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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

TESE

EFEITO DA PASTEURIZAÇÃO POR AQUECIMENTO ÔHMICO NAS CARACTERÍSTICAS QUÍMICAS, FÍSICAS E REOLÓGICAS EM BEBIDA LÁCTEA DE ACEROLA.

Leandro Pereira Cappato

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Orientador:
Profº D.Sc., Adriano Gomes da Cruz

Tese submetida como requisito parcial para obtenção do grau de **Doutor em Ciência e Tecnologia de Alimentos,** no Curso de Pós-Graduação em Ciência e Tecnologia de Alimentos, Área de Concentração em Tecnologia de Alimentos.

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RESUMO

CAPPATO, Leandro Pereira. **Efeito da pasteurização por aquecimento ôhmico nas características químicas, físicas e reológicas em bebida láctea de acerola**. RJ, 2018. 66p. Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ

O aquecimento ôhmico (AO) é uma tecnologia térmica emergente que consiste na passagem de corrente elétrica no próprio alimento, promovendo um rápido e homogêneo aquecimento, devido à conversão de energia elétrica em térmica. Em virtude da rápida taxa de aquecimento, o processo ôhmico apresenta vantagens em relação aos processos convencionais, como: a maior manutenção de compostos termossensíveis, compostos bioativos, redução da degradação da cor e de produtos da reação de Maillard (RPM’s), fatores importantes no processamento de produtos lácteos. Além do efeito térmico do AO, pode existir um efeito adicional não térmico (eletroporação) nas células microbianas, reduzindo a resistência térmica de microrganismos e possibilitando redução da intensidade térmica do processo. Assim, devido ao rápido e homogêneo aquecimento e a possível existência da eletroporação, o AO apresenta como uma tecnologia promissora de para o desenvolvimento de produtos lácteos. Neste sentido, o aquecimento ôhmico, sob diferentes condições de voltagem (45, 60 e 80 V à 60 Hz) e frequência (10, 100, 1000 Hz – 25 V), foi aplicado para pasteurizar bebida láctea de acerola em comparação ao processo convencional, sob mesmo perfil de temperatura (65°C/30 min), com o intuito de avaliar o efeito do AO sobre características físicas e químicas e reológicas da bebida láctea de acerola. Em geral os resultados foram afetados diretamente pelos parâmetros do tratamento. Baixas frequências (≤ 100 Hz) e baixas voltagens (< 45 V) resultaram em menores taxas de degradação do ácido ascórbico e da cor da bebida, contudo, em relação aos compostos bioativos, o AO à 1000 Hz resultou em maior manutenção dos compostos fenólicos e peptídeos bioativos. O AO pode promover aumento da viscosidade das bebidas, perfis lipídeos similares ao processamento convencional e menor formação de produtos da reação de Maillard no tratamento à 45 V – 60 Hz. No geral, o AO pode ser uma opção interessante para o processamento de bebidas lácteas de acerola.

Palavras-chave: Produtos lácteos, tecnologia emergente, aquecimento ôhmico.

ABSTRACT

CAPPATO, Leandro Pereira. **Effect of pasteurisation by ohmic heating on the chemical, physical and rheological characteristics of the acerola dairy beverage.** RJ, 2018. 66p. Thesis (PhD in Food Science and Technology). Institute of Technology, Department of Food Technology, Federal Rural University of Rio de Janeiro, Seropédica, RJ

Ohmic heating (OH) is an emerging thermal technology that consists of the passage of electric current in the food itself, promoting a rapid and homogeneous heating, due to the conversion of electric energy into thermal. Due to the rapid heating rate, the ohmic process presents advantages over conventional processes, such as: the higher maintenance of thermosensitive compounds, bioactive compounds, reduction of color degradation and the Maillard reaction products (MRP's), important factors in product processing dairy products. In addition to the thermal effect of OH, there may be an additional non-thermal effect (electroporation) in the microbial cells, reducing the thermal resistance of microorganisms and the thermal intensity of the process. Thus, due to the rapid and homogeneous heating and the possible existence of electroporation, the OH presents as a promising technology for the development of dairy products. In this sense, the ohmic heating under different voltage conditions (45, 60 and 80 V - 60 Hz) and frequency (10, 100, 1000 Hz - 25 V) was applied to pasteurize whey acerola-flavoured drink in comparison to the conventional process , under the same temperature profile (65 °C/30 min), in order to evaluate the OH effect on physical and chemical and rheological characteristics. In general the results were directly affected by the treatment parameters. Low frequencies (≤ 100 Hz) and low voltages (< 45 V) resulted in lower degradation of ascorbic acid and beverage color; however, in relation to bioactive compounds, OH at 1000 Hz resulted in greater maintenance of phenolic compounds and peptides bioactive. OH may promote increase in beverage viscosity, similar lipid profiles with conventional processing, and lower formation of Maillard reaction products (PRM's) in the treatment at 45 V - 60 Hz. In general, OH may be an interesting option for processing of whey acerola-flavoured drink.

Key-words: Dairy products, emerging technology, ohmic heating

SUMÁRIO

INTRODUÇÃO

A acerola (*Malpighia emarginata* D.C) é um fruto bastante conhecido pelo seu elevado valor nutricional, principalmente em relação ao teor de ácido ascórbico presente no fruto. Além disso, apresenta elevado teor de vitamina A, compostos antociânicos, ferro, cálcio e vitaminas do complexo B. No Brasil a acerola apresenta grande aceitação, sendo consumida in natura ou na forma de sucos, ou pela adição de leite.

O soro de queijo, gerado pelas indústrias queijeiras, apresenta coloração amarelo-esverdeada e constitui como importante subproduto da indústria. É um produto de valor agregado amplamente utilizado no enriquecimento de produtos alimentares, contendo proteínas com propriedades funcionais importantes quando hidrolisadas, propriedades antioxidantes e anti-hipertensiva. Atualmente, o soro produzido tem sido utilizado na formulação de produtos lácteos, como na elaboração de bebidas lácteas, no qual a base láctea deve representar pelo menos 51% (m/m) do total de ingredientes do produto. Além disso, o reaproveitamento do soro constitui uma importante alternativa para redução da emissão de resíduos.

No entanto, essas propriedades do soro e da acerola podem sofrer modificações drásticas durante o processamento térmico, como: degradação de compostos nutricionais da fruta, alteração da estrutura das proteínas do soro, desnaturação e agregação de proteínas e aumento de reações de escurecimento, como reação de Maillard, fatores de grande impacto na qualidade do produto final. Assim, o uso de tecnologias emergentes, como o aquecimento ôhmico, apresenta grande destaque no desenvolvimento de produtos lácteos. A crescente demanda mundial por produtos lácteos tem impulsionado as grandes indústrias e centros de pesquisa para desenvolvimento de novas tecnologias visando minimizar os efeitos deletérios do processamento nos compostos nutricionais, garantindo a segurança microbiológica e aumentando a vida de prateleira dos produtos.

O aquecimento ôhmico (AO) é definido como um processo onde a corrente elétrica passa através dos alimentos com o objetivo de promover seu aquecimento. O aquecimento é gerado no interior do alimento, em virtude da resistência elétrica dos alimentos, onde ocorre a conversão de energia elétrica em energia térmica (Efeito Joule), resultando em uma transferência de calor para o alimento de maneira rápida e homogênea. Este fenômeno consiste em uma das grandes vantagens desta técnica, pois possibilita o desenvolvimento de processos mais rápido e mais efetivo, garantindo maior capacidade de retenção de nutrientes e dos atributos sensoriais, devido à menor carga térmica ao qual os produtos são expostos. Além do efeito térmico, o AO pode provocar efeito adicional não térmico capaz de provocar danos leves na membrana celular dos microrganismos (eletroporação), promovendo assim a redução da resistência térmica, o que poderia resultar no desenvolvimento de processos com menor intensidade térmica. No entanto, a eletroporação ainda não é totalmente entendida e mais pesquisas são necessárias para elucidá-la.

Neste contexto, o conhecimento dos efeitos das diferentes condições do processo, como frequência e voltagem sobre os alimentos, apresenta grande destaque para o desenvolvimento e otimização dos parâmetros relativos ao processo. Desta forma, o presente trabalho apresenta grande importância para o desenvolvimento e avaliação dos efeitos do processamento ôhmico em produtos lácteos. O Capítulo I consta um artigo de revisão sobre os principais fatores e parâmetros envolvidos na aplicação do AO, destacando seus efeitos na qualidade, inativação microbiana e na redução do fouling durante o processamento de produtos lácteos. O Capítulo II aborda o efeito da pasteurização da bebida láctea de acerola

(65 °C/30 min) pelo Aquecimento Ôhmico (AO) sob diferentes condições de processo (10, 100 e 1000 Hz – 25 V; 45, 60 e 80 V a 60 Hz) comparado ao processamento convencional, nas propriedades reológicas (índice de consistência e comportamento fluxo), degradação da cor (h°, C^*, ΔE), microestrutura e na cinética de degradação do ácido ascórbico. No Capítulo 3, as mesmas condições de processo descritas anteriormente, foram aplicados na bebida láctea de acerola a fim de avaliar o efeito do AO nos compostos bioativos (fenólicos e peptídeos bioativos), capacidade antioxidante (DPPH e FRAP), comportamento térmico, mobilidade de água, perfil de ácidos graxos e compostos voláteis.

CAPÍTULO I

OHMIC HEATING IN DAIRY PROCESSING: RELEVANT ASPECTS FOR SAFETY AND QUALITY

OHMIC HEATING IN DAIRY PROCESSING: RELEVANT ASPECTS FOR SAFETY AND QUALITY

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Review

Ohmic heating in dairy processing: Relevant aspects for safety and quality

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ABSTRACT

Background: Ohmic Heating (OH) technology is a heating process wherein electric current is passed through the food acting as an electrical resistor, which conversion of electrical energy into thermal energy. This fact results in shorter heating times compared of conventional processes, promoting some advantages, like maintenance highest levels of nutritional compounds, uniforms heating and reduces fouling formation. The success of process depend of parameters relate of food and process, like frequency, electrical field strength, residence time and electrical conductivity.

Scope and approach: This review aims to describe the main factors and parameters involved in this technology, highlighting its effects on quality, microbial inactivation, and fouling during processing of dairy products.

Key findings and conclusions: Due to the more rapid and uniform heating, the OH technology has advantages over the conventional processes, such as maintenance of nutritional compounds and reduction of fouling, which are important factors in the dairy products processing. In addition, there are additional effects of electroporation on microbial cells, which promotes further microorganisms inactivation which are problematic for safety of dairy foods.

Key words: ohmic heating; dairy products; quality; safety.

1 INTRODUCTION

The conventional thermal processing (HTST pasteurization and UHT sterilization) stands out as the most used technique to ensure microbiological safety of processed foods (Goullieux & Pain, 2005). However, due to the heat transfer mechanisms involved (conduction and convection), such processes have certain disadvantages, including overheating, loss of nutritional compounds, and sensory changes; moreover, the combustion of fossil fuels to generate heat causes economic and energy losses. These drawbacks can be avoided with emerging technologies, such as ohmic heating (OH) (Goullieux & Pain, 2005; Kaur & Singh, 2015; Sakr & Liu, 2014; Sudhir, 2004; Varghese, Pandey, Radhakrishna, & Bawa, 2012).

Despite being considered a new thermal technology, the concept of OH applied to foods is known since the 19th century, being first applied to milk pasteurization (De Alwis & Fryer, 1990). The process fell into disuse because of the high cost of the electricity, lack of suitable inert materials to make the electrodes and difficulties to control the process parameters (Fryer, De Alwis, Koury, Stapley, & Zhang, 1993). However, over the years this technology has been studied by many scientists, and lots of improvements were made. Nowadays, it is applied in different fields, including blaching, evaporation, dehydration, fermentation, extraction, thawing foods, sterilization and pasteurization (Allali, Marchal, & Vorobiev, 2008; Duygu & Ümit, 2015; Guida, et al., 2013; Stancl & Zitny, 2010; Varghese, Pandey, Radhakrishna, & Bawa, 2014).

OH is defined as a process wherein electric current is passed through materials with the primary purpose of heating them through the conversion of electrical into thermal energy, resulting generally in a rapid and uniform temperature increase within the food (Leizerson & Shimoni, 2005). This phenomenon is the greatest advantage of this technique as it allows the development of faster and more effective processes, ensuring greater nutrient retention capacity and sensory attributes (Guida, et al., 2013; Jaeger, et al., 2016; Mercali, Schwartz, Marczak, Tessaro, & Sastry, 2014; Pellegrino, De Noni, & Resmini, 1995; R. Pereira, Martins, & Vicente, 2008; R. Pereira, et al., 2015).

The knowledge of the mechanisms during OH is an important factor for the correct application of this technology for thermal processing. Although the main mechanism involved in the OH microbial inactivation is based on thermal effects, several studies reported the existence of an additional non-thermal effect, which consists of pore formation in the microorganism cell membrane. This phenomenon, known as electroporation, changes the cell permeability and can disrupt the membrane, leading to cell death (USA-FDA, 2000, Jaeger, et al., 2016; Loghavi, Sastry, & Yousef, 2009; I.-K. Park & Kang, 2013; YOON, YUNG, LEE, & LEE, 2002). However, electroporation is still not fully understood and more research is necessary to elucidate it.

Few studies have reported the effect of OH on the intrinsic quality parameters and microbiological stability in dairy products. Jermann, Kuchma, Margas, Leadley, & Ros-Polski (2015) have reported the trends of using emerging technologies for food processing, involving researchers and CEOs (Chief Executive Officer) of large companies, and found that the OH is a promising technology for the dairy sector, with great commercial interest in the next five years. In this context, this paper aims to describe the main factors and parameters necessary for the application of this technology during processing of dairy products, considering its effects on products` quality, microbial inactivation and fouling.

2 FUNDAMENTALS AND PROCESS PARAMETERS

The basic principle of OH is given by the passage of alternating electric current (AC) via two electrodes inserted in the food, as shown in **Fig. 1**. The electrical energy conducted through the food is converted into thermal energy due to the electrical resistance of the food (phenomenon known as Joule effect), leading to a volumetric and instantaneous heating. Power generation is directly proportional to the square of the electric field applied (E, V/cm) and the electrical conductivity of the food (σ, S/m) (Ruan, Ye, Chen, Doona, & Taub, 2001). The use of electricity as the energy source is an important advantage, like, high efficiency (more than 90% of electrical energy converted into thermal energy) and produces fewer pollutants when compared to the ones used in conventional thermal processes (Allali, Marchal, & Vorobiev, 2010; Sakr & Liu, 2014).

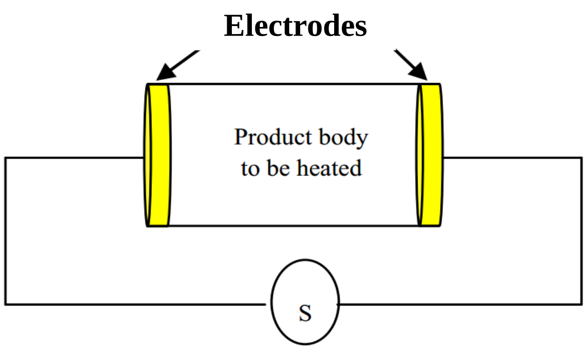

Fig 1. Basic Scheme of Ohmic Heating

Table 1 shows the main parameters of the OH concerning the variables inherent of the process, equipment, and product. The most important processing variables are the intensity of the electric field, which vary according to the voltage applied, and the electrical conductivity, which depends on temperature, ionic dissociation, viscosity, texture, solid content, cell structure and the presence of non-conductive components, such as fat, sugar and gases. Among them, temperature is the most significant factor because it affects ions mobility in the product (Sarang, Sastry, & Knipe, 2008; Zareifard, Ramaswamy, Marcotte, & Karimi, 2014a). Alike the conventional heating treatment, the main parameter during the OH to be controlled is the temperature-time profile (USA-FDA, 2000). The effectiveness of the process is strictly related to OH rate, which depends on the electric field and the electrical conductivity of the product. The electrical conductivity measures the material ability to conduct electricity through a unit of area, per unit of potential gradient and time (Goullieux & Pain, 2005) and has units of Siemens per meter (S/m), being determined generally by **Equation 1** (Zell, Lyng, Morgan, & Cronin, 2009):

$$\sigma = \frac{L}{A} \times \frac{I}{V}$$ **Equation 1**

where, A is the cross sectional area perpendicular to the passage of electric current (m²), L is the distance between the electrodes (m), I is the alternating electric current (A) and V is the voltage applied (V).

Electrical conductivity (EC) is a key parameter in the OH process, which allows determining the best parameters and the intensity of process. With an optimized process, it is possible to reduce the processing time, avoiding economic losses, and nutrient and sensory changes (Castro, Teixeira, Salengke, Sastry, & Vicente, 2004, Mercali, et al., 2014; Varghese, et al., 2012). Electrical conductivities between 0.01 and 10 S/m at 25 ° C are considered suitable for OH process and these values usually increase with temperature in a linear relationship (Varghese, et al., 2012).

Table 1. Important parameters of the ohmic heating process

Parameters	Factors
Processing parameters	Electric field strength Time Temperature Frequency of the electric current
Product parameters	Electrical conductivity Viscosity Specific heat Homogeneous or solid-liquid systems Fouling formation tendency
Equipment parameters	Size of the ohmic cell Size and shape of the electrodes Electrode composition Bath or continuous configuration

* Adapted from: Chen, Li, Zhao, & Zheng, 2010; Crattelet, et al., 2013; Fillaudeau, et al., 2006; Varghese, Pandey, Radhakrishna, & Bawa, 2012

Table 2 shows the electrical conductivity values of dairy products at different temperatures. As can be seen, these values are in the range of those appropriate for OH processing. In food systems with distinct phases, liquid and solid, the this process has major advantages over the conventional heat treatment, since it allows heating both phases in the same rate if they have similar electrical conductivities (Chen, Li, Zhao, & Zheng, 2010; S. K. Sastry & Palaniappan, 1992).

