orange juice-milk beverages submitted to conventional and microwave heating.

Sample	Treatments	$L*$	$a*$	$b*$	$C*$	h_{ab}	$\Delta E*$
T1	Untreated	65.7 ± 0.015^{d}	0.083 ± 0.025^{a}	28.7 ± 0.012^{f}	28.7 ± 0.012^{f}	89.8±0.050	0.000±0.000
T2	CVH 75°C/15s	65.4 ± 0.010^{f}	-0.143 ± 0.015^{e}	28.6 ± 0.021^{f}	28.6 ± 0.021^{f}	90.3±0.030	0.369±0.026
T3	MWH 65°C/15s	65.7 ± 0.010^{d}	-0.117 ± 0.015^{de}	28.7 ± 0.006^{e}	28.7 ± 0.006^{e}	90.2±0.031	0.210±0.029
T4	MWH 65°C/30s	65.5 ± 0.015^{e}	-0.070 ± 0.030^{bc}	28.8 ± 0.059^{d}	28.8 ± 0.059^{d}	90.1±0.060	0.270±0.017
T5	MWH 65°C/60s	66.0 ± 0.010^{b}	-0.123 ± 0.031^{de}	29.0 ± 0.049^{c}	29.0 ± 0.049^{c}	90.2±0.060	0.469±0.045
T6	MWH 75°C/15s	66.0 ± 0.006^{c}	-0.053 ±0.031^{b}	29.3 ± 0.020^{b}	29.3 ± 0.020^{b}	90.1±0.060	0.716±0.029
T7	MWH 75°C/30s	66.0 ± 0.006^{bc}	-0.093 ± 0.012^{cd}	29.3 ± 0.038^{b}	29.3 ± 0.038^{b}	90.2±0.022	0.742±0.027
T8	MWH 75°C/60s	66.0 ± 0.017^{a}	-0.037 ± 0.006^{b}	29.5 ± 0.010^{a}	29.5 ± 0.010^{a}	90.1±0.011	0.876±0.010

$^{a–h}$ Results are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences among the treatments (T1–T8) at the 95% confidence level according to the Fisher's test ($P < 0.05$). $L*$, $a*$ and $b*$ are colour parameters. $L*$ for the lightness from black (0) to white (100), $a*$ from green (−) to red (+) and $b*$ from blue (−) to yellow (+). h_{ab} is the hue angle, while $\Delta E*$ is the total colour difference.

Table 2. Rheological parameters obtained from nonlinear regression of the flow curves of orange juice-milk beverages submitted to conventional and microwave heating.

Samples	Treatments	η_{ap} (mPa.s)	*K* (mPa.s^n)	*n* (-)	*R²*
T1	Untreated	13.3 ± 0.1^b	114 ± 3^d	0.500 ± 0.01^b	0.9395
T2	CVH 75°C/15s	11.4 ± 0.3^f	135.9 ± 0.1^a	0.438 ± 0.004^f	0.9126
T3	MWH 65°C/15s	11.7 ± 0.2^e	126 ± 3^b	0.461 ± 0.004^e	0.9240
T4	MWH 65°C/30s	12.13 ± 0.04^d	107.4 ± 0.1^e	0.503 ± 0.001^b	0.9317
T5	MWH 65°C/60s	12.53 ± 0.04^c	111 ± 3^f	0.500 ± 0.01^b	0.9334
T6	MWH 75°C/15s	16.15 ± 0.01^a	106.3 ± 0.2^e	0.562 ± 0.001^a	0.9628
T7	MWH 75°C/30s	12.09 ± 0.02^d	112.1 ± 0.1^{df}	0.492 ± 0.001^c	0.9276
T8	MWH 75°C/60s	11.7 ± 0.2^e	118 ± 3^c	0.474 ± 0.003^d	0.9231

[a–h] Results are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences among the treatments (T1–T8) at the 95% confidence level according to the Fisher's test ($P < 0.05$). η_{ap} = apparent viscosity, *K* = consistency index, *n* = behaviour flow index.

Table 3. Particle size parameters obtained from particle size distribution analysis of orange juice-milk beverages submitted to conventional and microwave heating.

Samples	Treatments	Span (-)	$D_{4,3}$ (μm)	$D_{3,2}$ (μm)	$d_{0.1}$ (μm)	$d_{0.5}$ (μm)	$d_{0.9}$ (μm)
T1	Untreated	1.77 ± 0.03^{g}	361 ± 1^{a}	162 ± 4^{a}	98 ± 5^{a}	327 ± 1^{a}	676 ± 1^{a}
T2	CVH 75°C/15s	1.9 ± 0.1^{f}	301 ± 1^{b}	109.0 ± 0.1^{b}	62 ± 2^{b}	275 ± 1^{b}	577 ± 2^{b}
T3	MWH 65°C/15s	2.8 ± 0.1^{a}	220 ± 3^{f}	65 ± 3^{f}	29 ± 2^{e}	166 ± 4^{d}	496 ± 1^{d}
T4	MWH 65°C/30s	2.6 ± 0.2^{c}	215 ± 1^{f}	63 ± 1^{f}	28 ± 1^{e}	170 ± 3^{d}	473 ± 2^{ef}
T5	MWH 65°C/60s	2.72 ± 0.01^{b}	243 ± 3^{c}	60.2 ± 0.2^{g}	24.7 ± 0.1^{f}	192 ± 4^{c}	546 ± 3^{c}
T6	MWH 75°C/15s	2.16 ± 0.01^{e}	229 ± 3^{de}	80.6 ± 0.1^{c}	41.3 ± 0.1^{c}	197 ± 2^{c}	467 ± 3^{f}
T7	MWH 75°C/30s	2.32 ± 0.02^{d}	224 ± 2^{ef}	67 ± 1^{e}	31 ± 1^{e}	190 ± 2^{c}	471 ± 3^{ef}
T8	MWH 75°C/60s	2.3 ± 0.1^{d}	235 ± 4^{d}	75 ± 1^{d}	38 ± 1^{d}	198 ± 7^{c}	490 ± 2^{de}

Results are expressed as mean ± standard deviation. [a–h] Different lowercase letters indicate significant differences among the treatments (T1–T8) according to the Fisher's test ($P < 0.05$). $D_{4,3}$ = particle volume mean diameter, $D_{3,2}$ = particle surface area mean diameter, $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ = correspond to the particle diameters at 10%, 50% and 90% cumulative distribution respectively. Span = $(d_{0.9} - d_{0.1})/d_{0.5}$.

