Food Research International 161 (2022) 111827

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Ohmic heating treatment in high-protein vanilla flavored milk: Quality, processing factors, and biological activity

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A R T I C L E I N F O

Keywords:
Dairy foods
Emerging technologies
Ohmic heating
Bioactive compounds
Microbial shelf life

A B S T R A C T

The processing of high-protein vanilla-flavored milk was performed under different electric field strengths of ohmic heating (5.22 V/cm, OH6; 6.96 V/cm, OH8; 8.70 V/cm, OH10; 10.43 V/cm, OH12) to evaluate the energy consumption, processing parameters, and microbiological, rheological, and biological aspects, compared with the sample submitted to conventional pasteurization (PAST, 72 °C/15 s). All samples showed higher than 12 g/100 mL of protein, consisting of high-protein content products. In addition, Ohmic Heating (OH) generated lower energy expenditure and more significant microbial inactivation of lactic acid bacteria, molds and yeasts, total mesophiles, and psychotropics. Furthermore, OH at lower electric field strengths, mainly OH8, improved anti-diabetic, anti-oxidant, and anti-hypertensive activities and rheological properties, and resulted in lower hydroxymethylfurfural contents, and higher whey protein nitrogen index. The results suggest that OH is a technology that can be used in flavored milk with high-protein content, being recommended an electric field strength of 6.96 V/cm. However, more studies are necessary to evaluate the effect of OH on high-protein dairy products, mainly by studying other OH processing parameters.

1. Introduction

The increased number of consumers concerned about their health and eating habits has been directly reflected in the industry products (Rocha et al., 2020b). In this way, industries are increasingly looking for alternatives to meet this new demand, thus producing foods with greater functional appeal and using technologies capable of reducing processing time and/or energy expenditure and preserving food nutrients (Silva et al., 2020).

Milk and dairy products offer many essential nutrients for maintaining health and preventing chronic diseases (Voutilainen et al., 2022). Therefore, several dairy products have been available in the market, such as flavored milk and dairy beverages, fermented products, and cheeses. Dairy proteins, mainly whey proteins, have health benefits such as lean mass gain (Griffen et al., 2022), immunomodulatory and anti-hypertensive actions, and anti-oxidant and anti-diabetic properties (Baba et al., 2020; Bustamante et al., 2021; Gomes et al., 2021; Rafiq et al., 2021). The health effects are mainly associated with bioactive peptides able to inhibit angiotensin-converting enzyme (ACE), α-amylase, and α-glucosidase activities and increase the anti-oxidant potential of the products (Garg & Kumar, 2021). In addition, dairy proteins may show important technological properties when used as a food ingredient (Wen-Qiong et al., 2019; Lesme et al., 2020). Therefore, drinks with high-protein content, such as flavored milk, have excellent market potential and have been among the most consumed dairy products by people seeking better eating habits (Cornall, 2021).

Emerging technologies are gaining increasingly prominence due to their various advantages compared to conventional heat treatment, such as more remarkable nutritional and sensory preservation and greater sustainability (Ribeiro et al., 2022). For example, Ohmic Heating (OH) is

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https://doi.org/10.1016/j.foodres.2022.111827
Received 4 June 2022; Received in revised form 28 July 2022; Accepted 19 August 2022
Available online 24 August 2022

an emerging thermal technology consisting of an electrical current passing through a food product (Waziiroh et al., 2022). It shows several benefits, such as rapid and uniform generation of heating in the product and preservation of nutritional value (Pires et al., 2020) by forming small peptides with higher digestibility (Rocha et al., 2020c), which is a good characteristic of high-protein products. Furthermore, it results in the lower formation of undesirable aromas and flavors (Cappato et al., 2017), improving consumer acceptance of dairy products (Rocha et al., 2020a; Silva et al., 2020; Silva et al., 2021). Finally, compared to conventional ones, it is a technology that causes minor environmental damage when coming from clean energy sources (Balthazar et al., 2022).

The use of OH as an alternative method for pasteurizing processed foods deserves attention from the scientific community (Silva et al., 2022). For dairy foods, different applications have been reported as cheese (Rocha et al., 2020), dairy beverages (Ferreira et al., 2019), infant formula (Pires et al., 2021), whey drinks (Pereira et al., 2020; Cappato et al., 2018) and dairy dessert (Kuriya et al., 2020). However, the application of OH to high-protein liquid dairy products has not been reported so far. OH may change the protein's functional properties and structure, impacting the products' functional and technological properties (Costa et al., 2018). The electric field strength has been considered the most critical process parameter to be evaluated in the OH process (Waziiroh et al., 2022). Therefore, this study assessed the impact of OH treatment at different electric field strengths on the physicochemical, microbiological, technological, and biological properties of high-protein vanilla-flavored milk.

2. Material and methods

2.1. High-protein flavored milk processing

The methodology used for the processing of flavored milk was based on El Khoury et al. (2019), with modifications. First, refrigerated raw milk (3% w/w fat, 3% w/w protein, Ateliê do Queijo, Casemiro de Abreu, Brazil) was added with 10% industrial whey protein isolate (90% total protein, Sooro Renner Nutrição S/A, Paraná), 3% sucrose (União, São Paulo) and 0.5% w/w vanilla flavor. The concentration of WPI was defined to obtain a product with 12 g of protein per 100 mL, classifying it as a high-protein drink (Brasil, 2012). The samples were then processed by conventional heat treatment or OH. The same time/temperature binomial was used for both processes.

2.2. OH and conventional heat treatment

Six formulations were prepared: raw product (CONTROL, no conventional heat treatment or OH), conventional pasteurization (72 °C/15 s; PAST), ohmic heating at 5.22 V/cm (OH6), ohmic heating at 6.96 V/cm (OH8), ohmic heating at 8.70 V/cm (OH10), and ohmic heating at 10.43 V/cm (OH12). The schematic diagram of ohmic heating system is presented in Fig. 1. All samples submitted to OH followed the same binomial time/temperature that the PAST sample, and for both processes the parameters were controlled by a clock and bookmarks to voltage, current, and/or temperature. In all samples, after reaching the temperature and waiting for 15 s, the samples immediately emerged in cold water solution for rapid cooling. The electric field strength values result from the calculation of the voltage applied to the process with the distance between electrodes, as described by Balthazar et al. (2022). The frequency used in the OH process was 60 Hz. Conventional heat treatment took place in a water bath (Marconi®). In OH, the system was constituted by a voltage generator (Variac®), a container of 15.5 cm in length and 11.5 cm in width, two 316 steel electrodes, and the amperage, time and temperature markers. Process parameters were recorded and stored for energy parameters calculations during OH.

2.3. Energy consumption and processing parameters

Energy expenditure (KJ) and electrical conductivity were calculated following Equations (1) and (2), respectively (Gavahian et al., 2018):

$$\sigma = IL / AV \tag{1}$$

$$E = VIt \tag{2}$$

where σ = represents electrical conductivity (S/m); I represents the electrical current (A); L represents the electrodes distance (m); V represents the voltage (V); A is the electrodes surface area (m^2); E is the energy consumption (J); t is the time interval (s).