Studies have reported a linear relationship between the electrical conductivity and the process temperature (Icier & Ilicali, 2005; Sarang, et al., 2008), expressed by **Equation 2** (Palaniappan & Sastry, 1991).

$$\sigma_T = \sigma_{ref}\left[1 + m\left(T - T_{ref}\right)\right] \qquad \textbf{Equation 2}$$

where σ_T and σ_{ref} is the electrical conductivity at temperature T and T_{ref}, respectively and m is the constant of proportionality.

However, the linear relationship between conductivity and temperature is not always observed. Several authors have reported that high temperatures may favor the formation and expansion of air bubbles inside the food, leading to a reduction of the electrical conductivity (Castro, et al., 2004). In addition to the presence of air bubbles (gas), the gradual boiling of water molecules increases solids concentration, reducing ions mobility (Castro, et al., 2004; Darvishi, Khostaghaza, & Najafi, 2013; Sarang, et al., 2008).

However, the linear relationship between conductivity and temperature is not always observed. Several authors have reported that high temperatures may favor the formation and expansion of air bubbles inside the food, leading to a reduction of the electrical conductivity (Castro, et al., 2004). In addition to the presence of air bubbles (gas), the gradual boiling of water molecules increases solids concentration, reducing ions mobility (Castro, et al., 2004; Darvishi, Khostaghaza, & Najafi, 2013; Sarang, et al., 2008).

Table 2. Electrical conductivity values (S/m) of dairy products at different temperatures*

Dairy Food	Temperature (ºC)					
	4	22	30	40	50	60
Chocolate milk (3% w/w fat)	0.332	0.433	0.483	0.567	0.700	0.800
Chocolate milk (2% w/w fat)	0.420	0.508	0.617	0.700	0.833	1.000
Chocolate milk (skimmed milk)	0.532	0.558	0.663	0.746	0.948	1.089
Lactose-free milk	0.380	0.497	0.583	0.717	0.817	0.883
Skimmed milk	0.328	0.511	0.599	0.713	0.832	0.973
Whole milk	0.357	0.527	0.617	0.683	0.800	0.883

* Adapted from Zhang, 2009

Few studies have evaluated the effect of OH on dairy products, thus little is known about the factors that influence the electrical conductivity of these products. In addition to temperature, fat content may be another important variable to be considered. (Kim & Kang, 2015b) studied the effect of milk fat content (0, 3, 7, and 10%, w/w) on the heating rate and the electrical conductivity during ohmic treatment. The authors found that the electrical conductivity and the heating rate increased as milk fat content decreases, with a significant difference between samples containing 0 and 3%, and 7 and 10% of fat, this behavior was not observed in the conventional process. Moreover, the authors found that the increase in fat content led to a non-homogeneous heat distribution within the samples, decreasing the heating rate and electrical conductivity.

Another critical parameter that has an effect on the electrical conductivity is the viscosity of the product, however this effect is still little reported in recent literature (Zareifard, Ramaswamy, Marcotte, & Karimi, 2014b). Viscosity is directly related to temperature and is an important factor, especially in foods that use gums, such as in dairy drinks and yogurts (Castro, Cruz, Bisinotto, et al., 2013).

Khalaf and Sastry (1996) studied the effect of viscosity on the electrical conductivity of fluid-particle mixtures, where the fluids had the same electrical conductivity and solid concentrations, but different viscosities. The results showed that during continuous processing, the fluid with the higher viscosity presented higher electrical conductivity and, consequently, a higher heating rate. This phenomenon can be explained by the reduction of the convective effect in high viscosity fluids, promoting a faster heating in this area. This phenomenon have a great interesting in OH processing (Fryer, et al., 1993; Khalaf & Sastry, 1996; Zareifard, et al., 2014b).

In dairy beverages containing fruit pulps, ionic concentration and product pH vary widely and may directly affect the electrical conductivity of the product (Castro, Cruz, Bisinotto, et al., 2013; Castro, Cruz, Rodrigues, et al., 2013). Therefore, the study of different fruit pulps and milk/whey combinations is critical to the development and definition of the process parameters.

3 OHMIC HEATING AND FOOD SAFETY

3.1 General Aspects

As with conventional process, non-uniformity heating could be observed in products, although the heating is generated directly in the product through the conversion of electric energy into thermal. This fact results in regions of high temperatures (hot spots) and low temperatures (cold spots), being the Knowledge of the distribution of the temperature profile a critical point for food quality and food safety. From the safety point it is important to determine the worst scenario associated with cold spots (Goullieux & Pain, 2005; Jaeger, et al., 2016; Tucker, 2014).

Cold spots are related by lowest electrical conductivity of individual fractions presents in the food composition, influence for example, by lipid concentrations, ionic content and viscosity. This zones can result in a sub processing of the product directly affecting the microbiological stability of the final product (Jaeger, et al., 2016). On the other hand, hot spots can be observed in the higher spherical conductivity of individual fractions, but it does not represent a food safety problem, but it is a food quality problem due to over processing (Tucker, 2014).

Because it is a heat treatment, the knowledge of influences factors of heating rate is crucial to understand the temperature profile distribution in food. Main importance factors relate of physical properties of the food and process can influence in heating rates, like: specific heat capacity, electric field, geometry chamber of treatment, but electrical conductivity is a key parameter of OH process (Jaeger, et al., 2016; Varghese, et al., 2014; Zareifard, et al., 2014a).

Another factor of extreme importance for food safety is due to the effect of processing on microbial inactivation and determination of kinetic parameters (D and z value). Because the main mechanism of microbial inactivation in OH are thermal nature, the control of the time x temperature profile in cold spots, are important parameters of the process to be controlled (USA-FDA, 2000). Temperatures profile in cold spots must be sufficient to inactivate target pathogens (USA-FDA, 2000).

In addition, the additional non-thermal effect from electroporation in the microbial cell should be considered and investigated, because the reduction of the kinetic values (D e z value) of the microorganism, it could allow a lower thermal intensity of the process, thus degrading less nutritional compounds without affect the microbiological stability of the product (Goullieux & Pain, 2005; Jaeger, et al., 2016; Varghese, et al., 2014). So, the identification of cold spots and the factors that affect the heating rates, the microbial inactivation and the mechanisms of inactivation are the key problem for ensure the food safety in OH process.

3.2 Ohmic Heating and Microbial Inactivation in Dairy Foods

The main mechanism of microbial inactivation caused by OH is the thermal effect on membrane structure and enzymes of the microorganisms. However, several studies have reported an additional non-thermal effect capable of causing slight cellular damage (Knirsch, Alves dos Santos, Martins de Oliveira Soares Vicente, & Vessoni Penna, 2010; Sun, et al., 2008). Recent researches show that the main reason for the additional non-thermal effect on microorganisms occurs by mild electroporation of te cell, leading to pore formation in the cell membrane, which reduces the thermal resistance of the microorganism (Lebovka, Praporscic, Ghnimi, & Vorobiev, 2005; I.-K. Park & Kang, 2013; Sun, et al., 2011; YOON, et al., 2002). This phenomenon occurs mainly by the application of low frequency alternating current (50-60 Hz) (USA-FDA, 2000).

Some studies, described in **Table 3,** have reported these non-thermal effects on microbial inactivation in dairy products. For comparison purposes, these studies used the same temperature profiles for both treatments (conventional and ohmic) to verify if the OH can cause this additional effect on microbial inactivation. Microorganisms indicators of the sanitary quality and those involved in fermentation processes (*Streptococcus thermophilus* and aerobic mesophilic bacteria), as well as important pathogens (*E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*), have been studied, with distinct results and conclusions.

According to Murinda, et al. (2004), 3.76, 6.51, and 0.72 % of milk samples collected in farms were contaminated by *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*, respectively. This highlight the importance of studying the effect of OH on the inactivation of these pathogens to ensure microbiological stability of milk and milk products. These studies were carried out in a laboratory scale; however, studies on a pilot and industrial scale are fundamental to obtain practical data, assisting the food industry to develop specific procedures to guarantee adequate inactivation of target microorganisms, ensuring the microbial stability and food quality (Ryang, et al., 2016).

In dairy products containing low fat contents (0 to 3%), higher microbial inactivation rates were observed during OH. A high fat content causes decrease of the electrical conductivity because the fat globules in the milk matrix work as an electrical insulator; thus, regions with lower heating rates around the globule are formed, resulting in smaller reductions of microorganisms (Kim & Kang, 2015b). Therefore, foods containing higher fat levels can be heated non uniformly, which can negatively affect the microbiological stability and product safety (Sastry & Barach, 2000).

It is important to point out that the studies regarding the non-thermal effect are still inconclusive, presenting different results concerning the microbial strain, culture medium, and process conditions (Sastry & Barach, 2000). Moreover, none microorganism with specific resistance against OH, to be considered as a target microorganism in the process, has yet been found. Therefore, conventional target microorganisms have been used to validate the process (USA-FDA, 2000; Sastry & Barach, 2000). In this context, *Coxiella burnetti* is the target microrganism for milk pasteurization, while *Geobacillus stearothermophilus* (reduction of at least 8*D*) or *Bacillus subtilis* (reduction of 10-12*D*) are the ones for sterilization (Bylund, 2003).

Table 3. Effect of ohmic heating on microbial inactivation in dairy products

Strain	Dairy	Assay and results	Conclusion	Reference
- *E. coli* ATCC 25922	-Goat Milk	Heat resistance determination by OH ($D_{55ºC}$ = 14.2 min; $D_{63ºC}$ = 1.9 min; $D_{65ºC}$ =0.86 min; *z* =8.4 ºC) under alternating current (AC) at 50 Hz, compared with conventional treatment ($D_{55ºC}$ = 10.9 min; $D_{63ºC}$ = 3.9 min; $D_{65ºC}$ = 3.5 min; *z* = 23.1 ºC) under same temperature profile.	Results indicated that OH provided a non-thermal additional effect at 63 and 65 ºC, reducing the kinetic parameters (*D* and *z* values), although no statistic method was applied.	Pereira (2008)
-*Streptococcus thermophilus* 2646 -Aerobic mesophilic bacteria	-Milk	Heat resistance determination by OH (20 kHz, 7.3-2 A; 70–12 V) compared to conventional process, under same temperature profile. For *S. thermophiles, D values of OH were 6.59, 3.09 and 0.16 min at temperatures of 70, 75 and 80°C, respectively. For* aerobic mesophilic, *D* values of at these same temperatures were 8.6, 6.18 and 0.38 min. On the other hand, for conventional heating, the D values for *S. thermophiles were 7.54, 3.30 and 0.20 min at 70, 75 and 80°C, respectively, and for* aerobic mesophilic the values were 11.25, 9.39 and 0.44 min.	OH resulted in microbial inactivation due to the decreased of thermal resistance for *S. thermophilus* and for the total aerobic mesophilic bacteria. However, there was no significant effect ($p < 0.05$) for *S. thermophilus* at 75ºC.	Sun et al. (2008)
-*Streptococcus thermophilus 21072*	- Condensed Milk	The effect of sublethal ohmic and conventional treatment followed by the conventional treatment (70 °C / 0-6 min), under the same temperatures profiles, was studied in order to find the reason of the lethal effect in microbial inactivation.	For sublethal treatments, there was no significant difference. However, after lethal treatment, was observed higher rate of microbial reduction by ohmic-sublethal treatment, due an additional non-thermal effect.	Sun et al. (2011)
- *E. coli* O157:H7. ATCC 35150, ATCC 43889, ATCC 43890; - *S. Typhimurium.* ATCC 19585, ATCC 43971, DT 104*;* - *L. monocytogenes.* ATCC 19111, ATCC 19115, ATCC 15313	- Skimmed milk - milk cream	Microbial reduction from a cocktail of some strains during OH (20 kHz, sine wave, and electric field from 32 to 9.6 V/cm after reaching the target temperature) was compared to conventional processing, under the same temperature profiles (55 °C, 60 °C, 65 °C, 70 °C for 1 min).	Additional non-thermal effect was observed ($p<0.05$) for a cocktail of *E.coli* O157:H7 and *S. Typhimurium* at 60 ºC, and for *L. monocytogenes* at 65 ºC in the skimmed milk. In milk cream, the effect was observed for *L. monocytogenes* at 60 ºC and *S. Typhimurium* at 65 ºC.	Kim & Kang (2015a)

- *E. coli* O157:H7. ATCC 35150, ATCC 43889, ATCC 43890; - *S. Typhimurium.* ATCC 19585, ATCC 43971, DT 104*;* - *L. Monocytogenes*. ATCC 19111, ATCC 19115, ATCC 15313.	- Milk Fat content (0; 3; 7; 10 %)	Microbial reduction from a cocktail of some strains during OH (20 kHz sine wave; electric field of 19.2 to 9.6 V/cm, after reaching the target temperature 60 ºC), was compared to the conventional treatment under the same temperatures profiles.	Higher rates of microbial inactivation were observed in milk with low fat content. (0 and 3% w/w of fat) compared to samples with higher fat content (7 and 10 % w/w), but the additional non-thermal effect of OH was not observed. Thus inactivating occurred only by the thermal effects.	Kim & Kang (2015b)

3.3 Electroporaction (Additional Non-Thermal Effect)

Electroporation is the main non-thermal mechanism of cell death, and consists of pore formation in the membrane, causing changes in cell permeability. The increased permeability may allow greater diffusion of materials, with leakage of cellular compounds, causing irreversible damage to the cell (Castro, Barbosa-Cánovas, Swanson, 1993; Knirsch, et al., 2010). However, few studies have successfully proved the electroporation mechanism and its effects on microbial cells.

When applied with non-lethal conditions, the electroporation may also affect microbial growth, since it can assist the transport of nutrients into the cells. Some studies have investigated the effect of OH on growing kinetic parameters of *Lactobacillus acidophilus* in culture medium (Cho, Yousef, & Sastry, 1996; Loghavi, Sastry, & Yousef, 2007; Loghavi, Sastry, & Yousef, 2008; Loghavi, et al., 2009). Cho, et al. (1996) evaluated the effect of OH, compared to the conventional process, at different temperatures (30, 35, and 40 °C) under stirring, using 60 Hz of frequency and voltage between 15 and 40 V. The results showed that the electric field promoted a reduction of the adaptation phase (lag) and an inhibition of microbial population in the final growth stages. The electroporation led to an increase of nutrient diffusion into the cell, reducing the time of the lag phase; in the final stages, electroporation allowed greater and more rapid absorption of metabolites present in the medium, thus inhibiting microbial growth. This hypothesis was corroborated by other studies (Loghavi, et al., 2007; Loghavi, et al., 2008).

Table 4 summarizes the main studies concerning the effect of electroporation on microbial cell. Since the electroporation reduces the thermal resistance of the microorganisms, the thermal intensity of the process can be reduced, leading to lower nutrient degradation, but with the same effectiveness in reducing microbial counts. Therefore, knowledge of the mechanism and the main parameters that favor the electroporation is a major challenge for the production of higher quality and safe products treated by OH.

4 OHMIC HEATING AND INTRINSIC QUALITY PARAMETERS IN DAIRY PROCESSING

The physicochemical and sensory characteristics of dairy products are important for their quality and preservation assessment, being influenced by different factors, such as the type and the intensity of the treatment applied (Esmerino, et al., 2015; Gaze, et al., 2015; Morais, Morais, Cruz, & Bolini, 2014). In order to evaluate the benefits of OH and to define the optimum process parameters, it is essential to evaluate the influence of this technique on quality and sensory factors of the product. The same thermal process indicators commonly employed in conventional treatments can be used to determine the intensity of the OH process (S. K. Sastry & Barach, 2000). Several indicators are reported in the literature, such as lactulose, furosine and hydroxymethylfurfural (HMF), FAST index (fluorescence of advanced Maillard products and soluble tryptophan), carboxymethyllisine, glycoxidation products, protein denaturation and aggregation (β-lactoglobulin), free fatty acids, among others (Claeys, Ludikhuyze, & Hendrickx, 2001; González-Córdova & Vallejo-Cordoba, 2003; Meyer, Al-Diab, Vollmer, & Pischetsrieder, 2011; Oldfield, Singh, & Taylor, 2005; R. Pereira, et al., 2015; Schamberger & Labuza, 2006).

Table 4. Studies regarding the electroporation effect on the microbial cell.

