UFRRJ

INSTITUTO DE TECNOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E

TECNOLOGIA DE ALIMENTOS

DISSERTAÇÃO

PROCESSAMENTO DE BEBIDA LÁCTEA DE FRAMBOESA POR AQUECIMENTO ÔHMICO.

Marcus Vinicius da Silva Ferreira

2019

UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
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MARCUS VINÍCIUS DA SILVA FERREIRA

Sob a orientação do Professor
D.Sc., Adriano Gomes da Cruz

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

Seropédica, RJ
Fevereiro, 2019

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DISSERTAÇÃO APROVADA EM: 20/02/2019

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AGRADECIMENTO

RESUMO

O aquecimento ôhmico (AO) é uma tecnologia emergente que se apresenta como uma excelente alternativa ao tratamento térmico convencional (CO), já que o calor é dissipado no alimento de forma homogênea (passagem de corrente elétrica pelo alimento) e não por convecção ou condução. Dentre as vantagens pode-se listar: redução da degradação de cor, preservação dos compostos termossensíveis incluindo os bioativos, dentre outros fatores reológicos que interferem nas características do produto. Adicionalmente a presença do efeito não térmico no AO (eletroporação) lhe confere um potencial de inativação microbiana maior comparado ao convencional. Desta maneira o aquecimento ôhmico foi aplicado para tratar termicamente a bebida láctea de framboesa nos seguintes parâmetros voltagem (45, 60 e 80 V à 60 Hz) e frequência (10, 100, 1000 Hz – 25 V), mantendo o mesmo perfil tempo e temperatura (65°C/30min), no sentido de avaliar seus efeitos comparados ao CO frente as características físico químicas e reológicas da bebida láctea de framboesa. As bebidas tradas pelo AO apresentaram um aumento na capacidade antioxidante para os ensaios (DPPH, FRAP, e Fenólicos Totais), apresentaram também um aumento na inibição de ECA e 10% de aumento na capacidade antihiperglicemica. As amostras tratadas por voltagem (45V,60V e 80V-60Hz) apresentaram uma maior preservação das antocianinas e uma alteração no perfil de ácido graxos. Os tratamentos 10Hz-25V e 1000Hz-25V apresentaram uma conservação na cor, um aumento na reologia, enquanto os demais tratamentos (45V,60V e 80V) apresentaram uma menor viscosidade e maior alteração colorimétrica, alterando suas características negativamente. Os resultados evidenciam a importância de se estabelecer os parâmetros adequados para o equipamento ôhmico. Contudo, de modo geral o AO apresenta uma alternativa interessante para o processamento da bebida láctea de framboesa.

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

ABSTRACT

FERREIRA, M.V.S **Whey raspberry-flavored drink processing by Ohmic Heating.** 2019. 66p. Dissertation (Msc. 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 technology that presents as an excellent alternative to the conventional heating (CH), as the heat is dissipated within the food in a homogenous way (electric current through the food matrix) and not by convection and conduction. Among the advantages it can be listed: the reduction in color change, thermosensitive compounds preservation, including the bioactive, and other rheological factors that interferes in the product characteristics. Additionally, the presence of the non-thermal effect in the OH (electroporation) confers to it great potential in a better microorganism inactivation compared to the conventional treatment. Thus, OH was used to pasteurize raspberry flavored whey-drink in the following parameters (45, 60 and 80 at 60 Hz) and frequency (10, 100, 1000 Hz – 25 V), maintaining the same time-temperature profile (65°C-30min), in the evaluation physical-chemical and Rheological effect compared to the CH in the whey-drink. All the treated beverages showed an increase in the antioxidant capacity for the (DPPH, FRAP, and TPC). Likewise, it was observed an increase for ACE and 10% of increase for antihyperglycemic activity. The OH treated samples showed better preservation of anthocyanins and a change in the fatty acids profile. The treatment 10Hz-25V and 1000Hz-25V showed the best characteristic regarding color, rheological behavior, while the others (treated varying voltage) showed less viscosity and higher color alteration in a negative way. The results highlighted the importance to establish the adequate parameters to the ohmic equipment. However, in general, the OH showed an interesting potential to the raspberry-flavored whey-drink.

Key words: Emerging technology, ohmic Heating, dairy products

LISTA DE SIGLAS E ABREVIATURAS

ΔE^*	Total variation in color
$\Delta H°$	Entalpia de Fusão/Cristalização
A	Amperagem
ACE	Angiotensin converting enzyme
AI	Atherogenic index
AO	Aquecimento ôhmico
c*	Chroma values
CO	Tratamento convencional (pasteurização)
$D_{[3,2]}$	Surface diameter
$D_{[4,3]}$	Volume diameter
DFA	Desired fatty acids
DPPH	2,2-diphenyl-1- picrylhydrazyl
DSC	Differential scanning calorimetry analysis
DW	Dry weight
ECA	Enzima conversora da Angiotensina
ERO	Espécies Reativas de Oxigênio
FRAP	Ferric reducing antioxidant power
FT	Fenolicos totais
g	Gram
GAE	Galic acid equivalent
$h°$	Hue values
HSFA	Hypercholesterolemic saturated fatty acids
Hz	Hertz
I	Corrente elétrica em Ampere
IA	Indice aterogênico
IT	Índice trombogênico
L	Os espaços entre os eletrodos (m)
LCFA	Long-chain fatty acids
MFCA	Medium-chain fatty acids
mL	Mililiter
mM	Milimolar
MUFA	Monounsaturated fatty acids
OH	Ohmic heating
PSD	Particle size distribution
S	Área em m^2
SCFA	Short-chain fatty acids
SD	Standard deviation
T	Temperatura em °C
TDN-MR	Time domain nuclear magnetic resonance
TE	Tecnologia emergente
TI	Thrombogenic indices
TPC	Total phenolics contents
V	Voltagem em (Volts)
VT	Voltagem e tensão que passa pelo circuito
μM	Micromolar
ε	Fator de absortividade molar (26900 L mol^{-1} cm^{-1})

SUMÁRIO

ESTRUTURA DA DISSERTAÇÃO

A dissertação está estruturada na seguinte ordem: No Capítulo I, é a apresentada uma revisão da literatura, contemplando o uso das tecnologias emergentes e em especial o aquecimento ôhmico suas aplicações em lácteos e mais especificamente em bebidas lácteas.

No Capítulo II foi avaliado o efeito de diferentes condições (10, 100 e 1000 Hz a 25 V; 45, 60, e 80 V a 60 Hz) de aquecimento ôhmico e sua ação nos compostos bioativos para os seguintes ensaios (DPPH, Fenólicos totais , FRAP), além de medir o potencial de inibição da Ezima conversora do ECA, Antihiperglicemica (α-glucosidase e α-amilase). Alem de quantificação das antocianinas ácidos graxos e compostos voláteis.

No capítulo III são apresentadas as análises quanto o comportamento Reológico, e outros aspectos físicos (microscopia, cor, Calorimetria de varrimento diferencial (DSC), diâmetro de partícula e Espectroscopia de ressonância magnética nuclear (ERM) das bebidas tratadas pelo aquecimento ôhmico e o tratamento convencional.

Com exceção do Capítulo I, os demais (II, III) estão apresentados em forma de artigo e por este motivo se econtra formatado de acordo com o escopo de cada revista a qual foram submetido, sendo elas Food Chemistry (F. Chem.) e Food Research International (FRI).

INTRODUÇÃO GERAL

Atualmente, novas tecnologias de processamento de alimentos, denominadas de tecnologias emergentes (TE), vêm sendo estudadas para substituir ou complementar os tratamentos térmicos convencionais, com o potencial de proporcionar produtos com melhores qualidades nutricionais e sensoriais. Entre elas pode-se citar a alta pressão, Micro-ondas, Ultravioleta, Ultrassom, Campo elétrico pulsado, Irradiação, Plasma frio, aquecimento ôhmico e CO^2 supercrítico (LI et al, 2017; JERMANN et al., 2015). As TE são denominadas tecnologias do futuro, que irão compor o mercado pelos próximos anos (SUN, 2014) e apresentam-se como uma forma de aplicação prática e preservação como controle microbiológico, extração de compostos de interesse e homogeneização (MISRA et al, 2017).

O Aquecimento Ôhmico (AO) se apresenta como uma excelente alternativa ao tratamento térmico convencional (CO), já que o calor é dissipado no alimento e não por convecção ou condução como na pasteurização. O processo Ôhmico se dá pela passagem de corrente elétrica diretamente no alimento (KAUR e SINGH,2016; CAPPATO et al, 2017), onde muitas vantagens são apresentadas dentre elas, a redução dos impactos causados pelo aquecimento, já que o aquece mais rápido. Da mesma forma, estudos mostram a sua eficácia no controle microbiológico (Segurança do Alimento) entre outras vantagens (ex. uniformidade de aquecimento, controle de processo, redução de incrustação e adição de efeitos não térmicos). (JAEGER et al., 2016; KAUR e SINGH, 2016; CAPPATO et al,2017), o que demonstra seu alto potencial para ser utilizado no tratamento de bebidas lácteas.

A bebida láctea constitui-se da junção de leite, soro de leite e outros componentes devendo, contudo, a base láctea (Leite + soro) perfazer no mínimo 51% no total do produto (m/m%) constituindo um produto de alto valor agregado e uma alternativa ao consumidor (CRUZ et al, 2017). Seu grande potencial se dá pelo fato do soro ser subproduto da produção de queijo, o que confere uma alternativa aos produtores deste segmento, para inclusive solucionarem um grande problema ambiental com descarte (CRUZ et al, 2017; CHAVAN et al, 2015 BRANDELLI, DAROIT e CORRÊA, 2015).

O mercado de bebidas lácteas teve um aumento significativo nos últimos anos, movimentando 40 Bilhões em 2014. Por apresentar textura e sabor agradáveis, o mercado brasileiro também está em pleno vapor com este produto lácteo, sendo no Brasil consumido sistematicamente por mais da metade da população devido a sua grande aceitação (CRUZ et al, 2017). Deste modo existe uma inclinação a utilização de sucos de frutas para confecção da bebida, sendo uma promissora forma de adição de nutrientes (ROHINI e SHITAL, 2012).

A framboesa tem se tornado muito popular na culinária devido a sua fácil aplicação na indústria de alimentos. São fontes de antocianinas, composto do grupo dos polifenóis,que conferem caráter antioxidante que pode inibir a formação de tumores e doenças cardiovasculares, já que desempenha um grande papel na neutralização das espécies reativas de oxigênios (ERO) (CHEN e KANG, 2014; CHEN, ZHANG, e ZHENG, 2016; TENG H et al, 2017). Em relação aos fenólicos presentes na fruta em pode-se citar o ácido elágico (38 a 270 mg/100g) e as antocianinas (89 mg/100g de fruta). (TODA FRUTA, 2016) As bebidas adicionadas de fruta (fontes de compostos bioativos) se apresentarem como promissores para o futuro (TENG H et al, 2017; CHAVAN et al, 2015), entretanto, a vida útil e outros fatores de conservação precisam ser considerados (ROHINI e SHITAL, 2012), o que abre caminho para aplicação de tecnologias em seu processamento.

O aquecimento ôhmico tem sido amplamente utilizado no processamento de alimentos funcionais enriquecidos com compostos bioativos (ex. cálcio, vitaminas e selênio) e ainda apresenta alto potencial em efeitos combinados (associação com outras tecnologias) (MORENO, et al, 2017). Quanto a degradação de compostos, o AO apresenta resposta semelhante ao tratamento térmico convencional, contudo tem a vantagem de oferecer efeitos não térmicos adicionais como, por exemplo, o de eletroporação, que potencializam a inativação de microrganismos (ex. *E. coli e S. aureus*), bacterias essas que apresentam risco potencial para bebidas lácteas, principalmente as voltadas para o público infantil (RODRIGUES et al, 2017; MERCALI et al,2012).

Neste sentido, a necessidade de se conhecer os efeitos de processo e aplicação da tecnologia em bebidas lácteas se tornam necessários para que se obtenha os parâmetros adequados de processo, uma vez que são escassos na literatura trabalhos com aplicação do AO nestes produtos.

CAPÍTULO II: **REVISÃO DA LITERATURA**

1. REVISÃO DE LITERATURA

1.1 Tecnologias Emergentes (TE)

Nos últimos anos tem se visto muitos trabalhos sobre tecnologias não térmicas aplicadas em alimentos e algumas estão implementadas em industrias, contudo muito ainda precisa ser lançado aplicado em larga escala (MISRA et al, 2017 e; ROTOLO D., HICKS D. e MARTIN B. R, 2015; DEEPIKA K et al, 2016). Muitos são os conceitos físicos que estão relacionados com estas tecnologias. Dentre elas pode-se destacar o aquecimento por Micro-ondas, Radio Frequência, Alta Pressão, Infravermelho, Campo Pulsado e Aquecimento Ôhmico (LEE; CHOI e JUN, 2016; MISRA et al, 2017). Ainda que estas tecnologias usem conceitos por muito tempo conhecido, o que se tem de novo é sua aplicação para outros fins na indústria, como a preservação e processamento de alimentos. As novas aplicações e as combinações de tecnologias que configuram essa vertente "emergente" (LEE; CHOI e JUN, 2016). Muito embora as tecnologias convencionais (refrigeração e congelamento), bem como (pasteurização e esterilização) sejam ainda muito empregadas, é descrito que os usos das novas tecnologias vêm desempenhando um grande papel e estão cada vez mais presentes nas indústrias, como a de alimentos (MISRA et al, 2017).

Algumas das TE ainda precisam ser elucidadas de modo a se entender de maneira holística os processos físico-químicos envolvidos , entretanto é observado em muitos estudos a eficácia destas tecnologias em promover a inativação de patógenos e algumas enzimas. Bem como agregar valor aos produtos de alto valor biológico (enzimas, vitaminas e proteínas) derivadas do leite, que são sensíveis a altas temperaturas (MISRA et al, 2017; KNORR D et al, 2011).