The heat and volumetric heat generation parameters were calculated using Equations (3) and (4) (Sabanci & Icier, 2017; Kuriya et al., 2020):

$$HG = \sigma^{*}|\Delta V|^{2}ut \tag{3}$$

$$VHG = \sigma^{*}|\Delta V|^{2} \tag{4}$$

where HG represents the heat generation (W), ΔV represents the applied voltage gradient (V/m); ut represents the sample volume (m^3), and VHG represents the volumetric heat generation (W/m^3).

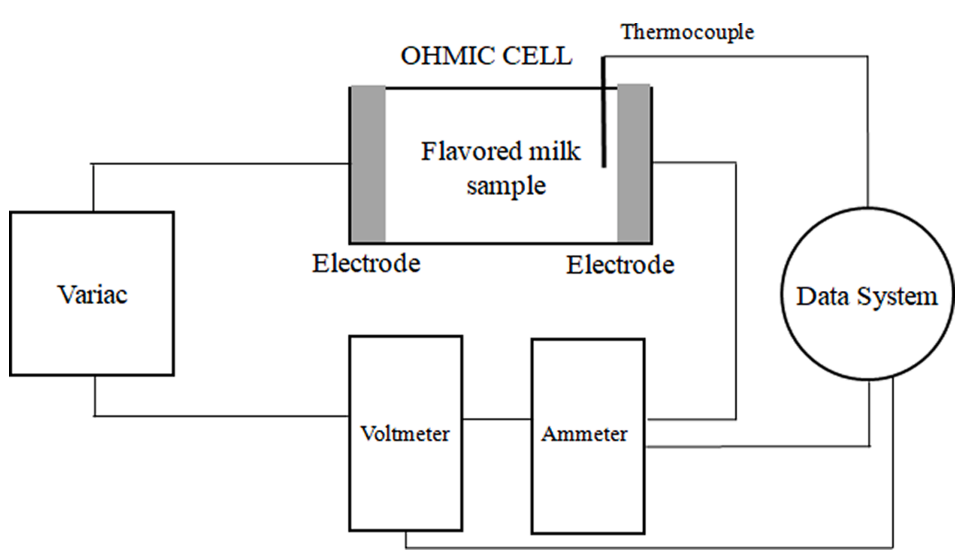

Fig. 1. Schematic diagram of ohmic heating system.

Finally, the electric field strength was calculated according to Equation (5) (Pires et al., 2020; Balthazar et al., 2022):

$$E = V/d \tag{5}$$

where d is the distance between the electrodes.

2.4. Gross composition

The moisture (constant weight at 100–105 °C), protein (Kjeldahl method), fat (Gerber method), and lactose (Lane-Eynon method) contents were determined according to Brasil (2006) and AOAC (2002).

2.5. Color measurements

The color parameters (L*, a*, and b*) were determined using the Colorimeter (Color Quest XE Hunter Lab, Northants, UK) (Silva et al., 2020). In addition to the L*, a*, and b* values, other parameters were calculated according to Equations (6), 7, 8, and 9, described by Pathare et al. (2013).

Chroma (C*) determines the difference between a hue and a gray color. The higher the C* value, the stronger the color perception. The C* value is calculated according to Equation (6):

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{6}$$

The hue angle (h) is an attribute related to the difference in absorbance at different wavelengths. It is calculated by Equation (7):

$$h^* = tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{7}$$

The whiteness index (WI) is essential for sensory acceptance of dairy products. In addition, it is a parameter that allows understanding of the consequences of heat treatment for the product. It is calculated according to Equation (8):

$$WI = \sqrt{(100 - L^{*2}) + a^{*2} + b^{*2}} \tag{8}$$

Yellowness (YI) is used to measure changes and degradations in the product due to thermal processing and exposure to light. It is calculated according to Equation (9):

$$YI = \frac{142.86b^*}{L^*} \tag{9}$$

2.6. Microbiological analysis

The samples were serially diluted in 0.1 g/100 mL buffered peptone water. Lactic acid bacteria (LAB) were counted using the Man, Rogosa, and Sharpe agar (MRS, Himedia®, India) added with cycloheximide (100 mg/L) and anaerobic incubation at 37 °C for 48 h (Anaerobac®, Probac, Brazil). Molds and yeasts were enumerated using potato dextrose agar (PDA, Himedia®, India) and aerobic incubation at 27 °C for 5 days. Aerobic mesophilic bacteria (AMB) and aerobic psychrotrophic bacteria (APB) were enumerated using plate counting agar (PCA, Himedia®, India) and aerobic incubation at 37 °C for 48 h and 7 °C for 10 days, respectively (Marshall et al., 2003; Alcántara-Zavala et al., 2021).

The enumerations were performed in the control product (untreated) and the formulations immediately after processing (microbial inactivation) and during storage (days 7, 14, and 21, microbial viability). The log reductions (γ) were calculated following Equation (10) (Guimarães et al., 2018):

$$\gamma = log10(N0) - log10(Nf) \tag{10}$$

N0 and Nf are the number of viable microorganisms of the control sample and samples after processing, respectively.

2.7. Rheology

A controlled stress rheometer (Anton Paar Instruments, Canada, model MCR 501) was used to obtain the flow curves. For that, stainless steel with plate-plate geometry, a 50-mm diameter, and 0.103-mm gap was used, and the temperature was set at 10 °C and controlled by a Peltier system. A sweep of shear rate (0.1 to 100 s^{-1}) was used, and the steady-state rheological properties of the flavored milks were calculated (Rocha et al., 2013, Patel et al., 2013). Finally, the data were fitted to the Power Law model (Equation 11).

$$\sigma = \sigma_0 + k\gamma^n \tag{11}$$

where σ represents the shear stress (Pa), k represents the consistency index (Pa s^n), n represents the flow behavior index, and γ represents the shear rate (s^{-1}).

2.8. Thermal load indicators

The hydroxymethylfurfural (HMF) content was measured using a spectrophotometric analysis and acidified medium, following the methodology described by Neves, Silva, and de Oliveira (2016). A 25 mL sample was deproteinated with trichloroacetic acid, filtered, and added with thiobarbituric acid. Then, absorbance readings were done at 443 nm. The concentration of 5-hydroxymethylfurfural, expressed in μmol/g, was determined from an analytical curve constructed with different standard concentrations of 5-hydroxymethylfurfural versus absorbance. At the same time, the whey protein nitrogen index (WPNI) was measured using a turbidimetric analysis (Neves et al., 2016).

2.9. Biological activity

The anti-oxidant capacity of the flavored milks was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the methodology described by Cappato et al. (2017) and Amaral et al. (2018). For that, 2850 μL of a methanolic solution of the DPPH radical (0.06 mM) was mixed to 150 μL of extract and held in the dark for 60 min, and the absorbance was read at 517 nm. The results were expressed as μg Trolox Equivalent/g sample. The anti-hypertensive activity was evaluated by the inhibitory activity of angiotensin-converting enzyme I (ACE) determined in a spectrophotometer, according to Konrad et al. (2014). For that, 20 μL of the ACE enzyme (0.1-unit/mL) was mixed with the extracts and incubated at 37 °C for 30 min. Then, hydrochloric acid (1 mol/L, 250 μL) were added, the vials dried, resuspended in deionized water and the absorbance read at 228 nm.