Strain	Dairy	Assay and results	Conclusion	Reference
-*Saccharomycies cerevisiae*	-Phosphate Buffer (0.02M; pH=7.0)	Observations of transmission electron microscopy (TEM) of the intracellular material displacement (nucleic acids, amino acids, protein and coenzymes) after different ohmic treatments, varying voltage (10, 15 and 20 V/cm) and frequency (60; 600 Hz; 6 ; 60 kHz), compared to the conventional process, under similar temperature profiles.	Intracellular material was moved at a higher rate to the phosphate buffer when compared to the conventional process; higher rates were obtained when the voltage (10 – 20 V/cm) and frequency (60 Hz – 60 kHz) increased.	Yoon, Yung, Lee & Lee (2002)
-*Lactobacillus acidophilus OSU133*	- Milk	Observation using fluorescence microscopy of *L. acidophilus cells* colored with two highlighted nucleotides in fluorescence (SYTO 9 and propide iodide (PI)). Ohmic treatment using 2 V/cm with different frequencies (45, 60, 1.000, 10.000 Hz) at 30 ºC were compared to the conventional treatment during the lactic fermentation.	Changes on the cellular permeability were observed at low frequencies, 45Hz (more evidence) and 60 Hz. The electroporation effect was observed in the lag (more evidence) and exponential phase. Those results proved that this effect can contribute to optimization of the fermentation process.	Loghavi et al. (2009)
-*S. thermophilus 21072*	- Phosphate buffer (1.19 S/m 20ºC)	Evaluation of the electroporation by comparison of the adenosine triphosfate (ATP) and lactate dehydrogenase (LDH) exudate, after sublethal treatments of OH (20kHz; 7 A; 27 - 18 V) with thermal settings of 10ºC up to 37ºC and cooling to 10ºC.	A significant increase ($p<0.05$) in the ATP content and LDH cell exudate presented in the buffer during the sublethal OH, demonstrating that the electrical current led to an increase in the cell permeability.	Sun et al. (2011)
- *E.coli* O157:H7. (ATCC 35150, ATCC 43889, ATCC 43890); - *S. Typhimurium.* (ATCC 19585, ATCC 43971, DT 104)*;* - *L. monocytogenes* (ATCC 19111, ATCC 19115, ATCC 15313);	- Phosphate Buffer (pH=7.2)	Evaluation of the electroporation effect in buffer by observations of transmission electron microscopy (TEM) (at 60ºC/30s) and quantification of iodine of propidium (viability indicator) at 60ºC for different times (0, 10, 20, 25, 30 s) of OH (30V/cm; 20KHz; sine wave) and conventional processing.	The leeching of the intracellular material was more evident in the OH when iodine of propidium in the buffer was , by quantified. The values found after 30 min treatment were 4, 4 and 12 °C for *E.coli*, *S. typhimurium* and *L. monocytogenes*, respectively.	Park & Kang (2013)

Table 5 shows the published studies on OH regarding the intrinsic quality parameters of dairy products. There is a lack of studies on processed dairy products, such as chocolate-flavored milk, cheese, butter, fermented milk and others, which opens up a range of research opportunities in this area. Roux, Courel, Ait-Ameur, Birlouez-Aragon, and Pain (2009) analyzed different quality indicators in milk-based infant formula (FAST index, Furosine and carboxymethyllisine (CML), and color changes) during OH (5 kW AC - 50 Hz, electric field from 0.1 to 3 kV/m) on a laboratory scale (100 mL). More recently, Roux, Courel, Birlouez-Aragon, Municino, Massa & Pain (2016) conducted a similar study on a pilot scale and found comparable results, indicating that it is possible to predict on a laboratory scale the effects of OH on nutritional compounds degradation in infant foods. Additionally, comparing OH with UHT steam injection results, the authors observed equivalent markers contents, showing that OH appears as a promising technology for the preservation of certain nutrients in dairy foods.

5 FOULING IN DAIRY PROCESSING

Fouling is a major problem in thermal processes of milk products, especially in those involving indirect heating, where the heat transfer is carried out from the fluid (generally steam or heated pressurized water) to the product, separated by the equipment wall (Bansal & Chen, 2006a). Two basic mechanisms are responsible for fouling in dairy processing plants: type A fouling, derived from the aggregates formed by the presence of whey protein (mostly β-Lg and α-La) at 75 and 110 °C; and type B fouling, which occurs at temperatures above 110 °C as a consequence of calcium phosphate precipitation (Bansal & Chen, 2006a; Visser & Jeurnink, 1997).

Ohmic heating has the disadvantage of promoting fouling on the surface of the electrodes, which occurs mainly in foods containing high mineral content, such as milk products (Ayadi, Leuliet, Chopard, Berthou, & Lebouché, 2005; Stancl & Zitny, 2010). Fouling causes many issues in dairy processing plants, which includes hindering the cleaning process, loss of quality and reduction of the heat transfer rates, thus compromising the microbiological stability of the final product. As reported by (Van Asselt, Vissers, Smit, & De Jong, 2005), about 80% of the costs of dairy industry corresponds to cleaning process to prevent fouling. Moreover, fouling leads to microorganism adhesion to the surface of the equipment, resulting in biofilm formation (Bansal & Chen, 2006b; Flint, Brooks, & Bremer, 2000). During OH, fouling also causes operational problems concerning the reduction and non-homogeneity of the electric field applied.

Although fouling can occur during the OH, it happens in a lower extension when compared to the conventional method, since the later is based on the heat conduction mechanism, resulting in overheating of the surfaces;. in contrast, the former is based on heat generation inside the food, with lower temperatures on the surface of the electrodes than those of the product (Ayadi, Leuliet, Chopard, Berthou, & Lebouche, 2004; Fillaudeau, et al., 2006).

Few studies have reported the effect of OH on fouling formation (Ayadi, Bouvier, Chopard, Berthou, & Leuliet, 2003; Ayadi, et al., 2004; Ayadi, et al., 2005; Bansal & Chen, 2006b; Mahdi, Mouheb, & Oufer, 2009). They indicated that fouling is directly related to the temperature increase; the higher the temperature, the greater the deposition, promoting a "snowball" effect (Crattelet, et al., 2013). Bansal and Chen (2006b) evaluated the effect of power frequency (10 kHz and 50 Hz) and found that fouling can be drastically reduced at high frequencies. Furthermore, a reduction of the current intensity at 50 Hz was observed, showing that fouling may have changed the OH process parameters.

Table 5. Studies regarding the evaluation of the OH effects on quality parameters in dairy products.

Parameter	Dairy	Assay and results	Conclusion	Reference
- Rancidity of the free fatty acids (FFA)	- Goat Milk	Characterization of short-chain free fatty acid content (FFA) medium during OH (14.4 V / cm, 5-30 A) and conventional process, under the same conditions (72 ° C / 15s).	The FFA after the OH did not show any change compared to the conventional process, which indicated that the ohmic treatment did not affect the product quality.	Pereira, et al. (2008)
-Protein denaturation from the FAST index	- Milk	Comparing the processing effect of OH (20 kHz; 7.3-2 A; 70 – 12 V) and conventional processing on protein denaturation using the same conditions (40, 50, 60, 65, 70, 75 and 80 ºC por 30 min).	There was no significant difference between the FAST index of the applied processes, indicating that there was no additional thermal effect on protein denaturation.	Sun, et al. (2008)
- Evaluation of the structural and physical properties	- Food grade film based on the whey protein.	Evaluation of OH effect (50 Hz; 10 V/cm) on the structural and physical properties of food grade films based on whey protein compared to the conventional treatment, both under 85 ºC for 30 min.	The OH resulted in a slimmer film and less permeable to water vapor, with lower levels of aggregation of protein and free thiol group. However, the authors suggest more studies to evaluate this technology in food grade films.	Pereira et al. (2010)
- Acidity - Fat content - Dry extract nonfat - Total solids Protein	- Buffalo Milk	Evaluation of OH (AC; 50 Hz; 0.6 A; 8.38 V/cm) and conventional treatment on the physical chemistry properties in the buffalo milk, under the same time vs temperature (72ºC/15 s).	The OH and conventional treatment showed similar physical chemistry property values; however no statistical analysis was performed. In contrast, the processing time showed a decrease of 18%.	Shivmurti, Harshit, Rinkita & Smit (2014)
- Aggregation and denaturation protein (% of free tiol group and the solubility loss)	- WPI (Whey Protein Isolate)	Characterization of the initial steps of protein aggregation denaturation in the WPI, by conventional and ohmic treatment (6 and 12 V/cm), under different rise time (10 and 100 s) to target temperature (90ºC).	Ohmic heating developed less aggregation and an increased the protein solubility on the first steps of heating. Treatment using 12 V/cm per 10 s promoted major protein retention, showing that the rapid come up time resulted in a better quality product.	Pereira, et al. (2016)
- FAST Index - Colour variation - Furosin formation and Carboxymethyllysine (CML) - Vitamin C	- Infant milk-based food.	Determination of the kinetic parameters (*K*, min^{-1}) of the different quality indicators, during OH (25 kHz 15 kW; 300 from 4000 V) and direct UHT processing, under the same temperature profile.	Results did not show a large difference between treatments; however, the OH presented higher values for *K* in the FAST index, Furosin and CML. Higher values of the retention in the vitamin C and color was also observed.	Roux et al. (2016)

Fouling can result in a further increase of the electrical resistance on the electrode surface, changing the operation conditions of the system (Bansal & Chen, 2006b; Fillaudeau, et al., 2006). To avoid this change, new process conditions should be applied, such as an increase of the electric field strength. Thus, mathematical methods were developed to describe the behavior of the OH at different fouling levels (Fillaudeau, et al., 2006). In addition, the study reported some factors that can contribute to reduce fouling, such as working in turbulent regime to promote convection phenomenon, reducing overheating of the electrode wall.

6 OHMIC HEATING AND ALLERGENICITY OF DAIRY FOODS

A food allergy is any adverse reaction to a food or to a specific component capable of sensitizing the immune system, where after repeated contact, it can trigger a severe allergic reaction. Due to the existence of various types of allergic reactions the diagnosis may be difficult to identify, but in some cases such allergies as "immediate hypersensitivity", detected rapidly due to reactions occurring after food intake (Anderson, 1986; EAACI, 2014; Huang, Hsu, Yang, & Wang, 2014).

Among the common symptoms related to food allergy are: changes in the gastrointestinal tract starting with swelling or itching of the lips, mouth and / or throat, and may progress to nausea, vomiting, colic and diarrhea. Other frequent symptoms are due to the onset of itching, hives, eczema and redness on the skin and problems in the respiratory system such as catarrh, asthma, laryngeal edema (Davis, 2009; EAACI, 2014).

The proteins present in foods are the main components related to the cause of allergies, and their conformational structures are the key to their allergenicity (Nowak-Wegrzyn & Fiocchi, 2009). More than 90% of allergies are related to 8 types of food, with bovine milk being one of them. The milk has several proteins, where approximately 30 of them, present allergenic potential. However, casein and β-lactoglobulin are the proteins with the greatest allergenic potential in bovine milk because they are not present in human milk (Miciński, et al., 2013; Shandilya, Kapila, Haq, Kapila, & Kansal, 2013).

Bovine milk proteins can be grouped into 2 groups, i.e. caseins and whey proteins. Caseins are the most present proteins in milk (2.6-2.8% total volume), which represents 80% of the protein content. There are 5 casein fractions in bovine milk, αs1, αs2, β, κ, containing 30, 9, 28, 10 and 2% of total casein, respectively (Farrell, et al., 2004). Casein causes inflammatory reactions in the instestinal mucosa of celiac patients, with the αs1 fraction being the most responsible for the allergenic picture (Cabrera-Chavez & de la Barca, 2009; Miciński, et al., 2013; Nowak-Wegrzyn & Fiocchi, 2009).

In relation to whey proteins (0.6% milk composition), they are of great importance due to their functional and nutritional characteristics, being composed in higher concentrations of β-lactoglobulin (β-Lg) e α-lactoalbumin (α–La), 51% and 22% of total whey proteins, respectivamente. At lower concentrations, lactoferrin, lactoperoxidase, lysozyme, bovine serum albulmin (BSA), Immunoglobulins, transferrin and proteose-peptones. Despite showing different proteins, the potential allergenic comprise β-Lg and α–La, and the β-mainly responsible for allergic reactions present in bovine milk. About 80% of the population has allergenic effects in relation to the presence of β-Lg (Miciński, et al., 2013; Y. W. Park & Nam, 2015).

The primary aim of the heat treatment is in the inactivation of microorganisms, aiming to guarantee the microbiological stability of the product. However, the application of heat also promotes a reduction in the allergenic potential of milk and milk products, due to the loss of the tertiary structure leading to protein denaturation and the formation of aggregates (Shandilya, et al., 2013). In contrast, thermal processing may lead to increased allergenicity, due to the formation of neoallergenic compounds, such as those formed in the Maillard Reaction (Jaeger, et al., 2016; Nowak-Wegrzyn & Fiocchi, 2009).

Although a thermal technology, the impact of OH process in allergenicity of the milk proteins can not be compared or estimated from results obtained by connvecional process, even under same temperatures profiles, due to the resulting nonthermal additional effect in OH process. Electricity and process parameters, such as frequency and electric field, can influence casein micelles and / or protein conformational structures, resulting in different results than the conventional ones (Jaeger, et al., 2016).

Due to the additional non-thermal effect of electroporation, the rate of heating may be reduced during treatment, resulting in reduced formation of neoallergenics compounds (from the Maillard reaction, for example). In contrast, with the reduction of total thermal food load, the treatment may promote less reduction of the allergenicity of proteins that would previously have been denatured by the treatment (Jaeger, et al., 2016).

However, until now, no studies were found assessing the allergenic properties of milk proteins treated by OH. This evaluation of the effect of the processing and the OH variables, both in the reduction of allergenicity and the formation of neoallerginic compounds, presents as one of the great challenges in the application of the OH process in milk and derivatives.

7 ADVANTAGES AND DISADVANTAGES

The application of OH has several advantages over conventional processing, with the possibility of promoting fast and even heating in the product. Due to the rapid heating of processing caused by internal energy generation, it is possible to reduce the impact of treatment on food quality (C value) without compromising food safety (same F_o value). So, Oh process results in a better product quality with reduced processing time avoiding economic losses (Jaeger, et al., 2016; Kaur & Singh, 2015; S. Sastry, Heskitt, Sarang, Somavat, & Ayotte, 2014) Additionally, OH have others vantages, like:

(I) Uniformity heating: OH process can promote the heating of both phases (solid and liquid) at the same time, which is impossible for conventional heating.

(II) Heating of particulate foods, liquid–particle mixtures and higher viscosity foods.

(III) It makes the control of the process easier with instant switch-on and shut-down.

(IV) Higher Energy efficeny: Due the internal energy generation, in OH process 90% of the electrical energy is converted into heat energy. Energy Efficiency is even better compared to other emerging technologies, like microwave, which is typically only 50% efficient. In addition, energy efficiency is a critical criterion in long-duration space missions, so OH have potential interesting for application in long-duration space missions.

(V) Fouling Reduction: The OH process reduces fouling and the risk of burning food layers because there are no hot surfaces for heat transfer. This fact also reduces the cost of the cleaning process, thus reducing processing time.

(VI) Nonthermal addition effects (electroporation): The effect of electroporation on the microbial cell promotes cellular damage, thus reducing the thermal resistance of the microorganism to the treatment. The reduction of kinetic parameters (D and z value) allows the accomplishment of a treatment with lower thermal intensity guaranteeing the same effectiveness for food safety.

When analyzed, as any other technology, OH presents some disadvantages, such as, the difficulty to control the heating rate in the product. Despite the advances in the studies regarding the effects that interfere in the electrical conductivity, it is still hard to control the OH heating rate (Kaur & Singh, 2016). The electrical conductivity is a parameter that undergoes interference in several factors, being at the temperature a critical factor, due to the conductivity changes with an increase in the temperature during the process, which makes difficult its measurement and control (Jaeger et al., 2016). Finally, another drawback is the application of the OH process in foods, which contains non-conductive substances in the composition, such as, fat globules, which promotes non-uniformity in heating generation (Goullieux & Pain, 2005). In addition, high concentration of lipids in the dairy foods may represent a risk, due to the appearance of cold zones, which affects the microbiological stability in final product (Sastry, 1992).

Finally, as dairy food is a class of processed food, the OH should take in account the material used in the electrode design. While materials such as titanium, stainless steel, platinized-titanium, aluminum and graphite are usually selected based on financial aspects, the use of stainless steel are mandatory and recommend for dairy applications. Simultaneously, is important to work higher frequency values of the power supply to prevent corrosion and metal dissolution (Sakr & Liu, 2014)

8 PERSPECTIVES

The growing global demand for dairy products has driven the major industries and research centers to develop new technologies to minimize the deleterious effects of processing, ensuring the microbiological safety and increasing products shelf life. However, the cost and difficulties to control the process parameters, as well as lack of appropriate legislation, are the main issues for the implementation of these technologies. In this context, several aspects need to be investigated:

- Understand the effects of the OH process and parameters on the physical-chemical properties of food;
- Development of sophisticated controls to ensure heating rates;
- Identification and measurement of cold spots during multiphase food processing;
- Understand the phenomenon of electroporation of microbial cells and identify the parameters of the process that promote these effects;
- Determination the electrical effects of enzymes inactivations;
- Determination of kinetics of inactivation of the relevant microrganism;
- Determination the effects of OH parameters in fouling formation;
- Identification of the impact of the OH process on the allergenic potential;

Although studies about OH with dairy products are scarce in the literature, the benefits of its application has been already reported, which makes it a promising technology to be used in the dairy processing units.

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CAPÍTULO II

WHEY ACEROLA-FLAVOURED DRINK SUBMITTED OHMIC HEATING PROCESSING: IS THERE AN OPTIMAL COMBINATION OF THE OPERATIONAL PARAMETERS?

WHEY ACEROLA-FLAVOURED DRINK SUBMITTED OHMIC HEATING PROCESSING: IS THERE AN OPTIMAL COMBINATION OF THE OPERATIONAL PARAMETERS?

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Whey acerola-flavoured drink submitted ohmic heating processing: Is there an optimal combination of the operational parameters?

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ABSTRACT

A whey acerola-flavoured drink was treated using ohmic heating (OH) at 65°C for 30 min to evaluate different frequencies (10, 100 and 1000 Hz with 25 V) and voltages (45, 60 and 80 V at 60 Hz) and was treated by conventional heating (CH) with the same temperature profile (65°C/30 min). Measurements of rheology parameters, color changes (h°, C*, ΔE) microstructure (optical microscopy), and ascorbic acid (AA) degradation kinetics were performed. AA degradation rates ranged from 1.7 to 29.3% and from 2.8 to 24.8% for OH and CH, respectively. The beverages treated with both processes exhibited a pseudo-plastic behavior ($n < 1$), higher saturation (C^*), lesser reddish color ($h°$), and higher color variations (ΔE^*). In microstructure analysis, OH (1000 Hz-25 V and 80 V-60 Hz) was able to rupture the cell structure. In a practical sense, OH processes on whey acerola-flavoured drinks should be performed at low frequencies and voltages (≤ 100 Hz and 45 V).