The two-dimensional map and confidence ellipses of the PCA of OJMB are shown in Figure 5a and b respectively. The first two principal components (F1–F2) accounted for 78.44% of data variability in which the first and second components explained 50.05% and 28.40%, respectively. The high variability obtained suggests that the physical analysis provided relevant information and summarised the impact of the thermal treatments on the physical properties of OJMB. In addition, the untreated raw beverage, the conventionally treated sample and the microwave-treated samples at 65 and 75°C were well distinguished from each other, as can be seen by the confidence ellipses shown in Figure 5b. The untreated sample (T1) was positively associated with larger particle sizes and redness (+*a**), whereas the CVH-treated sample (T2) was correlated to a higher consistency index. The MWH-treated samples at 65°C (T3–T5) were associated with more pronounced hue angles and spans. Additionally, the MWH-treated samples at 75°C (T6–T8) had a more intense pseudoplastic behaviour, lightness, chroma, colour difference and yellow colour. These findings summarise the main parameters affected by the microwave processing of the OJMB, indicating a positive impact of microwaves on the physical properties of the OJMB, especially for those processed at 75°C.

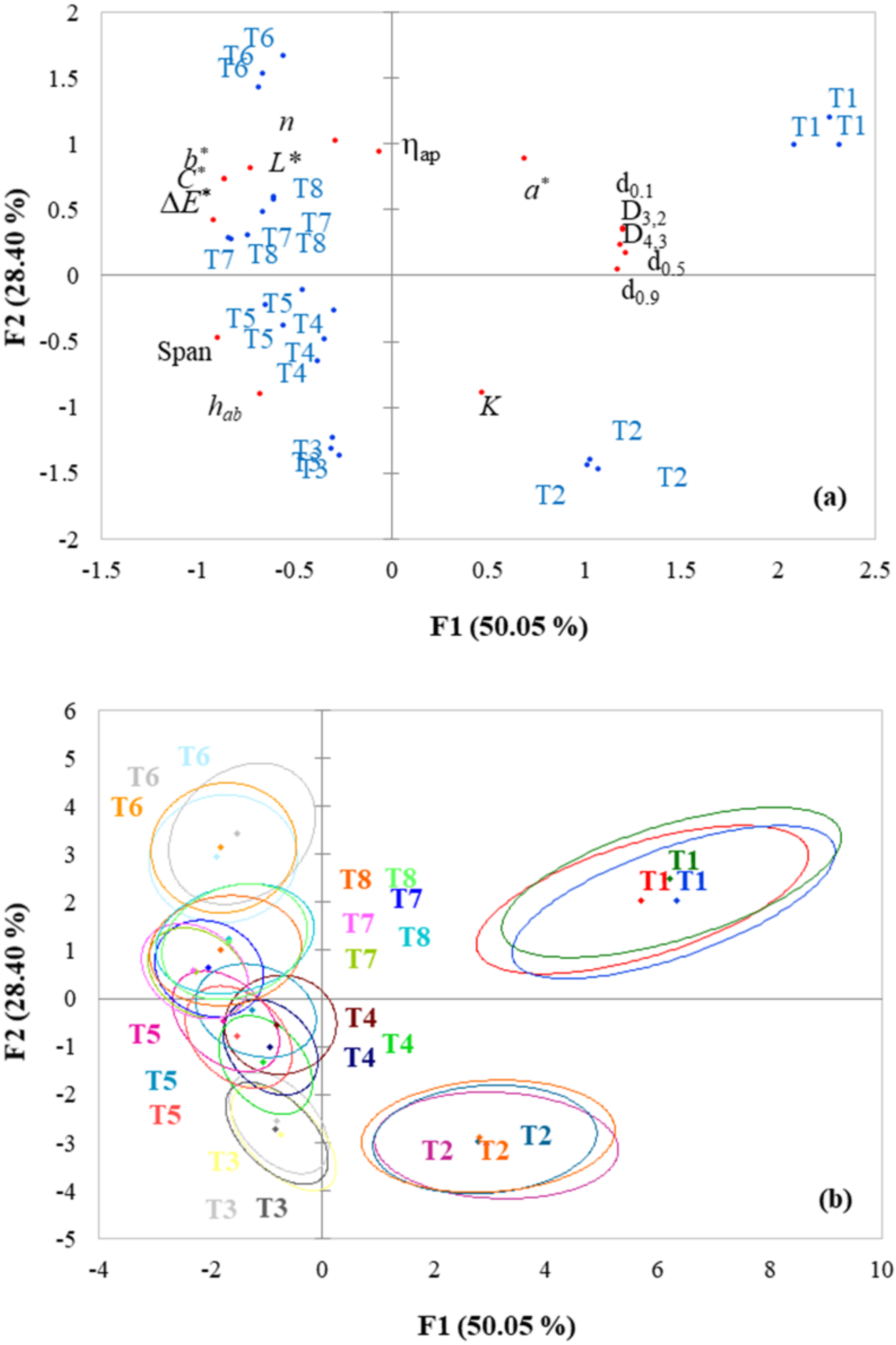

Figure 5. Two-dimensional map (a) and confidence ellipses (b) of the principal component analysis of orange juice-milk beverage. T1 (untreated beverage), T2 (CVH, 75°C/15 s), T3 (MWH, 65°C/15 s), T4 (MWH, 65°C/30 s), T5 (MWH, 65°C/60 s), T6 (MWH, 75°C/15 s), T7 (MWH, 75°C/30 s) and T8 (MWH, 75°C/60 s).

4 CONCLUSION

This work aimed to evaluate the impact of MWH on the physical characteristics of OJMB. Firstly, the dielectric properties of the beverages were measured to determine the ideal conditions of MWH. In general, the dielectric constant decreased with the temperature at both frequencies (915 and 2450 MHz). Thus, as the beverage is heated, it will gradually lose its ability to store electromagnetic energy. In contrast, higher values of loss factor and penetration depth were observed at 915 MHz, indicating that the microwave processing of OJMB at 915 MHz can be more effective in terms of heat dissipation and temperature distribution respectively.

The OJMB has presented significant differences in the physical properties between the heat treatments. For example, the CVH-treated beverages exhibited the highest consistency, while the untreated drink had considerable particle sizes. On the other hand, the MWH-treated samples presented a slightly more pronounced pseudoplastic behaviour and a little more intensity in lightness and yellow colour, which may positively appeal to the consumer's acceptance. In summary, MWH can be considered an effective alternative for processing mixed beverages of orange juice and milk, yielding products with similar or enhanced physical properties compared with pasteurisation.