The anti-diabetic activity was determined through the inhibition of α-amylase and α-glucosidase, according to Lavelli et al. (2016). For α-glucosidase activity assay, a total of 50 μL of flavored milk extract was placed in a tube containing 650 μL of 0.02 mol/L sodium phosphate buffer L (pH 6.8) and pre-incubated for 5 min at 37 °C. For the α-amylase assay, 650 μL of 0.02 mol/L sodium phosphate buffer L (pH 6.8) was mixed with 200 μL of the enzyme solution and 50 μL of flavored milk extract and pre-incubated for 5 min at 37 °C. For both samples, the assay mixture was centrifuged at 10,000 g for 3 min and the absorbance was measured at 405 nm using a spectrophotometer.

2.10. Statistical analysis

The experiment was repeated three times following a completely randomized design. The analyses were performed on day 1 of storage and in triplicates, except for microbiological analyses that were evaluated during the storage time (days 1, 7, 14, and 21). The data were submitted to the Analysis of variance (ANOVA) and Tukey test (significance level of $p < 0.05$) using the XLSTAT software version 2020 (Adinsoft, Paris, France).

3. Results and discussion

3.1. OH and conventional processing performances

Fig. 2 presents the heating performance of flavored milk subjected to OH and conventional pasteurization. Formulations of OH12 and PAST took shorter times (4.16 and 4.58 min, respectively) to achieve the processing temperature than OH6, OH8, and OH10 (20, 12, and 6 min, respectively). In addition, the increase in the electric fields (OH12: 10.43 V/cm and OH10: 8.70 V/cm) resulted in shorter processing times, in the same proportion that heat generation increased (10.8 mV (OH12) and 5.3 mV (OH10), respectively). In practice, heat generation is related to increased amperage during the process, so the increase in the electric fields increases the heat generation, a common phenomenon described by Rui et al. Furthermore, when the conductivity of the product is sufficiently high and uniform, as is the case of milk (Bonestroo et al., 2022), little changes in electric fields are sufficient to provide high changes in processing time, which possibility to promote fast and even heating (Suebsiri et al., 2019), and consequently reduce the processing times (Hashemi et al., 2019, 2020). These observations have already been highlighted by Kuriya et al. (2020) and Pires et al. (2020). In addition, the electrical conductivity values increase along with the rising temperature, i.e., samples subjected to higher electric fields achieve higher conductivity in a shorter time (Suebsiri et al., 2019). These results suggest that OH may be an alternative to conventional pasteurization, but reduced processing times may be achieved if high electric field strengths are used. Other authors also verified that the increased electric field strength resulted in a shorter processing time in different dairy matrices (Kuriya et al., 2020; Pires et al., 2021; Balthazar et al., 2022).

Electrical conductivity increased in all samples processed by OH (Fig. 3A). At the beginning of processing, all formulations showed similar values, ranging from 0.31 (OH6) to 0.41 S/m (OH12, $p > 0.05$). Electrical conductivity was also similar at the end of the process (0.80 and 0.79 S/m, $p > 0.05$). Electrical conductivity is a crucial factor as it directly affects the heating rate and mainly depends on the food's composition and structure. In foods, the conductivity range is usually between 0.1 and 10 S/m. In addition, electrical conductivity can be controlled by adjusting the water content or by adding acids and salts. Furthermore, the more significant the mobility of the electrical charge, the more conductive the food will be (Fadavi & Salari, 2019). These results suggest that OH did not change the gross composition of the flavored milks.

Table 1 presents operational parameters related to OH processing and conventional pasteurization. The energy consumption in all OH treatments was lower than in conventional heating (Fig. 4). The energy expenditure of samples submitted to OH ranged from 121.3 KJ (OH10) to 166.9 KJ (OH8). Results suggest that the greater the electric field intensity, the lower the energy expenditure due to the shorter processing time (Balthazar et al., 2022; Silva et al., 2020). This statement partly follows the present study; however, in this study, OH8 showed the highest energy consumption (166.9 KJ), followed by OH 12 (155.4 KJ), among samples processed by OH. This event could be related to applying higher electrical energy necessary to promote the voltage, as Al-Hilphy et al. (2021) described. The results are essential to demonstrate that other factors can alter energy expenditure during processing by OH. OH processing also consumed much less energy than conventional pasteurization (Fig. 4), according to previous researches (Balthazar et al., 2022; Ghnimi et al., 2021; Pires et al., 2021; Silva et al., 2020).

The heat generation of the OH treatments increased in parallel with the applied electric field strength (Fig. 3B). As a result, OH6 had the lowest heat generation, which justifies the longer time to achieve 72 °C (Fig. 2) and the less energy consumption (134.1 KJ). The same metric was observed in the volumetric heat, in which there was a gradual increase according to the greater electric field strength (Fig. 3C). Therefore, a shorter process time results from the higher heating capacity of milk beverages generated by higher electric field strength (Hashemi et al., 2019).

The results demonstrate that the PAST sample showed a shorter processing time together with OH12. Ohmic heating is effective because the heat is dissipated more uniformly than in conventional heating, and the possibility of using clean energy sources, with no harm to the environment (Balthazar et al., 2022; Rocha et al., 2020). Despite the

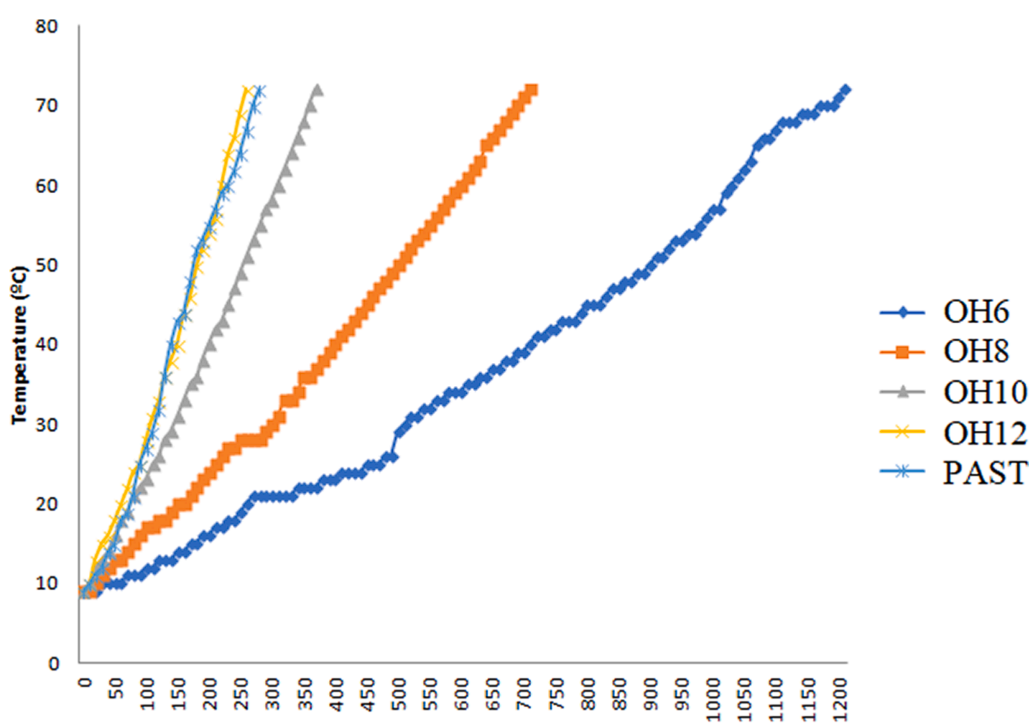

Fig. 2. Behavior of the time/temperature binomial of samples during ohmic heating processing and conventional treatment. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively. PAST: Conventional pasteurization.