Keywords: Ohmic heating; whey dairy beverages; color parameters; rheology; microstructure; ascorbic acid degradation

1 INTRODUCTION

Cheese whey is a by-product of cheese-making technology, and its use in dairy beverage manufacturing is an important alternative reuse because it is a product with a high biological oxygen demand (Castro et al., 2013). In addition, whey has excellent nutritional value, containing components such as lactose, various minerals, vitamins and approximately 20% total milk proteins (Svanborg et al., 2015). Cheese whey offers health benefits such as anti-diabetic (Akhavan et al., 2014) and anti-obesity (Freudenberg et al., 2013), antihypertensive (Cheung et al., 2015), anti-inflammatory (Kerasioti et al., 2013) and anticancer properties (Patel, 2015). For regulatory purposes, dairy drinks must contain at least 51% dairy base (milk and whey mixture) and can be fermented or unfermented, Pasteurized or sterilized, and contain fruit preparations and/or vegetable fat (Brasil, 2005). The addition of fruit pulp to beverages might be an interesting approach because it supplies desirable sensory characteristics and additionally contributes to increasing the nutritional value (Castro et al., 2013; Siqueira et al., 2013).

Acerola (*Malpighia emarginata* DC) is a tropical fruit with a high ascorbic acid (AA) content (800-4000 mg/100 g) and other nutrients such as anthocyanins, carotenoids, iron, and calcium (Lima et al., 2005). However, AA degradation can change the color of the product due to non-enzymatic browning reactions and promotes a reduction in the nutritional value (Rufián-Henares & Pastoriza, 2016). Acerola has a juicy and refreshing pulp and an acidic taste, which are sensory characteristics suitable for beverages containing high whey levels, such as dairy beverages (Caetano et al., 2012; Freitas et al., 2014).

Ohmic heating (OH) technology consists of a heating process in which electric current passes through the food, promoting instantaneous and homogeneous heating by conversion of the electrical energy into thermal energy, and electrical conductivity is the key processing parameter (Cappato et al., 2017). The rapid heating rate of OH is one of the advantages of this technique over conventional processing, resulting in a reduction in the thermal load submitted to the products and leading to a greater retention of nutrients and sensory attributes (Sastry et al. 2014). Previous studies have reported that in addition to the thermal effects, OH promotes additional non-thermal effects, such as electroporation, which can cause slight cellular damage to plant tissues, leading to an increase in product consistency due to leaching of intracellular material (Kaur & Singh, 2016). This effect also causes a reduction in the microbial thermal resistance (Knirsch et al., 2010), allowing the development of processes with lower thermal intensity, with less degradation of compounds, and without affecting the microbiological stability of the product.

The current study was intended to evaluate the effect of different ohmic heating parameters (electric field frequency and voltage gradient) on selected properties of a whey acerola-flavoured drink compared with conventional Pasteurization

2 MATERIALS AND METHODS

2.1 Whey Acerola-Flavoured Beverage Processing

A dairy mix consisting of Pasteurized milk and sweet whey (65/35 % v/v, Castro et al., 2013) was used at a concentration of 60% v/v. A blend containing 9.9% w/v sugar (União, Rio de Janeiro, Brasil) and 0.1% w/w xanthan gum (DEOSEN - 200 mesh) was added to the milk base and stirred, followed by the addition of 30% w/w acerola pulp (*Mais Fruta*

Company, Jarinu, SP, Brazil). The samples were stored at 5°C until OH and conventional processing.

Ohmic processing was conducted in two different ohmic systems. The first system allowed frequency variation at a fixed voltage, and the second system applied voltage variation at a fixed frequency (60 Hz). Both systems were used with frequencies of 10, 100 and 1000 Hz at 25 V for the first system and 45, 60, and 80 V at 60 Hz for the second system. These parameters were based on studies of ascorbic acid degradation kinetics in acerola pulp (Mercali et al., 2012; Mercali et al., 2014b). For both systems, 400 mL Whey acerola-flavoured drink was used, where the ohmic cell had jacketed beaker shape, a cover with input for 2 temperature controllers, a central aperture for sampling, and 2 titanium electrodes. The electrodes were curved to fit the reactor dimensions, where the maximum inter-electrode gap was 7.5 cm and the minimum gap was 5.7 cm. As the distance between the electrodes of the ohmic apparatus was not uniform, the results were not expressed in electric field (V/cm), because there would be a maximum and minimum electric field value for each applied voltage parameter, making it difficult to visualize the parameters. Thus, the results were expressed in voltage to facilitate the visualization and presentation of the data. The temperature was measured using two T-type stainless steel thermocouples, and stirring was performed by a magnetic stirrer (model C-MAG HS10, IKA) positioned under the ohmic cell.

The first system consisted of a function generator (Tektronix Inc., AFG3252, Richardson, Tex, USA), a power amplifier (500A, Industrial Test Equipment), a data acquisition system (34972A, Agilent Technologies Inc.), a computer, and an ohmic cell. The second system consisted of a 0-220 V voltage regulator (Sociedade Técnica Paulista LTDA, Varivolt, Brazil), a stabilizer (Force line, EV 1000T/2-2, Brazil), a computer, and an ohmic cell. Additional details of the ohmic systems are described in (Mercali et al., 2012, 2013, Mercali et al., 2014b).

Ohmic and conventional processes were performed using the same temperature profile for comparison purposes (**Fig. 1**), and the ohmic system (time 0) was started when the temperature reached 65°C (Pasteurization temperature). In both the ohmic and conventional systems, the cell was connected in thermostatic baths (Polystat, ColeParmer, Lauda, RM 12, Brazil) to reach the desired temperature. The only difference between the conventional and ohmic system was the absence of electrodes in the ohmic cell.

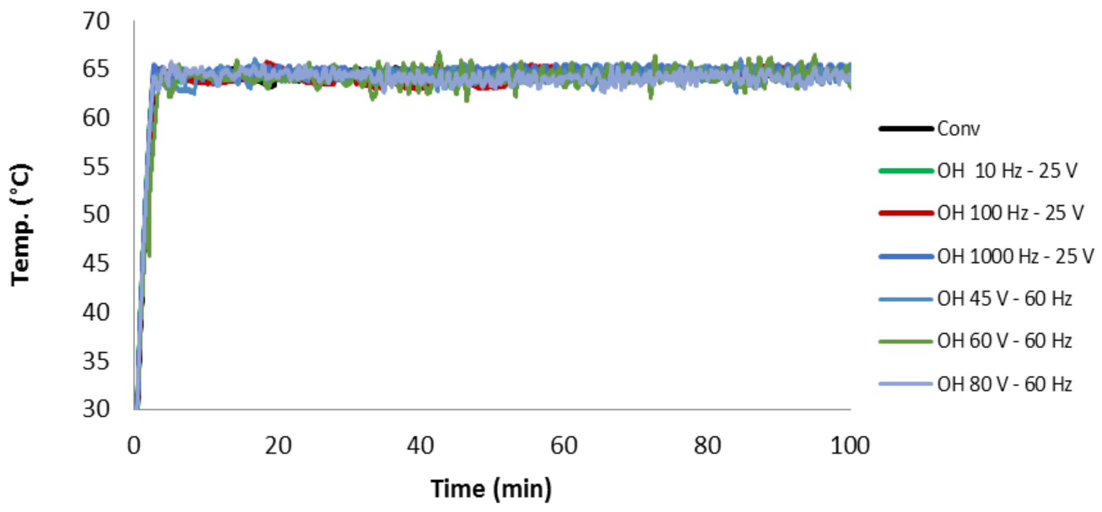

Fig. 1. Temperature profiles of conventional processing and Ohmic heating

2.2 Ascorbic Acid Content (Aa) and Degradation Kinetics

Determination of ascorbic acid was performed by the titration method using 2,6 dichlorophenol-indophenol (AOAC, 1990). For ascorbic acid extraction, 5 mL sample were mixed in 25 mL metaphosphoric acid solution. The sample was vortexed for 30 seconds and centrifuged at 6,000 rpm for 10 min. For ascorbic acid quantification, 5 mL of the supernatant were diluted in 15 mL extraction solution and titrated with indophenol solution with the samples protected from light. Aliquots of approximately 8 mL of whey acerola-flavoured drink were sampled at 0, 15, 30, 45, 60, 80, and 100 minutes. Time 0 was set as the time at which the temperature reached 64°C (Pasteurization time) for comparison between methods. The degradation kinetics of ascorbic acid are described by the first-order reaction kinetics parameters as shown below:

$$C = C_0 \times exp(-k.t)$$ **Equation I**

where *t* is the time (min), *k* is the rate constant (min^{-1}), and C_0 and C are the ascorbic acid concentrations at time *zero* and time *t*, respectively.

In addition, the half-life times ($t_{1/2}$) and the decimal reduction times (D-value) corresponding to the periods at which the AA levels were reduced by 50% and 90%, respectively, were calculated using the rate constant (K), according to the following equations:

$$D_{value} = \frac{\ln 10}{k}$$ **Equation II**

$$t_{1/2} = \frac{\ln 2}{k}$$ **Equation III**

The *z* value was calculated according to the model of Bigelow (1921) corresponding to the interval of voltage or frequency capable of promoting a 10x variation in the ascorbic acid degradation rate at 65°C. This parameter was determined using the D-values of the different ohmic treatments according to **Equation IV**:

$$z = \frac{(\gamma_2 - \gamma_1)}{(\log D_1 - \log D_2)}$$

Equation IV

2.3 Color Parameters and Degradation Kinetics

The color parameters a* (red-green), b* (blue-yellow), and L* (brightness) of the CIELAB scale were determined and were used to calculate hue angle (ºh), chroma (C*), and color variation (ΔE*) according to **Equations V, VI** and **VII**. Colorimetric analyses were performed in a ColorQuest XE (HunterLab) colorimeter equipped with a D65 illuminant and 10° viewing angle with specular reflectance included. Time zero was set as the initial process time at which the temperature reached 65 °C.

$$h^\circ = arctan(b^*/a^*)$$ **Equation V**

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$ **Equation VI**

$$\Delta E^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2}$$ **Equation VII**

where

$$\Delta a^* = a^* - a_0^*; \Delta b^* = b^* - b_0^*; \Delta L^* = L^* - L_0$$

2.4 Rheological Tests

The rheological tests consisted of determination of the flow curves at shear rates ranging from 10 to 250 ppm (Penna et al., 2001) using the rotational Brookfield Rheometer (USA). Measurements were performed with upward curves using Rheocalc software for data acquisition. The power law was used to describe the flow curves.

2.5 Microstructure

All samples submitted to ohmic heating and conventional processing were evaluated using optical microscopy. Approximately 20 μL of whey acerola-flavoured drink were deposited onto a glass slide surface and covered with a cover slip for observation under an optical microscope (Olympus, BX41, Japan) equipped with a digital camera.

2.6 Statistical Analysis

All processes were performed in triplicate, and all analyses were performed in duplicate. Statistical analyses were performed using Excel and Statistica software (7.0, Statsoft Inc., Tulsa, USA). The Fisher's means comparison test with a confidence level of 95% was used to compare the results.

3 RESULTS AND DISCUSSION

3.1 Acid Ascorbic Content and Degradation Kinetics

Fig. 2 shows the kinetics of ascorbic acid degradation over 100 minutes of OH and conventional processing using similar time and temperature profiles for both processes and **Table 1** shows the kinetic parameters obtained during the ohmic and conventional processing as well as the percentages of degradation of the whey acerola-flavoured drink. The ascorbic acid degradation kinetics in the different treatments were adjusted to the first-order equation with coefficients of determination (R^2) greater than 0.90, as also observed by other authors (Assiry et al., 2003; Assiry et al., 2006; Jiang et al., 2014; Mercali et al., 2014b; Zheng & Lu, 2011).

The AA degradation ranged from 2.8 and 24.8% and 1.7 and 29.3% for conventional and OH processing (1000 Hz-25 V), respectively. With respect to D-values (decimal reduction time), the highest D-value was observed for the treatment at 10 Hz-25 V ($D_{10\ Hz}$ = 870.4 ± 6.22), and the lowest value (worst process condition) was observed in the samples subjected to 1000 Hz-25 V ($D_{1000\ Hz}$ = 674.0). However, no differences were observed between the conventional process and the ohmic treatments at 100 Hz -25 V and 45 V-60 Hz ($p > 0.05$), and the treatment at 1000 Hz-25 V was significantly different from all treatments for this parameter. No significant differences were observed in the D-values of the treatments at different voltages (45, 60, and 80 V, $p < 0.05$).

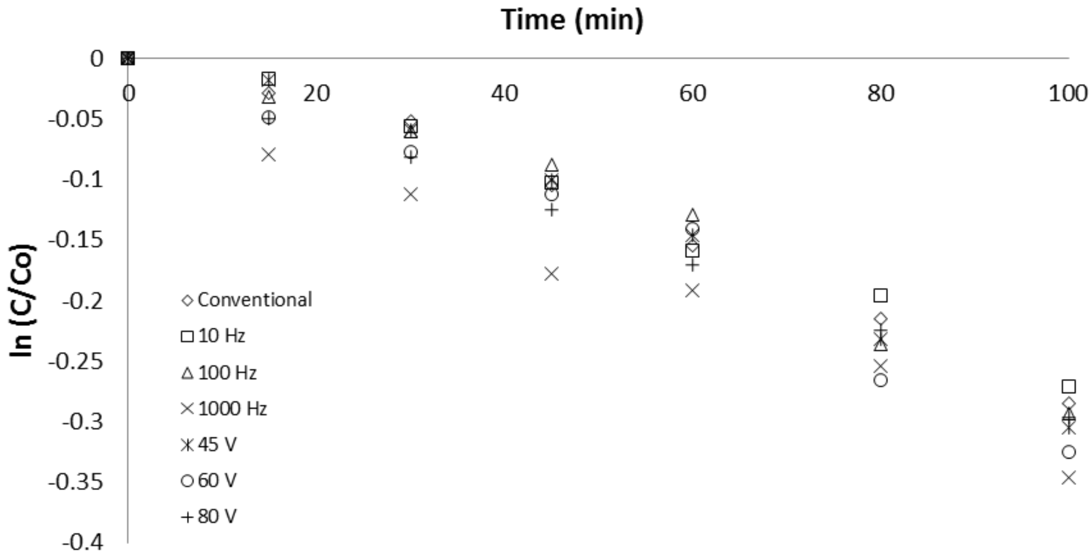

Fig. 2. Ascorbic acid degradation kinetic of Whey-acerola flavored drink during Ohmic Heating and conventional processing.

Thus, the results suggest that the use of low frequency (10 and 100 Hz) and low voltage (45 V) does not affect the degradation rate of AA because nutrient degradation occurred only by heating, with no significant differences ($p > .05$) compared with the conventional process. In contrast, the high frequency (1000 Hz) and high voltages (60 V and 80 V) applied resulted in an increase in the ascorbic acid degradation rate compared to conventional pasteurization ($p < .05$). The results show the presence of an additional non-thermal effect on the degradation of ascorbic acid, being more evident in the OH 1000 Hz–25 V (worst condition). According to the z values, at 65 °C, an increase of 8757 Hz–25 V or 2819 V–60 Hz is required to promote a 10-fold reduction in the degradation rate of AA, thus demonstrating the great stability of AA to the frequency and voltage parameters of OH at 65 °C. This hypothesis can describe the findings with respect to the halflife time ($t_{1/2}$) values.

Studies of ascorbic acid degradation in a whey acerola-flavoured drink subjected to ohmic heating are non-existent. However, similar results were obtained in previous studies using other food matrices (Athmaselvi et al., 2017; Chakrabortya & Athmaselvi, 2014; Mercali et al., 2012), primarily related to the effect of voltage on AA degradation during ohmic heating. Although acerola is a fruit with high vitamin C content, low rates of ascorbic acid degradation were observed. This effect may have occurred due to the limitation in oxygen availability in the medium, a reactant limiting to reaction. Some researchers (Assiry et al., 2003; Mercali et al., 2012; Palaniappan & Sastry, 1991) have reported that the increase of the applied electric field promotes greater degradation of ascorbic acid due to the electrolysis and corrosion of metal, producing compounds that catalyze the degradation pathways of ascorbic acid in the presence of oxygen. Probably, these effects may explain the increase of acid degradation at 60 and 80 V voltages compared to the conventional process and OH of low voltage (45 V) in the present work. In contrast, Castro, Teixeira, Salengke, Sastry, and Vicente (2004) reported that the electric field might not affect the degradation rate of AA during ohmic heating. Other authors have also found no differences in voltage in the kinetic parameters (k – rate constant and E_0 – activation energy) of ohmic-treated orange juice compared with conventional heating using a similar temperature profile (65–90 °C). However, neither study presented statistical data (Lima, Heskitt, Burianek, Nokes, & Sastry, 1999).

With respect to the effect of frequency on AA degradation, few studies exist in the literature (Mercali et al., 2014a, Mercali et al., 2014b). Other authors evaluated the influence of frequency variation (10 - 10^5 Hz at 25 V) on the degradation of AA in acerola pulp. The kinetic parameters (D, K, and $t_{1/2}$) were determined, and the results were compared with the conventional process under the same temperature profile. A similar trend was observed in both studies, which showed that the higher the frequency, the lower the degradation rate of AA, contrary to the findings of the current study in which an increase in the frequency of 10-1000 Hz created an increase in AA degradation.

It is likely that the difference between the results of the current study and the other results mentioned above is due to intrinsic factors related to the gross composition of the food. Despite following the same trend, Mercali et al. (2014b) observed different kinetic parameters for AA degradation in acerola pulp and whey once a significant difference was observed only for the pulp subjected to 10 Hz compared with the other treatments (including conventional Pasteurization), whereas significant differences were observed for ohmic-treated whey at 10, 100, and 1000 Hz. Thus, the composition of the food has a strong influence on the degradation rate and can present different responses to the different parameters used in ohmic heating.