Embora as tecnologias citadas acima sejam uma promessa, a pasteurização convencional (CO) ainda se encontra amplamente utilizada, deste modo vencer essa solidez depende de muitas pesquisas antes de sua implementação a nível industrial. Mesmo que algumas delas como por exemplo, a alta pressão, já tenham sido implementadas por grandes indústrias, ainda muito precisa ser estudado para que possam substituir as tecnologias convencionais. Entretanto, pode-se haver uma combinação de ambas (CO e TE) na otimização de processo, minimização de perdas nutricionais e sensoriais, redução de energia, entre outras. Esta combinação pode estar associada a um aquecimento moderado, compostos antimicrobianos, pH, alguns ácidos orgânicos, e as TE (LI.et al., 2017; KNORR D et al, 2011).

1.2 Aquecimento Ôhmico

O aquecimento é um ponto importante nas etapas da produção do alimento, neste cenário, o AO tem se mostrado como uma forma viável para se tratar termicamente os produtos alimenticios (KAUR e SINGH, 2016; CAPPATO et al, 2017). Muitas destas vantagens pelo fato do aquecimento ser dado pela passagem de corrente elétrica (I) pelo material(alimento), o qual oferece uma resistência a passagem da mesma (esse é efeito conhecido pelo nome de efeito joule), que resulta no aumento da temperatura do mesmo. Dentre as vantagens do AO se pode listar a diminuição dos impactos na degradação dos alimentos, visto que este aquecimento apresenta uma maior taxa de transferência de calor (aquece mais rápido). (GALLY T et al,2017; JAEGER et al., 2016; KAUR e SINGH, 2016; CAPPATO et al,2017). A Fig. 1 ilustra o processo.
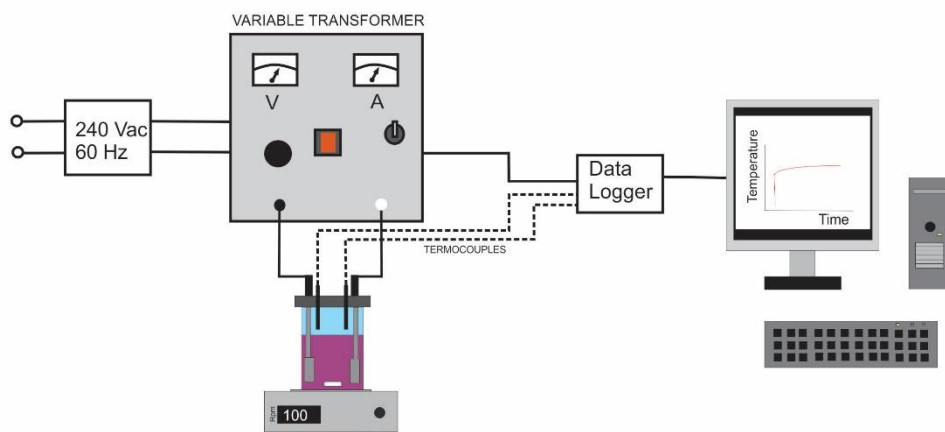
Figura 1: Equipamento Ôhmico completo, com Gerador de Frequência (Hz) e gerador de voltagem (V).

1.3 Aquecimento Ôhmico vs Aquecimento Convencional

O aquecimento convencional encontrados em indústrias geralmente ocorre pela combustão de algum material, geralmente fosseis (ex. carvão e gás natural) além de biomassa (ex. Celulose), e o calor é direcionado diretamente ao produto. Contudo este sistema é pouco eficiente em termos de aquecimento por unidade produzida (SAKR M, LIU S., 2014).

Desta forma, como alternativa, o aquecimento Ôhmico (AO) oferece uma técnica que tem ganhado visibilidade em aplicações em indústrias de alimentos, além de quando comparado ao CO, este oferece um menor tempo na passagem do sistema de aquecimento inicial para um estado continuo. Diferentemente do CO, o AO proporciona uma economia de energia na ordem de 89% em média, diminui o tempo de aquecimento em 90% e ainda proporciona uma eficiência na ordem de quase 100%, já que toda energia elétrica é convertida em calor (aquecimento), além de ainda proporcionar uma maior uniformidade no processo de aquecimento (SAKR M, LIU S., 2014; GALLY T et al, 2017). Contudo a aplicação da tecnologia ainda é onerosa em termos de custo inicial e difícil de ser implantada em grade escala devido aos parâmetros de processo. (KAUR e SINGH, 2016).

1.4 Campo Pulsado vs Aquecimento Ôhmico

Dentre as tecnologias não térmicas encontra-se o campo elétrico Pulsado (CEP), que também tem sido impulsionada como promissora nos últimos anos muito confundida com o aquecimento Ôhmico, contudo bem diferente. Seu funcionamento se resume em campos elétricos pulsados em pequenos intervalos de tempo na ordem dos microssegundos (1-100µS) e altas tensões na ordem de kV, gerando altíssimos campos elétricos que são gerados por dois eletrodos, similar ao aparato Ôhmico, com amplidutes amplamente maiores, porém. Desta forma, o CEP também operada, assim como o OH, com a eletroporação, porém o campo gerado e o impacto na estrutura celular é maior, atuando como potencial meio de inativação de microrganismos e também na liberação de bioativos como carotenoides e polifenóis (CAPPATO et al,2017; JIN et al, 2009; QUERALT A et al, 2015).

1.5 Conceitos do Aquecimento Ôhmico

O princípio físico do AO se dá pela passagem de uma corrente alternada (I) na matriz do alimento, esta corrente é gerada através dos eletrodos que estão em contato direto com a matriz a ser processada (alimento), como mostrado na fig.2. Através da passagem da energia elétrica pelo alimento, que funciona como resistência (R) o mesmo é aquecido. Processo pelo qual a energia elétrica é convertida em calor (aquecimento). (CAPPATO et al, 2017; SAKR M, LIU S., 2014). A condutiva elétrica é medida pela equação:

$$\sigma = L.I/S.V \qquad \text{Eq. (1)}$$

na qual, σ representa a condutividade elétrica na amostra ($S.m^{-1}$), L os espaços entre os eletrodos, S a sessão transversal do eletrodo (m^2), I a corrente que passa pelo alimento (A) e V a voltagem (V) (SARKIS J. R et al, 2015; CAPPATO et al, 2017; ZELL, M., et al, 2009).
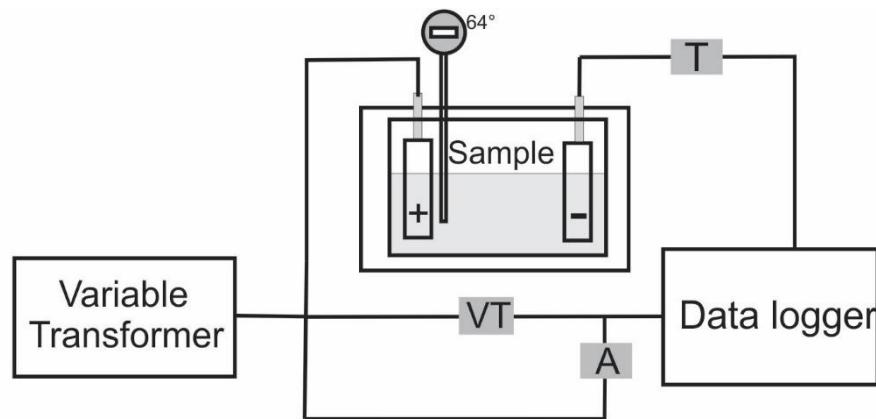
Figura 2 Equipamento Ôhmico simplificado
. VT (Voltagem e tensão), T (Temperatura) e A (Amperagem).

1.6 Parâmetros no Aquecimento Ôhmico

Alguns parâmetros importantes no AO em relação as variáveis de processo apresentam-se na Tabela 1. Dentre os principais fatores que afetam o processo encontram-se a condutividade elétrica, força do campo elétrico, tamanho de partícula, concentração da partícula, concentração iônica e localização da partícula e tempo de residência (KAUR e SINGH, 2016). Sendo a condutividade elétrica a mais crítica delas (VARGHESE, K. S, 2014).

Tabela 1: **Parâmetros Importantes no Aquecimento Ôhmico**

Parâmetros	Fatores
Processo	Força do Campo Elétrico Tempo de Residencia Temperatura Corrente Elétrica Condutividade Elétrica
Equipamento	Tamanho da célula Ôhmica Tamanho e Forma dos Eletrodos Composição do Eletrodo

Adaptado de VARGHESE, K. S, 2014; CAPPATO *et* al, 2017.

Alguns equipamentos na literatura incorporam os parâmetros de processo citados e alguns deles foram testados em alimentos líquidos e pastosos, que apresentaram comportamento não linear em relação a eletro condutividade (GALLY T et al,2017; SARKIS J. R et al, 2015; MARRA, F, 2014). A condutividade elétrica como já mencianada, apresenta papel importante e desta forma está bem definida em alguns produtos já processados na indústria de alimentos como pode ser observado naTabela 2 (SARKIS J. R et al, 2015).

Tabela 2: **Aquecimento Ôhmico na Indústria de Alimentos e os seus diferentes parâmetros de condutividade.**

Tipo de Material	Eletrocondutividade (25º C)
Cerveja	0.143
Café	0.182
Café com Leite	0.357
Chocolate	0.433
Suco de Tomate	0.169
Água do mar SST =44.00 mg	5.80
Água do mar SST =57.78 mg	6.780
Água do mar SST =62.82 mg	6.750

Adaptado de VARGHESE, K. S, 2014; CAPPATO *et* al, 2017.

Alguns parâmetros reológicos desempenham um grande papel no aquecimento ôhmico como o perfil Reológico, por exemplo (SINGH et al., 2014). Da mesma forma, a viscosidade também se apresenta como um ponto crucial no processo (ZAREIFARD et al., 2014). Ambos estão intimamente associados com a condutividade elétrica e consequentemente com o AO.

1.7 Aquecimento Ôhmico Aplicação em alimentos

Alguns ensaios com polpas de acerola e amora mostraram um comportamento não linear na condutividade elétrica que, segundos os autores, podem estar relacionados com a formação de bolha durante o processo. Este fenô em relação direta com alto teor de sólidos e o próprio campo elétrico, variando a temperatura do meio (SARKIS J. R et al 2015). Ensaios com frutos (tomates) também mostraram uma necessidade em se estudar mais os parâmetros (GALLY T et al,2017; NGASRI, P. W e SASTRY S. K., 2016).

1.8 Aquecimento Ôhmico aplicação em produtos Lácteos

O aquecimento ôhmico em produtos lácteos começou no início do século passado, mais precisamente na década de 20, quando através da passagem do fluido por um sistema de pratos, o mesmo era aquecido por uma voltagem aplicada aquele dispositivo. Atualmente a tecnologia é desempenhada em fluxo continuo de forma asséptica, preservando não só a qualidade sensorial e microbiológica do produto como os parâmetros reológicos, os quais são dependentes de muitos outros fatores ((KAUR e SINGH, 2016; Misra et al., 2017).

Um grande problema na indústria de leite é a formação de biofilme (microorganismos em superfície polimérica) em equipamentos, tendo o AO uma vantagem sobre o CO, uma vez que o primeiro demonstra um menor potencial de formação de particulado por se tratar de um aquecimento homogêneo e não limitado a superfície das placas de aquecimento (CAPATO et al, 2017). Este problema se agrava quando existe a presença de sais em solução, e também a caseína parece formar camadas mais extensas deste filme (HAUSMANN et al, 2013). Deste modo o AO se mostra com uma boa forma de se reduzir a formação de biofilmes (CAPATO et al, 2017).

A eletroporação (microfissuras na parede celular, que proporciona liberação de compostos) é outro fenômeno que ocorre com a aplicação desta tecnologia em produtos no geral e consequentemente nos produtos lácteos. Este evento não térmico é ocasionado pela passagem de corrente no alimento (semelhante ao campo pulsado) (KIM e KANG, 2015a). Este efeito nos produtos lácteos parece ter relação direta com a gordura presente no leite, sendo o efeito mais expressivo em produtos com menor percentual de gordura (KIM e KANG, 2015b). O mercado de bebidas lácteas tem se beneficiado muito com o desenvolvimento desta tecnologia para se contornar este problema (CRUZ, A. G et al., 2017).

1.9 Bebidas Lácteas

A bebida láctea é definida como pela mistura do soro de leite com outros ingredientes, na qual a base láctea não seja menor que 51% (m/m) da massa total. (BRASIL, 2005) Apresentando-se como uma excelente alternativa de aproveitamento soro de queijo e outros processos que o produzam. Além de gerarem receita, ajudam a diminuir os impactos ambientas reduzindo a carga orgânica descartada (CRUZ et al., 2017). Apresentam como uma opção economicamente viável desta forma para indústria por apresentarem uma boa aceitação sensorial e alta qualidade nutricional.

1.10 Bebidas Lácteas: Aspectos Gerais

As bebidas lácteas no Brasil é regida pela normativa n° 16 de 23 de agosto de 2005, podendo o leite para a composição da base láctea ser pasteurizado ou não. De maneira geral, as proteínas do soro do leite são utilizadas em concentrados em pó configurando ou polimerizadas (TORRES et al, 2011). Recentemente se verifica a tentativa de utilização concentrados líquidos para a produção de bebidas lácteas, sejam elas fermentadas ou não (PEREIRA et al 2015).

As bebidas estão classificadas em: com adição ou sem adição, para adição de produtos ou substratos; pasteurizada, esterilizada ou UAT para tratamento térmico; e fermentada ou não fermentada para presença ou não de processos fermentativos.