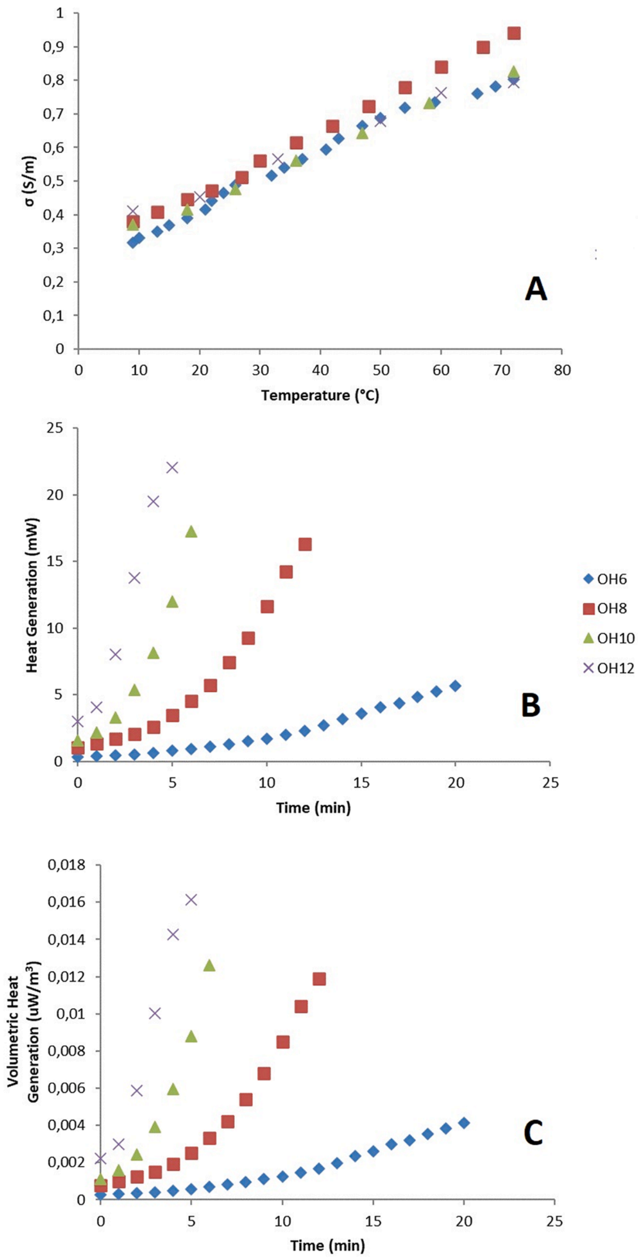

Fig. 3. Operating parameters for OH processing of flavored milk from 9 to 72 °C. (A) electrical conductivity (σ); (B) heat generation; (C) volumetric heat generation. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

Table 1
Operating conditions of Ohmic and Conventional Processing.

Samples	KJ	KWh	Heat Generation	Volumetric Heat Generation
OH12	155.4	0.043	10.879	0.008
OH10	121.3	0.034	5.391	0.004
OH8	166.9	0.046	4.552	0.003
OH6	134.172	0.037	1.730	0.001
PAST	503.5	0.130	–	–

*OH6, OH8, OH10, OH12, PAST: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm and Conventional pasteurization. Heat Generation, Volumetric Heat Generation are expressed as V/m^3.

fast-processing time, PAST was the sample with the highest energy consumption (503.5 KJ), emphasizing the advantage of using OH, regardless of the electric field strength. PAST used liquefied petroleum gas, a mix of propane and butane, which, when burned, contributes to the greenhouse effect. Thus, it harms the environment (Murshed et al., 2021).

3.2. Gross composition

The flavored milk beverages composition is shown in Table 2. There were no differences in the moisture, protein, and fat contents among the studied formulations ($p > 0.05$). The protein content in all samples was over 12 g/100 mL, which was expected since the addition of WPI was intended to increase the amount of this nutrient in the drink, allowing the denomination of “product of high-protein value” (Brasil, 2012). Thus, adding WPI in flavored milk formulations becomes an excellent alternative to be processed in OH. OH-treated samples showed higher lactose contents than PAST ($p < 0.05$, except OH8), which may be associated with higher rates of Maillard reactions in PAST (Nunes et al., 2019, Li et al., 2022).

The results of the proximate composition suggest that the use of OH in the processing of flavored milk with high-protein content is a viable and effective alternative since it did not change the values of essential nutrients such as protein and fat and maintained similar moisture standards. Furthermore, the addition of WPI in the beverage formulations did not interfere with the processing and, regardless of the electric field strength used (10.43 V/cm, 8.70 V/cm, 6.96 V/cm, and 5.22 V/cm), the protein content was maintained.

3.3. Color measurement

Color is one of the most important sensory quality attributes influencing consumers’ food choices and visual quality (Pathare et al., 2013). Conventional heating (PAST) and OH resulted in changes in the color parameters of the products compared to CONTROL, with increases in luminosity (L*), red color (a*), and WI, and decreases in yellow color (b*), C*, h* and YI ($p < 0.05$, Table 3). The milk color is influenced by plasma carotenoids, such as beta-carotene and lutein (Noziére et al., 2006). Therefore, the exposure of milk to increasing temperature may affect the concentration of lutein and beta-carotenoids, resulting in changes in milk color (Burgos et al., 2013). In this way, OH6 promoted the most important color changes, presenting the highest L* (89.98, $p < 0.05$) and WI (82.23, $p < 0.05$), probably due to a longer time of heating during processing (20 min). On the other hand, OH8 and PAST showed similar a*, b*, C*, h*, and YI values ($p > 0.05$), but OH resulted in minor changes in L* and WI values compared to CONTROL ($p < 0.05$).

The yellowness index (YI) is considered a good indicator of Maillard reaction during heating processing (Somjai et al., 2021), which comprises the nutritional value of whey protein products, blocking the functionality of essential amino acids (Xiang et al., 2021). The present study calculated YI for flavored milk drinks processed by OH to compare with PAST. There were no important YI differences between processed samples, ranging between 22.6 and 24.5. The results suggest that the yellow color of the products was provided by the ingredients used, and heating processing (by conventional heating or OH) did not impair it (Gómez-Narváez et al., 2017).

