

**UFRRJ**  
**INSTITUTO DE TECNOLOGIA**  
**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E**  
**TECNOLOGIA DE ALIMENTOS**

**DISSERTAÇÃO**

**EFEITO DO AQUECIMENTO ÔHMICO NAS CARACTERÍSTICAS**  
**FÍSICO-QUÍMICAS, MICROBIOLÓGICAS E SENSORIAIS DO SORO**  
**DE LEITE**

**NAIARA ROCHA DA COSTA**

**2017**



**UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO  
INSTITUTO DE TECNOLOGIA  
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA  
DE ALIMENTOS**

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DE LEITE**

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Dissertação de mestrado submetido como requisito parcial para o grau de **Mestre em Ciências**, no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, na área de concentração Tecnologia de Alimentos.

Seropédica, RJ  
Novembro de 2017

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Universidade Federal Rural do Rio de Janeiro  
Biblioteca Central / Seção de Processamento Técnico

Ficha catalográfica elaborada  
com os dados fornecidos pelo(a) autor(a)

C837e Costa, Naiara Rocha da, 1989-  
EFEITO DO AQUECIMENTO ÔHMICO NAS CARACTERÍSTICAS  
FÍSICO-QUÍMICAS, MICROBIOLÓGICAS E SENSORIAIS DO SORO  
/ Naiara Rocha da Costa. - 2017.  
53 f.

Orientador: Adriano Gomes da Cruz.  
Tese (Doutorado). -- Universidade Federal Rural do Rio  
de Janeiro, Pós-Graduação em Ciência e Tecnologia de  
Alimentos, 2017.

1. aquecimento ohmico. 2. soro de leite. 3.  
processamento. I. Gomes da Cruz, Adriano, 1978-  
orient. II Universidade Federal Rural do Rio de  
Janeiro. Pós-Graduação em Ciência e Tecnologia de  
Alimentos III. Título.

**NAIARA ROCHA DA COSTA**

Dissertação submetida como requisito parcial para obtenção do grau de **Mestre em Ciências**, no Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, na área de concentração Tecnologia de Alimentos.

DISSERTAÇÃO APROVADA EM 12/12/2017

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## AGRADECIMENTOS

Primeiramente a **Deus**, pelas bênçãos em toda minha trajetória;

Ao **Prof. Dr. Adriano Gomes da Cruz** pela oportunidade de desenvolver este trabalho, que quando tudo parecia perdido, soube me fazer o que um verdadeiro orientador faria, me “orientar” sem julgamentos.

Ao **PPGCTA, UFRRJ e todos os Professores, Técnicos e demais profissionais** pela oportunidade do mestrado e todas as oportunidades advindas do curso;

Ao **IFRJ** pelo espaço cedido para o desenvolvimento deste trabalho e por toda sua equipe que auxiliaram no desenvolvimento do mesmo.

A toda equipe maravilhosa que nos proporcionou um excelente trabalho sobre aquecimento ôhmico, **Leandro, Marcus, Roberto, Jeremias, Erick, Ramon, Roberto, Neto, Maria Inês, Mônica, Raimundo, Flávio, Rodrigo Bisaggio, Rodrigo Cavalcanti, Renata e Marcia,**

A **CAPES** pela bolsa de estudos concedida a qual possibilitou meus estudos;

Especialmente ao meu grande amigo **Leandro Cappato** que foi mais que um amigo, foi um mestre. Às amigas **Natalia Lengruher e Alyne Alves** pelo abrigo, café quentinho e cama macia para as poucas horas de sono.

## RESUMO

**DACOSTA, Naiara Rocha. Efeito do Aquecimento Ôhmico nas Características Físico-Químicas, Microbiológicas e Sensoriais do Soro de Leite.** 53 p. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ. 2017.

O aquecimento ôhmico (OH) consiste na passagem de corrente elétrica no alimento, promovendo seu aquecimento devido à conversão de energia elétrica em térmica. A quantidade de calor gerado é proporcional a corrente elétrica induzida. Em virtude da rápida taxa de aquecimento, o processo ôhmico apresenta algumas vantagens em relação aos processos convencionais (pasteurização e esterilização), como: a maior manutenção de compostos nutricionais e a redução do “fouling”, fatores importantes no processamento de produtos lácteos. Em adição, outra vantagem do OH se dá pela presença de um efeito adicional não térmico nas células microbianas ocasionando uma eletroporação (formação de poros na membrana celular), promovendo assim, redução na resistência térmica dos microrganismos. Este importante fenômeno permite a redução da intensidade térmica do processo, garantindo a mesma letalidade microbiológica e, conseqüentemente, menor degradação nutricional. Em virtude da presença de diversos compostos termossensíveis e a formação de produtos indesejáveis ocasionado pela ação do calor, o processamento ôhmico apresenta como uma tecnologia promissora na produção de alimentos, principalmente em produtos lácteos. Assim, o trabalho teve como objetivo avaliar o efeito do processamento ôhmico nas características físico-químicas, microbiológicas e sensoriais do produto, a fim de caracterizar e comparar o soro de leite tratado pelo processamento ôhmico e convencional. No Capítulo I deste trabalho foi realizada uma revisão de literatura visando avaliar os aspectos sensoriais e tecnológicos do uso do aquecimento ôhmico produtos lácteos. No Capítulo II foi realizado o estudo das propriedades físico-químicas, reológicas e sensoriais de soro de leite pasteurizado através do aquecimento ôhmico. Pode-se concluir que os efeitos do OH no soro de leite são dependentes da intensidade da tensão aplicada no tratamento. Baixas tensões proporcionaram maior variação de cor, enquanto que proporcionou melhor preservação dos compostos bioativos de capacidade antioxidante e anti-hipertensiva. Suas características físicas e sensoriais, entretanto, permaneceram similares ao soro de leite pasteurizado pelo método convencional. Estes resultados sugere que a tecnologia do aquecimento ôhmico é uma alternativa interessante para o processamento de soro de leite.

**Palavras-chaves:** aquecimento ôhmico, produtos lácteos, soro de leite

## ABSTRACT

**DA COSTA, Naiara Rocha. Study of alterations caused by conventional and conventional heating in reconstituted bovine serum.** 2017. 53p. Dissertation (Master in Food Science and Technology) Institute of Technology, Department of Food Technology, Federal Rural University of Rio de Janeiro, Seropédica, RJ, 2017.

The ohmic heating (OH) consists of the passage of electric current in the food, promoting its heating due to the conversion of electric energy into thermal. The amount of heat generated is proportional to the induced electric current. Due to the rapid heating rate, the ohmic process presents some advantages over conventional processes (pasteurization and sterilization), such as: the maintenance of nutritional compounds and the reduction of fouling, important factors in the processing of dairy products. In addition, another advantage of OH is the presence of an additional non-thermal effect on the microbial cells causing electroporation (pore formation in the cell membrane), thus promoting a reduction in the thermal resistance of the microorganisms. This important phenomenon allows the reduction of the thermal intensity of the process, guaranteeing the same microbiological lethality and, consequently, lower nutritional degradation. Due to the presence of several thermosensitive compounds and the formation of undesirable products caused by the action of heat, the ohmic processing presents as a promising technology in the production of foods, especially in dairy products. Thus, the objective of this work was to evaluate the effect of the ohmic processing on the physical-chemical, microbiological and sensorial characteristics of the product, in order to characterize and compare the whey treated by conventional and ohmic processing. In Chapter I of this work a literature review was carried out aiming at evaluating the sensorial and technological aspects of the use of the thermal heating of dairy products. In Chapter II the physical-chemical, rheological and sensorial properties of pasteurized whey were analyzed by means of ohmic heating. It can be concluded that the effects of OH on whey are dependent on the intensity of the voltage applied in the treatment. Low electrical fields provided greater color variation, while providing better preservation of the bioactive compounds of antioxidant and antihypertensive capacity. Its physical and sensorial characteristics, however, remained similar to whey pasteurized by the conventional method. These results suggest that the ohmic heating technology is an interesting alternative for the processing of whey.

**Keywords:** ohmic heating, dairy products, sweet whey

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## INTRODUÇÃO GERAL

As características sensoriais e nutricionais dos alimentos podem sofrer modificações drásticas durante o processamento térmico, sendo algumas modificações desejáveis e outras não. Para atingir o grau de modificação ideal, a tecnologia aplicada deve levar em consideração as características do alimento *in natura*, os níveis de desejo do consumidor, as condições de operação e demanda energética.

O soro de leite gerado pela produção de queijos e precipitação da caseína pelas indústrias é considerado como uma fonte de diversos produtos com valor agregado devido a suas características. Suas proteínas apresentam importantes propriedades funcionais quando hidrolisadas, como capacidade antioxidante, anti-hipertensivas e antibióticas (BRANDELLI *et al.*, 2015). Devido a suas características nutricionais, a demanda por produtos a base de soro de leite cresce juntamente com o desenvolvimento de tecnologias de processamento. Além das propriedades nutricionais, as proteínas do soro de leite são muito conhecidas pela versatilidade tecnológica como ingredientes em produtos alimentícios, principalmente pela sua elevada capacidade de gelificação (CAPITANI *et al.*, 2005).

O tratamento térmico do soro de leite pode ocasionar alterações negativas, por ser um produto sensível ao calor. Alterações como desaminação, desfosforilação, destruição parcial de aminoácidos e a reação de Maillard, modificando a características sensoriais (KORHONEN *et al.*, 1998; DA COSTA, 2004). Em alternativa ao tratamento térmico convencional, o aquecimento ôhmico (OH) tem se mostrado eficiente em sua utilização para pasteurização e esterilização comercial de soro de leite, causando menor desnaturação proteica (30 % menor, a temperaturas de 75 a 90°C) e resultando num produto de maior qualidade final (PEREIRA *et al.*, 2011).