1.11 Bebidas Lácteas: Componentes Bioativos

Ultimamente tem se visto muitas inovações para fidelização dos consumidores com diversos atrativos como apelos funcionais. As proteínas do leite, principalmente a caseína, está associada à inibição da enzima conversora da angiotensina (ACE-I), com o potencial de reduzir a pressão arterial (ÖZER B e KIRMACI, HY, 2010). Muitas enzimas proteolíticas têm sido utilizadas para a produção de soro, o qual apresenta estas propriedades terapêuticas como capacidade antioxidante, antimicrobiana, anti-hipertensiva. Além de conferir capacidade anti-inflamatória, antitumoral, imunoreguladora, e atuar no controle da obesidade e do diabetes (NEWSRX, 2015). Devido às diversas funções desempenhadas por esses compostos, pode-se dizer que que o soro é uma importante fonte de peptídeos bioativos (BRANDELLI A. et al, 2015).

1.12 Bebida láctea de Framboesa (Potencial Funcional)

Ainda, as bebidas lácteas são excelentes meios de inserção de probióticos, minerais como cálcio, magnésio e ferro, além dos bioativos já mencionados (ÖZER B e KIRMACI, HY, 2010). Desta forma, o mercado de bebidas lácteas com apelo funcional tende a ganhar impulso, sendo um dos pontos chaves de implementação a identificação destes bioativos e o efeito do processamento (AO) nestes compostos (CORBO et al, 2014).A framboesa pertence gênero *Rubus*, sendo a variedade vermelha da família (*Rubus Idaeus* L.) consumidas mundialmente. A Europa é a maior produtora do fruto chegando a tomar 75% do mercado. As bebidas de framboesa se destacam pelo seu potencial antioxidante como alta concentração de componentes polifenóis (ácido elágico (38 a 270 mg) e as antocianinas (89 mg) para cada 100 g da fruta *in natura*. (TODA FRUTA, 2016) e por suas propriedades terapêuticas, sendo geralmente utilizada em bebidas funcionais (CORBO et al, 2014). Apresentando-se como uma excelente opção para testes utilizando o Aquecimento Ôhmico.

2 OBJETIVOS
2.1 Objetivo geral

O objetivo geral deste trabalho é avaliar os efeitos de processo do Aquecimento ôhmico sobre a bebida láctea de framboesa para verificar sua influência sobre os

parâmetros de processo e então propor os melhores parametros para que se tenha uma manutenção da funcionalidade da bebida.

2.2 Objetivo Específico

O objetivo específico é :

- Determinar os parâmetros de processo (tensão, frequência, condutividade)

- Quantificar os componentes bioativos e suas alterações

- Avaliar os efeitos não térmicos associados ao OH.

- Determinar o perfil reológico

- Observar as alterações estruturais na (microscopia) , cor e fisico Quimica

- Definir as melhores configurações para o processamento da Bebida.

CAPÍTULO II: OHMIC HEATING FOR PROCESSING OF WHEY-RASPBERRY FLAVORED BEVERAGE

Manuscrito submetido para publicação na revista Journal of Dairy Science (Qualis Capes A1/ Ciência de Alimentos)

Ohmic Heating for processing of whey-raspberry flavored beverage

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1. Abstract

Ohmic Heating (OH) (10, 100, and 1000 Hz at25 V; 45, 60, and 80 V at60 Hz) for processing of whey-raspberry flavored beverage processing was performed. Bioactive compounds (alfa-glucosidase and amylase, Angiotensin-converting enzyme activity, antioxidant activity), fatty acid profile, and volatile compounds were determined. All ohmic treated samples presented lower anthocyanins values when compared to the conventional treatment (2.91± 0.23 mg/g). In general, the mild-intermediate conditions (10Hz and 100Hz at 60V; 45V and 60V at 60Hz) presented the best results of antioxidant activity, determined by the DPPH, FRAP and TPC assays, when compared to the extreme processing conditions (1000Hz-25V and 80V-60Hz). The ohmic heating led to an increase in 10% for both α -glucosidase (>99%) and α-amylase (≥ 70%) when compared to the conventional treatment. The lipids indexes (Atherogenic index, thrombogenic index, desired fatty acids, and hypercholesterolemic saturated fatty acids) showed to be less favorable in the ohmic treated samples. The presence of Furfural and 5-Hydroxymethylfurfural, one of the major intermediate products in the Maillard reaction, were found in all treatments. Overall, OH showed an interesting technology to be used in the whey-flavored raspberry beverage processing.**Keywords**: Ohmic heating, whey beverage, antioxidant, ECA.

2. Introduction

Health-conscious consumers have been searching for beverages with higher nutrients and bioactive compounds levels (Martínez-Monteagudo, Kamat, Patel, Konuklar, Rangavajla, e Balasubramaniam, 2017). Whey fruit beverages fit these recommendations. Brazilian Food regulation establishes a minimum 51% (w/w) of a dairy base in the total formulation, but it can contain other ingredients, such as fruits, and it can also be fermented (Brasil, 2005).

The conventional heat treatment used in whey fruit-flavored beverages processing has a negative effect on the bioactive compounds that are thermosensitive, thus affecting the sensory consumers' acceptance of the products (Amaral et al., 2018). Therefore, non-conventional technologies, such as Ohmic Heating (OH) may be a

potential alternative to be considered for processing functional beverages and dairy products, such as whey fruit beverages (Cappato et al., 2018a,b).

Ohmic heating (OH) technology consists of a heating process, in which the electric current passes through the food (Cappato et al., 2017) and promotes instantaneous and homogeneous heating due to the Joule effect. Overall it shows advantages over the conventional processing, resulting in a reduction in the thermal load, retention of nutrients, and improvement in the sensory attributes (Kaur e Singh, 2016). OH also provides additional non-thermal effects, e.g., electroporation, which can cause slight cellular damage to plant tissues and ultimately affecting the quality attributes, such as the rheological parameters and the bioactive compounds (Cappato et al., 2018a,b).

Raspberry fruits can be either consumed fresh or processed, and several studies have shown their functional potential and the benefits related to its ingestion, such as anti-carcinogenic, vasodilatory and diabetic effects (Gowd, Jia, e Chen, 2017), being an excellent ingredient for whey beverage formulations. The present study aimed to evaluate the effect of different OH conditions (10, 100, and 1000 Hz at 25 V; 45, 60, and 80 V at 60 Hz) on the whey-raspberry flavored beverage processing. The bioactive compounds (DPPH, total phenolics, FRAP), ACE inhibitory activity, antihyperglycemic inhibition (α-glucosidase and α-amylase, anthocyanin content, fatty acids profile (FAs), volatile compounds (VOCs) were determined. For comparison purposes, the whey raspberry flavored beverage was subjected to same temperature profiles (65 °C/30 min) for the OH and conventional processes.

3. Material and Methods

2.3 Whey-raspberry flavored beverage processing

The whey beverage was prepared according to Cappato, et al. (2018), containing 70-30 v/v% dairy mix (pasteurized milk + sweet whey) 9.9% w/v crystal sugar, and 0.1% w/v xanthan was added to the mix, and after complete dissolution, 30% of the raspberry pulp (Mais Fruta Company, Jarinu, SP, Brazil) was added. All samples were stored at 5°C until processing.

2.4 Ohmic heating and conventional processing

The OH processing was performed using two different configurations Fig 1, varying the frequencies (10,100, and 1000 Hz) at a constant voltage (25V), and varying the voltages (45, 60, and 80 V) at a constant frequency (60Hz), according to previous studies (Mercali, Schwartz, Marczak, Tessaro, e Sastry, 2014a; Sarkis, Jaeschke, Tessaro, e Marczak, 2013). Ohmic and conventional processes were performed using the same temperature profile Fig 2, and the ohmic processing (time 0) started when the temperature reached 65°C (pasteurization temperature). For both the ohmic and conventional systems, the cell was connected in thermostatic baths (Polystat, Cole Parmer, Lauda, RM 12, Brazil) to reach the desired temperature, and the only difference between the treatments was the absence of electrodes in the ohmic cell.
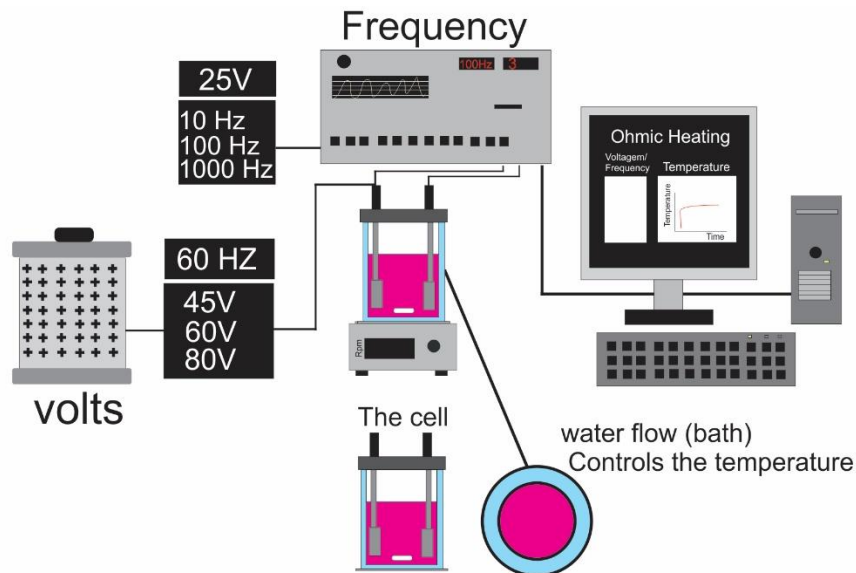
Figure 1. Ohmic Heating set-up
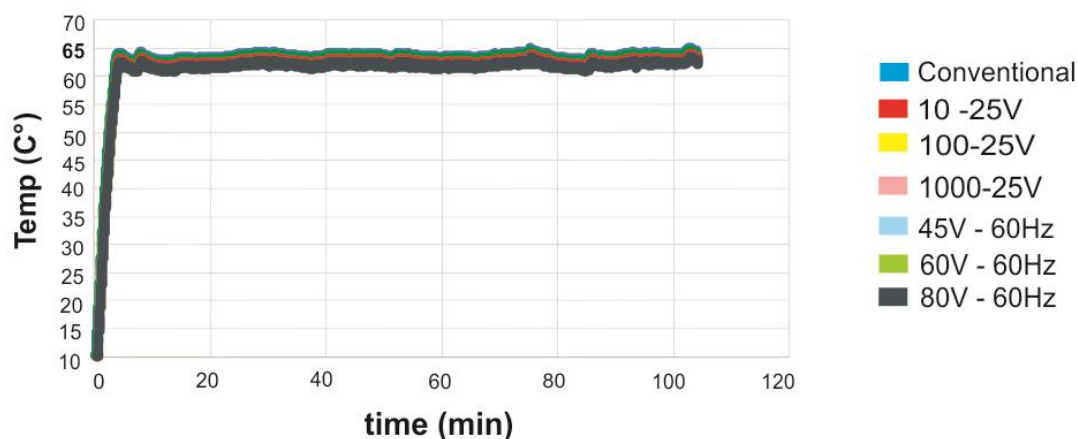
Figure 2. Time vs temperature profiles of conventional and ohmic heating processing.

4. Antioxidant capacity (FRAP and DPPH) and bioactive compounds (TPC and bioactive peptides)

The monomeric anthocyanin was measured with UV-visible spectroscopy utilizing the pH-differential method (Lee, Durst, e Wrolstad, 2005). The samples were centrifuged (Cientec, model 500R, Piracicaba, SP, Brazil) at 5°C (10 min, 3000xg). After that, two different dilutions were made with the supernatant in two buffer solutions (chloride pH1.0 and sodium acetate pH 4.5). for the absorbance readings, which was calculated using Eq. I, while the monomeric anthocyanin was calculated using Eq. II.

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{(pH.5)} \tag{I}$$

$$\text{Anthocyanin (mg/L)} = (A \times MW \times DF \times 10^3)/(\varepsilon \times l) \tag{II}$$

Where A_520 is the absorbance at 520 nm and A_700 at 700 nm, MW is the molar weight (g.mol-1), DF is the Dilution factor, ε represents the molar absorptivity (L mol-1 cm-1) and l the path length of the curvete (cm).

For DPPH, FRAP, and TPC, the extracts were prepared as described by Cappato et al., 2017. Where, approximately 1 g of the samples were weighted, and the compounds were extracted using 1:10 as a ratio sample/solvent (ethanol and water 1:1). Then it was paced at 200 rpm (SL180/D, Solab, Piracicaba, SP, Brazil) for 1 h at room temperature. Afterward the extract was filtered under vacuum and placed under refrigeration for further analysis.

The antioxidant capacity in terms of DPPH (2,2-diphenyl-1-picrylhydrazyl) was performed according to Amaral et al. (2018). Where 2.85 mL of a DPPH solution (0.006 μM) were reacted with 150μL of each extract. The tubes were then homogenized in absence of light for 60 min. The absorbance was measured at 545 nm and the results were expressed as Trolox Equivalent/g sample.

The Frap (Ferric Reducing Antioxidant Power) assay was performed according to Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006), where 2.7 mL of FRAP solution were mixed with 180 μL of distilled water and 90 μL of the sample. After homogenization, the mixed were placed in a bath(37°C) for 30 min and measured at 595 nm. The results were expressed in Trolox Equivalent/g sample.

The total phenolic compounds (TPC) evaluation were performed according to Cappato et al 2018. Briefly, an aliquot 1 mL of Folin-Ciocalteu reagent was added to a 10 mL flask and mixed with 1 mL of the extract. After 5 min 1.5 mL of 10% Na_2CO_3 was added. After 2 h of incubation at room temperature, the absorbance was determined at 725 nm, and the results expressed in Gallic acid equivalents (GAE) per 100 g of fresh weight.