3.4. Microbial inactivation

The results related to microbial inactivation are shown in Figs. 5 and 6. Compared to conventional pasteurization, the OH treatments generated lower microbial counts for all microbial groups at the end of the storage period (Fig. 5). For the PAST sample, the counts of AMB and APB on day 28 of storage were 5.27 and 5.91 log CFU/mL, respectively, while OH samples generated counts between 3.26 and 3.75 log CFU/mL. At the same time, the count of molds and yeasts in the sample treated by

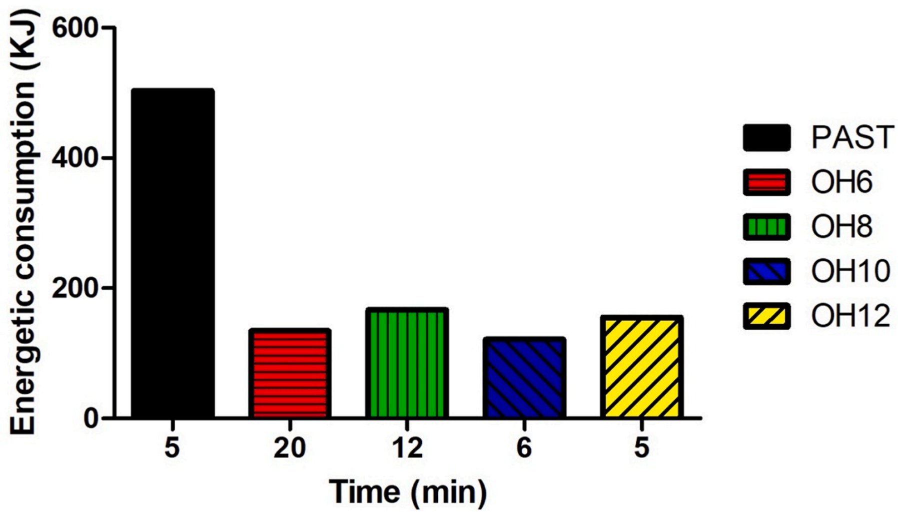

Fig. 4. Comparison of energy consumption (KJ) between conventional heating (PAST) and ohmic heating. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

Table 2
Gross composition of the flavored milk processed by ohmic heating.

Samples*	Moisture	Fat	Protein	Lactose
PAST	71.4 ± 0.46[a]	2.5 ± 0.2[a]	12.2 ± 0.15[a]	4.0 ± 0.1[a]
OH6	72.1 ± 0.32[a]	2.6 ± 0.15[a]	12.3 ± 0.15[a]	4.2 ± 0.01[bc]
OH8	72.1 ± 0.15[a]	2.3 ± 0.15[a]	12.1 ± 0.15[a]	4.1 ± 0.01[ab]
OH10	72.2 ± 0.11[a]	2.5 ± 0.17[a]	12.2 ± 0.11[a]	4.4 ± 0.01[d]
OH12	72.1 ± 0.15[a]	2.6 ± 0.15[a]	12.2 ± 0.2[a]	4.3 ± 0.01 [cd]

* Results are expressed as mean ± standard deviation. Different letters on the same column indicate a difference according to the Tukey test. ($p > 0.05$). OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively. PAST: Conventional pasteurization. Moisture, Fat, Protein and Lactose expressed as % g/100 g.

pasteurization was 5.27 log CFU/mL, while in the OH samples, the counts were between 3.27 and 3.69 log CFU/mL. Similar values were observed when considering the behavior of lactic acid bacteria.

In all evaluated microbial groups, there was a more significant log reduction (γ) in the OH samples than in the conventional pasteurized sample (Fig. 6). OH8 showed slightly higher log reductions than other intensities' ohmic treatments. The behavior of APB stands out in this case, wherein in conventional pasteurization, there was an inactivation close to zero (0.04 log CFU/mL). At the same time, in the OH treatments, the reduction varied from 0.87 to 1.04 log CFU/mL.

OH has a thermal effect but also a non-thermal effect, electroporation. This phenomenon is based on the change in the cell membrane permeability of microorganisms because of the formation of pores, generating the leakage of cellular material and consequent cell inactivation (Pires et al., 2021). Therefore, the occurrence of electroporation may justify the much higher desirable results compared to conventional treatment when the reduction in the population of the microbial groups studied is evaluated.

OH treatment has been continuously studied in foods, especially in dairy products, and the microbial inactivation effect has proved to be of interest for food safety (Pires et al., 2021; Pereira et al., 2020). However, this study indicates that the electric field variation has little influence on microbial inactivation since the variation of this parameter generated few differences in the behavior of the evaluated microbial groups.

3.5. *Rheology*

Fig. 7 shows the samples' shear stress and viscosity profiles obtained from the shear rate sweep (0.01–100 s^{-1}). The data showed an excellent adjustment to the Power-law model ($0.9781 < R^2 < 0.9940$). The estimated rheological parameters are shown in Table 4. The conventionally heated sample (PAST) exhibited the highest shear stress and viscosity profiles, numerically expressed by the highest consistency index (0.9985 Pa.s^n), implying a well-structured protein network with low mobility. On the other hand, ohmically-heated samples (OH) have lower consistency indexes (0.0144–0.4585 Pa.s^n, $p < 0.05$).

In recent years, whey protein has been widely used as an ingredient in food products, such as high-protein dairy beverages, mainly due to the high biological value and unique technological and functional properties of β-lactoglobulin, such as emulsifying, gelation, and foaming abilities. Protein gelation occurs mainly after a driving force, such as heat, resulting in the unfolding of the native protein, aggregation, and formation of a three-dimensional network (Pereira et al., 2016). The network structure is influenced by the balance between attractive and repulsive forces among denatured protein molecules due to denaturation and aggregation mechanisms (Rodrigues et al., 2015). In this context, the pronounced differences in the network mobility observed

Table 3
Color parameters of the flavored milk processed by ohmic heating.

Samples	*L**	*a**	*b**	*C**	*h*	WI	YI
CONTROL	81.9[e] ± 0.6	−4.0[d] ± 0.3	15.7[a] ± 0.6	16.3[a] ± 0.5	104.1[b] ± 1.2	75.7[e] ± 0.2	27.4[a] ± 0.9
PAST	85.9[b] ± 0.7	−3.2[b] ± 0.4	14.3[b] ± 0.5	14.7[b] ± 0.5	102.3[c] ± 1.2	79.6[b] ± 0.2	23.7[bc] ± 0.7
OH6	90.0[a] ± 0.6	−1.7[a] ± 0.1	14.6[b] ± 0.1	14.6[b] ± 0.1	96.8[d] ± 0.4	82.2[a] ± 0.2	23.1 [cd] ± 0.1
OH8	83.5[d] ± 0.3	−3.1[b] ± 0.1	14.3[b] ± 0.3	14.7[b] ± 0.2	102.3[c] ± 0.7	77.9[d] ± 0.1	24.5[b] ± 0.4
OH10	82.3[e] ± 0.5	−3.7[c] ± 0.2	13.0[c] ± 0.6	13.6[c] ± 0.5	106.1[a] ± 1.0	77.7[d] ± 0.6	22.6[d] ± 1.0
OH12	84.2[c] ± 0.6	−3.4[b] ± 0.1	13.6[c] ± 0.7	13.9[c] ± 0.7	104.3[b] ± 1.5	78.9[c] ± 0.2	23.1 [cd] ± 1.1

* Results are expressed as mean ± standard deviation. Different letters at the same column indicate a difference according the Tukey test ($p > 0.05$). OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively. PAST: Conventional pasteurization. Control = Without treatment. L, a*,b*,C*, h, WI, YI = indicates lightness, red/green coordinate, the yellow/blue coordinate, chroma, hue angle, whiteness index, yellowness.