O princípio do OH é a conversão de energia elétrica em energia térmica, resultando no aquecimento do alimento devido ao efeito joule (BUTZ e TAUSCHER, 2002; LEIZERSON e SHIMONI, 2005b). A quantidade de calor gerado é relacionada com a intensidade da corrente elétrica induzida, com a potência aplicada e a condutividade elétrica do alimento (JAEGER *et al.*, 2016). Portanto, o OH quando otimizado ao alimento de interesse pode oferecer menor tempo de processamento

minimizando impactos negativos como perda de nutrientes e características sensoriais (CAPPATO *et al.*, 2017).

Neste contexto, o conhecimento dos fatores que influenciam o processamento e os efeitos sobre os alimentos são importantes para o desenvolvimento dos parâmetros relativos ao processo. O presente trabalho teve como objetivo avaliar os efeitos do processamento através de aquecimento ôhmico em relação aos aspectos físico-químicos, microscópicos, reológicas e sensoriais do produto. Investigando a possibilidade de aplicação da tecnologia emergente como alternativa ao processo convencional de pasteurização de soro de leite ou derivados.

## **Objetivo Geral**

Avaliar o efeito do processamento ôhmico sobre as propriedades físico-químicas, microbiológicas, reológicas e sensoriais do soro de leite.

## **Objetivos Específicos**

- ✓ Determinação do efeito do OH na degradação da cor, perfil de voláteis, reologia e microbiologia;
- ✓ Avaliação do efeito da aplicação do OH nos compostos bioativos de capacidade antioxidante e anti-hipertensiva.
- ✓ Avaliação das características sensoriais do soro pasteurizado ôhmicamente em comparação ao pasteurizado convencionalmente;
- ✓ Determinação dos melhores parâmetros de OH (voltagem) para aplicação do OH para o soro de leite reconstituído;

## CAPÍTULO I

### REVISÃO BIBLIOGRÁFICA

#### 1. SORO DE LEITE

O soro de leite é um produto secundário da indústria láctea, produzido a partir da coagulação do leite destinado a fabricação de queijos ou de caseína (BRASIL, 2005). Para a produção de 1 kg de queijo, são gerados cerca de 9 litros de soro de leite, que, caso seja destinado de forma incorreta, como quando lançado *in natura* no solo e nos corpos hídricos, podendo ocasionar um grave impacto ambiental tendo em vista que este efluente é considerado cem vezes mais poluentes que os esgotos domésticos (DE PAULA *et al.*, 2012).

Este produto possui um alto valor nutricional, conferido pelas proteínas com elevado teor de aminoácidos essenciais. Além das propriedades nutricionais, as proteínas do soro de leite são muito conhecidas pela versatilidade de suas propriedades funcionais tecnológicas como ingredientes em produtos alimentícios, principalmente pela sua elevada capacidade de gelificação (CAPITANI *et al.*, 2005). Geralmente as indústrias utilizam o soro como ingrediente para elaboração de bebidas lácteas, suplementos e aprimoramento dos próprios produtos (IMAMURA *et al.*, 2012). . As propriedades funcionais das proteínas do soro mais relevantes na aplicabilidade em produtos alimentícios são a solubilidade em água, faixa de pH de trabalho, formação de espuma, capacidade emulsificante e de modulação de textura e gelificação (MULVIHILL e ENNIS, 2003; BANSAL e BHANDARI, 2016).

A composição do soro é de aproximadamente 93 % de água, 5 % de lactose, 0,9% de proteínas, 0,3 % de gordura, 0,2 % de ácido láctico e pequenas quantidades de vitaminas (BEN-HASSAN E GHALY, 1994). O soro contém 20 % das proteínas do leite. As duas principais proteínas são as  $\beta$ -lactoglobulina e a  $\alpha$ -lactoalbumina, que estão entre 70 a 80 % das proteínas do soro.

Além de apresentarem excelentes propriedades funcionais, as proteínas do soro de leite quando hidrolisadas, apresentam características antioxidantes, anti-

hipertensivas, e antibióticas (BRANDELLI *et al.*, 2015). Devido a estas características nutricionais, a demanda por produtos a base de soro de leite cresce juntamente com o desenvolvimento de tecnologias que agreguem valor. A aplicabilidade das proteínas do soro não se limita apenas a produtos lácteos, mas também em suplementos, produtos cárneos, alimentos congelados e fórmulas infantis (YADAV *et al.*, 2015).

Entretanto, o tratamento térmico do soro de leite pode alterar a conformação e estabilidade das proteínas causando sua desnaturação. A mudança na conformação das proteínas faz com que os resíduos de aminoácidos que estavam ocultos no interior da molécula sejam expostos, e com isso tornam-se susceptíveis a ação de proteases facilitando sua digestão. A intensidade do tratamento térmico pode também promover alterações negativas, como desaminação, desfosforilação, destruição parcial de aminoácidos e a reação de Maillard, alterando também as características sensoriais (KORHONEN *et al.*, 1998; DA COSTA, 2004). Em alternativa ao tratamento convencional, o aquecimento ôhmico (OH) tem se mostrado eficiente em sua utilização para pasteurização e esterilização comercial de soro de leite, causando menor desnaturação proteica (30 % menor, a temperaturas de 75 a 90 °C) e resultando num produto de maior qualidade (PEREIRA *et al.*, 2011).

## 2. AQUECIMENTO ÔHMICO (OH)

O tratamento térmico aplicado nas indústrias de alimentos visa eliminar os microrganismos, inativar enzimas e promover o cozimento dos produtos. Porém, são utilizadas altas temperaturas que podem degradar nutrientes importantes como vitaminas, aminoácidos e proteínas, além de alterações indesejáveis no sabor, aroma, textura e aparência dos alimentos. Baseando-se no fato que a inativação microbiana é mais sensível ao calor que os nutrientes, os métodos clássicos de tratamento utilizam altas temperaturas e curtos períodos de tempo. São eles o método LTLT (“Low Time Low Temperature”), HTST (“High Temperature Short Time”) e UHT (“Ultra High Temperature”) (RAMASWAMY e CHEN, 2002)

A transferência de calor nestes métodos ocorre essencialmente pela condução, convecção e radiação. E a desvantagem é a não uniformidade de aquecimento, pois o produto em contato com a superfície de aquecimento é aquecido em excesso até que o calor se transfira para parte mais fria do alimento, levando a perdas de qualidade

(RAMASWAMY e CHEN, 2002). Em contraste com os métodos convencionais de aquecimento, que podem causar danos à qualidade do alimento devido à baixa taxa de condução e convecção de calor, o aquecimento ôhmico aquece uniformemente o alimento a altas temperaturas em um tempo reduzido (BAYSAL e ICIER, 2010)

Seu princípio de aquecimento se dá pela conversão de energia elétrica em energia térmica, resultando no aquecimento do alimento devido ao efeito joule (BUTZ e TAUSCHER, 2002; LEIZERSON e SHIMONI, 2005b). A quantidade de calor gerado é relacionada com a intensidade da corrente elétrica induzida, com a potência aplicada e a condutividade elétrica do alimento (JAEGER et al., 2016). Portanto, o OH pode ser considerado uma tecnologia de geração de energia térmica interna, e não apenas de transferência de energia.

O alimento é constituído de componentes iônicos (ácidos e sais) que permitem que a corrente elétrica flua através dele, onde o aquecimento se dá, pela resistência elétrica do alimento sem envolver nenhum meio de aquecimento ou superfície de troca de calor. O princípio básico que governa a geração de calor no OH, descrita por (PALANIAPPAN e SASTRY, 1991) é dado pela Equação 1:

$$Q = I^2 \times \frac{L}{A \times \omega} \quad \text{Equação 1}$$

em que  $Q$  é a taxa de calor ou energia gerado [W],  $I$  é a intensidade da corrente elétrica através da amostra [A],  $L$  é o espaço entre os eletrodos ou o comprimento da amostra [m],  $A$  é a área dos eletrodos ou da seção transversal da amostra [m<sup>2</sup>] e  $\omega$ , a condutividade elétrica do produto a ser aquecido [S.m<sup>-1</sup>].

Aproximadamente 100 % da energia elétrica será convertidos em energia térmica, logo a quantidade de calor transferida ao alimento pode ser regulada em função dos parâmetros de voltagem e corrente elétrica aplicada ao equipamento, tornando-o fácil de manusear (ROUX *et al.*, 2016). Dependendo da condutividade do produto e das configurações da câmara de aquecimento, o tempo para aquecimento uniforme pode ser substancialmente mais baixo que o tempo para atingir de mesma temperatura utilizando-se o aquecimento convencional (LEIZERSON e SHIMONI, 2005b; BAYSAL e ICIER, 2010; JAEGER *et al.*, 2016).



Cappato *et al.* (2017) acrescentam que a condutividade do alimento é o parâmetro chave no OH, porém, ela depende da temperatura do material e de seu formato, não podendo ser considerado como uma constante. Entretanto, Castro *et al.* (2004) sugerem que a formação de bolhas de ar no alimento é favorecida a altas temperaturas diminuindo a sua condutividade. Porém é possível ajustar o equipamento de OH, evitando impactos negativos sobre o consumo energético e perdas nutricionais.

### 3. AQUECIMENTO ÔHMICO E INATIVAÇÃO MICROBIANA

A inativação microbiana se dá através do aquecimento, porém pesquisas sugerem que o OH operado a baixas frequências (50 a 60 Hz) permite o acúmulo de cargas elétricas formando poros nas paredes celulares causando sua destruição (eletroporação) (RICHARDSON, 2001). Porém, como qualquer outro tratamento térmico, a sua eficácia na inativação dos microrganismos depende do binômio tempo x temperatura aplicado no ponto mais frio do alimento (FDA, 2015).