3.2 Color Parameters

Fig. 3 shows the color parameters values (C*, h°, and ΔE*) after 100 min of OH and conventional processing under the following conditions: 10, 100, and 1000 Hz at 25 V; 60 Hz at 45, 60, and 80 V, 65 °C. The experimental error values were not plotted to facilitate visualization of data. **Table 2** shows the C*, h°, and ΔE* values after Ohmic heating and conventional treatment (65 °C/30 min). Both processes led to an increase in the parameters C*, h°, and ΔE*, i.e., during heating, the sample tended to present a higher saturation (higher C*), a less reddish color (h°), and higher color variations (ΔE∗). As shown in Table 2, after conventional processing, OH performed at 80 V–60 Hz showed the worst condition (higher C∗, h°, and ΔE∗ values), which presented a significant difference ($p < 0.05$) compared with the other treatments.

Table 2: Color mensurement after acerola whey drink pasteurization (64°C/30min) by Ohmic heating and conventional.

Treatments	Color mensurement after pasteurization (64°C/30min)		
	*C**	*h°*	Δ*E*
Conventional	16.60 ± 1.033 ^{b}	1,14 ± 0,016 ^{b}	1,31 ± 0,404 ^{b}
10 Hz - 25 V	18,48 ± 1.243 ^{ab}	1,14 ± 0,016 ^{b}	1,33 ± 0,645 ^{b}
100 Hz - 25 V	16,71 ± 0,435 ^{b}	1,14 ± 0,005 ^{b}	1,31 ± 0,584 ^{b}
1000 Hz - 25 V	17,51 ± 0,880 ^{ab}	1,13 ± 0,014 ^{b}	2,05 ± 0,529 ^{b}
45 V - 60 Hz	17,09 ± 0,684 ^{ab}	1,13 ± 0,006 ^{b}	1,21 ± 0,407 ^{b}
60 V - 60 Hz	18,03 ± 0,774 ^{ab}	1,14 ± 0,003 ^{b}	1,82 ± 1,079 ^{b}
80 V - 60 Hz	18,94 ± 0,279 ^{a}	1,25 ± 0,008 ^{a}	4,58 ± 0,482 ^{a}

Indeed, two factors can lead to greater changes in the color parameters and have an effect on the degradation rate of ascorbic acid: the increase in the voltage values with ohmic heating (Chakrabortya & Athmaselvi, 2014) and the reactions that can occur between the food and the electrode (Assiry et al., 2003). Relative to the effect of the frequency values (10, 100, and 1000 Hz) no difference was observed between the results, unlike the results obtained by Mercali et al. (2014b), who studied color changes using frequency values between 10 and 10^5 Hz and found higher color degradation in acerola pulp at 10 Hz. According to the authors, this result is due to the occurrence of greater electrochemical reactions at this frequency, which accelerate the degradation rate of the fruit pigments. Thus, it is important to highlight the need for additional studies involving OH in food matrices of different compositions, such as whey acerola-flavoured drinks.

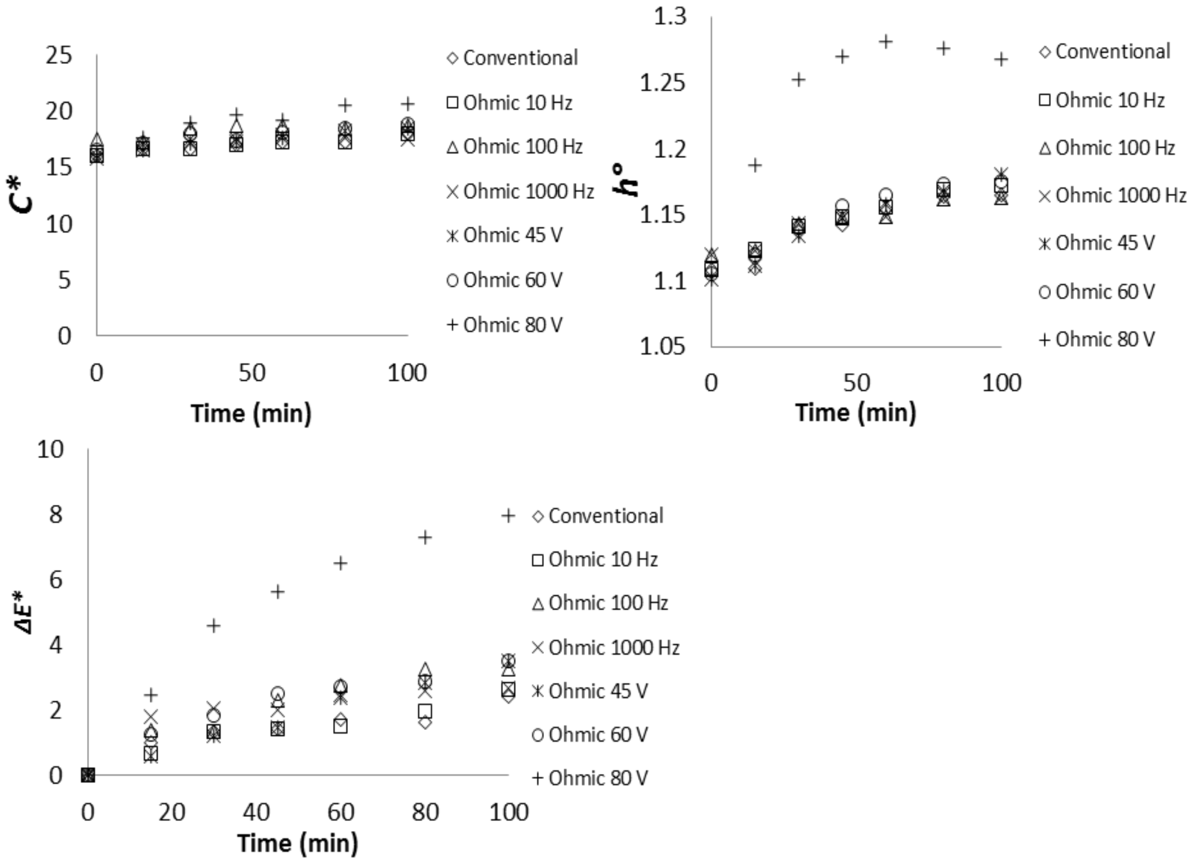

Fig. 3. Color parameters of whey-acerola flavored drink during Ohmic Heating and conventional processing

3.3 Rheological Parameters

Table 3 shows the rheological parameters (consistency index values and flow behavior index) of a whey acerola-flavoured drink after conventional and ohmic processing. The results were obtained using the power law model and were a good fit to the experimental data, with R^2 values > 0.98 for all samples. It was observed that all ohmic-treated samples exhibited a consistency index (k) lower than those of the samples subjected to the conventional treatment (12.66 mPa.sn), indicating a loss of viscosity, except for the treatments at 1000 Hz (44.72 mPa.sn) and 80 V (14.82 $mPa.s^n$) at which all the samples presented different behavior than the conventional treatment ($p < 0.05$). Relative to the flow behavior index (n), all samples presented pseudo-plastic behavior ($n < 1$), and the samples submitted to conventional processing and ohmic heating (1000 Hz and 80 V) presented n values of 0.704, 0.692, and 0.842, suggesting an increase and a direct relationship between the viscosity values and the strain rate.

Table 3. Rheological parameters of lactic beverages of acerola obtained by Power law model at 10 °C.

Samples	k (mPa.s^n)	n	R^2
Conv	12.66 ± 1.31[c]	0.704 ± 0.020[d]	0.9851
10 Hz – 25 V	7.21 ± 0.02[f]	0.955 ± 0.001[a]	1.0000
100 Hz – 25 V	7.60 ± 0.08[e]	0.939 ± 0.002[a]	0.9962
1000 Hz – 25 V	44.72 ± 2.31[a]	0.692 ± 0.010[d]	0.9999
45 V – 60 Hz	7.60 ± 0.08[e]	0.939 ± 0.002[a]	0.9999
60 V – 60 Hz	9.39 ± 0.17[d]	0.917 ± 0.003[ab]	0.9998
80 V – 60 Hz	14.82 ± 0.35[b]	0.842 ± 0.005[c]	0.9995

Data are expressed as mean ± standard deviation of at least 3 replicates. [a-g]: different letters at the same column indicates significant differences between samples (p > 0.05). k = consistency index; n = flow behavior index.

The results showed that the increase in the intensity of the ohmic parameters (frequency and voltage) significantly affected the viscosity of the ohmic-treated sample compared with other treatments, leading to an increase in the viscosity of the beverage. When analyzing the other samples submitted to OH processing (10 and 100 Hz at 25 V; 60 Hz at 45 and 60 V), a significant reduction in the consistency was observed compared with the conventional treatment. Therefore, the increase in voltage and frequency increased the viscosity of the whey acerola-flavoured drink, which can be explained by the microstructure images. As shown in **Fig. 4**, the treatments at 1000 Hz - 25 V and 60 Hz - 80 V promoted greater rupture in the cell structure, leading to a greater leaching of intracellular material into the medium, which might have increased the consistency of the beverage compared with other treatments.

No data were available in the literature that compared the effect of conventional and ohmic heating and the rheological behavior of dairy beverages, particularly for whey acerola-flavoured drinks. Icier & Tavman (2006) reported that the consistency of different types of ice cream (traditional and Turkish Maras ice cream) decreased significantly with increasing temperatures, but those authors did not study the effect of processing compared with the conventional process.

According to the current study, the best OH operation parameters for use during whey acerola-flavoured drink processing is low voltage (< 60 V) and low frequencies (< 100 Hz) because both the ascorbic acid and color degradation rates were similar to those obtained in conventional processing. However, it is interesting to note that an increase in the consistency of the product was observed, and the impact of these changes should be evaluated for consumers using an affective test and establishment of sensory profiling. In this sense, the findings of the current study results are of interest to the dairy industry and can serve as a guideline for implementation of OH for processing of whey dairy beverages, particularly whey acerola-flavoured drinks.

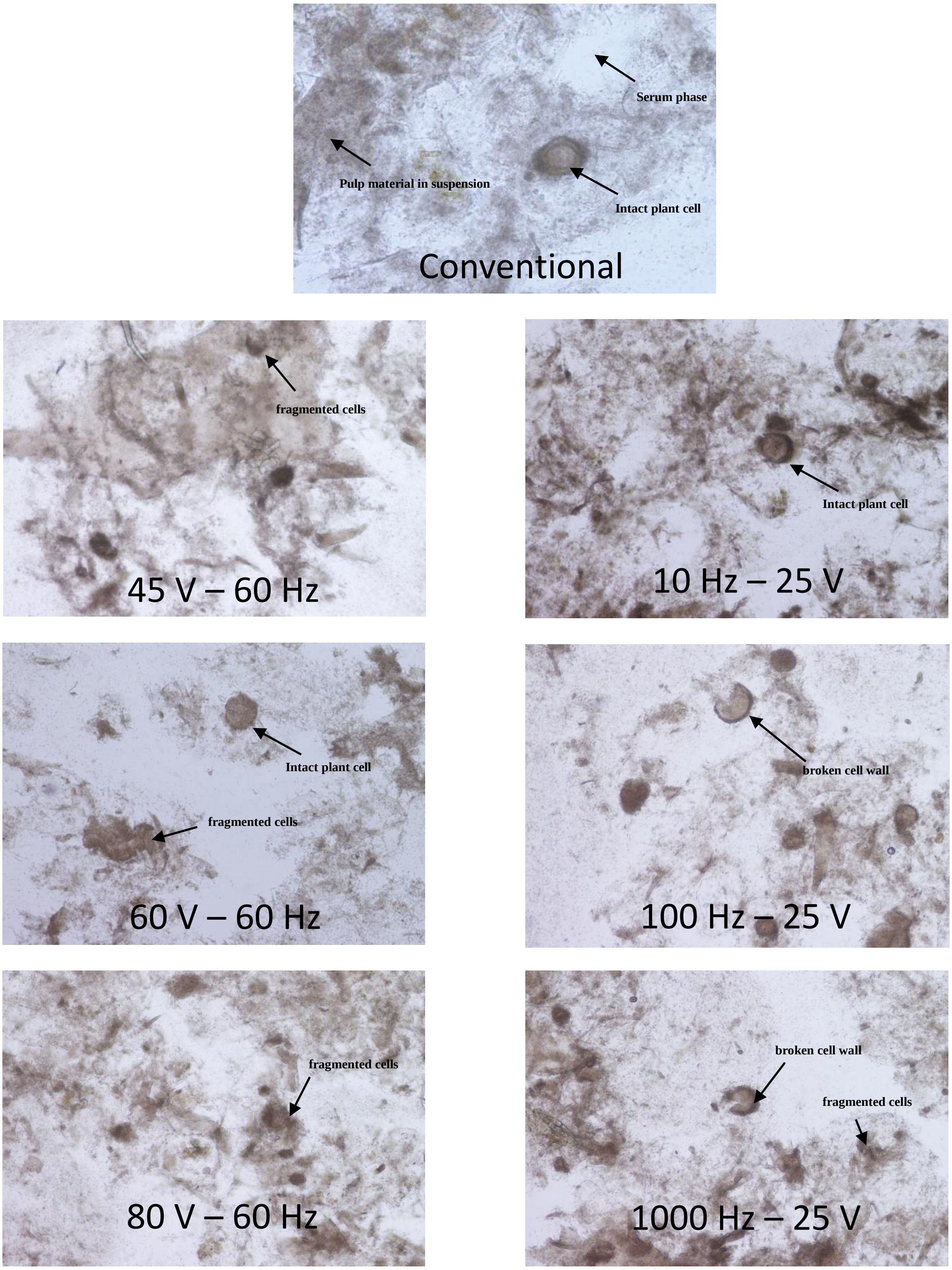

Fig. 4. Microstructures of Whey-acerola flavored drink during Ohmic Heating and conventional processing (optical microscopy, 10X objective).

3.4 Microstructure

Fig 4 shows the effect of the OH and the conventional process on the microstructure of the whey acerola-flavoured drink. The whey acerola-flavoured drink are composed of a serum phase consisting of milk, reconstituted whey and the components of acerola pulp. In plant cells are located intracellular substances, such as carotenoids, responsible for the coloring of the fruit, are located in the plant cell in different plastids, principally in chromoplasts (Meléndez-Martínez, Vicario, & Heredia, 2004). In general, intact cells were observed after Pasteurization with the conventional process, whereas a progressive increase in cell disruption was observed with ohmic processing, especially when higher frequencies (1000 Hz) and voltages (80 V) were applied.

The images can aid in understanding the ascorbic acid degradation profile, color, and rheological behavior, which can lead to both desirable and undesirable results. The desirable effects include an increase in viscosity of the ohmic-heated samples subjected to 1000 Hz and 80 V, probably due to the leaching of intracellular material caused by the rupture of the cellular structure, which can have a positive impact on the product's acceptance by consumers. However, greater cell disruption can result in undesirable changes, such as higher nutrient and color degradation rates. Absence of studies have correlated the impact of ohmic heating on the cell microstructure and the degradation rate of the physical and nutritional properties of dairy foods, in particular, whey dairy beverages. This observation is an interesting and novel result, which can help in understanding the effect of the electric field on processed food properties.

However, the influence of the best OH parameters on corrosion of the electrodes during OH pasteurization was not investigated. In addition to the corrosion of the electrodes, further investigations must be carried out in order to understand the effects of different OH parameters on the consumer acceptance and the establishment of the sensory profiling (Belsito et al., 2017; Gaze et al., 2015), as well as on conformational structure and the allergenic potential of proteins on dairy products (Davoodi et al., 2016).

4 CONCLUSION

The current results suggest that the ohmic heating operational parameters (frequency and voltage) might play an important role in the quality of the whey acerola-flavoured drink, affecting the rheological characteristics, ascorbic acid degradation, and optical characteristics of the product.

Considering practical and operational purposes for adoption by the dairy industry, the best ohmic heating conditions for a whey acerola-flavoured drink was processing at low voltage (< 45 V) and low frequency (≤ 100 Hz). Considering the high consumption of whey fruit-flavoured drinks worldwide, these results are relevant for the dairy industry from a technological point of view.

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CAPÍTULO III

WHEY ACEROLA-FLAVOURED DRINK SUBMITTED OHMIC HEATING: BIOACTIVE COMPOUNDS, ANTIOXIDANT CAPACITY, THERMAL BEHAVIOR, WATER MOBILITY, FATTY ACID PROFILE AND VOLATILE COMPOUNDS

WHEY ACEROLA-FLAVOURED DRINK SUBMITTED OHMIC HEATING: BIOACTIVE COMPOUNDS, ANTIOXIDANT CAPACITY, THERMAL BEHAVIOR, WATER MOBILITY, FATTY ACID PROFILE AND VOLATILE COMPOUNDS

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Whey acerola-flavoured drink submitted Ohmic Heating: Bioactive compounds, antioxidant capacity, thermal behavior, water mobility, fatty acid profile and volatile compounds

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1 INTRODUCTION

The consumption of whey-dairy beverages has grown every year, since it is an alternative to traditional yogurt, at a reduced cost due to the use of whey in its formulation (Janiaski, Pimentel, Cruz, & Prudencio, 2016). The addition of fruit pulps, such as acerola (*Malpighia emarginata* DC) provides desirable characteristics to the product, such as aromatic compounds, improving the flavor and consequently the acceptability of the product (Bosi, Bernabé, Della Lucia, & Roberto, 2013).