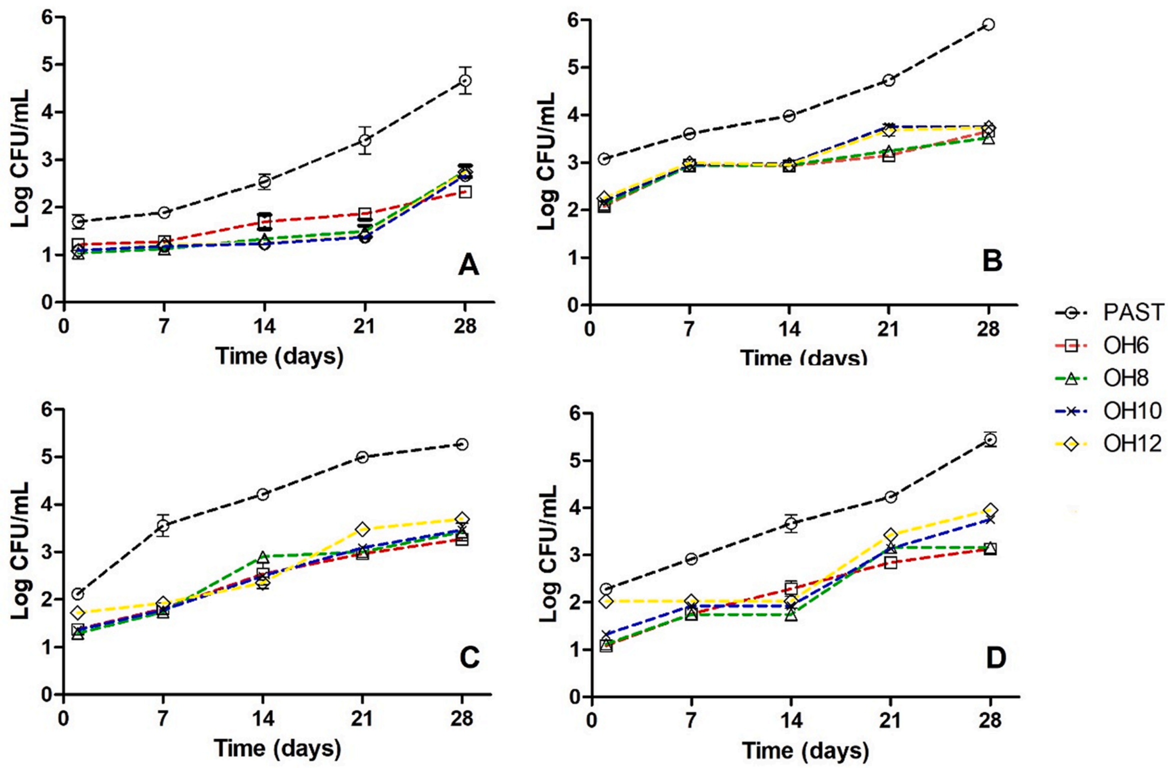

Fig. 5. Microbial viability in the samples submitted to different treatments along the storage: (A): Aerobic Mesophilic Bacteria. (B): Aerobic Psichrotrophic Bacteria. (C): Molds and yeasts. (D) Lactic Acid Bacteria. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

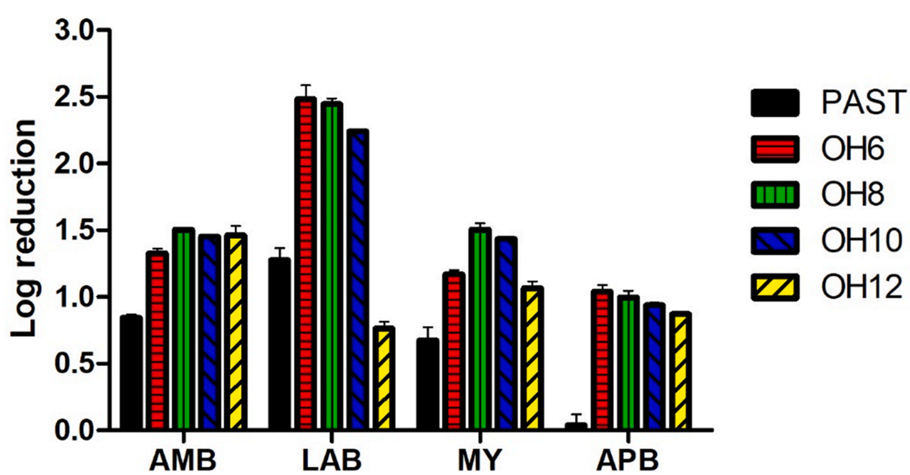

Fig. 6. Microbial inactivation (γ) in treated samples compared to the control. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively. AMB: Aerobic Mesophilic Bacteria. APB: Aerobic Psichrotrophic Bacteria. MY: Molds and yeasts. LAB: Lactic Acid Bacteria.

between conventional heating and OH can be mainly attributed to the different patterns of protein unfolding, conformation, interactions, and aggregation (Sereechantarerk et al., 2021; Rodrigues et al., 2020).

Conventional heating increased gel consistency due to a complete denaturation and denser protein aggregation. Otherwise, OH may induce more specific protein changes mainly due to thermal and non-thermal effects combined. The thermal effects are attributed to direct, rapid, and volumetric heating. In contrast, the non-thermal effects have been associated with changes in the polarity due to electric field alternation, resulting in alterations in molecular dipole orientation and molecular motions and rearrangements and, consequently, conformational changes in secondary and tertiary structures (Ferreira et al., 2021; Rodrigues et al., 2020). As the low viscosity characterizes flavored milks, the maintenance of lower consistency indices in OH-treated samples (more similar to CONTROL) is desired.

In addition, according to the nonlinear regression, all samples were characterized as non-Newtonian fluids with flow behavior indexes lower than one ($0.290 \leq n \leq 0.852$), indicating a shear-thinning behavior, where an increase in shear rate is followed by a decrease in viscosity due to the tendency of macromolecules to align towards the flow (Fig. 7B). Significant differences were observed for all samples, especially among the ohmically-heated beverages. It can be noticed that an increase in