A eletroporação é relacionada ao transporte de cargas resultantes das diferenças entre o potencial interno e externo das células submetidas a campos elétricos, formando assim, poros entre as camadas lipídicas e proteicas na membrana celular (CASTRO *et al.*, 1993; SITZMANN, 1995; PARK e KANG, 2013). A força do campo elétrico do OH aplicada isoladamente é ineficaz para promover efeito letal não térmico na estrutura e permeabilidade na membrana celular dos microrganismos, porém torna-se um fator letal quando associado ao aquecimento (PALANIAPPAN *et al.*, 1992). Os efeitos da eletroporação combinados com o aquecimento dão ao OH vantagem frente ao aquecimento convencional, oferecendo redução da resistência térmica de microrganismos importantes, reduzindo assim a carga térmica do processo (SUN *et al.*, 2008; SUN *et al.*, 2011; PARK e KANG, 2013).

Comparando o aquecimento ôhmico ao convencional, sob mesmo perfil tempo x temperatura, Sun *et al.* (2008) constataram que o tempo de redução decimal microbiana (valor D) para o *Streptococcus thermophilus* 2646 foi significativamente menor com o OH, sugerindo a existência de efeitos não térmicos que diminuiriam a resistência térmica do microrganismo. Posteriormente, para avaliar o mecanismo da eletroporação, Sun *et al.* (2011) avaliaram a quantidade de ATP (trifosfato adenosina) e LDH (lactase desidrogenase) exsudados das células de *S. thermophilus* após aquecimento subletal

com água quente e OH subletal (20kHz; 7 A; 70 a 45 V). Concluíram, a partir das concentrações de ATP e LDH encontradas no exsudado celular, que a permeabilidade das células foi maior durante o OH subletal do que a permeabilidade durante o aquecimento com água quente de maneira subletal. Sugerindo que a corrente elétrica aplicada durante o OH causa lesões não-térmicas as membranas celulares diminuindo significativamente a resistência térmica dos microrganismos.

#### **4. AQUECIMENTO ÔHMICO EM LEITE E DERIVADOS**

Tratamentos térmicos severos podem provocar efeitos negativos nas propriedades sensoriais, físico-químicas e nutricionais de produtos lácteos, como a desnaturação proteica, isomerização da lactose e a reação de Maillard. (FINOT *et al.*, 1981; FENAILLE *et al.*, 2006; ROUX *et al.*, 2016). O aquecimento uniforme a altas temperaturas sob tempos reduzidos, constitui uma vantagem do aquecimento ôhmico, diminuindo as perdas nutricionais e de qualidade dos alimentos. Na Tabela 1, encontram-se as últimas pesquisas relacionadas ao tratamento térmico de produtos lácteos, seus objetivos e as conclusões principais.

A degradação de compostos bioativos, reações de escurecimento enzimático e caramelização podem causar alterações significativas na cor do produto. Logo, a avaliação da cor pode indicar a degradação de compostos sensíveis a tratamentos térmicos elevados (BHARATE and BHARATE, 2014; FUSTIER *et al.*, 2011; SANT'ANNA *et al.*, 2013). Para avaliar a degradação de ácido ascórbico em bebida a base de soro de leite pelo aquecimento ôhmico, Cappato *et al.* (2018) analisaram a alteração de cor da bebida e observaram que o aumento da voltagem aplicada promoviam maior alteração nos parâmetros de cor da bebida.

Os parâmetros reológicos de bebidas lácteas também é um indicador de qualidade, visto que a viscosidade está relacionada a aceitabilidade dos produtos líquidos (PENNA *et al.*, 2001). Icier (2009) avaliou o efeito do aquecimento ôhmico em diferentes gradientes de tensão e não observou diferença significativa na reologia de soro de leite reconstruído tratado pelo aquecimento ôhmico e o convencional. Entretanto, Cappato *et al.* (2018), observou que o aumento da viscosidade da bebida a base de soro de leite pode ser relacionada ao aumento da intensidade da voltagem e frequência aplicadas ao OH.

As proteínas do soro de leite têm propriedades de formação de gel, que podem ser alteradas no tratamento térmico através de modificações estruturais. O tratamento por OH destas proteínas foi estudado por Pereira et al. (2016), que evidenciaram a presença de estruturas proteicas mais alongadas quando comparado ao aquecimento convencional. Posteriormente, avaliando a influencia da intensidade do campo elétrico, Pereira et al. (2017) observaram que o diâmetro de partícula de hidrogéis a base de proteínas de soro de leite tornam-se maiores a medida que a intensidade do campo elétrico aplicado aumenta.

Existem poucos estudos que relatam os efeitos do processamento ôhmico na microestrutura de produtos a base de soro de leite, tornando-se necessários mais estudos que relatem estes efeitos sobre as propriedades reológicas e sensoriais. A Tabela 1 sintetiza os principais estudos sobre os efeitos do aquecimento ôhmico em produtos a base de soro de leite.

**Tabela 1** Principais trabalhos utilizando o aquecimento ôhmico em produtos lácteos.

Objetivo	Pesquisa	Conclusão	Referencia
Estudar a aplicabilidade do aquecimento ôhmico no processamento do soro de leite, através do comportamento reológico a diferentes concentrações aplicando diferentes voltagens.	“Influence of ohmic heating on rheological and electrical properties of reconstituted whey solutions”	A temperatura e concentração do soro de leite são fatores determinantes a eletrocondutividade durante o aquecimento. As diferentes voltagens aplicadas não apresentaram diferenças significativas na reologia do soro.	(ICIER, 2009)
Comparar a desempenho do aquecimento ôhmico e da esterilização UHT por injeção de vapor quanto a degradação térmica das proteínas e da vitamina c e a produção de produtos da reação de Maillard.	“Comparative thermal impact of two UHT technologies, continuous ohmic heating and direct steam injection, on the nutritional properties of liquid infant formula”	Sob condições de aquecimento semelhantes ao tratamento UHT, o aquecimento ôhmico se mostrou eficiente na preservação dos componentes nutricionais estudados. Porém apresentou quantidades significativas quanto aos produtos da reação de Maillard, como furosina e CML, porém apenas nas condições de tempo/temperatura que não condizentes com o tratamento industrial (140°C).	(ROUX et al., 2016)
Avaliar o efeito das condições ôhmicas aplicadas (10, 100, 1000 Hz à 25 V e 45, 60, 80 V à 60 Hz) nessas propriedades durante a pasteurização ôhmica da bebida láctea de acerola, em comparação a pasteurização convencional.	Whey acerola-flavoured drink submitted ohmic heating processing: Is there an optimal combination of the operational parameters?	O comportamento reológico, degradação do ácido ascórbico e da coloração da bebida láctea de acerola foi maior em relação ao aumento da voltagem e da frequência aplicada em comparação ao processamento convencional.	(CAPPATO et al., 2018)
Caracterizar as etapas de desnaturação e agregação proteica provocadas pelo OH em proteína do soro de leite isolada.	Production of whey protein-based aggregates under ohmic heating	A proteína do soro de leite apresentou maior solubilidade e menor formação de agregados devido ao aquecimento mais rápido.	(PEREIRA, 2016)

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## CAPÍTULO II

### **OHMIC HEATING: A POTENTIAL TECHNOLOGY FOR SWEET WHEY PROCESSING**

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**Manuscrito publicado na Revista International Food Research International (Qualis A1- Ciência de Alimentos).**



## ABSTRACT

The use of Ohmic Heating (OH) for sweet whey processing was investigated in this study. Whey samples were subjected to both different OH parameters (2, 4, 5, 7 and 9 V·cm<sup>-1</sup> at 60 Hz, up to 72–75 °C/15 s) and conventional processing (72–75 °C/15 s). Physicochemical analyses (pH), color measurements (L\*, a\*, b\*), rheological properties (flow curves and particle size distribution), microstructure (optical microscopy), bioactive compounds (ACE and antioxidant capacity), microbiological characterization (mesophilic bacteria, total coliforms, and thermotolerant coliforms), water mobility (TD-magnetic resonance domain), and sensory evaluation (descriptive analysis) were carried out. The OH effects on sweet whey characteristics depended on the applied electric field intensity. Higher saturation, higher color variation ( $\Delta E^*$ ), and higher luminosity (L\*) were observed in low electric fields. For bioactive compounds, the increase of the electric field negatively affected the preservation of the antioxidant capacity and the ACE Inhibitory Activity of bioactive peptides. OH and conventional samples exhibited a pseudo-plastic behavior ( $n < 1$ ). OH performed at 4 and 5 V·cm<sup>-1</sup> was able to provide similar levels of sensory profile and higher volatile compounds levels. The results suggested the OH technology as an interesting alternative to whey processing.

**Key-words: ohmic heating; sweet whey; quality**

## INTRODUCTION

The whey produced in cheese making is a value-added by product that is widely used in the enrichment of food products, containing proteins with important functional properties when hydrolyzed, such as antioxidant and antihypertensive properties (Brandelli, Daroit, & Corrêa, 2015; Pate, 2015). However, these properties may undergo drastic modifications during thermal processing, thus changing the protein structure, such as protein denaturation and aggregation, protein-protein interactions (Roux et al., 2016), and browning reactions, such as Maillard reaction (Kim & Kang, 2015), causing great impact on the quality of the final product.

Among the emerging methods of food processing, Ohmic Heating seems to be an interesting alternative for dairy production (Cappato et al., 2017) as it provides fast and uniform heating, minimizing the generation of off-flavor compounds and providing minor changes in nutrients, when compared to the conventional heat treatment (Pataro, Donsi, & Ferrari, 2011), besides guaranteeing the microbiological safety of the product (Pereira, Martins, Mateus, Teixeira, & Vicente, 2007). Some authors evaluated the potential of OH application in the processing of whey-acerola flavored beverage, determining the frequency and electric fields intensity with effects similar to pasteurization (Cappato et al., 2018).

Research evaluating the emerging technology effect, such as HPP, Pulsed electric field and OH on the physico-chemical characteristics of whey is still scarce. The results found in the recent literature show that these technologies can affect conformation, physical stability, aggregation behavior and induce the denaturation of whey proteins, affecting their functional properties (Cruz et al., 2010; Krešić, Lelas, Jambrak, Herceg, & Brnčić, 2008; Pereira et al., 2016; Sharma, Oey, & Everett, 2014). Thus, the knowledge about the effects of new technologies in the whey presents great importance to the dairy sector in the development of products with sweet whey, such as dairy drinks and yogurts.