This study was limited to the physical aspects of the OJMB, while in a previous study of our group, the bioactivity and chemical composition were evaluated (Martins *et al*. 2021). In this way, future studies should assess consumers' sensory acceptance of the products and the differences in the sensory characteristics using descriptive tests.

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CAPÍTULO IV

HOW THE MICROWAVE TECHNOLOGY IS PERCEIVED A FOOD SAFETY CROSS-CULTURAL STUDY BETWEEN BRAZIL AND PORTUGAL

HOW MICROWAVE TECHNOLOGY IS PERCEIVED? A FOOD SAFETY CROSS-CULTURAL STUDY BETWEEN BRAZIL AND PORTUGAL

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How microwave technology is perceived? A food safety cross-cultural study between Brazil and Portugal

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ABSTRACT

This study aimed to verify the knowledge related to domestic microwave ovens by Brazilians (n = 494) and Portuguese (n = 460). A questionnaire with 24 questions about use, knowledge, safety, practice, and food safety attitude and concerns considering microwave oven and microwave-treated foods was used. Brazilian and Portuguese use microwave ovens mainly for reheating, defrosting, and cooking and using utensils labeled safe for microwaves. Furthermore, the migration of compounds from the container to the food and textural changes were the main concerns reported. Brazilians use more the microwave oven to reheat and cook frozen commercial foods. However, 3.6% of Brazilians still use metal containers, 19.7% do not read the instructions for reheating and 12.2% do not read the cooking instructions. Portuguese consumers had a higher understanding of the power levels applied in the microwave oven, a parameter closely linked to heating uniformity and the formation of cold spots, which can pose microbiological risks. Furthermore, they allow food to stand still before consuming it, promoting a more uniform heat distribution and minimizing the formation of cold spots. Safety indices when consuming microwave-treated foods were calculated at 5.7 for Brazilians and 6.4 for Portuguese on a 9-point scale, demonstrating that they were indifferent or considered microwaved-treated products as slightly safe, respectively. People with different levels of education showed different knowledge about the technology, indicating the greater need for information dissemination to reach the population with the lowest level of education.

Keywords: food safety; Brazil; Portugal; consumer perception; microwave

1 INTRODUCTION

Microwaves are a form of electromagnetic radiation widely used for heating food. These waves are reflected by metals but pass through other materials such as plastic and glass and are absorbed by food. Microwave heating is considered an emerging technology and can be more energy-efficient than conventional heating, as heating is faster and is directed only to the food, with no oven heating (USDA FSIS, 2020). However, among the problems in using this technology, non-uniform heating and the possible formation of cold spots stand out, associated with microbiological risk (Atuonwu & Tassou, 2018).

Domestic microwave ovens are currently present in most households. The magnetron is responsible for the production of microwaves, which cause vibration in the food's water molecules and consequent friction between them, generating an increase in temperature (USDA FSIS, 2020). It is estimated that the number of domestic microwave ovens in operation worldwide exceeds 1 billion. Domestic applications include general food cooking and reheating, pre-baking, food drying, defrosting, bleaching, and pasteurization (Atuonwu & Tassou, 2018).

Microwaved foods may have their safety impaired if consumers do not follow the instructions of use. The primary concern is the survival of pathogens due to insufficient heating, mainly in the cold spots (New *et al*., 2017). Recent study evaluated the consumer perception about microwave safety in Malaysia and reported that the respondents had a low level of microwave oven knowledge and safety practice (New *et al*., 2017). Considering that the knowledge about technologies is dependent on the cultural aspects, this study aimed to evaluate the knowledge of microwave technology, including microwave oven safety and microwaved food safety for Brazilians and Portuguese consumers.

2 MATERIALS AND METHODS

A questionnaire with 24 questions was used, adapted from the model proposed by New *et al*. (2017). The questionnaire was divided into four sections (Table 1): socioeconomic data (four questions, questions 1-4); microwave oven safety use and knowledge (seven questions, questions 5-11); microwave oven safety practice when reheating or cooking food (nine questions, questions 12-20); and food safety attitude and concerns about microwaved food (four questions, questions 21-24). The electronic questionnaire was built using Google Docs and sent to the general population in Brazil and Portugal through an email containing a direct link. There was a total of 954 respondents, 494 in Brazil and 460 in Portugal. No difference was observed in participants of the study considering the country (p>005).

Table 1. Questionnaire about microwave knowledge (Continued on next page).

Question	Answers
1. What is your age?	☐ 18 to 24 ☐ 25 to 34 ☐ 35 to 44 ☐ 45 to 54 ☐ 55 to 64 ☐ 65 to 74 ☐ 75 or older
2. What is the highest level of education you have completed?	☐ SPM ☐ Diploma/Advanced Diploma ☐ Bachelor's Degree ☐ Masters Degrees ☐ Doctorates Degree (PhD)
3. What is your gender?	☐ Female ☐ Male
4. Current location of residence	☐ Brazil ☐ Portugal
5. Do you have a microwave at home?	☐ Yes ☐ No
6. Have you used a microwave oven before?	☐ Yes ☐ No
7. Which functions do you use with the microwave oven? Please select all that apply.	☐ Reheating ☐ Cooking ☐ Grilling ☐ Defrosting/Thaw ☐ Others (please specify)

Table 1. Continued.

8. Do you use cookware labelled with 'microwave-safe?'	☐ Yes ☐ No
9. Do you use metal cookware or aluminium foil when you cook/reheat your food in a microwave oven?	☐ Yes ☐ No
10. Are you aware about the power levels of the microwave oven?	☐ Yes ☐ No
11. Do you have any knowledge about the power levels of the microwave oven and howdo they affect microwave reheating/cooking?	☐ Yes ☐ No
12. How long do you normally reheat your food?	☐ < 30 seconds ☐ 30 seconds ☐ 1 minute ☐ 2 minutes ☐ 3 minutes ☐ 4 minutes ☐ > 5 minutes ☐ Don't know
13. Do you reheat periodically?	☐ Yes ☐ No
14. If yes, do you stir your food midway through the microwaving time?	☐ Yes ☐ No
15. Do you allow your food to stand before you consume your food? (Stand meansleaving your food for about 2 to 5 minutes before consumption)	☐ Yes ☐ No
16. Have you tried reheating commercial frozen food products available in convenience stores/supermarkets/hypermarkets using microwave oven?	☐ Yes ☐ No
17. If yes, do you read the instructions on the food label before you start the reheatingprocess? If no, please proceed to Q20.	☐ Yes ☐ No

Table 1. Continued.