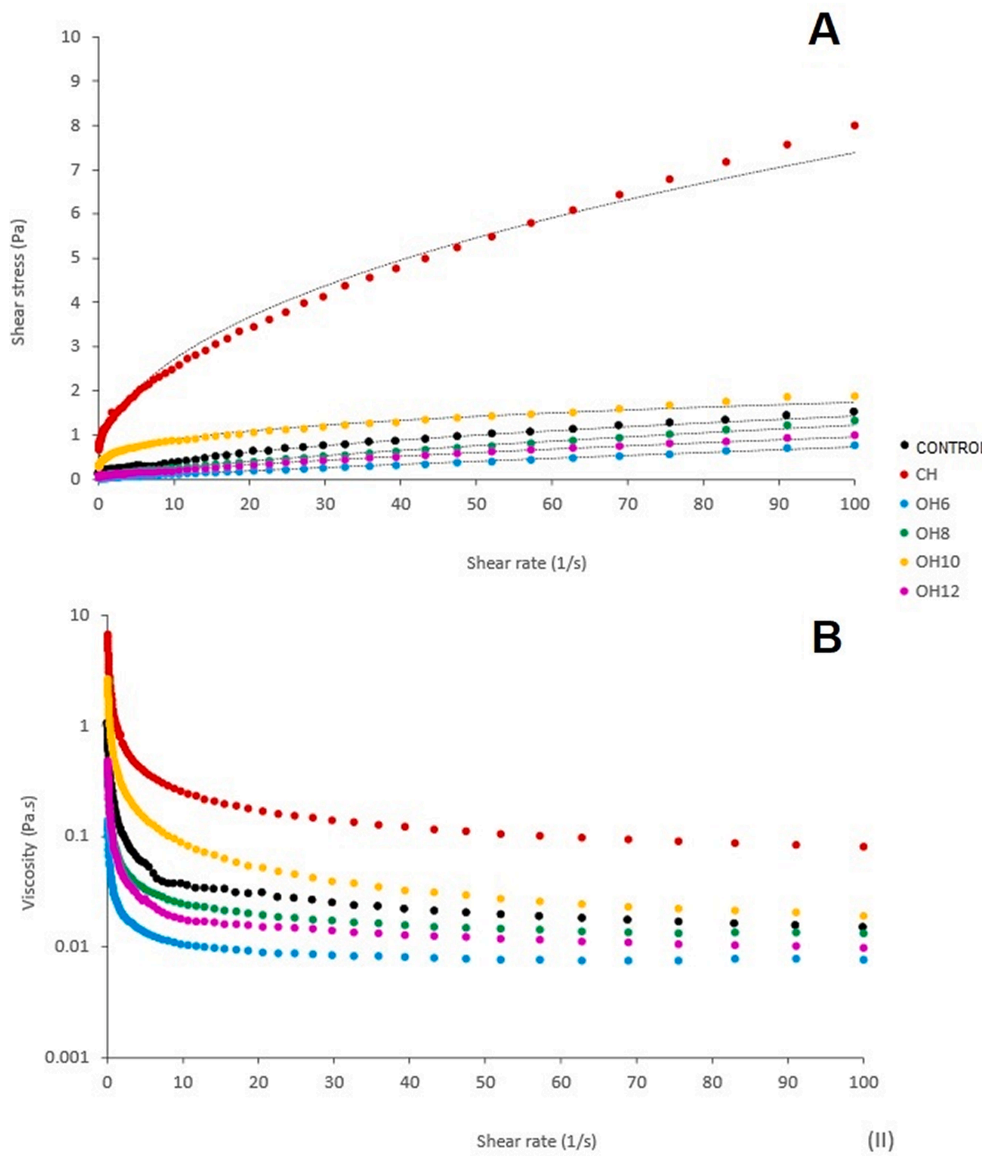

Fig. 7. Flow curves (A) and viscosity (B) obtained from the shear stress sweep tests of the samples. Full symbols represent experimental data; dotted lines represent Power law model. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

Table 4
Rheological parameters of the flavored milk processed by ohmic heating.

Sample	k (Pa.s^n)	n	R^2
CONTROL	$0.1368^c \pm 0.0061$	$0.5161^c \pm 0.0121$	0.9781
PAST	$0.9985^a \pm 0.0292$	$0.4345^d \pm 0.0079$	0.9852
OH6	$0.0144^f \pm 0.0007$	$0.8520^a \pm 0.0123$	0.9940
OH8	$0.0524^d \pm 0.0023$	$0.6846^b \pm 0.0111$	0.9914
OH10	$0.4585^b \pm 0.0058$	$0.2900^e \pm 0.0037$	0.9911
OH12	$0.0452^e \pm 0.0024$	$0.6612^b \pm 0.0131$	0.9865

*Results are expressed as mean ± standard deviation. Different letters on the same column indicate a difference according the Tukey test ($p > 0.05$).OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively. PAST: Conventional pasteurization. Control = Without treatment. K: consistency index. n: flow behavior index.

electric field strength has caused a decrease in the flow behavior index (up to OH10). In contrast, the consistency index has followed the opposite trend increasing with the electric field intensity (Table 4). The main hypothesis for this behavior is that the rapid heating and electric field following OH promoted a lower degree of denaturation and aggregation, consequently leading to a weaker and thinner protein network structure (Sereechantarerk et al., 2021; Rodrigues et al., 2015). However, the ohmically-treated sample at 12 V/cm has shown quite different values of consistency (0.0452 Pa sn) and flow behavior indexes (0.6612), indicating that a critical value of electric field strength might be achieved during the ohmic heating treatment progression.

3.6. Indicators of thermal load

HMF is a Maillard reaction byproduct commonly used to indicate severe heating, and its consumption should be avoided. In Fig. 8, it is possible to verify that the HMF content was close to zero in the control sample, which did not undergo any treatment. The sample treated by conventional heating showed the highest value of HMF (12.56 μmol/L), differing significantly ($p < 0.05$) from the samples treated by OH. The OH samples did not show significant differences (5.12 to 5.78 μmol/L; $p > 0.05$).

The WPNI value represents the denaturation of whey proteins as a processing function (Ribeiro et al., 2021). Fig. 8 shows that the highest value is associated with the control sample (10.24 mg WPN/mL), where

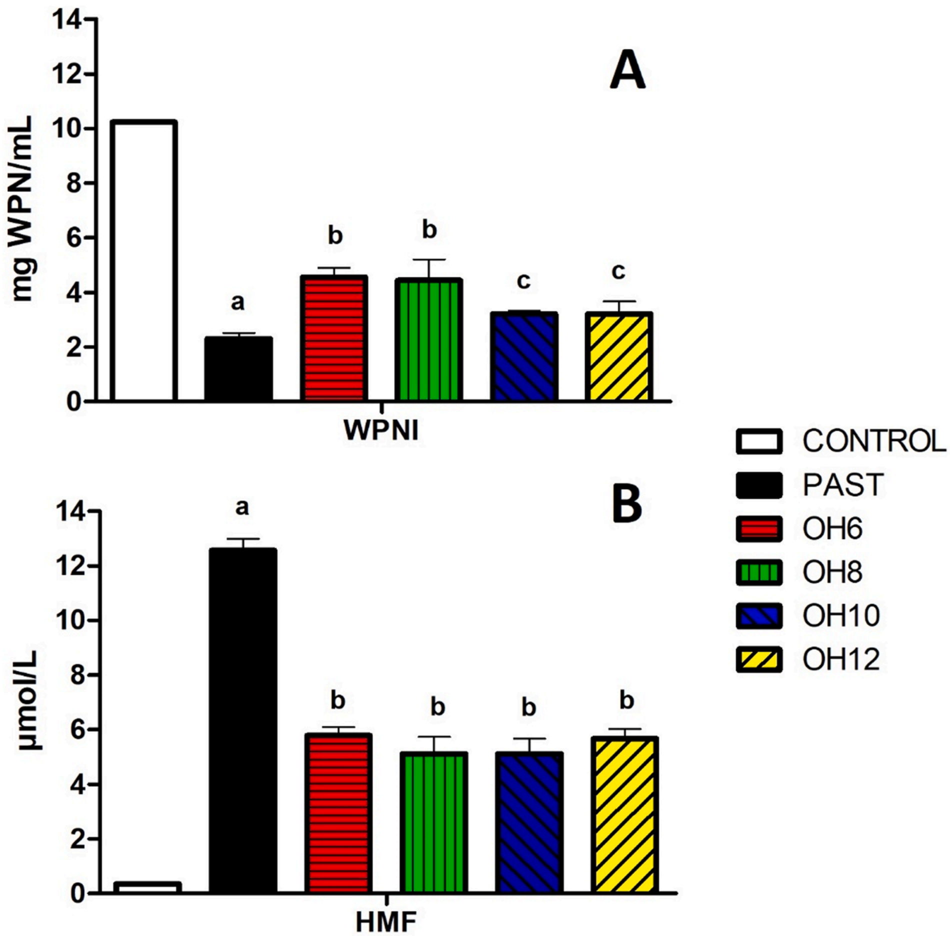

Fig. 8. Indicators of thermal load of the samples. Different letters mean significant difference between treatments ($p < 0.05$). (A) - WPNI: Whey Protein Nitrogen Index. (B) - HMF: total hydroxymethylfurfural level. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