Recent studies have shown that OH has great potential for application in whey processing, with lower protein denaturation and an improvement in product's quality (Pereira, Teixeira, & Vicente, 2011). OH can influence the unfolding, denaturation, and aggregation kinetics of whey proteins (Pereira et al., 2016). According to Pereira et al.

(2017), OH appears a promising technique in the development of innovative functional products, due to the effect of the electric field changes the properties of whey protein gels. However, further studies are needed to better understand the impact of OH on the physicalchemical, nutritional and sensory properties of whey.

Thus, the present work aims to evaluate the effects of conventional pasteurization and OH under different process parameters (2, 4, 5, 7, and 9 V·cm<sup>-1</sup> at 60 Hz) under the same temperature profile (72–75 °C/15 s), on the physico-chemical properties (pH, rheological properties, microstructure, volatile compounds and water mobility), on nutritional properties (bioactive compounds) and sensory profile of sweet whey.

## **1. MATERIAL AND METHODS**

### **1.1. Whey processing**

The OH system (Fig. 1) was located at Food Processing Laboratory of Federal Institute of Rio de Janeiro (IFRJ). It consisted of a voltage source associated with a data acquisition (volts). A polymeric jacketed tank coated with an insulating material (Styrofoam) was used to prevent heat loss. The electrodes were made of inert material (stainless steel) coupled to a Teflon support, together with the thermocouples and the specific thermometer for use in food. The voltage source (T) in Volts and a current meter (A) were arranged for data capture by the DataLogger, both in the output of the variable transformer (VT) and in the system as a whole. The temperature was measured by two stainless steel Type T transmitters and data were captured using a digital multimeter. The distance between the electrodes is 11.2 cm. For better heat distribution, a glass rod was used to promote stirring in the ohmic cell.

The whey samples were prepared by reconstituting whey powder (Alibra, Minas Gerais, Brazil) in water at 11% (w/v), according to the manufacturer's instructions and following a recent study (Barukčić, Lisak-Jakopović, Herceg, Karlović, & Božanić, 2015) about the effect of ultrasound on sweet whey. The samples were subjected to OH (2, 4, 5, 7, 9 V·cm<sup>-1</sup> at 60 Hz, named W2, W4, W5, W7, W9, respectively) to 72–75 °C for 15 s (HTST – High-Temperature Short-Time), and immediately cooled in an ice bath (~0 °C). Fig. 2 shows the temperature profiles during the pasteurization of whey by OH

and  
process.

conventional

## 1.2. Thermal Load

The thermal load was calculated using *Coxiella burnetii* as the reference microorganism, with a z value (temperature range required for the thermal destruction curve to cross a logarithmic cycle) of 4.34 °C (Cerf & Condron, 2006). The heating curves were constructed using the experimental data. The temperature profile was measured at intervals (5 s) during the conventional and OH until the target temperature range of 72 °C, remaining at this temperature for 15 s. The pasteurization value (F) was calculated according to Eq. (1), which can be calculated from the integration of the lethal rate as a function of time, as reported by Achir et al. (2016):

$$F = \int_{t_0}^{t_{end}} 10^{\frac{(T-T_{ref})}{z}} dt \quad (1)$$

where T is whey temperature, Tref is the reference temperature (72 °C) and z is the z-value of *C. burnetii*.

## 1.3. pH measurement and color parameters

The pH was measured using a pH meter (AKSO, AK103, São Leopoldo/RS, BR) calibrated with pH 7 and pH 4 buffer solutions, according to Amaral et al. (2018). The color measurements were performed in a Color Quest XE Spectrophotometer (Hunter Associates Laboratory, City, USA), using glass cuvettes with an optical path of 10 mm in diameter. The color parameters a\* (red-green), b\* (blue-yellow), and L\* (brightness) of the CIELAB scale were determined and were used to calculate, chroma (C), and color variation ( $\Delta E$ ) (Balthazar et al., 2015).

## 1.4. Bioactive compounds

The antioxidant capacity (1,1-diphenyl-2-picrylhydrazyl, DPPH radical) and ACE inhibitory activity of whey samples were evaluated in accordance with Amaral et al. (2018). For the antioxidant capacity,

0.1 mL sample and 2.85 mL of 0.06 mM DPPH, and a reaction mixture containing 0.1 mL ethanolic solution and 2.85 mL DPPH were used. The absorbance was measured at 515 nm after incubation for 60 min in the absence of light. Methyl alcohol was used as a blank for calibration of the spectrophotometer

### 1.5. Particle size distribution and rheology parameters

The particle size distribution was determined by laser diffraction technique using the Mastersizer 2000 equipment (Malvern Instruments Ltd., Malvern, UK) in accordance with a recent study (Rojas, Leite, Cristianini, Alvim, & Augusto, 2016). The measurements were performed at 25 °C immediately after treatments. The mean diameter was determined considering the mean diameter of a sphere of similar area, mean surface diameter ( $D_{3,2}$ , Eq. (2)), and the mean diameter of a sphere equal in volume, Brouckere's diameter ( $D_{4,3}$ , Eq. (3)). In addition, the particle dispersion index (span) was determined according to Eq. (4). The samples were analyzed in triplicate, by wet dispersion method, using a refractive index of 1.52.

$$D_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (2)$$

$$D_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (3)$$

$$span = \frac{(d_{90} - d_{10})}{d_{50}} \quad (4)$$

where  $d_i$  is the mean particle diameter,  $n_i$  is the number of particles, and  $d_{10}$ ,  $d_{50}$ , and  $d_{90}$  are the particle diameter at 10, 50, and 90% cumulative distribution, respectively.

Regards the rheology parameters, flow curves were obtained using a stress-controlled rheometer (AR1500ex, TA Instruments, England) with stainless steel flat plate geometry (4 cm) and a 1 mm gap (Cappato et al., 2018). The shear rate varied between 0 and 300 s<sup>-1</sup>, and the flow curves were obtained using an up-down-up steps program. The third

flow curve data were fitted to the models for the power-law model (Eq. (5)).

$$\sigma = k\dot{\gamma}^n \quad (5)$$

where  $\sigma$  is the shear stress (Pa),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>),  $k$  is the consistency index (Pa·sn), and  $n$  is the flow behavior index (dimensionless). The parameters  $k$  and  $n$  were estimated by non-linear regression using the Quasi-Newton method with a convergence criterion of 10<sup>-4</sup>, using Statistica software.

### 1.6. Time Domain Nuclear Magnetic Resonance (TD-NMR)

The samples were submitted to Time Domain NMR measurements (TD-NMR), to evaluate the effect of the type of processing (conventional and OH pasteurization) on the molecular dynamics of water. All measurements were performed using MARAN Ultra 0.54 T relaxometer (23 MHz for the 1H core) equipped with an 18 mm probe for 1H cores at 30 ± 2 °C, with a pulse duration of 90 °C (7.5 μs). All samples werethermal stabilized before measurements.

Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to determine the transverse relaxation times (with a time constant T<sub>2</sub>) of the samples. The time between the echoes (2.tau) was 600 μs, and the decay signal was acquired with 16,384 echoes, being only the even echoes taken for the exponential adjustment, according to Eq. (6):

$$M(t) = M \cdot \exp\left(-\frac{t}{T_2}\right) + k \quad (6)$$

where  $M(t)$  is the residual magnetization at a given time and  $k$  is the residual error. The monoexponential fit led to the lowest chi-square ( $\chi^2$ ) value for all samples. For the determination of the longitudinal relaxation time (T<sub>1</sub>), the CWFPT1 pulse sequence was used for the first time in dairy samples, which is faster when compared to the traditional Inversion Recovery (IR) sequence, idely applied to measure the longitudinal relaxations times, once CWFPT1 has an estimated measurement time of 40 min rather than 2 min in IR (Moraes, Monaretto, & Colnago, 2016). In addition, it is as accurate as inversion-recovery in liquid or quasiliquid systems, that is, in the region of  $\omega_0\tau_c < 1$ . For this sequence, 3 s of recycle delay and 4 repetitions (scans) for each point were used, with a receiver gain sufficient to provide a response with a signal/noise

ratio of about 20. The signal obtained was fitted with an exponential function for the determination of a single value T1, according to Eq. (7):

$$M_z(t) = M_0 \left[ 1 - 2 \cdot \exp\left(\frac{-t}{T_1}\right) \right] \quad (7)$$

where T1 and T2 values are calculated for each sample, and the ratio T1/T2 was correlated with the different intensities used in the OH and conventional pasteurized samples. The more this ratio approaches 1, the more it approaches the behavior of pure water, indicating lower viscosity or lower interactions of water molecules with the hydrogen from other molecules present in the system (i.e. protein or fat) (Song, 2009).

### **1.7. Microstructure**

A recent study by Cappato et al (2018) has reported the use of optical microscopy for similar purposes in the present study. The samples (~20 µL) were deposited and dispersed on glass slides, covered with a coverslip for observation under an optical microscope (Olympus model BX41, Japan) equipped with a digital camera and the images were captured in quintuplicate using 40x objective.

### **1.8. Microbiological characterization**

The whey samples were analyzed for the viable cells count (CFU mL 1186 ) and coliform bacteria by plate pouring method using appropriated culture media and standardized procedures (Jeličić, Božanić, Brnčić, & Tripalo, 2012).