Despite the health benefits of dairy beverages containing fruit pulps, thermal processing leads to the degradation of several thermosensitive nutrients, causing undesirable alterations (Pereira, Martins, & Vicente, 2008). The use of emerging technologies, such as Ohmic Heating (OH), has been increasingly studied to reduce the deleterious effects of conventional thermal processes (Cappato et al., 2017; Jaeger et al., 2016) and represents a promising technology for the dairy sector (Cappato et al., 2017). OH promotes rapid and homogeneous heating of the product, through the conversion of electric energy into thermal energy, resulting in a greater retention of thermosensitive compounds and sensory attributes due to exposure to a lower thermal load (Sastry, Heskitt, Sarang, Somavat, & Ayotte, 2014), which can reduce the degradation of the thermosensitive compounds such as bioactive compounds and fatty acids in whey acerola-flavoured drinks.

OH parameters can affect conformation, physical stability, induce the denaturation and aggregation and viscoelastic behavior of whey proteins (Pereira et al., 2016; Rodrigues et al., 2015). So, the knowledge about the effects of OH (Electric fields and Frequency) is essential for the development of whey dairy products, such as dairy drinks and yogurts (Costa et al., 2018; Cappato et al., 2017). However, researches that report the effect of the OH parameters on whey dairy products are still scarce in the literature.

Despite the scarce research, the results have shown that OH has great potential in the whey dairy products processing, promoting improvements in physical, rheological and chemical characteristics (Cappato et al., 2018; Costa et al., 2018; Pereira et al., 2016). Recently, OH has been reported with an alternative technology in processing whey fermented dairy products (Pereira et al., 2018). Costa et al. (2018) observed that the pasteurization of the sweet whey under low electric field (4–5 $V.cm^{-1}$) promotes positive effects on the rheological, major preservation of the antioxidant capacity, volatile compounds and the ACE Inhibitory Activity of bioactive peptides, without affecting the sensory profiles.

The objective of this study is to continue investigate the effects of OH under different process conditions (10, 100, 1000 Hz – 25 V, 45, 60, 80 V – 60 Hz) on the whey acerola-flavoured drink LTLT pasteurization compared with conventional pasteurization. In previous study, the effects of OH conditions on rheological parameters, color changes (h°, C*, ΔE), microstructure and ascorbic acid kinetics degradation were performed, and now, the present research proposed to investigate the effect of OH on the bioactive compounds (total phenolics and ACE Inhibitory Activity), antioxidant capacity (DPPH and FRAP) fatty acid profile (FAs), volatile compounds (VOCs), thermal behaviour and water mobility on the whey acerola-flavoured drink.

2 MATERIAL AND METHODS

2.1 Whey Acerola-Flavoured Drink Processing

The whey acerola-flavoured drink was prepared according to recent study, containing 60% dairy base (pasteurized milk+sweet whey, 70:30% v/v Parmalat, São Paulo, Brazil and Porto Alegre, Belo Horizonte, Brazil, respectively), 30% v/v acerola pulp (MaisFruta, Jarinu, São Paulo, Brazil), 9.9% w/w sugar (União, Rio de Janeiro, Brasil), and 0.1% w/w xanthan (Deosen – 200 mesh) (Cappato et al., 2018).

Ohmic processing was conducted in two different ohmic systems. The first system allowed frequency variation at a fixed voltage (25 V) and was consisted of a function generator (Tektronix Inc., AFG3252, Richard-son, Tex, USA), a power amplifier (500A, Industrial Test Equipment), a data acquisition system (34972A, Agilent Technologies Inc.), a computer, and an ohmic cell. The second system allowed voltage variation at a fixed frequency (60 Hz) and was consisted of a 0–220 V voltage regulator (Sociedade Técnica Paulista LTDA, Varivolt, Brazil), a stabilizer (Force line, EV 1000T/2-2, Brazil), a computer, and an ohmic cell.

For both systems, 400 mL Whey acerola-flavoured drink was used. The temperature was measured by two type-T thermocouples: one located close to the electrode and the other located away from the electrodes, which maximum difference between temperatures was 0.5 °C. The cylindrical electrodes had 5 cm of height with maximum and minimum inter-electrode gap was 7.5 cm and 5.7 cm, respectively. The product was stirred using a magnet inside the cell and a magnetic stirrer plate to promote the homogenization of the whey drink. As the distance between the electrodes of the ohmic apparatus was not uniform, the results were not expressed in electric field (V/cm) because there would be a maximum and minimum electric field value for each applied voltage parameter, making it difficult to visualize the parameters. Thus, the results were expressed in voltage to facilitate the visualization and presentation of the data. A schematic diagram of the OH setup is presented in **Fig**. **1**.

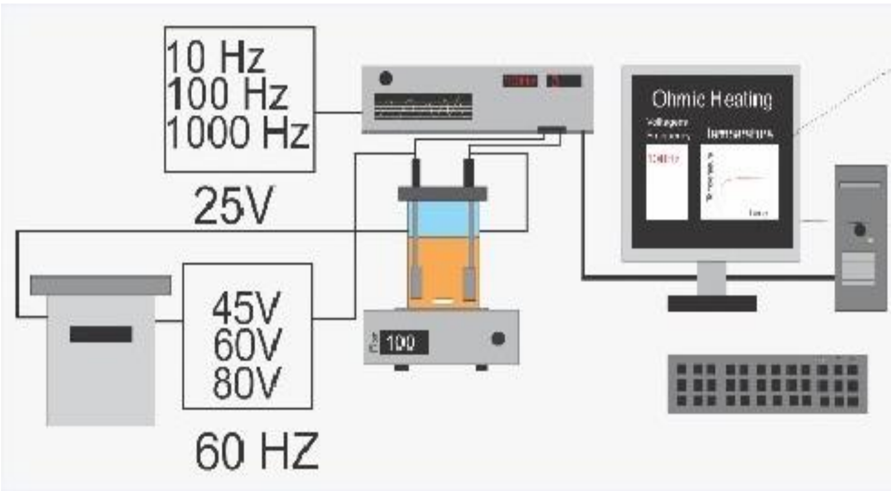

Fig. 1. Schematic diagram of the OH setup.

OH processing was carried out with frequencies of 10, 100, and 1000 Hz at 25 V for the first system, and 45, 60, and 80 V at 60 Hz for the second system. These parameters were based in previous study, which have been shown to directly influence the chemical and physical properties of acerola-based products (Mercali, Schwartz, Marczak, Tessaro, & Sastry, 2014; Cappato et al., 2018). The OH and conventional processes were performed at the same temperatures profiles (65 °C/30 min) as shown in **Fig**. **2**, aiming to evaluate the existence of an additional non-thermal effect through the variation of voltages and frequencies. The OH system (time 0) was started when the temperature reached 65 °C.

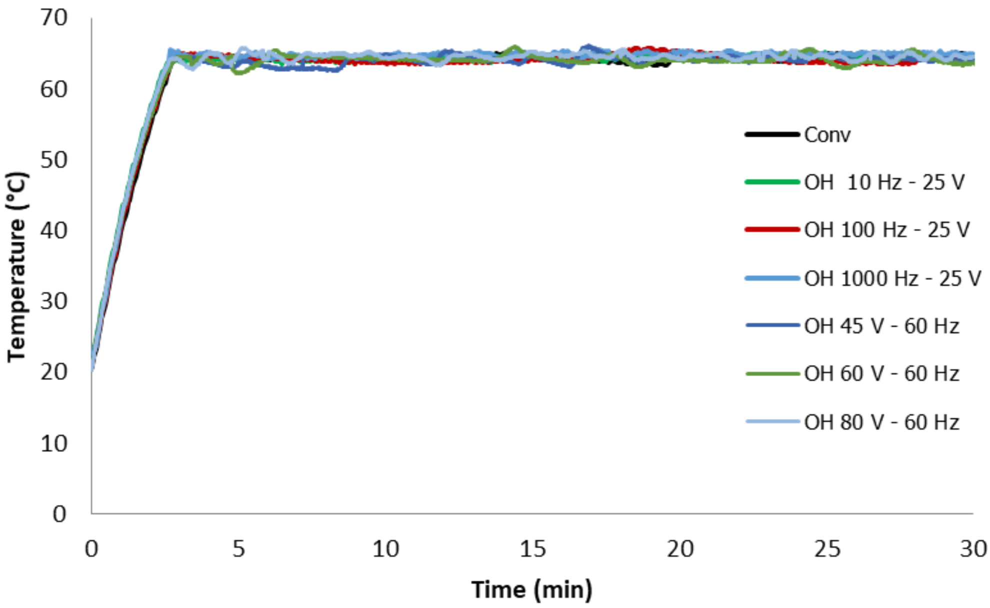

Fig. 2. Temperature profiles of Whey acerola-flavoured drink submitted to conventional processing and Ohmic Heating

2.2 Antioxidant Capacity (FRAP and DPPH) and Bioactive Compounds (TPC and Bioactive Peptides)

The extracts for analysis of total phenolic compounds (TPC), DPPH and FRAP assays were obtained in triplicate, as described by Vianna et al. (2018), with modifications. For that, approximately 1 g sample was weighed into beakers (200 mL), and 30 mL of a mixture of water and ethanol (50:50 v/v) was added and stirred at 200 rpm on an orbital table (SL180/D, Solab, Piracicaba, SP, Brazil) for 1 h. The resulting extract was filtered under vacuum.

The antioxidant capacity determined by the DPPH% (2,2-diphenyl-1- picrylhydrazyl) assay was performed according to Brand-Williams, Cuvelier, and Berset (1995) and FRAP assay (Ferric Reducing Antioxidant Power) in accordance with Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006). For the former, 2850 µL of a methanolic solution of the DPPH% radical (0.06mM–700 nm) was mixed to 150 µL of extract and held in the dark for 60 min. The results for both analysis were expressed as µg Trolox Equivalent/g sample.

Total phenolic compounds (TPC) was performed according to Swain and Hillis (1959) with modifications. The absorbance was measured at 725 nm in a spectrophotometer (Biospectro, SP-220), and the results were obtained using a calibration curve. The results expressed as gallic acid equivalents per liter of sample (EAG g/L). For the analysis, 1 mL of Folin reagent diluted in distilled water (1:10) was added to 1 mL of extract and agitated for 1 min.

ACE inhibitory activity were performed by filtration of the extracts, as described by Ramchandran and Shah (2010). The angiotensin Iconverting enzyme inhibitory (ACEI) was determined in a spectrophotometer and the ACE inhibitory activity was calculated as follows:

$$\text{ACEI activity}(\%) = [(B-A)/(B-C) \times 100].$$ **Equation I**

where, A – absorbance ACEI component in the presence of ACE; B – absorbance without the ACEI component; C – absorbance without ACE.

2.3 Fatty Acids Profile

Fat were extracted from the samples according to Batista et al. (2017). For the analysis of fatty acids profile, 1 g sample was weighed into 50 mL Falcon tubes using an analytical balance (Mars, AY220), and 50 µL of internal standard containing heptadecanoic acid and sorbic acid (6.0 mg.mL−1), 4 mL methanol, 2 mL dichloromethane and a little amount of BHT were added. Fatty acid identification and quantification was performed in a GC–MS (Agilent Technologies, 7890A-5975C), with a CTC PAL sampler (SPME 120, Agilent Technologies) using the following chromatographic conditions: injection volume of 1 µL; mobile phase ratio of 1:100; injector temperature of 240 °C; mobile phase flow rate of 0.5 mL.min−1; linear velocity of 36.796 cm.sec−1; oven temperature programmed to 70 °C for 1 min, with a temperature ramp from 45 °C.min−1 to 115 °C, followed by a new ramp at 40 °C.min−1 to 175 °C, and finally 30 °C.min−1 to 240 °C holding for 4.23 min; column – DB-FFAP CG 15m×0.10 mm, 0.10 µm; and MS detector with mass range 40–400 m/z. The identification of the samples was performed by comparing the retention times of the chromatographic peaks with reference standards (Sigma FAME 37 18919-1AMP) and the mass spectra were compared with the NIST 11 spectra library. Atherogenic (AT) and thrombogenic (TI) indices were also calculated, according to **Equations II** and **III** (Batista et al., 2017), as it was reported recently:

AI = (C12:0 + 4 × C14:0 + C16:0)/[Σ MUFA + Σ PUFA(n-6) and (n-3)] **Equation II**

TI= (C14:0 + C16:0 + C18:0)/[0.5 × Σ MUFA + 0.5 × Σ PUFA(n-6) + 3 × Σ PUFA(n-3) + (n-3)/(n-6)] **Equation III**

2.4 Volatile Profiling

The volatile compounds were extracted by solid phase microextraction (SPME), according to (Condurso, Verzera, Romeo, Ziino, & Conte, 2008) with modifications. The compounds were identified by gas chromatography (Agilent Technologies® 7890A GC) coupled to a mass spectrometer (Agilent Technologies® 5975C). The SPME extractions were performed using 50/30 µm thick Sulpeco® divinylbenzene/ carboxy/polydimethylsiloxane (DVB/CAR/PDMS) fibers and 20 mL headspace vials in an automated CTC PAL Sampler (Agilent Technologies SPME 120). The linear retention index (LRI) was calculated according to the equation proposed by Van Den Dool and Dec. Kratz (1963) and compared to the LRI of the C8-C40 alkane standards (Supelco, 40127-U) injected under the same chromatography and mass spectrometry conditions. Identification was enhanced using Agilent Mass Hunter Qualitative Analysis software (Agilent Technologies) in deconvolution mode, with signal to noise ratio above 10, left Δm/ z=0.3 AMU and right Δm/z=0.7 AMU, and hints obtained from the National Institute of Standards and Technology Library (NIST/EPA/NIH Mass Spectra Library, version 11, USA).

2.5 Differential Scanning Calorimetry Analysis (DSC)

The DSC analysis was performed according to Kavaz Yuksel (2015) with some modifications. The products were subjected to the following steps at a constant heating rate (10 °C/min): (1) holding for 1 min at −60 °C; (2) heating from −60 °C to −40 °C; 3) cooling from −40 °C to −60 °C; (4) holding for 1 min at −60 °C; (5) heating from −60 °C to 30 °C; (6) holding for 1 min at 30 °C; (7) cooling from 30 °C to −60 °C; (8) holding for 1 min at −60

°C; and finally (9) heating from −60 °C to 30 °C. Melting and crystallization behavior was evaluated by DSC, using a PYRIS Diamond DSC equipment (Diamond, Perkin–Elmer, Norwalk, PA), equipped with an intracooler and the software Pyris Manager. Calibration was performed using an indium standard and 15 mg of sample were sealed into aluminum pans (50 µL, Perkin–Elmer).

2.6 Time Domain Nuclear Magnetic Resonance (TD-NMR)

TD-NMR measurements were performed in a low field spectrometer operating at 23.4 MHz for proton (MARAN Ultra 0.54 T) with 7.5 µs and 90° pulse length. All samples were subjected to transversal relaxation time (T2) through the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with 4096 echoes, 600 µs between each echo and 32 scans with a waiting time of 4 s. Laplace Inverse Transformation was used to determine the distribution curve of relaxation domains using the exponential decay curve obtained by echo recording.

2.7 Statistical Analysis

Both processing and analyses were performed in triplicate. The results were presented as mean ± standard deviation, and analyzed by analysis of variance (ANOVA) and Fisher's test ($p \leq 0.05$), using Statistica software (7.0 Statsoft Inc., Tulsa, EUA).

3 RESULTS AND DISCUSSION

3.1 Antioxidant Capacity and Bioactive Compounds

Table 1 shows the bioactive compounds levels of the whey acerola-flavoured drink submitted to OH and conventional processing. Costa et al. (2018) observed that the increase of the electric field negatively affected the preservation of the antioxidant capacity and the ACE Inhibitory Activity of bioactive peptides.

Table 1. Antioxidant Capacity and Bioactive Compounds of whey acerola-flavoured drink submitted to Ohmic heating and conventional processing

Samples	TPC (µg Acid Gallic/g)	FRAP (µg Trolox Eq/g)	DPPH (µg Trolox Eq/g)	ACE (%)
Conventional	61.54 ± 1.30^{b}	248.15 ± 36.28^{a}	8.48 ± 0.18^{c}	65.86 ± 4.94^{c}
10 Hz - 25 V	49.35 ± 2.52^{f}	211.34 ± 8.56^{c}	8.47 ± 0.25^{c}	71.05 ± 0.11^{c}
100 Hz - 25 V	56.13 ± 1.56^{c}	232.07 ± 8.49^{b}	8.30 ± 0.09^{f}	93.19 ± 2.03^{a}
1000 Hz - 25 V	63.08 ± 0.99^{a}	226.99 ± 4.08^{b}	8.62 ± 0.28^{b}	97.31 ± 3.04^{a}
45 V - 60 Hz	54.87 ± 1.45^{d}	197.41 ± 32.76^{cd}	8.48 ± 0.37^{c}	57.62 ± 0.63^{d}
60 V - 60 Hz	45.66 ± 3.27^{g}	209.02 ± 31.18^{c}	8.60 ± 0.14^{b}	66.40 ± 3.42^{c}
80 V - 60 Hz	52.04 ± 1.12^{e}	196.27 ± 10.27^{d}	8.88 ± 0.31^{a}	84.86 ± 2.15^{b}

* Data are expressed as mean ± standard deviation of at least 3 replicates. $^{a\text{-}c}$ Different letters at the same column indicates significant differences between samples (p < 0.05). Profile temperature = 65 °C/30 min.

Regards to the antioxidant capacity determined by the FRAP assay, lower retention was observed in the samples subjected to 80 V–60 Hz (196.27 μg Trolox equivalent/g). Highest retention was obtained in the sample pasteurized by the conventional process (248.15 μg Trolox equivalent/g), followed by the samples subjected to 100 Hz (232.07 μg Trolox equivalent/g) and 1000 Hz (226.99 μg Trolox equivalent/g), with no significant differences ($p > 0.05$) between 100 and 1000 Hz. For the DPPH assay, OH resulted in a lower degradation of the antioxidant capacity when compared to the conventional treatment (8.48 μM Trolox equivalent/g) in the samples subjected to 80 V–60 Hz (8.88 μM Trolox equivalent/g) ($p < 0.05$), 1000 Hz–25 V (8.82 μM Trolox equivalent/g) and 60 V–60 Hz (8.60 μM Trolox equivalent/g). According to Loypimai, Moonggarm, & Chottanom, 2009, the antioxidant capacity by the FRAP and DPPH assays is influenced by the electric field applied, with higher values when compared to the steamassisted solvent extraction.