2.5 ACE Inhibition Essay

The ACE-inhibitory activity was determined in the filtrates of whey beverages according to Konrad, Anna, Marek, Marta, Aleksandra, and Józefa, (2014), where 20μL of ACE enzyme (0.1-unit mL-1) was added in and the flasks were placed at 37°C for 30 min. Thereafter 250 μL of 1mol L-1 HCl was added to end the enzyme activity. The flasks were placed to dry and suspended in deionized water for further absorbance measuring at 228nm. The percent inhibition was determined using the following Eq. (III):

ACE-inhibition (%) = ⌊1-(C-D)/(A-B)⌋ x 100   (III)

A: represent the absorbance with the enzyme and no sample

B: no sample nor the enzyme

C: presenting both enzyme and sample

D: containing the sample but not the enzyme.

2.6 α-glucosidase and α-amylase essay

For the inhibitor α-glucosidase and α-amylase essay were performed according to L. Lavelli, Sri Harsha, Ferranti, Scarafoni, and Iametti (2016), where the α-glucosidase were determined using a spectrophotometric technique by taken 4-nitrophenyl-D-

glucopyranoside (pNPG) as a substrate, both the substrate and the enzyme (α-glucosidase) were prepared using a phosphate buffer (pH 6.8). Then 100µL of phosphate buffer (pH6.8), 100 µL of the enzyme solution (10 unit/mL) and 100 µL of the sample were mixed in an Eppendorf tube, being incubated at 37°C for 10 min. Followed by the addition of the 50 µL substrate (5.5 mmol/L) and incubation of 20 min at 37°C. The reaction was stopped by adding 1mL of glycine solution (pH 11). The absorbance was read at 400 nm. All the results were performed in triplicate and expressed as mean (n=3) ± SD.

The inhibition was calculated using the Equation (VI) as follows:

$$\alpha\text{-Glucosidase Inhibition}(\%)=\left[\ 1-(A_{sample}-A_{background})/A_{control}\ \right] \times 100 \qquad (IV)$$

Acontrol: Enzyme + Substrate

Asample: Enzyme + sample + subtrate

Abackground: Enzyme + sample

The assessment of α-amylase inhibitory activity was performed by mixing 100 µL of the enzyme (α-amylase) that was previously prepared in phosphate buffer 9pH 6.8) with 100 µL of the sample followed by 250 µL of starch solution 1%, then the tubes were incubated for 5 min at 37°C. Afterward the reaction continued with the addition of 150 µL dinitro salicylic reagent, the mixture was incubated again at 100°C for 30 min. After the heating process, the tubes were cooled in a cold bath where 2 mL of distilled water was added in. The absorbances were read at 540nm. All the results were performed in triplicate and expressed as mean ±SD.

The inhibition was calculated using the Equation (V) as follows

$$\alpha\text{-amylase Inhibition}(\%)=\left[\ 1-\ (A_{sample}-A_{control})/A_{control}\ \right] \times 100 \qquad (V)$$

Acontrol: Enzyme + Substrate

Asample: Enzyme + sample + substrate

Acarbose was used as a reference inhibitor for both enzymatic reactions. Dose-response inhibition percentage were made for samples and acarbose. For acarbose results were accessed in the same way the samples were, therefore, their response was expressed in % of inhibition.

2.7 Fatty Acids Profile

Fatty acids identification and quantification were determined according to Batista, Silva, Cappato, Ferreira, Nascimento, Schmiele, et al. (2017) using a GC-MS (Agilent Technologies, 7890A-5975 C, Santa Clara, Calif., USA), with a CTC PAL sampler (SPME 120, Agilent Technologies). The column (DB-FFAP CG 15 m x 0.10 mm, 0.10 µm) temperature were programmed to 70 °C for 1 min, followed by a temperature ramp ranging from 45 °C.min^{-1} to 115 °C, subsequently a new ramp at 40

°C.min^{-1} to 175 °C, and finally 30 °C.min^{-1} to 240 °C holding it for 4 min; and a MS detector with mass range 40-400 m/z.

The fatty acids identification 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. The quantification was performed according to ISO 5508:1990, using an Agilent Mass Hunter Quantitative Analysis software. Atherogenic (AT) and thrombogenic (TI) indices, desired fatty acids (DFA), and hypercholesterolemic saturated fatty acids (HSF) (Barłowska, Pastuszka, Rysiak, Król, Brodziak, Kędzierska- Matysek, et al., 2018) were also calculated according to Eq. I, II, III, and IV.

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / [\Sigma \text{ MUFA} + \Sigma \text{ PUFA}(n-6) \text{ and } (n-3)] \tag{I}$$

$$TI = (C14:0 + C16:0 + C18:0)/[0.5 \times \Sigma \text{ MUFA} + 0.5 \times \Sigma \text{ PUFA}$$
$$(n-6) + \times \Sigma \text{ PUFA}(n-3) + \Sigma \text{ PUFA}(n-3)/\Sigma \text{ PUFA}(n-6)] \tag{II}$$

$$DFA = \text{MUFA} + \text{PUFA} + C18:0 \tag{III}$$

$$HSFA = C12:0 + C14:0 + C16:0 \tag{V}$$

2.8 VolatileCompounds

The volatile compounds were extracted by a solid phase microextraction (SPME) and performed using a gas chromatograph (Agilent Technologies® 7890A GC) coupled to a mass spectrometer (Agilent Technologies® 5975C). The extracts were obtained by 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 volatile compounds were identified using the Agilent Mass Hunter Qualitative Analysis software (Agilent Technologies) and then comparing the experimental spectra with the National Institute of Standards and Technology Library (NIST/EPA/NIH Mass Spectral Library, version 11, USA) using linear retention indices (LRI).

2.9 StatisticalAnalysis

The processing was repeated three times, andthe analyses were performed in triplicate. The results were presented as mean ± standard deviation, and data were analyzedby 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

Antioxidant capacity (FRAP and DPPH) and bioactive compounds (TPC and bioactive peptides)

The results of the determination of bioactive compounds are shown in Table 1. Regarding the anthocyanins content, all ohmic treated beverages had lower values when compared to the conventional treatment (2.91± 0.23 mg/g). The samples subjected to 45V and 60V showed similar results (2.88± 0.74 mg/g) for the anthocyanin levels, with no significant differences from the conventional sample (p>0.05). In contrast, significant

differences were observed for the samples subjected to all frequencies when compared to the conventional treatment and different voltages. Only the treatment at 80V (2.86± 0.75 mg/g) differed from the conventional treatment and the different frequencies. These findings suggest that the frequency parameters applied in OH had a negative impact on the anthocyanins content, decreasing its levels.

Similar values were found in previous studies (Bochi, Barcia, Rodrigues, e Godoy, 2015). The anthocyanin profile of all treatments suggests that the OH conditions were not capable of drastically decrease its antioxidant potential. Some protective behavior due to the presence of casein in dairy products has also been suggested. A study on anthocyanins concentrations and stability has proven its dependence on casein and other b-lacto globulin proteins, which confers protection against anthocyanins degradation (Liang, Zhang, Zhou, e Subirade, 2013).

The DPPH results showed higher scavenging power for almost all treatments, with better antioxidant capacity when compared to the conventional treatment; however, no significant difference was observed between the conventional treatment and the treatment at 80V-60Hz, with values of 8.64 ± 0.08 and 8.69 ± 0.23 µg Trolox Eq/g, respectively. The treatment at 100Hz-25V showed the best DPPH value (8.87 ± 0.09 Trolox Eq/g), followed by the treatment at 60V, which shows the hypothesis of a mild degradation behavior in these parameters. Other studies have reported that the processing conditions are related to the increase in the antioxidant capacity due to the release of certain bioactive compounds such as small peptides (Costa et al., 2018). In addition, it is suggested that the release of anthocyanin from raspberry has also contributed to increase the beverage antioxidant potential. Overall, all treatments showed higher DPPH values when compared to the conventional treatment ($p<0.05$).

Regarding the ferric reducing antioxidant power, the treatments at 10Hz-25V presented similar results, with values of 35.49 ± 0.40; 37.72 ± 0.34; and 34.16 ± 0.12 Trolox Eq/g when compared to the conventional treatment (32.63 ± 0.08 Trolox Eq/g). On the other hand, the mild and two more intense treatments at 45V-60Hz, 80V-60Hz, and 1000Hz-25V presented the higher antioxidant power, with FRAP values of 43.73 ± 0.04; 41.04 ± 1.27; and 46.53 ± 1.07 Trolox Eq/g, respectively. This behavior may be due to the electroporation phenomena releasing the bioactive compounds through the matrix membrane, once previous studies investigated different extraction methods using Ohmic Heating and found an increase in the bioactive compounds levels (Pereira et al., 2016).

Concerning the phenolic compounds, all treatments presented equal or higher level, except for the treatments at 10Hz-25V, and 80V and 60V-60Hz. The treatment at 10Hz, 60V, and 80V exhibited lower phenolic compounds levels, with values of 6.72 ± 0.52; 7.35 ± 0.06; and 6.83 ± 0.07 µg gallic acid /g, respectively, when compared to the conventional treatment (7.78 ± 0.02 µg gallic acid/g) ($p < 0.05$), with the lowest value for the treatment at 80V-60Hz. On the other hand, the treatment at 100Hz-25V presented the higher values (8.02 ± 0.04 µg gallic acid /g) when compared to the conventional treatment (7.78 ± 0.02 µg gallic acid /g). In general, lower TCP levels were observed for the extreme processing condition (80V), while mild treatment at 10Hz exhibited the lowest ones. However, other studies have found an increase in TPC during the Ohmic heating either for pretreatment or extraction, which shows a great potential of its use (Loypimai, Moongngarm, e Chottanom, 2015).

Table 1. Bioactive compounds of whey-flavored raspberry beverages submitted to Ohmic heating and conventional processing

Samples	ANTHOCYANIN	TPC	FRAP	DPPH	ACE	α - amylase	α - glucosidase
	mg/g	**(µg Gallic Acid /g)**	**(µg Trolox Eq/g)**	**(µg Trolox Eq/g)**	**(%)**	**(%)**	**(%)**
Conventional	2.91 ± 0.00^a	7.78 ± 0.02^{ab}	32.63 ± 0.08^d	8.64 ± 0.08^d	59.68 ± 0.34^b	60.28 ± 0.01^b	89.30 ± 0.08^b
10 Hz	2.76 ± 0.34^c	6.72 ± 0.52^d	35.49 ± 0.40^{cd}	8.79 ± 0.55^{bc}	74.46 ± 0.32^{ab}	71.98 ± 0.01^a	99.33 ± 0.01^a
100 Hz	2.77 ± 0.74^c	8.02 ± 0.04^a	37.72 ± 0.34^{bcd}	8.87 ± 0.09^a	80.65 ± 1.43^{ab}	73.52 ± 0.04^a	99.31 ± 0.08^a
1000 Hz	2.78 ± 0.75^c	7.70 ± 0.09^b	46.53 ± 1.07^a	8.76 ± 0.28^c	87.37 ± 1.04^a	70.60 ± 0.01^a	98.38 ± 0.08^a
45 V	2.88 ± 0.74^{ab}	7.78 ± 1.05^{ab}	43.73 ± 0.04^{ab}	8.78 ± 0.07^{bc}	92.47 ± 0.93^{ab}	74.54 ± 0.02^a	99.58 ± 0.07^a
60 V	2.88 ± 0.74^{ab}	7.35 ± 0.06^c	34.16 ± 0.12^d	8.81 ± 0.14^b	75.54 ± 2.12^{ab}	71.58 ± 0.02^a	99.68 ± 0.34^a
80 V	2.86 ± 0.75^b	6.83 ± 0.07^d	41.4 ± 1.27^{abc}	8.69 ± 0.23^d	57.26 ± 1.05^b	70.56 ± 0.01^a	99.74 ± 0.30^a

* Data are expressed as mean ± standard deviation of at least 3 replicates. [a-d] Different letters at the same column indicates significant differences between samples ($p < 0.05$). Profile temperature = 65°C/30 min. The voltage was fixed in 25V for the frequency tests and the frequency held in 60Hz for the voltage ones.

The mild-intermediate conditions presented the best antioxidant activity when compared to the extreme conditions. The electric field plays an important role in explaining the release of bioactive compounds from the cells and the effect on the molecules (peptides) itself by changing their structure (Mercali, Schwartz, Marczak, Tessaro, e Sastry, 2014b). It is shown that increasing the frequencies can cause different responses in polarity of the chemical structure, changing its configuration in the same way as the field direction, which can have an impact on the relaxation time and energy assimilation (Mercali, Schwartz, Marczak, Tessaro, e Sastry, 2014a). Thus, the electric field plays an important role in the cell components behavior during the heating process.

3.1 ACE Inhibition Essay

Regarding the ACE values, all treatments presented higher inhibition when compared to the conventional treatment, and only the treatment at 80V-60Hzexhibited configuration below the conventional value (57.26 ± 1.05 %). Tavares, Contreras, Amorim, Martín-Álvarez, Pintado, Recio, et al. (2011) performed tests in vitro and found some bioactive peptides fragments from α-lactalbumin and whey protein concentrate substrates. The authors reported that protein hydrolysates showed good potential to anti-thrombotic activity within different amino acid sequences and molecule size. Other studies also reported similar findings regarding the hydrolysis of β-lactoglobulin in pumpkins, even though its action mechanism has not been evaluated (Konrad, Anna, Marek, Marta, Aleksandra, e Józefa, 2014). The ohmic heating process in this study seemed to improve the protein hydrolysis, which had an impact on the small peptides breaking them into smaller ones. The electric field promoted by OH (Mercali, Schwartz, Marczak, Tessaro, e Sastry, 2014b) may have affected the protein molecules, by fractioning α-lactalbumin (α-La) and β-lactoglobulin (β -La) that has approximately 14-16KDa (B. Hernández-Ledesma, Recio, e Amigo, 2008), which confers more inhibition potential towards the ACE activity. Even though the interaction with other components, such as sugar and lipids, the effect of the treatment on these molecules should be investigated. Cappato, et al. (2018) reported that the OH conditions (frequency and voltage) led to an increase in ACE inhibition of whey acerola-flavored beverages, with positive effects in the product, probably due to the protein hydrolysis promoted by OH treatment in the small fragments of whey proteins, which conferred the inhibition effect.