18. Do you follow the instructions on the food label to reheat your food using microwave oven?	☐ Yes ☐ No
19. Have you tried cooking commercial frozen food products available in supermarkets/hypermarkets using microwave oven?	☐ Yes ☐ No
20. If yes, do you read the instructions on the food label before you start the cooking process? If no, please proceed to the next page.	☐ Yes ☐ No
21. Do you feel safe eating food that are reheated/cooked using microwave oven?	☐ Extremely unsafe ☐ Very unsafe ☐ Moderately unsafe ☐ Slightly unsafe ☐ Neither safe nor unsafe ☐ Slightly safe ☐ Moderately safe ☐ Very safe ☐ Extremely safe
22. What are your concerns eating microwave heated foods? Please select all that apply.	☐ No concerns ☐ Presence of microorganisms (bacteria, viruses, fungi) in food that can cause foodborne illness ☐ Migration of chemicals from food packaging into food ☐ Change in the taste and texture of the food ☐ Food is not cooked properly and adequately ☐ Other (please specify): _______
23. How important do you consider food labelling to be?	☐ Very important ☐ Somewhat important ☐ Neutral ☐ Somewhat not important ☐ Not important
24. Do you think that it is important to follow the food preparation instructions on the foodlabelling to reheat/cook your food using microwave oven?	☐ Very important ☐ Somewhat important ☐ Neutral ☐ Somewhat not important ☐ Not important

In the socioeconomic data section, consumers were asked about their age, education level, gender, and current location of residence (questions 1-4, Table 1). Regarding the use and knowledge about microwave safety, knowledge about the microwave oven and the safety instructions that must be followed when using the appliance were evaluated. There were seven questions (question 5-11, Table 1), and four were explicitly about the safe use of the microwave oven, with answers necessarily yes or no (question 8-11). Each correct answer received one point, while incorrect ones received no points. Scores ranging from 0 to 4 were added, and the pass rate was set at 50%.

The section on microwave oven safety practice when reheating or cooking food covered the time needed to reheat food, the safe practice of reheating food, and following labeling instructions, with yes or no answers (question 12-20). Two questions were used to measure how often consumers practice safety when reheating/cooking food in the microwave oven. Each correct answer received one point, while incorrect ones received no points. Scores ranging from 0 to 2 were added, and the pass rate was set at 50%.

Regarding food safety attitudes and concerns about microwaved food, a scale of 1 (extremely unsafe) to 9 (extremely safe) was used. The respondents were asked to rate their perception of the safety of eating microwaved food, where an average score was calculated (question 21). They were asked to highlight concerns about eating food heated in the microwave oven through a multiple-choice question, highlighting as many answers as they wanted (question 22). Furthermore, two questions were explicitly used to measure consumer attitudes towards food safety (questions 23 and 24). Points were assigned according to the Likert scale, ranging from 1 to 5, with a maximum of 10 points. Cumulative scores were grouped according to the following three levels: 0-5 negative attitude, 6-7 neutral attitude, and 8-10 positive attitude.

All statistical analyzes were performed using XLSTAT software for Windows Excel® version 2019.0.0 (Addinsoft, Paris, France). The chi-square test and Monte Carlo simulation were used to test the independence between the variables. The chi-square test for larger contingency tables was valid according to the terms: (1) At least 80% of expected frequencies exceed 5; and (2) All expected frequencies exceed (1). The Monte Carlo simulation was used based on the 95% confidence interval if both terms are not in agreement. Findings with a p-value of less than 0.05 were considered statistically significant. Cramer's Phi and V tests were used to test the strength of the association.

3 RESULTS AND DISCUSSION

The sociodemographic results of the survey are shown in Table 2. Of the consumers, 75.1% (371) were female, and 24.9% (123) were male in Brazil, and 87.0% (400) were female, and 13.0% (60) were male in Portugal. In both countries, the majority had completed graduation, 56.4% (279) in Brazil and 47.6% (219) in Portugal, and were under 35 years old, 55.9% (276) in Brazil and 65.0% (299) in Portugal. Regarding the use and knowledge of microwave oven safety, the results showed that 86.6% (428) of Brazilians and 97.6% (449) of Portuguese respondents have a microwave oven at home. Furthermore, 98.8% (488) of Brazilians and 93.0% (428) of Portuguese had previously used the microwave oven.

Table 2. Socioeconomic profile of respondents.

	Brazil		**Portugal**	
Age	**(%)**	**(n)**	**(%)**	**(n)**
18 - 24	24.7	122	22.2	102
25 - 34	31.2	154	42.8	197
35 - 44	26.3	130	21.3	98
45 - 54	11.3	56	9.6	44
55 - 64	4.3	21	2.8	13
65 - 74	1.6	8	1.1	5
≥75	0.6	3	0.2	1
Education level				
Elementary School	2.6	13	0.2	1
High School	28.5	141	6.5	30
University graduate	25.3	125	40.9	188
Specialization	17.0	84	14.1	65
Master's degree	11.5	57	17.4	80
Doctorate degree	10.5	52	10.9	50
Post doctoral	4.5	22	10.0	46
Gender				
Female	75.1	371	87.0	400
Male	24.9	123	13.0	60
Total Respondents	494		460	

Most respondents use the microwave oven to reheat (Brazil: 94.9%, 469; Portugal: 98.0%, 451) and defrost (Brazil: 69.8%, 345; Portugal: 82.8%, 381) the food. The microwave oven was also used for cooking (Brazil: 28.5% 141; Portugal: 25.9%, 119) and grilling (Brazil: 3.4% 176; Portugal: 6.3%, 29). In addition, other ways (3.6%, 18) in Brazil, such as baking food and heating towels, dish towels, and water, were also mentioned.

Figure 1 shows the result regarding the safe use of the microwave oven. Most of the consumers interviewed used utensils labeled as safe for microwaves (Brazil: 73.5%, 363; Portugal: 77.80%, 358) and did not use metal or aluminum pans (Brazil: 96.4%, 476; Portugal: 100%, 460). The use of metal or aluminum pans was reported only among Brazilian consumers (3.6%; 18). Food containers made of plastic that can be microwaved are usually marked at points of sale. The use of this type of container proves to be safe from an operational point of view, but studies have not been able to verify the long-term effect of contact of these materials with food concerning consumer health. Other types of plastics not suitable for microwaves are not recommended, as there may be migration of compounds from the container to the food, considering the high operating temperatures (New *et al*., 2017). Although this information is widely disseminated, a small percentage of Brazilian consumers indicated using aluminum containers in microwaves. The use of metals, especially aluminum, prevents the passage of microwaves and can damage the device due to the reaction with the metal surface and the possible production of sparks (New *et al*., 2017).