In spite of the phenolic compound, higher degradation was observed for OH at 60 V–60 Hz (45.66 μg gallic acid/g) (worst condition), followed by OH at 10 Hz–25 V (49.35 μg gallic acid/g) and 80 V–60 Hz (52.04 μg gallic acid/g), while OH applied at high frequencies resulted in higher preservation, where OH at 1000 Hz–25 V (63.08 μg gallic acid/g), followed by the conventional process (61.54 μg gallic acid/g) and OH 100 Hz–25 V (56.13 μg gallic acid/g).

With the exception of the antioxidant capacity by the DPPH method, the results of TPC and FRAP presented higher degradation in the OH treatments using low voltages and low frequencies. These results can be explained by the possible existence of electrochemical reactions in electrode/solution interface. Electrochemical reactions promote corrosion of the electrode by releasing metallic ions capable of catalyzing the oxidation process (Samaranayake & Sastry, 2014). In addition, these reactions may promote the generation of reactive oxygen species (ROS), such as hydroxyl radicals (%OH) and hydroperoxyl (%OOH) and hydrogen peroxide (H2O2) (Schafer, 2001; Samaranayake & Sastry, 2014). ROS can react with food components causing oxidative degradation of these nutrients.

Electrochemical reactions are more intense at low frequencies in AC because the time interval necessary for the field to revert its polarity is long, allowing the accumulation and consequently the saturation of charges on the surface of the electrodes. Once fully charged or “saturated”, it becomes a “leaky” capacitor initiating electrochemical (ie, faradaic) reactions (Samaranayake & Sastry, 2014; Mercali et al., 2014).

On the other hand, at high frequencies the electrochemical reactions are reduced, because the reverse motions in the field polarity are high, preventing the movement of the loads in the orientation of the field and Faradaic current. At high frequencies, molecules remain in their random, steady-state orientations (Içier & Baysal, 2004).

ACE-inhibitory peptides are amino acids that exhibit positive physiological effects on human health, such as blood pressure control. Dairy products, such as dairy drinks and cheeses, are important sources of bioactive peptides, due to the hydrolysis of whey proteins by the enzymatic action or fermentation process (Yadav et al., 2015). However, Studies on the effect of OH on the bioactive compounds on dairy products are scarce in the literature.

In the present study, the findings showed that OH contributed to an increase in the enzyme inhibition when compared to the conventional treatment (65.86%), except for the 45 V–60 Hz (57.62%). OH performed on high frequencies, like 1000 and 100 Hz, showed the greater inhibition (93.19 and 97.31%, respectively), which had a significant effects ($p < 0.05$) when compared to the conventional pasteurization. Regarding the voltage, only the treatment at 80 V–60 Hz exhibited a greater inhibition (84.86%) as compared to the conventional treatment.

Enzymes (which are proteins) possess net charges and dipole moments when in an aqueous environment and move in response to an electric field. In according of Castro, Macedo, Teixeira, & Vicente, 2004, Moderate Electric Fields (MEF) applied during OH (< 100 V/cm, 50–60 Hz) have been shown to exhibit nonthermal effects on enzyme activities, which may contribute to a change in the amplitude of aggregation, unfolding, denaturation and conformation of whey proteins (Pereira et al., 2016; Pereira, Souza, Cerqueira, Teixeira, & Vicente, 2010). Due to the presence of net charge and dipole moment of enzymes, recent researches have been hypothesized that the electrophoretic motion resulting from MEF exerts either an activation or inactivation effect on enzyme molecules, (Samaranayake & Sastry, 2016a, 2016b). According to Samaranayake and Sastry (2018), the frequency may promote an increase in the electrophoretic movement of the enzyme and, consequently, the reaction rate of the enzyme. Another hypothesis is that the applied frequency can promote alterations in the enzymatic conformation, and may result in the increase or reduction of the enzymatic activity. So, such effects may contribute to the formation of bioactive peptides; however, future investigations about the effect of OH on the formation of bioactive peptides are required.

3.2 Fatty Acid Profile

Table 2 shows the fatty acid profile of the whey acerola-flavoureddrink after OH and conventional pasteurization. Overall, OH parameters promoted different behaviors on the fatty acid profile and the type of fatty acids. Although the results presented significant differences ($p < 0.05$), the values were very close, indicating the presence of a slight additional non-thermal effect on the fatty compounds. Thus, the results suggesting that degradation of the fatty acid is predominantly due to the thermal effect of the pasteurizing process. Studies reporting the effects of OH on the fatty acid profile in dairy products are scarce in this sense this research brings interesting contribution.

The fatty acids are classified as short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA) in relation to the chain length, and as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in relation to the degree of instauration in the carbon chain. Concerning the short-chain fatty acids (SCFA), only butanoic acid was identified, with the highest concentration obtained in the treatment at 10 Hz–25 V (5.56 ± 0.09), and the lower levels observed in the OH samples at 60 V–60 Hz (4.73 ± 0.20), followed by the conventional treatment (4.96 ± 0.43). In relation to the medium-chain fatty acids (MCFA), a higher concentration was observed in the OH samples at 60 V–60 Hz (17.69 ± 0.41), followed by 10 Hz–25 V (17.20 ± 0.69) ($p < 0.05$), while the lower concentration was observed in the samples subjected to the conventional pasteurization (14.13 ± 0.35). In all treatments, with the exception of OH at 60 V–60 Hz, decanoic acid (C10:0) was the majority, followed by hexanoic (C6:0), dodecanoic (C12:0) and octanoic acid (C8:0). In contrast, Pereira et al. (2008) found no differences ($p > 0.05$) in the fatty acid levels (SCFA and MCFA) in HTST goat's milk (72 °C/15 s) when compared to OH and conventional pasteurization. Probably, these conflicting results are due to the exposure time of LTLT and HTST pasteurization.

Short chain (SCFA) and medium-chain fatty acids (MCFA) presents important health benefits and maintenance of homeostasis. The SCFA intake is associated with a reduction in the risk of inflammatory bowel diseases, diarrhea and cardiovascular diseases, once butyrate plays an important role in colonic mucosa and prevention of colon cancer (Hijova & Chmelarova, 2007; Huda-Faujan et al., 2010), while the MCFA intake has an effect against obesity and metabolic disorders (Nagao & Yanagita, 2010).

Table 2. Fatty acid profile (g/100 g fat) of whey acerola-flavoured drink submitted to Ohmic heating and conventional processing

Fatty Acids	Samples						
	Conventional	10 Hz – 25 V	100 Hz – 25 V	1000 Hz – 25 V	45 V – 60 Hz	60 V – 60 Hz	80 V – 60 Hz
Short-Chain Fatty Acids (SCFA)	4.96 ± 0.43^{ab}	5.56 ± 0.09^{c}	5.48 ± 0.22^{c}	5.11 ± 0.24^{abc}	5.24 ± 0.40^{bc}	4.73 ± 0.20^{a}	5.10 ± 0.05^{abc}
Butanoic (C4:0)	4.96 ± 0.43^{ab}	5.56 ± 0.09^{c}	5.48 ± 0.22^{c}	5.11 ± 0.24^{abc}	5.24 ± 0.40^{bc}	4.73 ± 0.10^{a}	5.10 ± 0.05^{abc}
Medium-Chain Fatty Acids MFCA)	14.13 ± 0.35^{a}	17.20 ± 0.69^{cd}	16.11 ± 0.07^{bc}	15.61 ± 0.59^{b}	16.15 ± 0.46^{bc}	17.69 ± 0.41^{d}	16.31 ± 0.95^{bc}
Hexanoic (C6:0)	3.73 ± 0.15^{a}	4.70 ± 0.14^{d}	4.52 ± 0.00^{cd}	4.01 ± 0.42^{abc}	4.40 ± 0.32^{bcd}	3.91± 0.08^{ab}	4.35 ± 0.26^{bcd}
Octanoic (C8:0)	1.79 ± 0.10^{a}	2.17 ± 0.20^{cd}	2.02 ± 0.03^{bc}	1.90 ± 0.08^{ab}	2.11 ± 0.02^{bc}	2.34± 0.8^{d}	2.09± 0.11^{bc}
Docanic (C10:0)	5.17 ± 0.20^{a}	6.11 ± 0.48^{b}	5.66 ± 0.10^{ab}	5.71 ± 0.12^{ab}	5.68 ± 0.04^{ab}	6.76 ± 0.10^{c}	5.84 ± 0.42^{b}
Dodecanoic (C12:0)	3.43 ± 0.07^{a}	4.23 ± 0.14^{c}	3.91 ± 0.01^{b}	3.99 ± 0.14^{bc}	3.96 ± 0.13^{b}	4.68 ± 0.08^{d}	4.03 ± 0.15^{bc}
Long-Chain Fatty Acids (LCFA)	80.91 ± 0.16^{d}	77.23 ± 0.60^{a}	78.41 ± 0.15^{abc}	79.28 ± 0.36^{c}	78.61 ± 0.86^{bc}	77.58 ± 0.16^{ab}	78.59 ± 1.00^{bc}
Myristic (C14:0)	12.87 ± 0.05^{a}	14.63 ± 0.04^{c}	14.37 ± 0.06^{c}	14.22 ± 0.26^{c}	13.45 ± 0.39^{ab}	14.10 ± 0.08^{bc}	14.10 ± 0.62^{bc}
Myristoleic (14:1(w-5))	0.25 ± 0.03^{a}	0.31 ± 0.03^{b}	0.26 ± 0.00^{a}	0.30 ± 0.02^{b}	0.28 ± 0.03^{ab}	0.39 ± 0.00^{c}	0.28 ± 0.01^{ab}
Palmitic (C16:0)	35.36 ± 0.09^{c}	33.75 ± 0.43^{b}	35.54 ± 0.00^{c}	34.13 ± 0.34^{b}	35.33 ± 0.21^{c}	31.82 ± 0.08^{a}	33.84 ± 0.88^{b}
Palmitoleic (C16:1(w-7))	0.62 ± 0.02^{a}	0.67 ± 0.07^{a}	0.63 ± 0.01^{a}	0.68 ± 0.02^{a}	0.63 ± 0.07^{a}	0.99 ± 0.00^{b}	0.66 ± 0.15^{a}
Stearic (C18:0)	18.49± 0.10^{b}	15.49 ± 0.08^{a}	15.98 ± 0.63^{a}	16.02 ± 0.47^{a}	16.38 ± 0.37^{a}	16.41 ± 0.25^{a}	16.37 ± 1.11^{a}

Oleic (C18:1(w-9))	11.50 ± 0.20[bc]	10.89 ± 0.23[ab]	10.33 ± 0.34[a]	12.32 ± 0.35[d]	11.09 ± 0.52[b]	11.89 ± 0.15[cd]	11.52 ± 0.03[bc]
Linoleic (C18:2(w-6))	1.40 ± 0.06[cd]	1.16 ± 0.08[ab]	1.02 ± 0.07[a]	1.27 ± 0.12[bc]	1.15 ± 0.09[ab]	1.56 ± 0.06[d]	1.42 ± 0.12[cd]
Linoleinic (C18:3(w-3))	0.41 ± 0.05[bc]	0.33 ± 0.01[abc]	0.29 ± 0.00[a]	0.34 ± 0.03[abc]	0.31 ± 0.09[ab]	0.42 ± 0.03[c]	0.41 ± 0.08[bc]
Saturated Fatty Acid (SFA)	85.81 ± 0.02[bd]	86.64 ± 0.06[ab]	87.47 ± 0.42[a]	85.09 ± 0.49[de]	86.54 ± 0.66[bc]	84.75 ± 0.09[e]	85.72 ± 0.38[cd]
Monounsaturated Fatty Acid (MUFA)	12.37 ± 0.30[b]	11.87 ± 0.13[b]	11.22 ± 0.35[a]	13.30 ± 0.35[c]	12.00 ± 0.48[b]	13.27 ± 0.32[c]	12.46 ± 0.18[b]
Polyunsaturated Fatty Acid (PUFA)	1.82 ± 0.08[cd]	1.49 ± 0.07[ab]	1.31 ± 0.07[a]	1.61 ± 0.14[bc]	1.46 ± 0.18[ab]	1.98 ± 0.06[d]	1.82 ± 0.20[cd]
Atherogenic Index (AI)	6.36 ± 0.02[d]	7.22 ± 0.03[ab]	7.74 ± 0.24[a]	6.37 ± 0.31[d]	6.93 ± 0.45[bc]	6.09 ± 0.02[d]	6.60 ± 0.05[cd]
Thrombogenic Index (TI)	7.92 ± 0.01[bd]	8.20 ± 0.02[bc]	9.07 ± 0.29[a]	7.50 ± 0.29[cde]	8.40 ± 0.65[ab]	6.96 ± 0.01[e]	7.63 ± 0.53[be]

*Values are expressed as mean ± standard deviation. Analysis performed in triplicate. [a-e] Means with different lowercase superscripts in the same row indicate presence of statistical difference ($P < 0.05$) among treatments by Fisher Test. SFA = saturated fatty acid. MUFAs = medium-chain fatty acids; PUFA = polyunsaturated fatty acid. The FA composition was classified as reported by Batista et al. (2017) who described SCFA as C2 to C4. MCFA as C6 to C12 and LCFA as C14 to C24. See text for codes. AI= (C12:0 + 4 × C14:0 + C16:0) / [Σ MUFA + Σ PUFA (n−6) and (n−3)]. IT= (C14:0 + C16:0 + C18:0) / [0.5 × Σ MUFA + 0.5 × Σ PUFA (n−6) + 3 × Σ PUFA (n−3) + (n−3) / (n−6)].

High SFA levels were observed in all samples (84.75–87.47 g/100 g fat), with the higher levels in the OH samples at 100 Hz–25 V and lower levels at 60 V–60 Hz. Palmitic acid was the majority of SFA in the OH samples at 100 Hz–25 V (35.54 ± 0.00), followed by the conventional treatment (35.36 ± 0.09) and OH at 45 V–60 Hz (35.33 ± 0.2) ($p > 0.05$). Other long-chain SFAs identified in the present study were stearic acid (15.49–18.49 g/100 g fat) and myristic acid (12.87–14.63 g/100 g fat). SFAs, especially the long-chain fatty acids, directly affect the factors involved in cholesterol metabolism and are associated with increased adipose cells, triglycerides and insulin resistance, especially palmitic and stearic acids (Ma et al., 2015).

In relation to the monounsaturated acids (MUFA), high oleic acid levels were found in the samples, with the higher levels in the OH samples at 1000 Hz–25 V (12.32 ± 0.35) and lower levels at 100 Hz–25 V (10.33 ± 0.34), followed by 10 Hz–25 V (10.89 ± 0.23), with no statistical difference between the OH treatments at 100 and 10 Hz ($p > 0.05$). MUFAs have several health benefits, such as reducing the risk of cardiovascular disease and preventing obesity and may be a useful tool in eating disorders (Schwingshackl, Strasser, & Hoffmann, 2011). Oleic acid has beneficial effects on the immune system, the prevention of autoimmune and inflammatory diseases, and ability to facilitate wound healing (Sales-Campos, Reis de Souza, Crema Peghini, Santana da Silva, & Ribeiro Cardoso, 2013).

The long-chain polyunsaturated fatty acids (PUFAs) are divided into two categories according to the position of the double bond in the fatty acid molecule, omega-6 and omega-3. PUFAs are considered essential fatty acids because they are not synthesized by humans and have several properties, such as prevention of cardiovascular disease, cancer, rheumatoid arthritis, ulcerative colitis, and cholesterol reduction (Piepoli et al., 2016). In this study, PUFA levels varied from 1.31 to 1.98 g/100 g fat, with the higher retention observed for the OH at 60 V–60 Hz, and the higher degradation for OH at 100 Hz–25 V. For all samples, linoleic acid was more abundant than linolenic acid. Higher linoleic acid and linolenic acids levels were obtained in OH treatment at 60 V–60 Hz (1.56 ± 0.06, 0.42 ± 0.03), followed by the conventional pasteurization (1.40 ± 0.06; 0.41 ± 0.05) and OH 80 V - 60 Hz (1.42 ± 0.12; 0.41 ± 0.08), with no differences between them ($p > 0.05$).

Fatty acids composition and the related health lipid indices (IA, atherogenic and IT thrombogenic). These indices are linked to the different effects that fatty acid results in human health and in particular of increasing the formation of atheromas and thrombus formation (Garaffo et al., 2011). In relation to IA, the highest indexes, or worse conditions, were obtained in the OH samples at 10 Hz–25 V (7.22 ± 0.03) and 100 Hz–25 V (7.74 ± 0.24) ($p > 0.05$). Among the other treatments (conventional, 1000 Hz–25 V, 45, 60 and 80 V–60 Hz) there was no significant difference between them. On the other hand, in relation to IT, OH at 60, 80 V–60 Hz and 1000 Hz–25 V, respectively (6.96 ± 0.01, 7.63 ± 0.53, 7.50 ± 0.29, $p > 0.05$). OH at 60, 80 V–60 Hz resulted in lower indices showing a healthier lipid profile compared to the other treatments.

3.3 Volatile Compounds

Table 3 shows the organic acids identified in whey acerola-flavoured drink subjected to the OH and conventional pasteurization. Esters were the most identified class of compounds among the treatments (13 compounds), followed by alcohols (9 compounds) and carboxylic acids (6 compounds). The variation of the OH parameters (voltage and frequency) influenced the identification of the volatile compounds when compared to the conventional pasteurization.