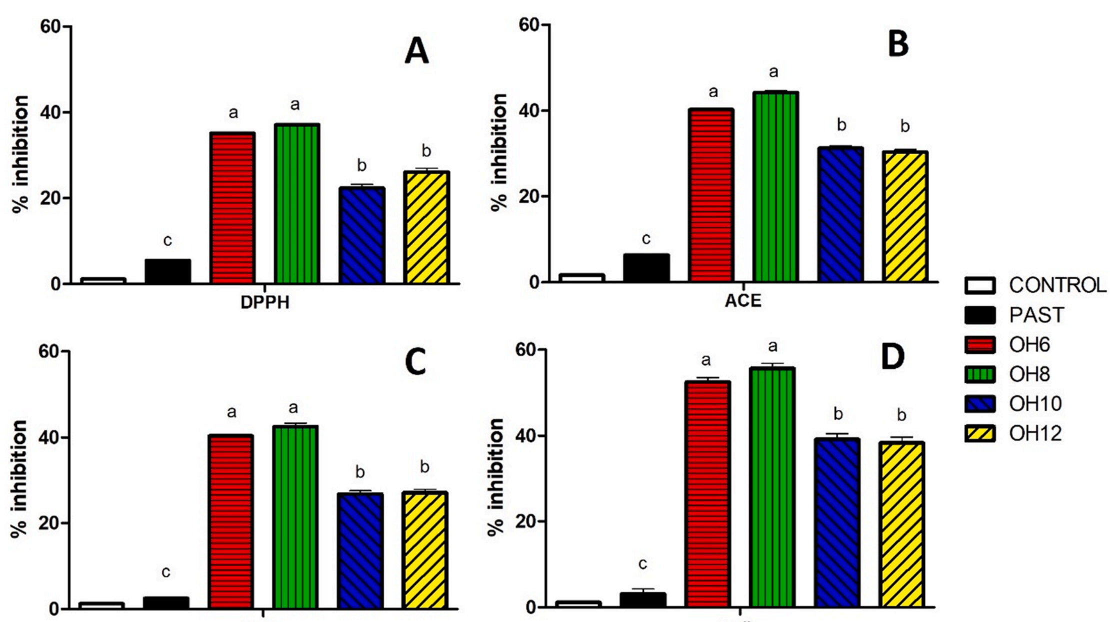

Fig. 9. Biological activities of the samples. Different letters mean significant difference between treatments ($p < 0.05$). (A) - DPPH: 2,2-diphenyl-1-picrylhydrazyl. (B) - ACE: Angiotensin converting enzyme. (C) - α-a: α-amylase. (D) - α-g: α-glucosidase. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

there was no processing. The conventional treatment generated the lowest WPNI value (2.30 mg WPN/mL), indicating that pasteurization was the treatment where the greatest denaturation occurred. Among the OH treatments, the lower electric fields (OH6 and OH8) showed greater preservation of whey proteins ($p < 0.05$) when compared to treatments with higher electric fields (OH10 and OH12). Similar results were obtained in a study evaluating the treatment of infant milk formulas by OH (Pires et al., 2021). Other emerging technologies applied to dairy products, such as cold plasma (Ribeiro et al., 2021), also showed similar results to this study, indicating that these technologies are promising in lower production of undesirable substances, such as HMF, and more excellent preservation of whey proteins.

3.7. Biological activity

The results related to the biological activity are shown in Fig. 9. Processing by OH generated higher values ($p < 0.05$) for ACE inhibitory activity, DPPH, and α-glucosidase and α-amylase inhibitions compared with the product treated by conventional pasteurization and the control product. Furthermore, in all parameters evaluated, treatments with the lowest electric fields (OH6 and OH8) showed better results than treatments with higher electric fields (OH10 and OH12; $p < 0.05$), indicating that OH processing using a lower intensity electric field is capable of promoting greater preservation of the evaluated compounds. This finding can be related to increased time to performing OH in these treatments, which can contribute to more generation of peptides with biological activity due to the breaking of protein bounds.

The higher values of biological activity can be related to the more significant number of bioactive peptides generated by ohmic heating, a result of the kinetics of milk proteins, which seems to be improved in emerging technologies, as indicated in recent studies (Cappato et al., 2018; Kuriya et al., 2020; Oliveira et al., 2022). However, the higher electric fields in this study (OH10 and OH12) indicate a lower formation of bioactive compounds related to anti-oxidant, anticancer, and anti-diabetes activity, emphasizing the importance of evaluating processing parameters to optimize food processing. Indeed, biological activity seems better preserved during OH due to the lower heating intensity than conventional methods, as the degradation mechanism is mainly related to the thermal effect (Salari & Jafari, 2020).

OH seems an exciting technology to process high-protein dairy foods, as with high-protein vanilla flavored milk. However, further studies must consider other quality parameters, such as volatile compounds, fatty acid profile, and proteomic findings. In addition, the sensory and consumer perception must also be checked.

4. Conclusion

Ohmic heating seems an interesting technology to be used to process high-protein dairy foods, as with high-protein vanilla flavored milk. All samples showed higher than 12 g/100 mL of protein, consisting of high-protein content products. OH generated lower energy expenditure and more significant microbial inactivation of lactic acid bacteria, molds and yeasts, total mesophiles, and psychotropics. Furthermore, OH at lower electric field strengths, mainly OH8 (6.96 V/cm), resulted in improved anti-diabetic, anti-oxidant, and anti-hypertensive activities and rheological properties, lower hydroxymethylfurfural contents (5.12 μmol/L; $p > 0.05$), and higher whey protein nitrogen index (4.14 mg WPN/mL). The results suggest that OH, mainly at 6.96 V/cm electric field strength, is a viable alternative in the processing of flavored milk with high-protein content, given its ability to process in low energy expenditure and the preservation of nutrients essential for maintaining health without compromising microbiological and technological properties. However, more studies are necessary to discuss the application of OH in flavored milk, mainly by using other processing parameters.

CRediT authorship contribution statement

Ramon S. Rocha: Methodology, Data curation, Writing – original draft. **Ramon Silva:** Methodology, Data curation, Writing – original draft. **Gustavo L.P. Ramos:** Methodology, Data curation, Writing – original draft. **Louise A. Cabral:** Methodology, Data curation, Writing – original draft. **Tatiana C. Pimentel:** . **Pedro H. Campelo:** . **Patricia Blumer Zacarchenco:** . **Mônica Q. Freitas:** . **Erick.A. Esmerino:** Conceptualization, Supervision. **Marcia C. Silva:** Writing – review & editing. **Adriano G. Cruz:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to IFRJ, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo a Pesquisa no Rio de Janeiro (FAPERJ) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) financing code 001, in Brazil, for their financial support and the productivity grants.

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