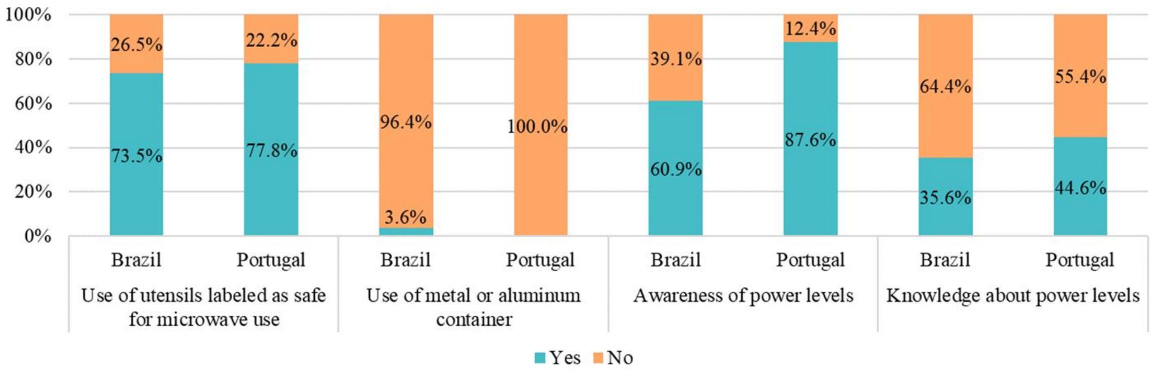

Figure 1. Consumer knowledge about safe microwave oven use.

The survey indicated that 60.9% (301) of Brazilian consumers interviewed were aware of microwave power levels (Figure 1). Still, only 35.6% (176) understood the effects of microwave power levels during reheating and cooking of food. Portuguese consumers interviewed indicated more excellent science and understanding between microwave power levels with 87.6% (403) and 44.6% (205), respectively. The power level is a variable available for the consumer to adjust when using the microwave oven, and it is the parameter that determines the number of microwaves produced. Thus, the power used in the cooking or reheating process must follow the manufacturer's instructions. In the case of reheating, higher power levels should be chosen to ensure heating within the programmed time and provide the product's microbiological safety concerning reaching a safe temperature at all points of the food (New *et al*., 2017).

The microwaves' inactivation of important food pathogens, such as Salmonella sp., Escherichia coli, and Listeria monocytogenes, has been evidenced in several studies addressing different food matrices (Valero *et al*., 2014; Sung & Kang, 2014; Song & Kang, 2016). The power used in microwave treatment directly influences the inactivation of microorganisms, requiring a combination of defined operating parameters. In general, higher potencies cause faster inactivation. Still, it should be noted that exaggeratedly high powers generate more elevated temperatures, leading to the evaporation of a part of the water in the food and consequently to a lower heat transfer, causing less microbial inactivation (Portela *et al*., 2019).

Regarding consumer compliance concerning following the labeling of food for reheating and cooking using the microwave oven (Table 3), it is observed that among Brazilian consumers, 77.9% (385) of respondents reheat commercial food in the microwave. Among these, 80.3% (309) indicated that they read the rewarming instructions, and among those that did, 96.8% (299) stated that they followed them. Meanwhile, 53.0% (262) of respondents cook commercial food in a microwave oven. Among these, 87.8% (230) indicated that they read the cooking instructions, and among those who did, 92.2% (212) stated that they followed them.

Table 3. Safety practice and compliance with food labeling instructions for reheating/cooking using microwave oven.

	Brazil	Portugal	p-value
Reheating using microwave			
Reheat frozen commercial food using a microwave oven	77.9 % (385)	56.7% (261)	< 0.0001[a]
Read label instructions before reheating	80.3% (309)	93.1% (242)	< 0.0001[a]
Follow label instructions before reheating	96.8% (299)	92.1% (223)	0.016[a]
Allows food to stand still before consuming it	24.3% (120)	35.4% (163)	0.000[a]
Cooking using microwave			
Cooking commercial frozen food using a microwave oven	53.0% (262)	41.3% (261)	0.000[a]
Read label instructions before cooking	87.8% (230)	93.2% (177)	0.060[a]
Follow label instructions before cooking	92.2% (212)	95.6% (172)	0.163[a]
Stores microwave-cooked food for later use	23.7% (117)	18.9% (87)	0.073[a]

[a] Assessed using Pearson's Chi-square.

In comparison, among Portuguese consumers, 56.7% (261) of respondents reheat commercial food in a microwave oven. Among these, 93.1% (242) indicated that they read the rewarming instructions, and among those that did, 92.1% (223) stated that they followed them. Meanwhile, 41.3% (190) of respondents cook commercial food in a microwave oven. Among these, 93.2% (177) indicated that they read the cooking instructions, and among those who did, 95.6% (172) stated that they followed them.

Heat is transferred through the food by conduction. For example, when thicker food is subjected to microwave heating, the outermost layers are heated first, and the innermost part is heated by conduction. The rate at which the phenomenon happens depends on the food's geometry, composition, and homogeneity (Muthukumarappan & Swamy, 2019; USDA FSIS, 2020). Also, in industrialized foods specifically for microwave heating, specific packages with particularities must be followed according to the label instructions. For example, active packages aim to promote browning, crisping, and a high-temperature steam atmosphere or even target microwaves to a particular part of the food (Bhunia *et al.*, 2013). Therefore, it is essential to follow the food packaging guidelines, where the manufacturer has tested the main associated parameters to obtain the best result after heating.

The main problem associated with microwaves is the non-uniform distribution of temperature in the food, resulting in regions that have not reached adequate temperatures in the product (cold spots). Therefore, the label instructions, once again, must be followed, as the manufacturer performs heat penetration tests with temperature measurements to verify the most critical points of the food and the combination of microwave heating parameters necessary to avoid these zones. In addition to affecting the sensory characteristics of the food, this problem can pose a microbiological risk, as there is not the necessary temperature range for an eventual microbial inactivation (Portela *et al.*, 2019).