3.2 α-amylase and α-glucosidase essay

The whey raspberry drink presented good potential to the inhibition of α-amylase (≥ 70%), and the Ohmic treated samples showed advantages when compared to the conventional process (≥60%). Martinez-Gonzalez, DíAz-SáNchez, De La Rosa, Bustos-Jaimes, and Alvarez-Parrilla (2019) investigated five well-known flavonoids (hesperidin, luteolin, quercetin, catechin, and rutin) with the salivary and pancreatic enzyme using molecular potential surface analyses and reported that catechin did not present a good interaction potential. Despite those findings, the fact that flavonoids lead to an increase in the alpha-amylase inhibitor level, and can be used as a hypoglycemic compound has been elucidated (L. Zhang, et al., 2011). Moreover, the presence of the bioactive compounds in the whey fraction also contributes as a binding component, showing affinity with the enzyme active site. Finally, the alpha-glucosidase levels showed an excellent potential to inhibit the alpha-glucosidase enzyme (>99%) when compared to the conventional process (89%), with an increase of approximately 10%. The voltage treatments at 45, 60, and 80 V at 60Hz showed higher alpha glucosidase levels, with

values of 98.38± 0.08; 99.68± 0.34; 99.74± 0.30 %, respectively, with no differences observed for the ohmic treatments (p>0.05).In vitro studies have shown that certain flavonoids and proanthocyanins presented a higher potential to inhibit the digestive enzymes when compared to acarbose (Lavelli, Sri Harsha, Ferranti, Scarafoni, e Iametti, 2016)., even though the effect within the body may be different since acarbose can be boosted by other interactions with different molecules, with a hypoglycemic effect (Chen, Wu, Zou, e Gao, 2016). For the antihyperglycemic analysis, the bioactive compounds of the beverages showed a great capacity towards the suppression of the enzyme when compared to the acarbose assay, with 69% and 75% inhibition for α-amylase and α-glucosidase, respectively. In addition, the ohmic heating increased in 10% the α-amylase inhibition when compared to the conventional treatment, while a higher inhibition of α-glucosidase was observed for the voltage treatments, which suggests lower effects of the different voltages on the bioactive compounds when compared with the different frequencies and the conventional treatment.

3.3 Fatty Acid Profile

The fatty acids profile of the samples is shown in table 2. Almost all OH treatments showed significant differences in fatty acids profile when compared to the conventional processing (p <0.05). Studies have shown that when the long-chain fatty acids (LCFA) are submitted to certain temperatures, they may experience decarboxylation, therefore changing their molecule structure to a polar one (Kimura e Endo, 2017). Significant differences were observed for myristic (C14:0), palmitoleic (C16:1(ω-7)), oleic (C18:1(ω-9)), linoleic (C18:2(ω-6)) and Linolenic (C18:3(ω-3)) acids, with no differences for all the others (myristoleic(14:1(ω-5), palmitic (C16:0) and stearic (C18:0)) when compared to the standard treatment, probably due to the mild temperature used in this assay (65°C). Both myristic and oleic fatty acids showed a difference in the 45 and 60V-60Hz treatments when compared to the conventional treatment, while linoleic and linolenic acids presented differences along with all ohmic treatments when compared to the conventional treatment, with values of 2.07 ± 0.06 g/100g and 0.95 ± 0.05g/100g, respectively. In general, the ohmic treated samples showed slightly different fatty acid profile when compared to the samples subjected to the pasteurization process, probably due to the changes in fatty acids composition when an electric field is applied.

3.4 Fatty Acid Profile

The fatty acids profile of the samples (released from the milk fat present in the beverage) is shown in table 2. Almost all OH treatments showed significant differences in fatty acids profile when compared to the conventional processing (p <0.05). Significant differences were observed for myristic (C14:0), palmitoleic (C16:1(□-7)), oleic (C18:1(□-9)), linoleic (C18:2(□-6)) and Linolenic (C18:3(□-3)) acids, with no differences for all the others (myristoleic (14:1(□-5), palmitic (C16:0) and stearic (C18:0)) when compared to the standard treatment, probably due to the mild temperature used in this assay (65°C). Both myristic and oleic fatty acids showed a difference in the 45 and 60V-60Hz treatments when compared to the conventional treatment, while linoleic and linolenic acids presented differences along with all ohmic treatments when compared to the conventional treatment, with values of 2.07 ± 0.06 g/100g and 0.95 ± 0.05g/100g, respectively. In general, the ohmic treated samples showed slightly different fatty acid profile when compared to the samples subjected to the pasteurization process, probably due to the changes in fatty acids composition when an electric field is applied.

As for the short-chain fatty acids (SCFA), the butanoic acid stood out, with significant differences only for the treatment at 60V-60Hz (4.55 ± 0.15 g/100g) when compared to the conventional treatment (2.70 ± 0.23 g/100g) (p<0.05). The SCFA plays an important role to reduce the risk of developing some types of cancer (Faraz et al., 2018).

For the medium-chain fatty acids (MFCA), the hexanoic and dodecanoic acids showed the greatest difference when compared to the conventional treatment, with values of 2.33 ± 0.15g/100g and 2.56 ± 0.07 g/100g, respectively, while all the samples subjected to ohmic treatments presented higher values when compared to the conventional treatment. These results suggest that the MFCA profile of the treated beverages depends on the OH processing conditions (voltage or frequency).

Previous study on ohmic heating showed some changes and an increase in the fatty acid profile during the process (Kumari, Mudgal, Viswasrao, e Srivastava, 2016)

No effects of Ohmic heating were observed for the octanoic and dodecanoic acids levels, except for the treatment at 45V-60Hz (2.12 ± 0.02 g/100g), with a significant difference from the conventional treatment. However, all the Ohmic tested conditions showed an increase in those fatty acids, which is a positive result since octanoic acid plays an important role on the stimulation of insulin within the pancreatic β-cells and prevention of diabetes type 2 (Leem, Shim, Cho, e Park, 2018).

The saturated fatty acids (SFA) play an important role in cholesterol metabolism, insulin resistance, and molecular mechanisms within the body, and the SFA levels are associated with obesity-related diseases, including osteoarthritis (Ma et al., 2015; Sekar, Wu, Friis, Crawford, Prasadam, e Xiao, 2018). Higher saturated fatty acids levels were observed for all ohmic treatments (82.09 - 83.24 g/100g) when compared to the conventional treatment (68.82 ± 0.02 g/100g). The higher SFA values of the OH treated samples may be due to the stability of the fatty acid molecule subjected to the Ohmic process.

Regarding the monounsaturated fatty acids (MUFA), all treatments were different from the conventional treatment (28.14 ± 0.30 g/100g), with the lowest level observed for the treatment at 45V-60Hz (13.60 ± 0.48 g/100g). Probably, the electric field may have broken the double bonds, thus changing the molecular structure of MUFA.

The traditional treatment exhibited higher polyunsaturated fatty acids level (3.03 ± 0.08 g/100g), when compared to the treatments at 45 V-60Hz (1.78 ± 0.18 g/100g), 60V (1.68 ± 0.06 g/100g), and 80V-60Hz (1.84 ± 0.20 g/100g). Although some authors have reported that the electric field has no impact on the unsaturated fatty acids, those presenting a double bond seemed to have an alteration on its molecule, which led to a fatty acids degradation (Ariza-Ortega, Ramírez-Moreno, Díaz-Reyes, e Cruz-Cansino, 2014).

With respect to the atherogenic (AI) and thrombogenic indices (TI), significant differences (p < 0.05) were observed for the ohmic treatment, with a two-fold increase for both indexes, when compared to the conventional treatment. Regarding the desired fatty acids (DFA), a decrease in 27% was observed, followed by an increase in 13% hypercholesterolemic saturated fatty acids (HSFA), probably due to the non-thermal application and other components of the food matrix that may had contributed to a conversion and consequently change in the molecule structure, breaking down the double bonds. The best DFA and HSFA levels were observed for the treatments at 100Hz, and 1000Hz, and 10Hz at 25V, respectively. The ohmic treatment resulted in whey beverages

with less favorable indices when compared to the pasteurized beverage, with an increase in AI, TI, and HSFA levels ($p < 0.05$) for all samples. All the findings must be carefully analyzed since free fatty acids contribute to the rancid flavor of dairy products.

Table 2. Fatty acid profile (g/100 g fat) of whey-flavored raspberry beverages 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)	2.70 ± 0.23^b	4.13 ± 0.02^{ab}	3.97 ± 0.02^{ab}	4.39 ± 0.44^{ab}	4.25 ± 0.10^{ab}	4.55 ± 0.15^a	3.93 ± 0.08^{ab}
Butanoic (C4:0)	2.70 ± 0.23^b	4.13 ± 0.02^{ab}	3.97 ± 0.02^{ab}	4.39 ± 0.44^{ab}	4.25 ± 0.10^{ab}	4.55 ± 0.15^a	3.93 ± 0.08^{ab}
Medium-Chain Fatty Acids (MFCA)	9.96 ± 0.35^b	12.42 ± 0.69^{ab}	12.18 ± 0.07^{ab}	14.40 ± 0.59^a	15.90 ± 0.46^b	15.58 ± 0.41^a	13.69 ± 0.95^{ab}
Hexanoic (C6:0)	2.33 ± 0.15^b	3.49 ± 0.14^a	3.50 ± 0.00^a	3.54 ± 0.42^a	3.83 ± 0.32^a	3.82 ± 0.08^a	3.65 ± 0.26^a
Octanoic (C8:0)	1.35 ± 0.10^b	1.68 ± 0.20^{ab}	1.58 ± 0.03^{ab}	1.82 ± 0.08^{ab}	2.12 ± 0.02^a	1.89 ± 0.8^{ab}	1.77 ± 0.11^{ab}
Docanic (C10:0)	3.7 ± 0.20^b	4.32 ± 0.48^{ab}	4.20 ± 0.10^{ab}	5.13 ± 0.12^{ab}	5.78 ± 0.04^a	5.78 ± 0.10^a	4.86 ± 0.42^{ab}
Dodecanoic (C12:0)	2.56 ± 0.07^c	2.91 ± 0.14^{ab}	2.89 ± 0.01^{ab}	3.90 ± 0.14^{ab}	4.14 ± 0.13^a	4.08 ± 0.08^{ab}	3.40 ± 0.15^{ab}
Long-Chain Fatty Acids (LCFA)	87.33 ± 0.16^a	83.44 ± 0.60^{ab}	83.83 ± 0.15^{ab}	81.19 ± 0.36^a	79.84 ± 0.86^a	79.86 ± 0.16^a	82.37 ± 1.00^a
Myristic (C14:0)	9.97 ± 0.05^c	10.83 ± 0.04^{cb}	11.13 ± 0.06^{cb}	12.10 ± 0.26^{abc}	13.33 ± 0.39^{ab}	13.71 ± 0.08^a	11.64 ± 0.62^{abc}
Myristoleic (14:1($\square$-5))	0.45 ± 0.03^a	0.22 ± 0.03^a	0.20 ± 0.05^a	0.32 ± 0.02^a	0.33 ± 0.03^a	0.38 ± 0.02^a	0.28 ± 0.01^a
Palmitic (C16:0)	30.55 ± 0.09^a	36.26 ± 0.43^a	36.13 ± 0.04^a	34.57 ± 0.34^a	34.48 ± 0.21^a	34.39 ± 0.08^a	35.45 ± 0.88^a
Palmitoleic (C16:1($\square$-7))	1.89 ± 0.02^a	0.72 ± 0.07^b	0.88 ± 0.01^{ab}	1.14 ± 0.02^{ab}	0.85 ± 0.07^{ab}	1.05 ± 0.00^{ab}	0.82 ± 0.15^{ab}

Stearic (C18:0)	15.65± 0.10[a]	19.93 ± 0.08[a]	18.66 ± 0.63[a]	16.84 ± 0.47[a]	16.63 ± 0.37[a]	15.63 ± 0.25[a]	18.26 ± 1.11[a]
Oleic (C18:1($\square$-9))	25.78 ± 0.20[a]	13.63 ± 0.23[ab]	14.87 ± 0.34[ab]	14.06 ± 0.35[ab]	12.42 ± 0.52[b]	12.99 ± 0.15[b]	14.03 ± 0.03[ab]
Linoleic (C18:2($\square$-6))	2.07 ± 0.06[a]	1.23 ± 0.08[b]	1.28 ± 0.07[b]	1.40 ± 0.12[ab]	1.18 ± 0.09[b]	1.10 ± 0.06[b]	1.25 ± 0.12[b]
Linolenic (C18:3($\square$-3))	0.95 ± 0.05[a]	0.61 ± 0.01[b]	0.65 ± 0.00[ab]	0.72 ± 0.03[ab]	0.60 ± 0.09[b]	0.58 ± 0.03[b]	0.59 ± 0.08[b]
Saturated Fatty Acid (SFA)	68.82 ± 0.02[d]	83.56 ± 0.06[b]	82.09 ± 0.42[c]	82.33 ± 0.49[c]	84.60 ± 0.66[a]	83.87 ± 0.09[ab]	83.00 ± 0.38[bc]
Monounsaturated Fatty Acid (MUFA)	28.14 ± 0.30[a]	14.58 ± 0.13[d]	15.96 ± 0.35[b]	15.53 ± 0.35[bc]	13.60 ± 0.48[e]	14.43 ± 0.32[d]	15.14 ± 0.18[c]
Polyunsaturated Fatty Acid (PUFA)	3.03 ± 0.08[b]	1.84 ± 0.07[ab]	1.94 ± 0.07[a]	2.13 ± 0.14[ab]	1.78 ± 0.18[a]	1.68 ± 0.06[a]	1.84 ± 0.20[a]
Atherogenic Index (AI)	5.27 ± 0.02[d]	11.63± 0.03[b]	10.71 ± 0.24[a]	10.79 ± 0.31[c]	12.69 ± 0.45[a]	12.18 ± 0.02[a]	11.28 ± 0.05[bc]
Thrombogenic Index (TI)	3.5 ± 0.01[d]	7.68 ± 0.02[a]	6.79 ± 0.29[bc]	6.62 ± 0.29[c]	7.85 ± 0.65[a]	7.42 ± 0.01[a]	7.28 ± 0.53[ab]
desired fatty acids (DFA)	46.63 ± 0.01[a]	36.33 ± 0.01[b]	36.50 ± 0.01[b]	34.44 ± 0.01[d]	32.05 ± 0.01[e]	31.62 ± 0.01[f]	35.23 ± 0.00[c]
hypercholesterolemic saturated fatty acids (HSFA)	43.27± 0.02[f]	49.82 ± 0.02[d]	50.16 ± 0.02[cd]	50.52 ± 0.02[c]	51.80 ± 0.02[b]	52.29 ± 0.02[a]	50.40 ± 0.00[c]

*Values are expressed as mean ± standard deviation. Analysis performed in triplicate. [a-f] Means with different lowercase superscripts in the same row indicate presence of statistical difference ($P < 0.05$) among treatments (OH and conventional pasteurization) 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)] DFA = MUFA + PUFA + C18:0 HSFA = C12:0 + C14:0 + C16:0.