Table 3. Volatiles compounds of whey acerola-flavoured drink submitted to Ohmic heating and conventional processing

Groups	Compounds	RI*	Conventional	10 Hz - 25 V	100 Hz - 25 V	1000 Hz - 25 V	45 V - 60 Hz	60 V - 60 Hz	80 V - 60 Hz
	Total of VOC's Identified		33	28	26	33	35	30	30
Esters	Pentanoic acid. ethyl ester	107	-	-	-	-	58230	-	37148
	Hexanoic acid. methyl ester	164	2743131	2032254	2891829	3644511	2815644	4269914	2433151
	Hexanoic acid. ethyl ester	215	9245030	7314570	9142862	11961106	9527583	10349757	8353434
	2-Propenoic acid. 2-methyl-. ethenyl ester	277	-	-	-	-	-	38069	-
	2(3H)-Furanone. dihydro-4-hydroxy-	575	537708	-	-	543368	924731	-	-
	3-Methyl-3-buten-1-ol. acetate	174	10176478	8813472	11015321	12880677	11015885	12063518	9301908
	Prenyl acetate	232	-	-	-	-	117772	116411	74892
	Hex-4-enoic acid. ethyl ester	285	50446	29451	43554	57753	49419	-	29156
	3-Hexen-1-ol. acetate. (Z)-	302	79094	-	-	117252	64179	89974	-
	Heptanoic acid. ethyl ester	319	61253	32402	53820	70633	40557	45055	35370
	Butanoic acid. 4-pentenyl ester	326	3272921	2943058	3235516	4219117	3591252	3654293	2978217
	2-Hexenoic acid. ethyl ester	333	-	-	-	49317	-	39741	-
	Hexanoic acid. 4-pentenyl ester	525	506409	497893	479666	627135	534476	-	455591
	Ethyl 3-furoate	610	69263	76210	69659	89663	74215	74236	61271
	Octanoic acid. ethyl ester	424	749541	694780	681766	881059	776405	827410	633157
	Total of Esters		**11**	**9**	**9**	**12**	**13**	**11**	**11**
Alc ohols	1-Butanol	129	-	-	22689	49206	-	-	-
	1-Hexanol	338	932205	1017195	976917	1157147	1063090	1046140	870036
	3-Hexen-1-ol. (E)-	348	117833	134344	91958	151197	144385	137944	119509

	3-Hexen-1-ol. (Z)-	370	2272647	2638373	2419715	2852305	2694655	2770922	2317711
	3-Octanol	379	155456	148130	148095	203846	118555	106694	117554
	2-Hexen-1-ol. (E)-	391	186920	239367	207227	262286	179971	145724	170925
	1-Octen-3-ol	435	377300	433342	-	516814	418364	447792	334260
	3-Buten-1-ol. 3-methyl-	235	926070	934002	816682	1031031	909148	898691	852446
	2-Furanmethanol	643	47689	-	-	107785	276315	89177	172424
	1-Hexanol. 2-ethyl-	475	-	20544	-	-	-	-	-
	Total of Alcohols		**8**	**8**	**7**	**9**	**8**	**8**	**8**
Carboxylic acids	Acetic acid	440	515782	486238	336274	468135	755012	989785	774827
	Butanoic acid	615	117151	168507	143682	160849	182075	168067	160114
	Octanoic acid	041	414958	449531	450491	546457	453860	445384	369137
	Decanoic acid	251	152059	128268	104366	195766	138153	154550	103273
	Benzoic acid	411	192291	144034	66696	32237	73813	38603	70010
	Hexanoic acid	829	529723	609157	535073	676089	635960	593942	474329
	Total of Carboxylic acids		**6**	**6**	**6**	**6**	**6**	**6**	**6**
Aldehydes	2-Hexenal. (E)-	204	-	151605	69490	144548	190401	-	-
	Furfural	453	71420	37203	-	137023	293803	163605	167753
	Benzaldehyde	517	112946	134556	114093	-	121341	152726	134331
	5-Hydroxymethylfurfural	475	351349	-	-	133004	457990	142200	122483
	Total of Aldehydes		**3**	**3**	**2**	**3**	**4**	**3**	**3**
etones	2-Butanone. 4-(acetyloxy)-	575	28415	-	24159	24642	-	-	-
	Acetoin	277	154811	162154	145953	165094	192324	-	172436

	2H-Pyran-2.6(3H)-dione	977	26642	-	-	-	30810	48316	-
	Total of Ketones		**3**	**1**	**2**	**2**	**2**	**1**	**1**
erpenes	Limonene	171	36950	36501	-	53863	104765	198408	39561
	Total of Terpenes		**1**	**1**	**0**	**1**	**1**	**1**	**1**
thers	Pyranone	246	42696	-	-	-	47948	-	-
	Total of Terpenes		**1**	**0**	**0**	**0**	**1**	**0**	**0**

*LRI – Linear Retention Index. VOCs- volatile organic compounds.

OH processing at 45 V–60 Hz presented the highest number of VOC's (35 VOC's), being 13 esters, 8 alcohols, 6 carboxylic acids, 4 aldehydes, 2 ketones, 1 terpene and a Pyranone, followed by OH at 1000 Hz–25 V and conventional treatment, both with 33 VOC's detected. Most of the compounds identified come from the acerola pulp used in the preparation of the drink, while a small fraction, mainly carboxylic acids and aldehydes, are related to milk. Vendramini and Trugo (2000) identified 31 compounds in acerola, while Boulanger and Crouzet (2001) identified 46 VOCs, and alcohols (3-methyl-but-3-en-1- ol, 3-methyl-butan-1-ol and 2-methyl-butan-1-ol) were the predominant compounds. Pino and Marbot (2001) identified 150 compounds, and the most abundant compounds were furfural (2.19 ppm), hexadecanoic acid (0.58 ppm), 3-methyl-3-butenol (0.72 ppm), and limonene (0.68 ppm), which were also identified in the present study.

Due to high protein and sugar contents of dairy products, thermal processing (pasteurization/UHT) can induce the Maillard reaction and produce Maillard reaction products (MRPs), such as furfural, 5-hydroxymethylfurfural, furanones, and pyranones (Cadwallader & Singh, 2009). The formation of MRPs may have positive effects, such as antioxidant activity, antibacterial activity, and ACE inhibitory activity (Arihara, Zhou, & Ohata, 2017), and recent studies have shown that MRPs have positive effects on the bioactive peptides. Indeed, MRPs may enhance the ACE inhibitory activity of casein hydrolysate under certain conditions.However, despite the health benefits, the formation of MRPs may adversely affect the sensory characteristics of dairy products, due to the formation of undesirable compounds responsible for off-flavors (Cadwallader & Singh, 2009). In addition, the Maillard reaction may produce toxic substances, such as advanced glycation end products (AGEs), which can participate in the development of various diseases such as diabetes, degenerative diseases, atherosclerosis and chronic kidney disease (Bastos, Monaro, Siguemoto, & Séfora, 2012). In addition to the MRPs, heating of dairy products can favor the auto-oxidation generating rancid off-flavors, including octanal and nonanal from the oxidation of oleic acid; hexanal and 2-octenal from linoleic acid, and propanal and 3-hexenal from the degradation of linolenic acid (Cadwallader & Singh, 2009). However, no auto-oxidation was observed in the present study.

OH presents great potential in controlling the MRPs due to lower thermal load during processing (Jaeger, Janositz, & Knorr, 2010). However, besides the thermal effect of OH, the present work observed the presence of an additional non-thermal effect capable of affecting the formation of these compounds. Were identified 6 probable Mallard reaction products (MRPs), including furfural (conventional treatment; OH – 10 Hz–25 V; 1000 Hz–25 V; 45 V–60 Hz; 60 V–60 Hz; 80 V–60 Hz), 2-furanmethanol (conventional; OH – 1000 Hz–25 V; 45 V–60 Hz; 60 V–60 Hz; 80 V–60 Hz), 5-hydroxymethylfurfural (conventional; OH – 1000 Hz–25 V; 45 V–60 Hz; 60 V–60 Hz; 80 V–60 Hz), pyranone (conventional; OH – 45 V–60 Hz), 2(3H)-furanone dihydro-4-hydroxy (conventional; OH – 1000 Hz–25 V; 45 V–60 Hz), 2H-pyran-2.6(3H)- dione (conventional; OH – 45 V–60 Hz; 60 V–60 Hz). According to these results, the treatments at low frequency (10 Hz) provided lower MRP levels, suggesting that OH performed at low frequency resulted in a product of superior quality, concerning the nutritional aspect.

3.4 Differential Scanning Calorimetry (DSC)

The enthalpies (ΔH°) and the peak crystallization and melting temperatures (T°C) are presented in **Table 4**. The DSC thermograms showed an exothermic peak, corresponding to the crystallization process and an exothermic peak corresponding to the melting process. The results showed different behavior as a function of the different OH conditions. For crystallization, all results differed from each other ($p < 0.05$), except for the samples processed at 100 and 1000 Hz. Only the OH at 10 Hz–25 V (ΔH°=−13.6 ± 0.58) presented lower enthalpy than the conventional treatment (ΔH°=−154.7 ± 0.42). Peak crystallization temperatures ranged from −7.3 °C (60 V–60 Hz) to −19.8 °C (conventional). Concerning the melting behavior, different results were observed for OH at 10 Hz–25 V when compared to the conventional treatment (ΔH°=173.7 ± 0.37 against 172.5 ± 0.63, p > 0.05) while the melting enthalpy values ranged from ΔH°=82.3 ± 0.99 (100 Hz–25 V) to ΔH°=218.5 ± 0.68 (45 V–60 Hz).

Table 4. Differential Scanning parameters (Enthalpies and peak temperatures, ΔH°, T°C, respectively) of whey acerola-flavoured drink submitted to Ohmic heating and conventional processing

	Crystallization		Fusion	
Samples	T °C	ΔH°	T °C	ΔH°
Conventional	-19.8 ± 0.04	-154.7 ± 0.42^{e}	4.9 ± 0.01	172.5 ± 0.63^{b}
10 Hz - 25 V	-17.2 ± 0.13	-13.6 ± 0.58^{f}	5.3 ± 0.01	173.7 ± 0.37^{b}
100 Hz - 25 V	-14.6 ± 0.35	-163.4 ± 0.78^{d}	5.6 ± 0.01	82.3 ± 0.99^{f}
1000 Hz - 25 V	-16.5 ± 0.03	-163.3 ± 0.41^{d}	5.6 ± 0.01	84.3 ± 0.48^{e}
45 V - 60 Hz	-18.6 ± 0.27	-170.5 ± 0.75^{c}	4.4 ± 0.01	218.5 ± 0.68^{a}
60 V - 60 Hz	-7.3 ± 0.21	-178.7 ± 0.99^{b}	4.6 ± 0.01	97.5 ± 0.67^{d}
80 V - 60 Hz	-16.2 ± 0.01	-195.7 ± 0.41^{a}	---	102.8 ± 1.22^{c}

* Data are expressed as mean ± standard deviation. Analysis performed in triplicate. $^{a\text{-}c}$ Different letters at the same column indicates significant differences between samples ($p < 0.05$) by Fisher test.

The crystallization and melting behavior of milk can help in understanding the composition, physical properties, and functionality of milk fat, since they are influenced by the intensity of the heat treatment applied (Herrera & Hartel, 2000). DSC can be an interesting approach for evaluating the intensity and the impact of the emerging technologies, such as OH, on fouling behavior and biochemical modifications of milk proteins

3.5 Time Domain Nuclear Magnetic Resonance (TDN-MR)

As shown in **Fig. 3**, two different relaxation environments (domains) were observed for all samples. The region of 0.1–100 ms (T2.1) comprises the protons of water molecules in highly restricted or confined environments, and sugar molecules or other structures may be associated by hydrogen bonding, such as proteins. The majority and greater relaxation time domain (T2.2) of 100 to 700 ms correspond to the free water hydrogen molecules.

NMR has some advantages over the traditional methods (viscosity, sedimentation, syneresis) because it is a non-destructive method. Thus, TD-NMR can help in understanding the effect of OH on the product's stability concerning the parameters texture and syneresis. Regardless of the operating conditions, OH led to a reduction of the relaxation time when compared to the conventional treatment. The sample subjected to OH treatment at 100 Hz showed the lowest value for T2.2, suggesting to be slightly more viscous than the other samples. Thus, OH can assist in the elaboration of more viscous dairy drinks and solve problems with the texture of the products. However, no statistical tests were applied to determine the existence of significant between samples. So, further studies are required for a better understanding and confirmation of these results.

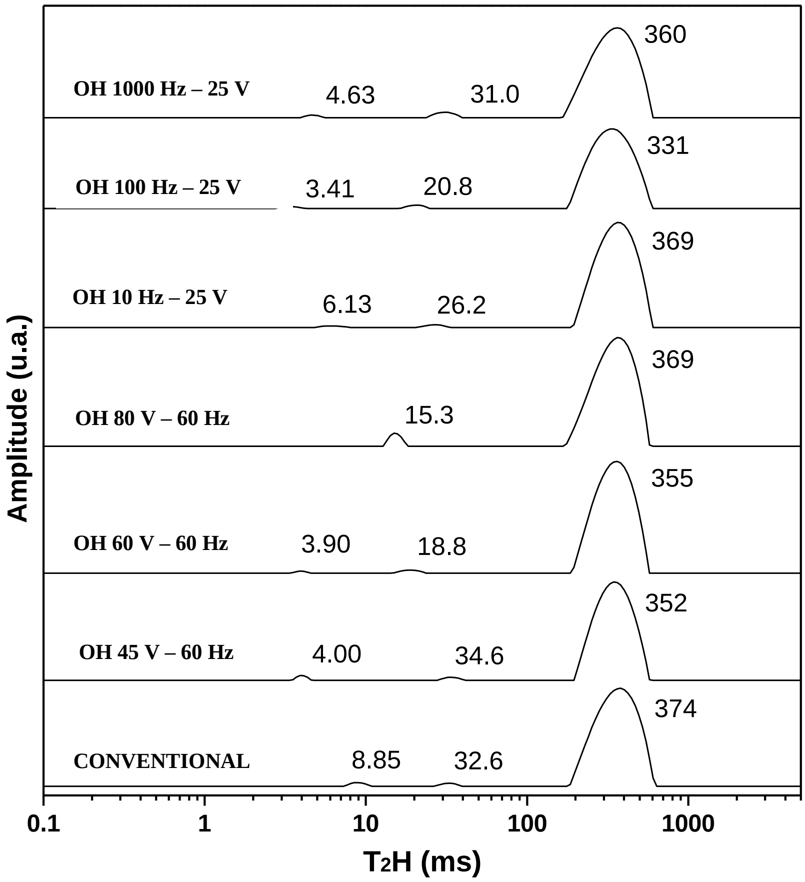

Fig. 3. Distribution curves of transverse relaxation domains of TD-NMR of Whey acerola-flavoured drink submitted to conventional processing and Ohmic Heating.

4 CONCLUSION

Whey acerola-flavoured drink submitted to OH presented effects on the generation of bioactive compounds (total phenolics, DPPH, FRAP, ACE levels) as well as thermal parameters and the fatty acid profile and volatiles profiling, which depended on the operational parameters used. OH promote small reduction of the relaxation time, when compared to the conventional treatment, regardless of the operating conditions while proportionate the lower formation of MRP's and the small modifications in the fatty acid profile, maintaining the nutritional characteristics of the processed product. Electric fields effects applied promoted small modifications on nutritional aspects while that frequency influence more intensive the quality product. High frequencies (1000 Hz and 100 Hz) resulted in better results of bioactive compounds and antioxidant capacity presenting similar fatty acid profile compared conventional treatment, while low frequency (10 Hz) provided lower MRP levels. Overall, OH has proven to be a promising technology for the whey beverages processing, in particular whey acerola-flavoured drink.

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CONCLUSÃO

O AO resultou em efeitos positivos para o processamento da bebida láctea de acerola, promovendo melhorias nos parâmetros de qualidade e nas características reológicas, físicas e químicas do produto processado. Os parâmetros de processo (frequência e tensão) podem desempenhar um papel importante na qualidade da bebida. Os efeitos das tensões aplicadas promoveram pequenas modificações nos aspectos nutricionais, contudo baixas tensões (< 60 V) favoreceu melhores resultados na qualidade da bebida da bebida, como degradação da cor e do ácido ascórbico. Diferentemente da tensão, a variação da frequência influenciou intensamente as propriedades da bebida. Altas frequências (1000 Hz e 100 Hz) resultaram em melhores resultados nos compostos bioativos, menores degradações na capacidade antioxidante, sem promover alterações no perfil lipídico similar da bebida. Em contrapartida, resultou em maiores degradações do ácido ascórbico. Em Baixas frequências (< 100 Hz) menores níveis de PRM foram detectados, maior preservação do ácido ascórbico e menor alteração das propriedades de cor comparado ao processo convencional.

Assim, o AO provou ser uma tecnologia promissora para o processamento de bebidas de soro de leite, promovendo resultados relevantes para a indústria de um ponto de vista tecnológico, principalmente com o aumento no consumo de bebidas à base do soro e a crescente busca de novas tecnologias que auxiliem na preservação das características nutricionais de produtos processados. Contudo, mais estudos devem ser realizados buscando avaliar os efeitos dos melhores parâmetros de processos, visando otimização do processo. Adicionalmente, vários aspectos precisam ser investigados a fim de tornar esta tecnologia viável para o processamento de produtos lácteos, como: avaliação da existência do fenômeno da eletroporação e seus efeitos na cinética de inativação microbiana, o impacto desta tecnologia no potencial alergênico das proteínas e a avaliação dos efeitos do AO nas características sensoriais do produto e na formação do Fouling.