### **1.9. Volatile compounds**

The volatile compounds were extracted by solid phase microextraction (SPME) and identified by gas chromatography (Agilent Technologies® 7890A gas chromatograph) coupled to mass spectrometry (Agilent Technologies® 5975C mass spectrometer), as described by (Condurso et al., 2008) with modifications. The SPME extractions were performed using 50/30 µm thick Sulpeco® divinylbenzene/carboxy/polydimethylsiloxane (DVB / CAR / PDMS) fibers and 20mL headspace vials in an automated CTC PAL sampler

(Agilent Technologies Sampler 120). For that, 3 g sample was mixed with 3 mL saturated NaCl solution maintaining the vial at 40 °C, with a 20 min equilibration time and 30 min extraction time. Samples were stirred continuously at 750 rpm during equilibration and extraction. After extraction, SPME fiber was introduced into the GC-MS for 30 minutes under the following conditions for the thermal desorption of the analytes: injector temperature of 240 °C; splitless injection mode; column HP-5MS 30 m, 0.25 mm id, 0.25 µm film thickness; oven temperature programmed from 45 °C for 5 min, then increased to 80 °C at a rate of 10 °C min<sup>-1</sup>, then to 290 °C at 5 °C min<sup>-1</sup>; Helium gas flow at constant 1 mL min<sup>-1</sup>; transfer-line temperature of 240 °C, electron impact ionization energy at 70 eV, acquisition mass range of 40-400 m/z. For the identification of the volatile compounds, the linear retention index (LRI) of the samples was calculated according to the equation proposed by Van den Dool and Kratz (1963) and compared with the LRI of C8-C40 alkanes standard (Supelco, 40127-208 U) injected under the same chromatographic and mass spectrometry conditions. Enhance identification was performed with Agilent Mass Hunter Qualitative Analysis software (Agilent Technologies) in deconvolution mode, with Signal to Noise ratio above 10, left  $\Delta m/z = 0.3$  AMU and right  $\Delta m/z = 0.7$  AMU, according to the procedures of the National Institute of Standards and Technology Library (NIST/EPA/NIH Mass Spectra Library, version 11, USA).

#### **1.10. Sensory Profiling**

The sensory evaluation was performed using descriptive analysis. Eleven participants with extensive experience in descriptive sensory analysis of dairy products were recruited (5 male and 6 female, 19-35 years). The sensory descriptors were obtained through the repertory Grid method, and 10 training sessions of 1 hour each, in the morning and the afternoon, were performed 3 times a week. The samples (5 °C) were presented in plastic cups (30 mL) and coded with 3 random digits. All samples were evaluated in a single session, with a balanced order of presentation. The samples were evaluated using the Intensity Scale method using a 9 cm unstructured scale, anchored at the extremes by intensity descriptors (Oliveira et al., 2017).

#### **1.11. Statistical analysis**

All processing was repeated three times, and the analyses were performed in triplicate. The results were analyzed by Analysis of Variance (ANOVA) one way considering the



sample as a fixed effect, followed by Tukey's test ( $p$ -value  $\leq 0.05$ ) using the software Statistica (StatSoft®, Tulsa, OK, EUA).

## 2. RESULTS AND DISCUSSION

### 2.1. Comparison of heating curves

The electric field intensity applied to OH exerts a significant increase in heating rate. In contrast, the temperature increase inside the sample depends on its electrical resistance, which is related to the food composition and electrical conductivity (Sakr & Liu, 2014). An observation of heating curves obtained in the different samples shows the OH processing was capable to heat the food at a shorter time when compared to the conventional heating, thus preserving nutrients and the heat-sensitive quality parameters. The highest heat input (W9) provided a faster heating of the sample, taking 85 s to reach 72–75 °C, while the lower electric fields treatment (W2) provided a slower heating, 4475 s, with little viability for industrial applications. Thus, the treatment time was affected by the electric field strength in the OH.

Several authors have reported this phenomenon when studying the electric fields used in OH treatments (Icier, 2009; Sarkis, Jaeschke, Tessaro, & Marczak, 2013). Icier and Ilicali (2005) studied peach puree samples subjected to OH processing at 70 V and 20 V and found a higher time to reach the desired temperature when lower electric fields were applied, with a 7-fold higher heating time for lower electric fields. On the other hand, Icier (2009) studied the effect of OH on whey powder reconstituted at different concentrations, and showed that higher electric fields provided a lower heating time regardless of whey concentration. These different results can be due to different configurations and operational parameters used during OH. However, it is worth emphasizing that the pasteurization parameters, i.e., the thermal load (F-value) must meet the criteria established for dairy products. In this sense, the treatment W9 was effective against *C. burnetti*, once an Fvalue of 21 s was obtained, which is above the value of 15 s established by law (Codex Alimentarius, 2003). However, this value increased considerably in lower electric field treatments (W7, W5, W4, and W2), due to the long time to reach the target temperature (72–75 °C).

## 2.2. pH values and color parameters

Table 1 shows the pH and color measurements of whey samples subjected to OH and conventional heat treatment. From pH, no significant difference ( $p > 0.05$ ) was observed in the samples, suggesting that the electric fields applied were not affect the pH of whey. Few studies report the effect of OH on pH, however the results show that the electric field does not affect the pH of the products (Chakrabortya & Athmaselvi, 2014; Darvishi, Khostaghaza, & Najafi, 2013).

Regarding the optical parameters, differences were observed between  $L^*$  values (58.2 to 55.7,  $p > 0.05$ ), indicating that the higher intensity current of OH reduced the luminosity, while the negative  $a^*$  values (-6.1 to -6.4,  $p > 0.05$ ) indicated that the samples approximated the green color, which is consistent with the literature, which refers to whey as yellow/green liquid (Yadav et al., 2015). Considering the sample Wconv as a reference, no significant changes were observed for the parameter  $C^*$  (Chroma) of the treatments W9, W7, W4, and a higher saturation was observed in the treatments W5 and W1,8. The sample W2 exhibited the higher color variation ( $\Delta E^*$ ), and higher luminosity ( $L^*$ ), probably due to the higher heating time since this sample was exposed to heat for a longer time due to the low electric field applied.

In addition to the electric field, another factor that may affect food staining is the possible reactions between the electrode and the food (Assiry, Sastry, & Samaranayake, 2003). To reduce this possibility, the present work used inert electrodes, such as stainless steel, thus minimizing electrochemical reactions that could affect the color properties (Tola, Rattan, & Ramaswamy, 2014). Thus, it is important to highlight the need for additional studies to evaluated the existence of electrochemical reactions that can occur between the food and the electrode and their effects on the chemical and physical properties of food and higher luminosity ( $L^*$ ), probably due to the higher heating time since this sample was exposed to heat for a longer time due to the low voltage applied.

## 2.3. Bioactive compounds

Table 2 shows the ACE and DDPH values of whey samples after OH and conventional heat treatment. In relation of ACE Inhibitory Activity, OH was capable to provide the release of bioactive peptides in whey. The greater inhibition was observed in samples submitted to OH at 2 and 4 V (93.19 and 97.31%, respectively –  $p > 0.05$ ), which had

a significant result ( $p < 0.05$ ) compared to the conventional pasteurization (33.7%). In contrast, the increase in intensity of electric field (5, 7 and 9 V·cm<sup>-1</sup>), resulted in the reduction of ACE Inhibitory Activity.

Probably, there is a relationship between the heat exposure time and denaturation of whey proteins and release of bioactive peptides (Yadav et al., 2015). These results corroborated the findings of Costa, Gontijo and Netto (2007), who reported that whey hydrolysates heated treated at 65 °C exhibited higher ACE-inhibitory activity. Some studies report that OH can promote changes in unfolding, denaturation, and aggregation kinetics of whey proteins (Pereira et al., 2011; Pereira et al., 2016), which could have induced the formation of bioactive peptides. However, no studies were found in the literature that reported the effect of OH on bioactive peptides.

The results of the antioxidant capacity by the DPPH method showed that OH at 2 V resulted in a lower degradation of the bioactive compounds compared to conventional pasteurization ( $W_2 = 48.2$  and  $W_{conv} = 45.2\%$ ,  $p < 0.05$ ). However, the increase of the applied electric field intensity, resulted in greater degradations of the antioxidant capacity in relation to the conventional pasteurization, except for OH at 4 V (42.3%) where it was not observed significant difference ( $p > 0.05$ ).

In respect of antioxidant capacity, previous studies have confirmed that high-intensity parameters of OH can increase the degradation of bioactive compounds with antioxidant capacity, such as anthocyanin and ascorbic acid (Assiry et al., 2003; Mercali, Jaeschke, Tessaro, & Marczak, 2012; Sarkis et al., 2013). In a recent research, Loypimai, Moongngarm, and Chottanom (2016) evaluated the antioxidant capacity by DPPH method in natural food colorant prepared from black waxy rice bran by OH and conventional process. The authors observed that the electric field applied to OH resulted in lower antioxidant capacity degradations.

Thus, from the results of the ACE-inhibitory activity and antioxidant capacity, it can be observed that the electric field intensity directly affects the bioactive compounds, where lower intensities result in greater preservation of bioactive compounds.

## 2.4. Particle size distribution and rheology parameters

Table 3 shows the particle size distribution through  $D[4,3]$ , which is based on the volume mean diameter, and widely used to indicate the presence of larger particles. The OH-treated samples showed higher  $D[4,3]$ , which demonstrated that the treatments W9, W7, and W5 resulted in particles of larger diameters when compared to the samples subjected to lower electric fields (W4 and W20) and those subjected to the conventional heating (Wconv). The effect of electric field on the formation of whey protein-based hydrogels was recently studied by Pereira et al. (2017), who observed that lower electric field intensities (between  $2 \text{ V}\cdot\text{cm}^{-1}$  and  $6 \text{ V}\cdot\text{cm}^{-1}$ ) resulted in gels with smaller diameters, whereas higher intensities (above  $10 \text{ V}\cdot\text{cm}^{-1}$ ) resulted in larger particle diameters.