3.5 Volatile compounds

Table 3 shows the results of the volatile compounds of the samples. Overall, OH treated samples exhibited more diverse compounds when compared to the conventional processing. The treatments at 1000Hz-25V, followed by 80V and 45V-60Hz, presented the higher number of compounds, being 33, 31, and 29, respectively, and alcohols, ester, and carboxylic acid which represented 50% of the total volatile profiling. Regarding the esters, only the hexanoic acid ethyl ester was found in the conventional treatment. Although furan derivatives are found in berries, conferring a strong flavor to the fruits (Song, Hong, Zhao, Liu, Schulenburg, Huang, et al., 2016), in this study, only the compounds 2(5H)-furanone, methyl α-furoate, 2(3H)-furanone, dihydro-4-hydroxy-,2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one were detected in the treatments at 1000Hz and the voltage settings 45, 60, and 80 V-60Hz, which demonstrates a tendency of these treatments to promote the formation and preservation of these compounds.

The ether component Ethyl-1-propenyl ether was found only in the treatment at 80V, thus suggesting that the higher voltage may have favored the formation of these compounds.

Concerning the alcohols, acetol was detected in the samples subjected to 1000Hz-25V and different voltages. Although β-Butoxyethanol was not observed at 1000Hz-25V, 45, and 60V-60Hz, it was detected in the other treatments. The compounds isomaltol, 2-furan methanol, maltol, and 2-hydroxy-gamma-butyrolactone were not observed in both the standard treatment and those subjected to different frequencies. Either alcohols and esters represent a great part of the VOC´s in the beverage, once they come from the raspberry used to manufacture the beverage (Aprea, Biasioli, e Gasperi, 2015), thus these compounds are extremally important for their health benefits and sensory profile.

In relation to the carboxylic acids, most of them i.e. (acetic acid, butanoic acid, hexanoic acid, and octanoic acid) were present in all treatments.

Table 3. Volatiles compounds of whey-flavored raspberry beverages submitted to Ohmic heating and conventional processing

Groups	Compounds	LRI*	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		**12**	**13**	**12**	**33**	**29**	**25**	**31**
Ester	Hexanoic acid. methyl ester	1168	-	-	31631	-	-	-	-
	Hexanoic acid. ethyl ester	1213	25239	28625	-	37198	1824547	36869	52519
	2(5H)-Furanone	1747	-	-	-	63180	56659	-	32598
	Methyl α-furoate	1993	-	-	-	248767	-	-	42943
	2(3H)-Furanone, dihydro-4-hydroxy-	2576	-	-	-	569588	2214171	597874	282626
	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1518	-	-	-	209347	-	-	-
	Total of Eters		**1**	**1**	**1**	**5**	**3**	**2**	**4**
	Ethyl-1-propenyl ether	2154	-	-	-	-	-	-	50638
	Total of Eters		**0**	**0**	**0**	**0**	**0**	**0**	**1**
Alcohols	2-Heptanol	1313	137985	136911	122562	141622	167645	23942	135580
	Acetol	1320	-	-	-	66407	132192	94830	125405
	β-Butoxyethanol	1393	21582	26471	41859	-	-	-	47399
	1-Hexanol, 2-ethyl-	1476	146517	93663	50543	83279	98412	68613	38128
	Linalool	1534	39599	88015	-	62751	84673	80805	-
	Isomaltol	1610	-	-	-	47010	11784	-	20110'
	2-Furanmethanol	1644	-	-	-	54759	20039	44883	12294
	Maltol	1951	-	-	-	36798	24961	-	55545
	2-Hydroxy-gamma-butyrolactone	2155	-	-	-	55213	14192	-	-

	Total of Alcohols		**4**	**4**	**3**	**8**	**8**	**5**	**7**
Carboxylic acids	Acetic acid	1442	667999	1030032	847012	19228564	3774837	3262974	2670944
	Butanoic acid	1616	206590	-	62751	84673	80805	-	
	Hexanoic acid	1830	439148	390750	410863	655665	654215	586394	492938
	Octanoic acid	2042	168061	164581	170114	739148	1038569	490418	403420
	Decanoic acid	2250	-	-	-	237914	241769	192375	168319
	Benzoic acid	2409	-	-	-	501823	824795	275854	245233
	Formic acid	1502				741841	1605423	448932	829983
	Total of Carboxylic acids		**4**	**3**	**4**	**7**	**7**	**6**	**6**
Aldehydes	Furfural	1455	-	-	-	3599115	2301151	410810	702209
	Benzaldehyde	1515	84129	70899	49755	127703	-	132941	164234
	5-Hydroxymethylfurfural	2480	-	-	-	28097315	16998177	6052565	5329611
	2-Furancarboxaldehyde, 5-methyl-	1565				493918	310281	123450	129108
	2,5-Furandicarboxaldehyde	1967				459933	450604	250718	209204
	Total of Aldehydes		**1**	**1**	**1**	**5**	**4**	**5**	**5**
Ketones	Dihydroxyacetone	2068	-	-	-	342942	222545	20682	231568
	Acetoin	1279		31447	29520	-	-	-	-
	Total of Ketones		**0**	**1**	**1**	**1**	**1**	**1**	**1**
Terpenes	**Limonene**	**1172**	**-**	**-**	**-**	**-**	**-**	**20070**	**-**
	o-Cymene	1253	-	74327	51997	78323	74167	76704	60714
	α-Phellandrene	1133	26105	26057	-	32030	52301	68872	30887
	β-Phellandrene	1172	-	-	-	-	-	20070	-
	Total of Terpenes		**1**	**2**	**1**	**2**	**2**	**4**	**2**
Pyrans	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	2247	-	-	-	4785060	4048559	1373778	1512577

	2H-Pyran-2,6(3H)-dione	1976	-	-	-	174891	-	-	42943
	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	2267	-	-	-	109802	95185	-	32198
	Total of Pyrans		**0**	**0**	**0**	**3**	**2**	**1**	**3**
Other	2-Cyclopenten-1-one, 2-hydroxy-	1756	-	-	-	66407	95726	-	47942
	Total of others		**1**	**1**	**1**	**2**	**2**	**1**	**2**

*LRI – Linear Retention Index

As reported by other authors, these compounds have good thermal and electrical stability (Maya, Benavente, e De Abajo, 2012). On the other hand, some compounds, including decanoic acid, benzoic acid, and formic acid were observed only in the voltage treatments, which suggests that the voltage can affect in some extent the formation of these compounds.

Regarding the aldehydes, the furfural and 5-hydroxymethylfurfural, one of the major intermediate products in the Maillard reaction, were found in all the voltage treatments. Besides the Maillard and caramelization reactions, these compounds are also formed during heating processes (Chávez-Servín, de La Torre Carbot, García-Gasca, Castellote, e López-Sabater, 2015; Kocadağlı e Gökmen, 2016). The results demonstrated that the voltage condition had an impact on the formation of these compounds, either by heating or other reactions (Maillard or caramelization). Concerning the Ketones, dihydroxyacetone presented a similar profile of aldehydes, which was not found in the standard sample and those subjected to 10 and 100Hz; however, they were detected in all the voltage treatments.

The terpene o-cymene were found only in the ohmic treatments, probably due to the electric field applied to the process. Limonene was observed only in the treatment at 60V-60Hz, which suggests the conversion of o-Cymene into this molecule. Terpenes can be easily found in raspberry fruits (Aprea, Biasioli, e Gasperi, 2015). Pyrans (4H-Pyran-4-one, 2,3-dihydro-3,5; 2H-Pyran-2,6(3H)-dione-dihydroxy-6-methyl and and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl) were also present only in the treatment at 1000Hz-25V and the voltage settings.

4   Conclusion

Ohmic Heating had an impact on the concentrations and availability of the bioactive compounds in the whey-flavored raspberry beverage, with an increase in these compounds, as shown by the antioxidant capacity assays (DPPH, FRAP, and TPC), as well as the ACE inhibition. An increase in 10 % of the antihyperglycemic inhibition was also observed, probably due to the compound release favored by the electric field. The anthocyanins seemed to have a better response within the voltage treated samples, with no significant differences when compared to the conventional treatment. In contrast, the frequency treatments showed a negative effect on anthocyanins, decreasing its levels. Changes in the fatty acids profile were observed in the OH treated samples, with less favorable results for the AI and TI indices and DFA and HSFA levels, when compared to the conventional treatment, thus further studies are required to better understand the modifications within its compounds.

In summary, OH showed a great potential to be used in the whey-flavored raspberry beverage processing, representing a prominent technology. That can aid the industry to establish the OH equipment when manufacturing whey products.

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CAPÍTULO III: **RASPBERRY-FLAVORED WHEY DRINK SUBMITTED OHMIC HEATING PROCESSING: IMPLICATIONS ON THE PHYSICAL AND THERMAL PARAMETERS, MICROSTRUCTURE AND WATER MOBILITY BY TIME DOMAIN NUCLEAR MAGNETIC RESONANCE**

Raspberry-flavored whey drink submitted ohmic heating processing: Implications on the physical and thermal parameters, microstructure and water mobility by time domain nuclear magnetic resonance

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1 Abstract

This study evaluated the effect ohmic heating (OH) on physical (color, rheology, particle size diameter), thermal (differential scanning calorimetry, DSC), physico-chemical (time domain nuclear magnetic resonance) and microstructural (optical microscopy) aspects of a raspberry-flavored whey drink. The samples were submitted to ohmic systems: (10,100,1000 Hz - 25V; 45,60,80 V - 60Hz) until 65°C-30 min and a conventional pasteurization (65°C-30 min). Both processing led to increases in the color parameters (c*, h° and ΔE*) in the first 30 minutes. For the treatments 10 Hz-25V and 1000 Hz-25V, more viscous, and consequently increased D [4,3]) (D [3,2]) values were observed, and similar results were obtained for the DSC behavior. The microstructure confirmed aggregation of cell structure in those configurations (10Hz and 1000Hz-25V). This study showed that the ohmic heating parameters for both ohmic configurations have an impact in all the evaluated parameters (color, rheology, PSD, DSC, and TDN-MR) for raspberry-flavored whey drink. The findings can aid the industry in manufacturing high quality whey beverages through ohmic heating.

Keywords: Ohmic heating; whey dairy beverages; physical aspects; microstructure; Time domain nuclear magnetic resonance, thermal aspects

2   Introduction

Emerging Technologies (ET) have been studied to complement or substitute the conventional treatments in the food and beverage industries, with the potential in preserving better sensorial and nutritional attributes in the products. (Jermann, Koutchma, Margas, Leadley, e Ros-Polski, 2015; Li, Chen, Zhang, e Fu, 2017). These procedures have also demonstrated great potential in controlling pathogenic and spoilage microorganisms, compounds extraction and homogenization procedure (Cappato et al., 2017; HANSEN, et al., 2017).

The Ohmic heating (OH) shows as an excellent alternative to the conventional processing (CP), since the heat is dissipated within the food and not by convection or conduction as in the CH. This process is operated due to the electrical current being applied directly to the food (Cappato et al., 2017; Kaur e Singh, 2016) and has been successfully applied to functional foods enriched with bioactive compounds e.g. (calcium, vitamins, and selenium). The OH shows potential to combined effects with other technologies e.g. (cold plasma, ultrasound etc. (Moreno, et al, 2017), where the electric field plays an important role in determining the food behavior during OH processing (Mercali, Schwartz, Marczak, Tessaro, e Sastry, 2014b).

The whey dairy beverage market had slight growth over the last few years, which was responsible for 40 Billion in 2014 and there is an increasing consumer demand for whey-based beverages (Brockman, 2014). Raspberry is a popular fruit among consumers due to their nutritional and potential therapeutic effects Further, food applications of raspberry is convenient and economical in new product development at the industry level (Teng et al., 2017). This scenario leads to an interest in investigating the rheological behavior and other OH processing aspects since many of these emerging technologies, including OH still face challenges in adopting advanced processing procedures at the industry level (Sun, 2014).

The current study evaluated the effect of different OH parameters (electric field frequency and voltage gradient) on rheological and other physical properties of a raspberry-flavored whey drink compared to CP. For comparative purposes, the raspberry-flavored whey drink was submitted with same temperature profiles (65 °C/30 min) for OH and CP.