Among consumers, although most respondents indicated compliance with the reheating and cooking instructions, there are still among Brazilian consumers, 19.7% (76) who do not read the instructions for reheating and 12.2% (32) who do not read the cooking instructions. Among Portuguese consumers, 6.9% (18) do not read the instructions for reheating, and 6.8% (13) do not read the instructions for cooking.

As noted in Table 3, most respondents do not allow the food to standstill after reheating (Brazil: 75.7%, 374; Portugal: 64.6%, 297). On the other hand, most do not store cooked food in microwave oven for later consumption (Brazil: 76.3%, 377; Portugal: 81.1%, 373). The act of keeping the food stand after reheating is related to the possibility of more uniform heat distribution, minimizing the formation of cold spots (USDA FSIS, 2020). However, as seen in this study, most consumers are not aware of this information, mainly Brazilian.

When respondents were asked about the microwave reheating time, 44.3% (219) preferred one minute among Brazilians, and 34.8% (172) chose two minutes. At the same time, 55.9% prefer two minutes among the Portuguese, and 23.9% prefer three minutes. The inactivation kinetics of food pathogens by microwaves in different food matrices must be established in the scientific literature to define a period of domestic use that offers microbiological safety to the consumer. Consumers use the shortest possible heating time for convenience, practicality, and agility in daily life, generating a potential microbial risk. Existing studies such as the establishment of microwave Salmonella inactivation kinetics in potato omelet and infant formulas are of great value in this regard (Portela *et al*., 2019; Valero *et al*., 2014). Although there are no scientific records of outbreaks related to foods prepared by microwaves in Brazil and Portugal, there are reports from other locations in the literature (CDC, 2008; Evans *et al*., 1995).

Assessing the attitude of food safety and concerns about foods prepared in the microwave, most consumers consider food labeling essential (Brazil: 90.7%, 448; Portugal: 95.7%, 440), while a lower percentage considers it very important to follow what is described in food labeling (Brazil: 84.6%, 418; Portugal: 76.3%, 351). There is a considerable difference of about 20 percentage points between the importance of labeling and the act of following the preparation instructions on the label among the Portuguese. According to a local study, the Portuguese have a strong habit of checking the expiration date of products but not giving the same importance to storage and preparation instructions (Toscano, 2006).

Respondents were asked if they felt safe eating food prepared in a microwave oven. The average score obtained from the rating response was 5.7 for Brazilian consumers, who felt neither secure nor insecure, and 6.4 for Portuguese consumers, who felt slightly safe. The percentages of the distribution of responses are shown in Figure 2.

The most common concerns about eating food prepared in the microwave oven are indicated in Figure 3. As noted, most respondents indicated their concerns about the migration of chemical products from packaging (261 Brazilians and 287 Portuguese), followed by changes in the flavor and texture of food (206 Brazilians and 200 Portuguese); the food is not cooked correctly and completely (100 Brazilians and 108 Portuguese); and as the slightest concern, the presence of microorganisms (93 Brazilians and 63 Portuguese). In contrast, 24.3% (120) Brazilians and 11.2% (83) Portuguese reported having no concerns about consuming foods prepared in the microwave oven. Microwaves are a type of non-ionizing radiation. However, it should be noted that microwave radiation can heat the human body, causing possible burns if contact is at high levels. Thus, there must be no microwave leakage from the device, which must be sealed in this sense (USDA FSIS, 2020). Furthermore, regarding the migration of substances from the container to the food, there are many concerned consumers due to the constant misinformation about microwaves broadcast on social networks and concerns associated with radiation and cancer (New *et al*., 2017).

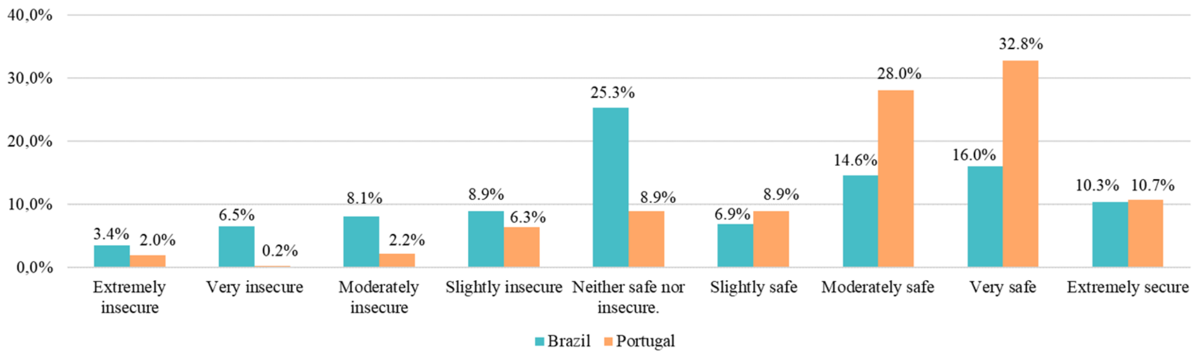

Figure 2. Insecurity in consuming microwave-prepared foods.

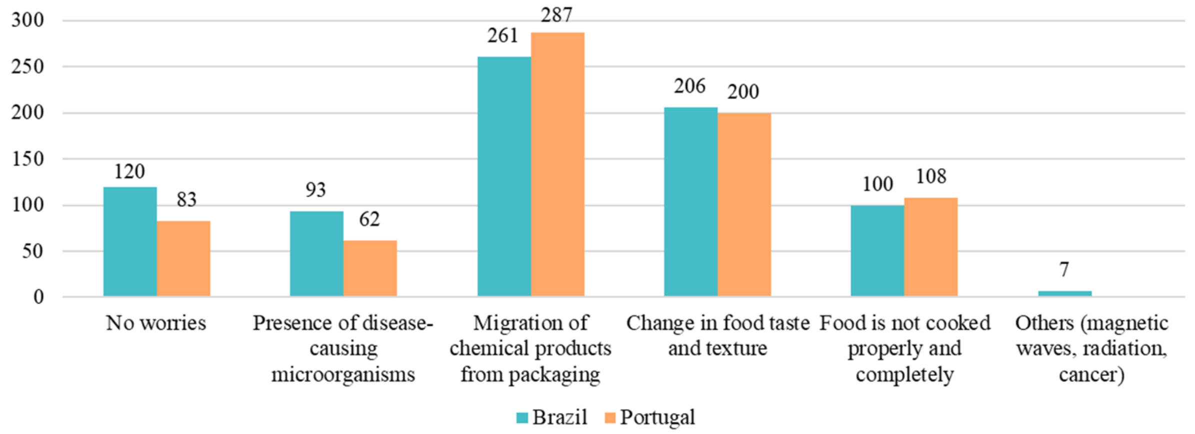

Figure 3. Consumer concerns related to the consumption of microwaved foods.