Concerning the parameter  $D[3,2]$ , which represents the surface area of the particles, the OH-treated samples subjected to higher electric field intensities presented higher  $D[3,2]$  and span, indicating a greater heterogeneity in the particle distribution for the treatments subjected to higher electric field intensities (W9, W7, and W5), while the samples Wconv presented greater homogeneity. Heating of whey protein concentrate can lead to the formation of small and reversible bonds between the protein particles due to the weak Van der Waals interactions (Simmons, Jayaraman, & Fryer, 2007). However, Rodrigues et al. (2015) observed protein aggregates of smaller diameters using moderate electric current (MEF) after heating whey protein isolate, and presence of native forms  $\beta$ -Lactoglobulin and  $\alpha$ -lactalbumin when compared to the conventional treatment at  $85 \text{ }^\circ\text{C}$ . Our results confirm these studies and suggest that the nonthermal effects of electricity provided by OH can alter the form of the aggregates in the denaturation of whey proteins, with different properties of gel formation by changing the particle distribution. In this study, stainless steel electrodes were used, thus the possibility of electrochemical or fouling reactions cannot be ruled out, which may contribute to a decrease in consistency (Singh, Rattan, & Raghav, 2014).

Table 3 shows the rheological parameters by adjusting the power law model ( $0.990 < R^2 < 0.997$ ) and Fig. 3 shows the flow curves of the sweet whey samples submitted to OH and conventional processing. Absence of differences were observed in the consistency index ( $k$ ) among the samples W4, W5, and W7, with values of 6.89, 6.84, and 6.83  $\text{mPa}\cdot\text{sn}$ , respectively ( $p > 0.05$ ). However, the sample W9 (9.49  $\text{mPa}\cdot\text{sn}$ ) showed an increase in consistency of about 50% when compared to the control (6.00  $\text{mPa}\cdot\text{sn}$ ). Despite the high values of behavior index ( $n$ ), all samples presented a pseudoplastic behavior ( $n < 1$ ).

Although no difference was observed in  $n$  values among the samples ( $p > 0.05$ ), the sample W9 showed a slightly more pseudoplastic behavior, suggesting a higher viscosity and more intense protein denaturation.

When comparing the results of the aggregates formed during heating, and thus influence the consistency of the final product. In fact, the non-thermal effects of electricity provide secondary effects to the rheological properties of whey due to the triggering of chemical reactions (Icier, 2009). On the other hand, when higher intensities are applied, the food was heated faster, which can be an advantage in the industrial application of OH.

## 2.5. TD nuclear magnetic resonance parameters

Table 4 shows the calculated  $T_1$  (s),  $T_2$  (s),  $T_1/T_2$  and  $\tau_c$  (s) from the TD-magnetic resonance. A minimum  $T_1/T_2$  ratio was observed for sample W4, which means a decrease in viscosity, with a drop from 2.27 (Wconv) to 1.55. This behavior indicates a lower distribution of the other whey constituents (casein, fat, lactose, and minerals) (Gad, Emam, Mohamed, & Sayd, 2013), with a larger fraction of free water in the medium or a low chemical interaction between the water molecules and those constituents.

The same principle can be used for the samples W9, W7, and W2, which exhibited similar  $T_1/T_2$  ratios. A higher viscosity was verified as a function of dipole interactions between the hydrogen from water and whey constituents. It was observed that the  $T_1/T_2$  ratios were close from each other for almost all samples, with the exception of W7. In this system, there was a greater deviation between the correlation time and the  $T_1/T_2$  ratio, indicating a chemical environment in which the water molecules need more time to rotate by one radian. Therefore, although the samples W9, W7, and W2 have very similar  $T_1/T_2$  ratios, which refer to similar viscosity; on the other side, W7 and Wconv presented a similar behavior, which can be due to the new interaction created between the water molecules and the other whey solution, moving away from the  $\tau_c$  value of pure water.

## **2.6. Optical microscopy**

The optical microscopy showed structural changes in the particles from the different treatments (Fig. 4). The samples subjected to higher electric field intensities (W7 and W9) presented a great number of particles with a larger structure, suggesting deformation of whey protein structure rather than protein breakdown. In addition, the decrease in electric field led to the formation of aggregates with lower diameter, which may be bioactive peptides. These characteristics can be compared to the particle size distribution, which shows that the lower electric fields produced aggregates with smaller diameter  $D[3,2]$  and volume  $D[4,3]$ . The present results corroborate a recent study on whey proteins subjected to OH (Pereira et al., 2016), using electron transmission microscopy to capture images of whey proteins aggregates formed during OH and conventional heating. The presence of more open protein structures was observed in the OH-treated samples, while particles with denser and compact structures were found in the conventional treatment.

## **2.7. Microbiological characterization**

All samples, regardless of the processing conditions, exhibited counts  $< 3$  MPN/mL for total and thermotolerant coliform, and values between 2.3 log CFU/mL and 3.5 log CFU/mL for mesophilic bacteria, which is in agreement with the technical regulation of whey quality, which determines maximum counts of 5 log CFU/mL of mesophilic bacteria, and absence of coliforms (Brasil, 2013). In this sense, OH has proven to be an effective technology to providing high-quality products.

## **2.8. Volatile profiling**

Table 5 shows the volatile profiling of the sweet whey samples submitted to different ohmic heating conditions and the conventional processing. Intense heat treatments can cause loss of volatile compounds, changes in the sensory or functional characteristics of the product (Amaral et al., 2018), as well as the formation of off-flavor compounds (Cappato et al., 2017). The presence of 1-octen-3-ol alcohol in the sample W5 can indicate lipid oxidation, which is frequently related to rancid or sour flavor and aroma, as also observed for decanoic acid fatty acid (Wright, Carunchia Whetstine, Miracle, & Drake, 2006). This same sensory defect can be due to the presence of

ketones such as 2-,onanone from the oxidation of saturated fatty acids, which was found in sample W5. Contarini and Povolo (2002) have reported that these compounds are associated with the intensity of heat treatment, which was observed in milk processed at high temperatures (UHT). Although the presence of these compounds can differentiate the sample W5 from the others in terms of lipid oxidation, no significant differences ( $p > 0.05$ ) were observed for the flavor attribute among all samples. However, only the sample W2 contained 2, 4-dimethyl-1-heptene, which is a hydrocarbon whose origin is still not well understood (Pereda et al., 2008).

### **2.9. Sensory profiling**

Table 6 shows the sensory profiling of the sweet whey samples submitted to different ohmic heating conditions and the conventional processing. Among the twelve attributes raised, differences were observed only for the attributes milk flavor and burnt milk flavor in all treatments. The OH-treated samples did not differ among themselves in the other attributes, presenting characteristics similar to the conventionally pasteurized sample used as a control. However, the samples subjected to OH presented lower intensity of these attributes when compared to the samples subjected to conventional heat treatment. The burnt milk flavor may be related to products of the Maillard reaction or caramelization, which can release compounds by exposing sulfhydryl groups of whey proteins, such as DMS, sulfates, and sulfides (Simon & Hansen, 2001). However, these compounds were not observed in this study, probably due to the parameters used in OH.

## **CONCLUSION**

The OH was effective for whey processing, with an important role in the quality parameters, with positive effects on the rheological, microscopic, and sensory characteristics and water mobility of the processed product. For a practical and operational application of OH in dairy industries, the electric field intensity should be between 4 V and 5 V, which provided a great release of bioactive peptides. OH-treated samples, provides minimal damage to the rheological and sensory characteristics. Overall, the OH technology has proven to be an effective alternative for the industrial sweet whey processing.

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## ANEXOS

### Tabelas

**Table 1.** pH values and color parameters (L,a,b) of sweet whey after conventional processing and Ohmic Heating

Samples	pH	L	A	b
W <sub>past</sub>	6.12±0.01 <sup>a</sup>	56.4±0.26 <sup>cd</sup>	-6.2±0.03 <sup>ab</sup>	5.6±0.28 <sup>bc</sup>
W <sub>20</sub>	6.12±0.0 <sup>a</sup>	58.7±0.52 <sup>a</sup>	-6,3±0.09 <sup>bc</sup>	7.4±0.43 <sup>a</sup>
W <sub>40</sub>	6.12±0.01 <sup>a</sup>	55.7± 0.11 <sup>de</sup>	-6.1±0.07 <sup>ab</sup>	5.2± 0.29 <sup>c</sup>
W <sub>60</sub>	6.13±0.02 <sup>a</sup>	57.9±0.07 <sup>ab</sup>	-6.4±0.05 <sup>c</sup>	6.7±0.24 <sup>ab</sup>
W <sub>80</sub>	6.12±0.02 <sup>a</sup>	57.25±0.26 <sup>bc</sup>	-6.1±0.77 <sup>a</sup>	5.5±0.32 <sup>c</sup>
W <sub>100</sub>	6.12±0.01 <sup>a</sup>	55.2±0.24 <sup>e</sup>	-6.1±0.24 <sup>ab</sup>	5.0±0.25 <sup>c</sup>

\* Results are presented as the mean ± standard deviation. Analysis performed in triplicate. pH, L, a,b are dimensionless.abcde Different letters in the same column denote difference according the Tukey test (p<0.05). W<sub>past</sub>, W<sub>20</sub>, W<sub>40</sub>, W<sub>60</sub>, W<sub>80</sub>, W<sub>100</sub> = see text

**Table 2.** DPPH and ACE values of sweet whey after conventional processing and Ohmic Heating

Samples	ACE	DDPPH
W <sub>past</sub>	33.7±0.67 <sup>b</sup>	46.2±0.05 <sup>ab</sup>
W <sub>20</sub>	39.2±0.89 <sup>a</sup>	48.2±0.07 <sup>a</sup>
W <sub>40</sub>	38.1± 0.90 <sup>a</sup>	42.3±0.02 <sup>bc</sup>
W <sub>60</sub>	32.8±0.54 <sup>bc</sup>	36.1±0.01 <sup>5c</sup>
W <sub>60</sub>	32.8±0.54 <sup>bc</sup>	36.1±0.01 <sup>5c</sup>
W <sub>80</sub>	29.6±0.78 <sup>c</sup>	40.7±0.03 <sup>cd</sup>
W <sub>100</sub>	30.1±0.68 <sup>c</sup>	40.8±0.03 <sup>de</sup>