3   Materials and Methods
3.1   2.1 Raspberry-flavored whey drink processing

The whey drink was prepared according to Cappato et al. (2018), where 70-30 % of the dairy mix (pasteurized milk + sweet whey), 9.9% w/w crystal sugar, and 0.1% w/v xanthan gum were included to produce the first mix. After complete dissolution, 30% w/v of the raspberry pulp (Mais Fruta Company, Jarinu, SP, Brazil) was mixed with the above preparation. All samples were stored at 5°C until processing. Two ohmic systems were used to process the samples, one with frequency variation, by fixing the voltage (10,100,1000 Hz - 25V) and a second with voltage variation with a fixed frequency (45,60,80 V - 60Hz) (Mercali, Schwartz, Marczak, Tessaro, e Sastry, 2014b; Cappato et al., 2018). Ohmic and conventional processes were performed using the same temperature profile (Figure 1).
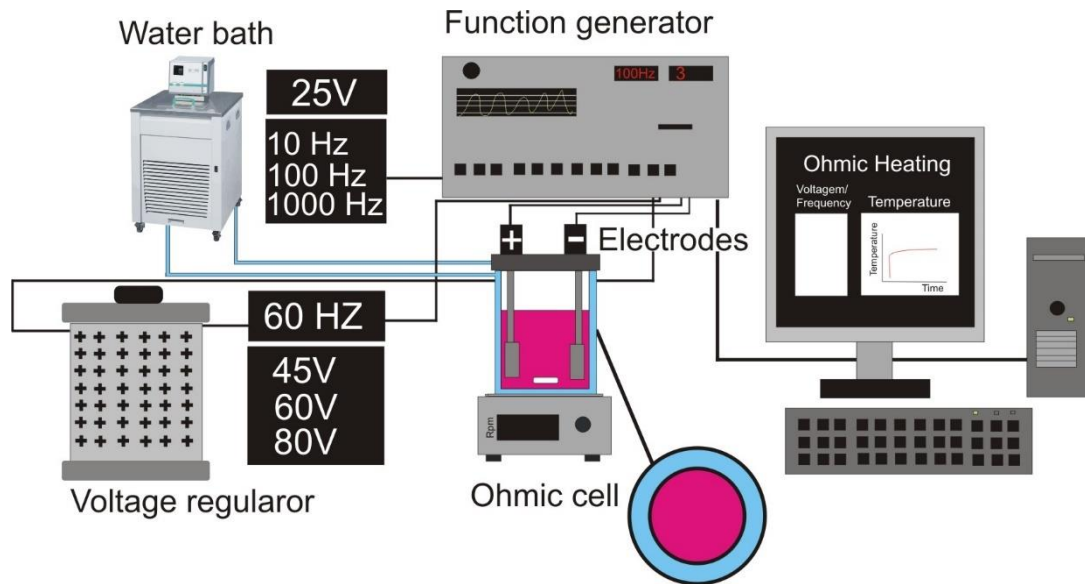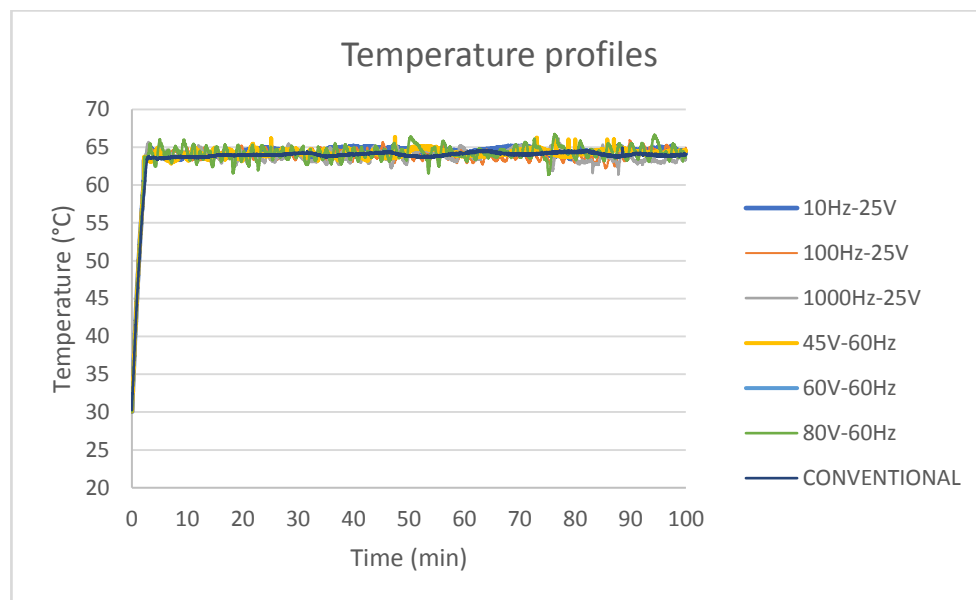
Figure 1. Ohmic heating Set-up.

Figure 2. Time vs temperature profiles of conventional and ohmic heating processing.

The apparatus shown in Figure 2, consists of power supply, a variable transformer and a stabilizer using a computer to store and monitor the data. The voltage was measured using current transducers while the temperature was monitored within two sensors (Novus, model pt-100, Brazil). The Ohmic cell could fill 400mL and the electrodes were made of platinum. As an important part the electrodes cylindric shaped (5cm height), the distance between the electrodes were 7.5 and 5.7, which the former was the maximum distance and the later the minimum due to its shape. The sample was stirred using a magnetic stirrer in order to homogenize the whey drink. The results were expressed in voltage and not in electric field (V/cm) due to the snag of the electrodes not being uniform, which would make it difficult to elucidate the parameters.

3.2 Color parameters

The color parameters a* (red-green), b* (blue-yellow), and L* (brightness) of the CIELAB scale were evaluated and used to generate the hue angle (°h), chroma (C*), and color variation (ΔE*) using the Eq. 1,2 and 3. The Colorimetric analyses were performed using a ColorQuest XE (HunterLab) colorimeter equipped with a D65 illuminant and 10° viewing angle with specular reflectance. Time zero was set as the initial one, once the temperature reached 65 °C.

$$h° = arctan(b*/a*) \tag{I}$$

$$C* = \sqrt{(a*)^2 + (b*)^2} \tag{II}$$

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

Where

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

3.3 Rheological parameters

The apparent viscosity of the beverages was determined according to Cheong et al. (2015) by using a controlled-stress rotational Brookfield Rheometer (USA). For the deamination of the flow curves rates, which ranged from 10 to 250 rpm, all measurements were performed by upward curves employing Rheocalc software for data acquisition. The power law choice of the model used was determined after considering the highest regression value of $R^2 \geq 0.98$.

3.4 Particle Size Distribution (PSD)

Particle size distribution analysis was determined as per Coutinho et al. (2019) using a Laser Diffraction Particle Size Analyzer (MasterSizer 2000, Malvern Instrument Ltd., Worcestershire UK). Particle size calculations were based on the Mie-Scattering theory, where the $D_{[4,3]}$ value represents the mean diameter over volume i.e. (DeBroukere mean Eq. 4) whereas the $D_{[3,2]}$ comprehend the surface mean diameter i.e (Sauter mean Eq. 5). The span was also calculated laser diffraction results. Eq. 6 The percentage of a volume corresponding to each observed population and the Calculations were done with the software provided with the equipment (Mastersizer 2000 V. 5.40).

$$D_{[4,3]} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \tag{IV}$$

$$D_{[3,2]} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \tag{V}$$

$$Span = \frac{D_{v0.9} - D_{v0.1}}{D_{v0.5}} \tag{VI}$$

where, d_i is the mean particle diameter, and n_i is the number of particles. The span represents particle diameter corresponding to 10%, 50%, and 90% of the cumulative distribution.

3.5 Microstructure

All the samples subjected to ohmic heating and conventional processing were evaluated using optical microscopy. For that 20 µL of raspberry-flavored drink was placed in a glass slide and was sent to optical microscope analysis (Olympus, BX41, Japan) connect with a camera (Coutinho et al., 2019).

3.6 Differential scanning calorimetry analysis (DSC)

The DSC analysis was performed according to Kavaz Yuksel (2015), with some modifications. The melting and crystallization behavior were estimated by DSC with the use of PYRIS Diamond DSC equipment (Diamond, Perkin–Elmer, Norwalk, PA), with an intercooler attached and controlled by Pyris Manager software. Calibration was performed using mercury and indium as a standard. Therefore, 15 mg of the sample were placed in aluminum pans (50 µL, Perkin–Elmer). The whey drinks 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.

3.7 Time domain nuclear magnetic resonance (TDN-MR)

The TD-NMR test used a spectrometer (23.4 Mhz 7.5µL and 90° pulse length) (MARAN Ultra o.54 T). The samples were carried out using a transversal relaxation time (T2) and Carr-Purcell-Meiboom-Gill (CPMG) (4096 echoes, 600 µs gap). In which all the samples passed through a number of 32 scans, having its time held for 4 s. Laplace Inverse transformation was used in the exponential decay curves from the samples, in order to assay the distribution curve of related domains.

3.8 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.

4 Results and Discussion
4.1 Color parameters

Color parameters values for (c*, h° and ΔE*) during 100 min of OH and CP are shown in Figure 3. The experimental error was suppressed to better show the curve response. Table 1 shows the same parameters (c*, h° and ΔE*) after the OH and CP (65°C/30min). Both treatments (OH and CP) led to increase in the parameters (c*, h° and ΔE*) in the first 30 minutes, however, the curves show different behavior after the half-hour period. The results showed a decrease in the c* (less saturation), however, it showed an increase in reddish color (h°) and higher color variation (ΔE*). Comparing the OH treatments the 80V-60Hz showed the highest variation in color (ΔE*) 9.85 ± 0.11 and reddish color (h°) 0.536 ± 0.07. The 100Hz-25V did not differ to the conventional for c* 15.39 ± 0.05 and h° 0.157 ±0.01 (P>0.05), which suggests that the 1000Hz-25v treatment preserved the color for the two parameters (c* and h°). A study on the effect of Ohmic

heating on the stability of fungal red pigment demonstrated a higher impact in color degradation when compared to the conventional processing (Aguilar-Machado et al., 2017). It is suggested form several studies that over time the OH changes the color of pigments present in fruits (R. Kaur, Gul, e Singh, 2016), and at low frequencies (10Hz) the color changes were more intense, due to electrochemical reactions (Mercali, Schwartz, Marczak, Tessaro, e Sastry, 2014a).
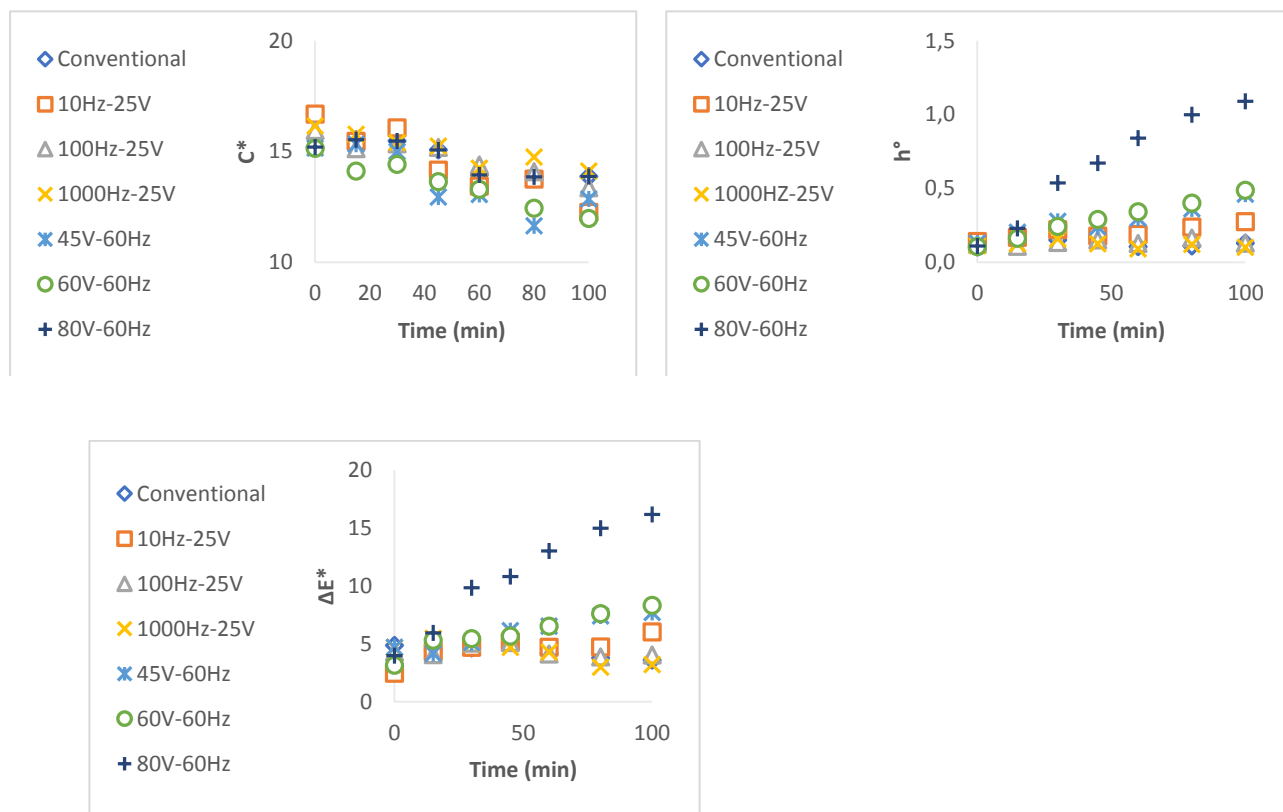

Figure 3. Color parameters of raspberry-flavored whey drink during ohmic heating and conventional processing.