Texture changes in food are, in fact, a problem in using microwaves for reheating and cooking. The difficulty of crust formation and significant changes in products with high starch content are the main obstacles faced. In addition, moisture loss, steam generated internally in the food, and amylose crystallization is associated with these textural problems (Mizrahi, 2012).

According to local studies, Brazilians show considerable concerns regarding good hygiene practices in food production, but for the most part, they do not have more profound knowledge about microbiological risks (Auad *et al*., 2019; Baptista *et al*., 2020). Positively, it was observed that during the COVID-19 pandemic, there was an increase in the concerns of Brazilian consumers regarding food safety and hygiene practices (Rodrigues *et al*., 2021).

There was no statistically significant association between knowledge of the microwave oven and gender (Brazil: p=0.72; Portugal: p=0.55); and between the attitude of food security concerning gender (Brazil: p=0.41; Portugal: p=0.11) and the education level (Brazil: p=0.25; Portugal: p=0.26), as shown in Table 4, where the p-value was more significant than 0.05. Thus, both genders are statistically independent of microwave oven knowledge and food safety attitude, and there is no significant effect on food safety attitude based on education level. However, a relationship was observed between the microwave oven knowledge and education level (Brazil: p=0.00; Portugal: p<0.0001); and between the microwave oven safety practice during reheating and gender (Brazil: p=0.03; Portugal: p=0.04) and education level (Brazil: p=0.04; Portugal: p=0.01).

Pearson's Chi-square and Cramer's V values were calculated for the measures of association. Knowledge about microwave ovens concerning gender was 0.016 for Brazilians and 0.028 for Portuguese, and the food security attitude towards gender was 0.055 for

Brazilians and 0.093 for Portuguese. Values were interpreted as an insignificant relationship. The attitude of food safety and education was 0.122 for Brazilians and 0.116 for Portuguese. Values were interpreted as a weak relationship, thus not rejecting the null hypothesis of association. However, the values were not equal to 0, which indicates that there may have been some association to affect statistical significance.

Data collection on the population's habits and behavior on emerging technologies, such as microwaves, is necessary for development and consolidation. The percentages of the people that perform a particular practice or procedure that influence the microbiological quality of the product are significant for the development of quantitative microbial risk assessment models, which can offer accurate risk estimates applied to a given population and allow actions towards mitigating the perceived risks (Ramos *et al*., 2021).

Table 4. Association between socioeconomic profile (gender and education level) with knowledge about microwave ovens. safety practices during reheating/cooking of food in microwave ovens. and food safety attitude (Continued on next page).

	Knowledge about microwave oven					
	Brazil			**Portugal**		
	Disapproved	**Approved**	**p-value**	**Disapproved**	**Approved**	**p-value**
Genre						
Female	31.0% (153)	44.1% (218)	0.72[a]	38.5% (177)	48.5% (223)	0.55[a]
Male	10.7% (53)	14.2 (70)		6.3% (29)	6.7 (31)	
Education level						
Elementary School	2.2% (11)	0.4% (2)	0.00[a]	0.0% (0)	0.2% (1)	<0.0001[b]
High School	16.0% (79)	12.6% (62)		2.6% (12)	3.9% (18)	
University graduate	13.6% (67)	11.7% (58)		22.2% (102)	18.7% (86)	
Specialization	8.1% (40)	8.9% (44)		7.0% (32)	7.2% (33)	
Master's degree	3.4% (17)	8.1% (40)		4.1% (19)	13.3% (61)	
Doctorate degree	2.8% (14)	7.7% (38)		2.4% (11)	8.5% (39)	
Post doctoral	0.8% (4)	3.6% (18)		1.5% (7)	8.5% (39)	
	Safety practices					
	Brazil			**Portugal**		
	Disapproved	**Approved**	**p-value**	**Disapproved**	**Approved**	**p-value**
Genre						
Female	45.5% (225)	29.6% (146)	0.03[a]	54.1% (249)	32.8% (151)	0.04[a]
Male	12.3% (61)	12.6% (62)		6.3% (29)	6.7% (31)	
Education level						
Elementary School	1.6% (8)	1.0% (5)	0.04[a]	0.2% (1)	0.0% (0)	0.01[b]
High School	15.8% (78)	12.8% (63)		3.7% (17)	2.8% (13)	
University graduate	17.4% (86)	7.9% (39)		27.4% (126)	13.5% (62)	
Specialization	8.5% (42)	8.5% (42)		7.0% (32)	7.2% (33)	
Master's degree	7.5% (37)	4.0% (20)		11.1% (51)	6.3% (29)	
Doctorate degree	5.1% (25)	5.5% (27)		5.2% (24)	5.7% (26)	
Post doctoral	12.0% (10)	2.4% (12)		4.6% (21)	5.4% (25)	

Table 4. Continued.

	Food safety attitude							
	Brazil				Portugal			
	Negative	**Neutral**	**Positive**	**p-value**	**Negative**	**Neutral**	**Positive**	**p-value**
Gender								
Female	0.2% (1)	1.4% (7)	73.5% (363)	0.41[b]	0.2% (1)	1.1% (5)	85.7% (394)	0.11[b]
Male	0.2% (1)	0.8% (4)	23.9% (118)		0.2% (1)	0.4% (2)	12.4% (57)	
Education level								
Elementary School	0.0% (0)	0.0% (0)	2.6% (13)	0.25[b]	0.0% (0)	0.0% (0)	0.2% (1)	0.26[b]
High School	0.2% (1)	1.6% (8)	26.7% (132)		0.2% (1)	0.2% (1)	6.1% (28)	
University graduate	0.0% (0)	0.4% (2)	24.9% (123)		0.0% (0)	0.9% (4)	40.0% (184)	
Specialization	0.2% (1)	0.0% (0)	16.8% (83)		0.2% (1)	0.2% (1)	13.7% (63)	
Master's degree	0.0% (0)	0.0% (0)	11.5% (57)		0.0% (0)	0.0% (0)	17.4% (80)	
Doctorate degree	0.0% (0)	0.2% (1)	10.3% (51)		0.0% (0)	0.2% (1)	10.7% (49)	
Post doctoral	0.0% (0)	0.0% (0)	4.5% (22)		0.0% (0)	0.0% (0)	10.0% (46)	

[a] Assessed using Pearson's Chi-square.