\* Results are presented as the mean ± standard deviation. Analysis performed in triplicate. pH is adimensional. ACE (angiotensin-converting enzyme activity) is expressed in %. DPPH (1,1-diphenyl-2-picrylhydrazyl radical activity) is expressed in %. abcde Different letters in the same column denote difference according the Tukey test (p<0.05). W<sub>past</sub>, W<sub>20</sub>, W<sub>40</sub>, W<sub>60</sub>, W<sub>80</sub>, W<sub>100</sub> = see text

**Table 3.** Particle size and rheological parameters of sweet whey after conventional processing and Ohmic Heating

Samples	D <sub>3,2</sub>	D <sub>4,3</sub>	Span	K	n	R <sup>2</sup>
W <sub>100</sub>	2.43 ± 0.26 <sup>c</sup>	0.74 ± 0.12 <sup>ab</sup>	3.59 ± 0.05 <sup>cd</sup>	9.49±0.37 <sup>a</sup>	0.813±0.006 <sup>ab</sup>	0.997
W <sub>80</sub>	2.43 ± 0.26 <sup>c</sup>	0.74 ± 0.12 <sup>ab</sup>	3.59 ± 0.05 <sup>cd</sup>	6.83±0.19 <sup>b</sup>	0.861±0.000 <sup>a</sup>	0.997
W <sub>60</sub>	2.90 ± 0.20 <sup>c</sup>	1.05 ± 0.35 <sup>ab</sup>	3.67 ± 0.44 <sup>d</sup>	6.84±1.31 <sup>b</sup>	0.841±0.032 <sup>ab</sup>	0.991
W <sub>40</sub>	1.18 ± 0.01 <sup>a</sup>	0.45 ± 0.03 <sup>a</sup>	2.52 ± 0.01 <sup>a</sup>	6.99±0.07 <sup>b</sup>	0.820±0.000 <sup>ab</sup>	0.993
W <sub>20</sub>	1.54 ± 0.07 <sup>ab</sup>	0.48 ± 0.01 <sup>a</sup>	3.09 ± 0.10 <sup>bc</sup>	5.24±0.07 <sup>c</sup>	0.860±0.001 <sup>a</sup>	0.971
W <sub>past</sub>	1.72 ± 0.18 <sup>b</sup>	0.55 ± 0.05 <sup>a</sup>	2.97 ± 0.05 <sup>ab</sup>	6.00±1.02 <sup>bc</sup>	0.836±0.025 <sup>a</sup>	0.990

\* Data are expressed as mean ± standard deviation. a-g Different letters at the same column indicates significant differences between samples ( $p > 0.05$ ) according to the Tukey test ( $p < 0.05$ ). k = consistency index; n = flow behavior index. D<sub>3,2</sub> and D<sub>4,3</sub> and span are expressed in  $\mu\text{m}$ . K is expressed in (mPa.sn). n is dimensionless; W<sub>past</sub>, W<sub>20</sub>, W<sub>40</sub>, W<sub>60</sub>, W<sub>80</sub>, W<sub>100</sub> = see text.

**Table 4.** Time-domain magnetic resonance parameters of sweet whey after conventional processing and Ohmic Heating

Samples	T <sub>1</sub> (s)	T <sub>2</sub> (s)	T <sub>1</sub> /T <sub>2</sub>	$\tau_c$ (s)
W <sub>100</sub>	2.81	1.48	1.89	1.33x10 <sup>-11</sup>
W <sub>80</sub>	2.45	1.28	1.91	1.53x10 <sup>-11</sup>
W <sub>60</sub>	2.73	1.55	1.76	1.26x10 <sup>-11</sup>
W <sub>40</sub>	2.90	1.86	1.55	1.06x10 <sup>-11</sup>
W <sub>20</sub>	2.69	1.46	1.84	1.34x10 <sup>-11</sup>
W <sub>past</sub>	2.72	1.20	2.27	1.64x10 <sup>-11</sup>

**Table 5.** Volatile profiling of sweet whey after conventional processing and Ohmic Heating

Compound	LRI	W <sub>100</sub>	W <sub>80</sub>	W <sub>60</sub>	W <sub>40</sub>	W <sub>20</sub>	W <sub>past</sub>
<b>Hydrocarbon</b>							
2,4-Dimethyl-1-heptene	831	-	-	-	-	X	-
<b>Acids</b>							
Hexanoic acid, methyl ester	920	-	X	X	X	-	-
Hexanoic acid, ethyl ester	997	-	X	-	X	-	X
Decanoic acid	1368	-	-	X	-	-	-
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester	1513	-	-	X	-	X	-
<b>Alcohols</b>							
1-Octen-3-ol	976	X	-	X	-	-	-
(±)-Menthol	1172	X	X	X	X	X	X
2-Ethylhexanol	1026	-	-	-	-	X	-
<b>Ketones</b>							
2-Nonanone	1090	-	-	X	-	-	-
Isovalerone	966	X	X	X	X	X	X
Hemimellitene	988	X	X	X	-	X	X

\* LRI/= Linear Retention Index on CP-Wax 52 CB according to the Van der Dool and Kratz equation. Organized by families. x= presence. ----- = absence. Wpast. W20. W40. W60. W80. W100 = see text.

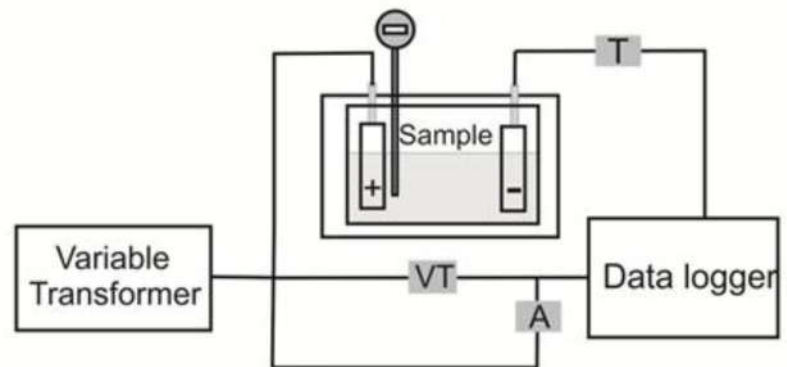


**Table 6.** Sensory Profiling of sweet whey after conventional processing and Ohmic Heating

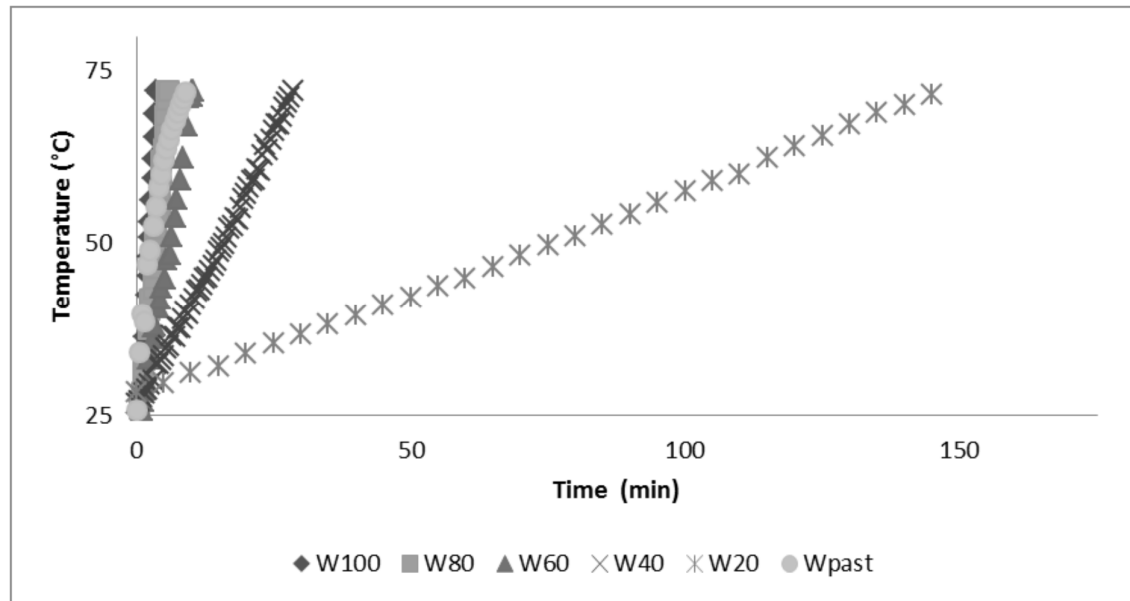
Samples	W <sub>100</sub>	W <sub>80</sub>	W <sub>60</sub>	W <sub>40</sub>	W <sub>20</sub>	W <sub>past</sub>
Yellow color	2.5 <sup>a</sup> ±0.84	2.8 <sup>a</sup> ±1.11	1.9 <sup>a</sup> ±1.05	3.0 <sup>a</sup> ±1.60	2.4 <sup>a</sup> ±1.59	2.5 <sup>a</sup> ±1.22
Milk aroma	2.0 <sup>a</sup> ±1.49	1.1 <sup>a</sup> ±1.14	0.6 <sup>a</sup> ±0.59	1.5 <sup>a</sup> ±1.66	1.8 <sup>a</sup> ±1.31	2.4 <sup>a</sup> ±1.84
Cheese aroma	2.3 <sup>a</sup> ±2.23	0.6 <sup>a</sup> ±0.45	0.4 <sup>a</sup> ±0.39	1.0 <sup>a</sup> ±0.94	0.7 <sup>a</sup> ±0.82	1.9 <sup>a</sup> ±2.13
Butter aroma	1.2 <sup>a</sup> ±1.76	0.7 <sup>a</sup> ±1.19	0.4 <sup>a</sup> ±0.43	1.1 <sup>a</sup> ±1.19	0.7 <sup>a</sup> ±0.68	1.6 <sup>a</sup> ±2.58
Boiled milk aroma	1.6 <sup>a</sup> ±1.68	0.5 <sup>a</sup> ±0.80	0.4 <sup>a</sup> ±0.58	1.0 <sup>a</sup> ±1.75	1.3 <sup>a</sup> ±0.99	0.8 <sup>a</sup> ±1.06
Sweet flavor	2.2 <sup>a</sup> ±2.02	2.3 <sup>a</sup> ±1.54	2.2 <sup>a</sup> ±1.73	4.0 <sup>a</sup> ±1.77	3.5 <sup>a</sup> ±1.92	2.9 <sup>a</sup> ±1.97
Milk flavor	1.9 <sup>ab</sup> ±1.52	1.5 <sup>a</sup> ±1.01	1.0 <sup>a</sup> ±1.11	2.1 <sup>ab</sup> ±1.06	2.2 <sup>a</sup> ±1.59	3.6 <sup>b</sup> ±2.37
Bitter flavor	0.8 <sup>a</sup> ±1.26	0.6 <sup>a</sup> ±0.73	0.4 <sup>a</sup> ±0.62	0.4 <sup>a</sup> ±0.87	0.7 <sup>a</sup> ±1.23	0.8 <sup>a</sup> ±0.92
Boiled milk flavor	1.9 <sup>ab</sup> ±2.26	0.8 <sup>a</sup> ±1.25	1.3 <sup>a</sup> ±0.98	1.7 <sup>ab</sup> ±1.89	1.7 <sup>a</sup> ±1.68	3.7 <sup>b</sup> ±2.07
Residual sweet flavor	1.4 <sup>a</sup> ±1.44	1.7 <sup>a</sup> ±1.59	1.7 <sup>a</sup> ±1.79	2.3 <sup>a</sup> ±2.07	1.9 <sup>a</sup> ±2.02	1.1 <sup>a</sup> ±1.27
Brackish flavor	4.4 <sup>a</sup> ±2.89	4.5 <sup>a</sup> ±2.29	2.3 <sup>a</sup> ±2.15	2.7 <sup>a</sup> ±2.35	2.7 <sup>a</sup> ±2.73	3.6 <sup>a</sup> ±2.50
Viscous	0.8 <sup>a</sup> ±0.99	0.5 <sup>a</sup> ±0.41	0.3 <sup>a</sup> ±0.29	1.1 <sup>a</sup> ±1.14	0.5 <sup>a</sup> ±1.16	0.3 <sup>a</sup> ±0.24

\* Data are expressed as mean ± standard deviation. a-g Different letters at the same column indicates significant differences between samples ( $p > 0.05$ ) according the Tukey test ( $p < 0.05$ ). W<sub>past</sub>. W<sub>20</sub>. W<sub>40</sub>. W<sub>60</sub>. W<sub>80</sub>. W<sub>100</sub> = see text.

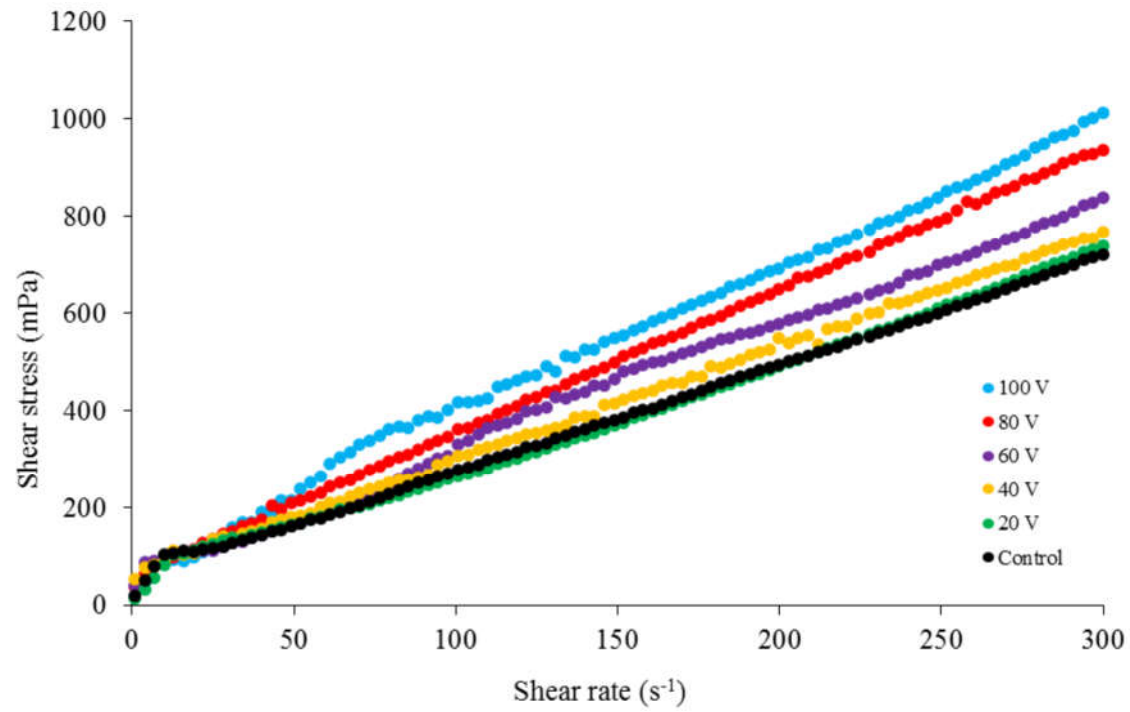
### Figuras



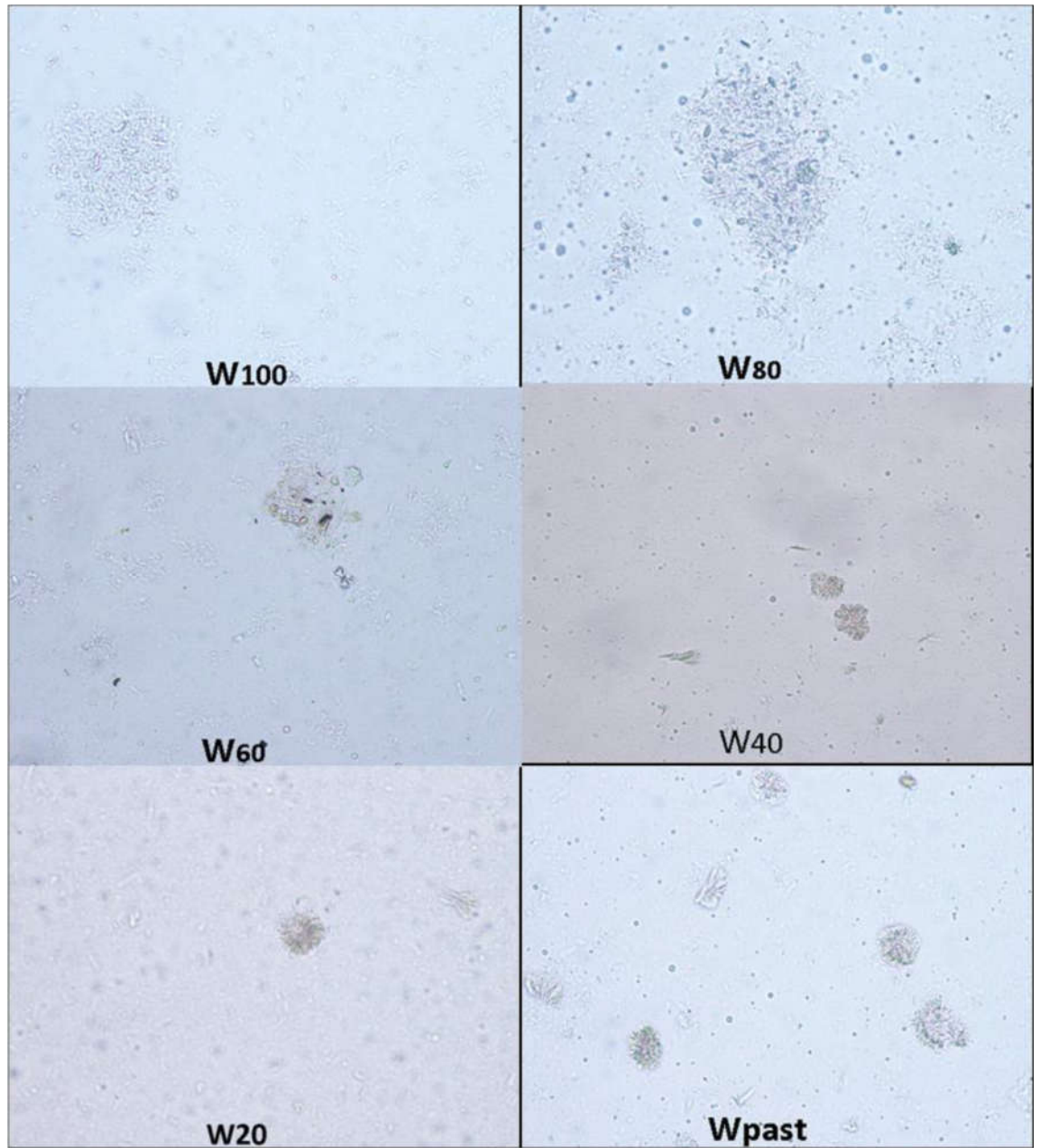
**Figure 1.** The Ohmic heating system



**Figure 2.** Time x Temperature profile of whey samples submitted to OH heating and conventional processing



**Figure 3.** Flow curves of of sweet whey submitted to ohmic heating and conventional processing. Wpast, W20, W40, W60, W80, W100 = see text



**Figure 4.** Optical microscopy of sweet whey submitted to ohmic heating and conventional processing (40X increase). Wpast, W20, W40, W60, W80, W100 = see text