[b] Assessed using Pearson's Chi-square test based on Monte Carlo 95% confidence interval significance.

4 CONCLUSION

Based on the data extracted from this study, the portion of Brazilians who still use inappropriate materials in the microwave oven (3.6%) and who do not follow label instructions for reheating specific foods (19.7%) is noteworthy. These facts indicate safety risks in the handling of the equipment and microbiological safety, as the non-uniform reheating enables the occurrence of cold spots. In this sense, it is essential to encourage studies of microbial inactivation kinetics using microwaves to establish safe parameters (time and power) for the inactivation of the primary food pathogens in different matrices.

The Portuguese exhibit a higher percentage related to knowledge of microwave power levels, and in both populations studied, the level of education influenced knowledge about the technology. Thus, a significant disparity is evidenced concerning access to knowledge, revealing the need for greater dissemination of information that can reach the population with the lowest level of education, providing better operational safety of the microwave oven, and more excellent knowledge of associated microbiological risks.

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CONCLUSÃO

O aquecimento micro-ondas apresentou impacto nas concentrações dos compostos bioativos da bebida de suco de laranja e leite, com maior preservação do ácido ascórbico, dos compostos fenólicos e dos carotenoides, e melhor atividade antioxidante, inibição da enzima conversora de angiotensina I, da α-amilase e da α-glicosidase em comparação ao tratamento convencional, além disso, possibilitou menor degradação relacionada ao escurecimento.

A análise de componente principal indicou que o tratamento T5 (65 °C/60 s) ficou mais próximo de T1, seguido por T7 (75 °C/ 30 s) e T3 (65 °C/15 s), sendo essas as condições mais semelhantes ao produto não tratado. O dendrograma sugeriu três grupos diferentes: I (T2), II (T4, T5, T6 e T8) e III (T1, T3 e T7), ou seja, as amostras T3 e T7 (65 °C/15 s e 75 °C/30 s, respectivamente) apresentaram características de qualidade semelhantes à amostra não tratada (T1), o que indica a preservação dos compostos bioativos. No entanto, as amostras do grupo II apresentaram resultados mais interessantes para o processamento de bebidas, uma vez que apresentaram maior retenção de compostos fenólicos, atividade antioxidante, inibição α-amilase e menor índice de escurecimento, principalmente a amostra T5, já que seus parâmetros (65 ºC por 60 s) podem ser utilizada para a manutenção e/ou melhoria na concentração dos compostos bioativos da bebida.

Durante o armazenamento, o aquecimento micro-ondas apresentou um comportamento semelhante tanto ao produto submetido à pasteurização convencional quanto ao produto não tratado. No geral, houve redução do teor de ácido ascórbico, compostos fenólicos, carotenoides, atividade antioxidante, inibição da ECA-I, da α-amilase e da α-glicosidase, bem como um aumento no índice de escurecimento. No entanto, as amostras tratadas por micro-ondas apresentaram melhores resultados quando comparado as amostras pasteurizadas convencionalmente (T2).

O aquecimento micro-ondas apresentou maior concentração de ácidos graxos de cadeia média e menores concentrações de ácidos graxos de cadeia longa e ácidos graxos monoinsaturados e, consequentemente, maior índice aterogênico e menor teor de ácidos graxos desejados quando comparado ao produto pasteurizado convencionalmente (T2). Entretanto, o perfil dos ácidos graxos dos produtos tratados por micro-ondas foi semelhante ao produto não tratado (T1).

A pasteurização convencional (T2) possibilitou a formação de compostos orgânicos voláteis que podem contribuir negativamente para o aroma do produto. O tratamento T5 (65 ºC por 60 s) promoveu a formação de compostos importantes responsáveis pelo aroma doce e frutado que não constavam no produto não tratado (T1), entretanto sem a formação de compostos prejudiciais ao aroma da bebida.

Foram verificadas diferenças significativas nas propriedades físicas entre os tratamentos térmicos. As amostras tratadas por micro-ondas (T3-T8) apresentaram um comportamento pseudoplástico ligeiramente mais pronunciado do que a amostra tratada convencionalmente, além de índices de fluxo e consistência mais próximos da bebida não tratada (T1), indicando preservação das propriedades reológicas da bebida. As amostras de micro-ondas aquecidas a 65 °C por 60 s (T5) e 75 °C (T6-T8) apresentaram valores ligeiramente maiores de luminosidade e cor amarela em comparação com as demais amostras, o que pode afetar positivamente para a aceitação do consumidor. A amostra não tratada (T1) e tratada convencionalmente (T2) apresentaram distribuição de partícula mais estreita, e as bebidas tratadas por micro-ondas mostraram uma distribuição mais ampla indicando maior heterogeneidade. Além disso, houve um declínio mais acentuado no tamanho das partículas para as amostras tratadas por micro-ondas, entretanto nenhum efeito significativo foi observado. De modo geral, impactos positivos do micro-ondas nas propriedades físicas da bebida foram verificados, especialmente para as processadas a 75 °C.

A tecnologia micro-ondas provou ser uma alternativa interessante para o processamento da bebida mista de suco de laranja e leite, apresentando resultados semelhantes ou até melhores do que a pasteurização convencional, podendo ser considerada uma tecnologia de destaque na obtenção de produtos inovadores.

Brasileiros e portugueses usam o equipamento principalmente para reaquecer, descongelar e cozinhar, geralmente com o auxílio de utensílios rotulados como seguros para micro-ondas. Brasileiros ainda utilizam utensílio de metal, além de serem maioria em não ler as instruções de reaquecimento e cozimento. Os consumidores portugueses têm um maior conhecimento dos níveis de potência do equipamento, além de permitirem que os alimentos fiquem parados antes de consumi-los. A migração de compostos do recipiente para o alimento, e as alterações de textura e sabor foram as principais preocupações relatadas. Os índices de segurança ao consumir alimentos tratados por micro-ondas indicaram que os brasileiros eram indiferentes e que os portugueses estavam pouco seguros.

O nível de escolaridade influenciou o conhecimento sobre a tecnologia. Assim, evidencia-se uma disparidade significativa no acesso ao conhecimento e revela-se a necessidade de uma maior disseminação das informações para que possam atingir a população com menor escolaridade, proporcionando melhor segurança operacional do forno micro-ondas e conhecimento dos riscos microbiológicos associados.