Table 1. Color parameters of whey-flavored raspberry beverages submitted to Ohmic heating

Samples	C*	h*	ΔE
Conventional	15.47 ± 0.01^b	0.152 ± 0.01^e	4.65 ± 0.28^d
10Hz	16.050 ± 0.00^a	0.219 ± 0.01^d	4.70 ± 0.14^d
100Hz	15.360 ± 0.09^b	0.133 ± 0.02^f	5.06 ± 0.42^c
1000Hz	15.39 ± 0.05^b	0.157 ± 0.01^e	5.03 ± 0.42^c
45V	14.99 ± 0.13^c	0.279 ± 0.02^b	5.09 ± 0.02^c
60V	14.40 ± 0.04^d	0.245 ± 0.04^c	5.46 ± 0.08^b
80V	14.68 ± 0.01^c	0.536 ± 0.07^a	9.85 ± 0.11^a

*Data are expressed as mean $\pm$ standard deviation of at least 3 replicates. [a-f] Different letters at the same column indicates significant differences between samples (p < 0.05). Conventional Processing =65 °C/30 min.

In milk, the hydroxymethylfurfural from the Maillard reaction is responsible for the reduction in whiteness (L*) and an increase in (a*) and (b*) even though the change does not lead to a visible alteration (Parmar, Singh, Meena, Borad, e Raju, 2018). Alternatively, other studies in dairy beverages showed that minimum alterations in color are sensorially noticeable by consumers (Arancibia, Costell, e Bayarri, 2011), which shows the importance of studying this parameter in an industry context.

4.2 Rheological analysis

From the rheological behavior results (Table 2) it was possible to observe that for the consistency indexes (K), the samples treated with OH were superior to the conventional treatment (4.74 ± 0.03 mPa.s^n) (p<0.05), where the 10Hz-25V and 1000Hz-25V showed the best values (7.73 ± 0.06 mPa.s^n and 7.70 ± 0.07 mPa.s^n) respectively, indicating an increase in the viscosity, except for the samples treated with 60 V- 60Hz (3.00 ± 0.02 mPa.s^n). In addition, all the samples showed a pseudoplastic behavior, which was indicated by the flow behavior (n<1). Based on the data most of the OH treated samples showed an increase in the viscosity compared to the CP, where the 10Hz and 1000Hz showed the greatest viscosity levels. This behavior can be explained by the microstructure (Figure 5) and particle size (Table 3), where the two configurations 10Hz-25V and 1000Hz-25V showed bigger particle sizes and aggregation of cells in the microstructure images Figure 5.

Table 2. Rheological parameters of whey-flavored raspberry beverage submitted to Ohmic heating

Samples	k (mPa.s^n)		n			R^2
Conventional	4.74	$\pm$ 0.03[d]	0.953	$\pm$	0.001[ab]	0.9999
10 Hz-25V	7.73	$\pm$ 0.06[a]	0.922	$\pm$	0.002[b]	0.9997
100 Hz-25V	4.69	$\pm$ 0.24[d]	0.811	$\pm$	0.006[c]	0.9970
1000 Hz-25V	7.70	$\pm$ 0.07[a]	0.963	$\pm$	0.003[a]	0.9996
45 V-60Hz	5.80	$\pm$ 0.04[b]	0.929	$\pm$	0.001[ab]	0.9999
60 V-60Hz	3.00	$\pm$ 0.02[e]	0.926	$\pm$	0.001[ab]	0.9999
80 V-60Hz	5.46	$\pm$ 0.04[c]	0.943	$\pm$	0.002[ab]	0.9999

*Data are expressed as mean $\pm$ standard deviation of at least 3 replicates. [a-f] Different letters at the same column indicates significant differences between samples ($p < 0.05$). Rheological parameters were obtained by Power law model at 10 °C. k=consistency index; n = flow behavior index. Profile temperature= 65 °C/30 min. Conventional Processing =65 °C/30 min.

The model used in describing the rheological behavior in liquid dairy beverages is complex; therefore, it requires attention (Kelleher, O'Mahony, Kelly, O'Callaghan, e McCarthy, 2018). Even though studies can be found regarding dairy drinks and its rheological properties correlating casein and amount of whey (Castro et al., 2013), which can suggest that the change on those structures may occur due to heat treatment. Even though it is still lacking the impact of OH in the change of the rheological behavior, the study by Cappato et al. (2018) showed an impact of OH on the rheological behavior for whey acerola-flavored drink, which was shown the greatest impact on 1000Hz and 80V treatments. Since the Rheological behavior is also sensorially evidenced by consumers (Arancibia et al., 2011), the findings highlight the importance of better understand the parameters for the OH settings.

4.3 Particle Size Distribution (PSD)

The particle size results are shown in Table 3 and Figure 4. For the D [4,3] values ranged from 160.7 ± 4.30c to 235.1 ± 5.30, where all the frequency (45V-60Hz, 60V-60Hz, 80V-60Hz) and 100Hz-25V OH treaded samples were statistically similar and did not differ from the CP (172.3 ± 6.20) (P>0.05).

Table 3. Particle Size parameters (D $_{[4,3]}$ D $_{[3,2]}$) of whey-flavored raspberry beverage submitted to Ohmic heating and conventional processing.

Samples	Particle Size Parameters		
	D $_{[4,3]}$ (µm)	D $_{[3,2]}$ (µm)	Span (µm)
Conventional	172.3 ± 6.20[c]	29.4 ± 1.00[cd]	4.18 ± 0.05[c]
10 Hz-25V	235.1 ± 5.30[a]	43.4 ± 2.00[a]	3.40 ± 0.20[e]
100 Hz-25V	161.7 ± 6.50[c]	26.7 ± 0.80[de]	4.26 ± 0.01[bc]
1000 Hz-25V	195.7 ± 11.4[b]	33.9 ± 1.00[b]	3.75 ± 0.08[d]
45 V-60Hz	174.6 ± 3.00[c]	30.2 ± 0.40[c]	4.08 ± 0.09[c]
60 V-60Hz	162.9 ± 4.10[c]	26.0 ± 0.20[e]	4.44 ± 0.10[ab]
80 V-60Hz	160.7 ± 4.30[c]	24.8 ± 0.50[e]	4.53 ± 0.01[a]

* Data are expressed as mean ± standard deviation of at least 3 replicates. [a-e] Different letters at the same column indicates significant differences between samples ($p < 0.05$) by Fisher test. Conventional Processing = 65°C/30 min.

The treatments that differed from the CP were the 10Hz-25V and 1000Hz-25V, showing bigger particles 235.1 ± 5.30 and 195.7 ± 11.4, respectively (P<0.05). The D [3,2] values did not follow the D [4,3] pattern, the 60 V-60Hz and 80 V-60Hz showed the lowest surface mean 26.0 ± 0.20 and 24.8 ± 0.50 respectively, while the 10 Hz-25V 1000 Hz-25V showed the biggest 3.40 ± 0.20 and 3.75 ± 0.08 respectively (Figure 4), all the 4 treatments differed from the conventional (29.4 ± 1.00) (P<0.05). The 100Hz-25V and 45V-60Hz did not differ from the conventional treatment (P<0.05). The electric field in certain OH configurations possibly affected the small structures (cells and other particles) i.e. (10Hz-25V and 1000HZ-25). Both treatments (10Hz and 1000Hz) showed bigger values for D $_{[4,3]}$ and D $_{[3,2]}$, which suggest that the non-thermal effect had an impact on how the structures were rearranged forming an aggregation. Other studies found a different outcome for a gel-like emulsion of lactoferrin. They found that the electric field promoted less aggregation in the OH treated samples possibly due to the hydrophobic portions of the molecules (De Figueiredo Furtado, Pereira, Vicente, e Cunha, 2018). However, a study testing OH in sweet whey found bigger values for D $_{[4,3]}$ and D $_{[3,2]}$ (Costa et al., 2018) that closely aligns with the findings in the present study. Thus, it is suggested that the electric field does have an impact on the particle size distribution either in the whey protein itself or in the fruit base added to the drink.
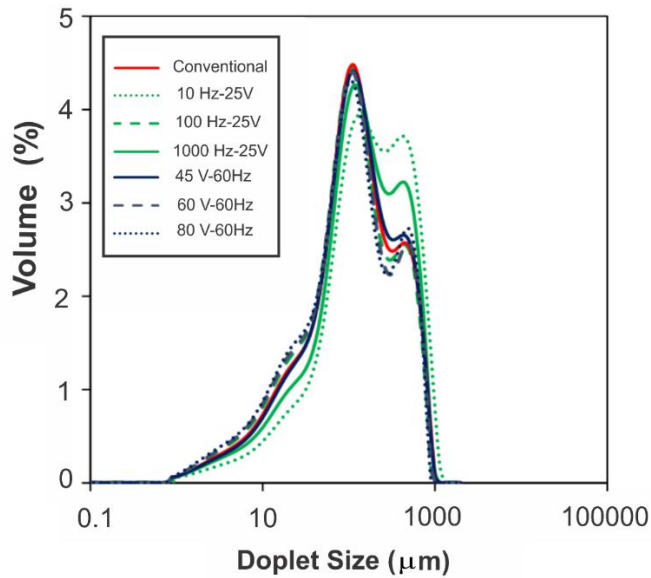
Figure 4. Particle Size distribution of raspberry-flavored whey drink submitted to ohmic heating and conventional processing.

4.4 Differential scanning calorimetry analysis (DSC)

Regarding the DSC, the enthalpies ($\Delta H°$) and peak for crystallization and melting temperatures (T, °C) are shown in Table 4. The negatives values are associated with the crystallization processes (exothermic behavior), whereas positives responses are correlated to the Fusion process (endothermic). For the crystallization all the voltage OH treated and the 10Hz-25V differed from the conventional (p<0.05). On the other hand, the 100Hz-25V ($\Delta H° = -190.4 \pm 0.59$) and 1000Hz-25V ($\Delta H° = -190.4 \pm 0.83$) did not differ from the conventional treatment ($\Delta H° = -192 \pm 0.21$). The results showed that within the 100Hz and 1000Hz-25V treated samples more heat is released and therefore, proving to be more exothermic. The fusion or melting behavior of all treatments differed from the conventional process ($\Delta H° = 110 \pm 0.57$), where the 80V-60Hz ($\Delta H° = 52.39 \pm 0.86$) presented the lowest temperature peak and consequently enthalpy.

Table 4. Differential Scanning parameters (Enthalpies and peak temperatures, $\Delta H°$, T°C, respectively) of whey-flavored raspberry beverages submitted to Ohmic heating and conventional processing

	Crystalization		Fusion	
Samples	**T °C**	**ΔH°**	**T °C**	**ΔH°**
Conventional	-10.57 ± 0.33	-192 ± 0.21[a]	4.67 ± 0.08	110.5 ± 0.57[c]
10 Hz - 25V	-14.31 ± 0.05	-97 ± 0.79[e]	4.49 ± 0.03	137.3 ± 0.95[b]
100 Hz - 25V	-9.81 ± 0.04	-190.4 ± 0.59[a]	4.93 ± 0.01	91.68 ± 0.96[e]
1000 Hz - 25V	-8.53 ± 0.45	-190.4 ± 0.83[a]	3.82 ± 0.09	87.72 ± 0.38[f]
45 V - 60Hz	-19.35 ± 0.07	-167.5 ± 0.70[d]	4.83 ± 0.08	223.5 ± 0.70[a]
60 V - 60Hz	-7.30 ± 0.14	-177.5 ± 0.70[b]	5.01 ± 0.28	97.5 ± 0.70[d]
80 V- 60Hz	-11.60 ± 0.50	-171.2 ± 1.05[c]	3.08 ± 0.01	52.39 ± 0.86[g]

* Data are expressed as mean $\pm$ standard deviation of at least 3 replicates. [a-g] Different letters at the same column indicates significant differences between samples ($p < 0.05$) by Fisher test. Conventional Processing = 65°C/30 min.

The crystallization and fusion properties are important to understand not only the milk but other major components of the dairy drink; therefore (DSC) is an important analysis. Further, it is fast and non-invasive and hence helps to maintain the identity of the product (Farah, Silva, Cruz, e Calado, 2018). Since they can predict changes in the physicochemical properties of food throughout the non-thermal processing, it is an excellent choice to observe modifications in protein, lipids and other components within the product. (Parniakov et al., 2018). In this scenario, DSC is an interesting alternative to better evaluate the impact of the OH and other emerging technologies regarding food composition.
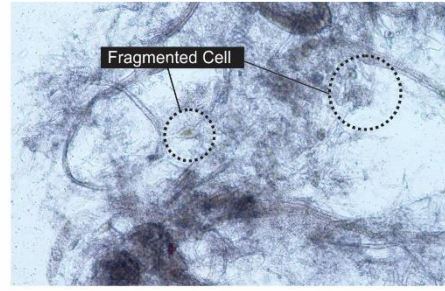
4.5 Microstructure

The effect of the Ohmic heating on the microstructure of the raspberry-flavored whey beverage is shown in Figure 5. The components presented in the images constitutes of raspberry pulp, whey, and milk components. In the plant phase (pulp) the plant cells and other plant components were present. The plant phase was responsible for the reddish color (Table 1) due to the anthocyanin pigments. The microscopy can provide substantial support in understanding other parameters, such as rheology and particle size. Overall the microscopy images show cell aggregation in the 10Hz-25V and 1000Hz-25V treatments. Different behavior is shown for all the voltage treated samples (45,60,80 V-60Hz) that showed more fragmented cells. The increase in the viscosity, as well as, in the diameter of particle size can be explained observing the aggregation in the 10Hz-25V and 1000Hz-25V. The images obtained from this study showed that 1000Hz-25V and 10Hz-25V OH treated samples have formed bigger particles, yet some cell fragmentation was also observed. However, other modifications were also noticed for other configurations (45, 60 and 80V-60Hz) such as more fragmented cells and more damage to other particles. Similar findings were observed in a previous study on whey beverage, where larger structure was observed with intense frequencies (Costa et al., 2018). Another study also found the creation of protein aggregates with high electric field (Pereira et al., 2016). Overall image analysis seemed to be a good method to distinguish micro-components in dairy systems (Silva, Legland, Cauty, Kolotuev, e Floury, 2015). In the present study, relevant structures were useful to confirm other physical parameters of interest. However, more studies are necessary to better understand the alteration on the complex molecules and cells in dairy-based products, such as whey beverages.
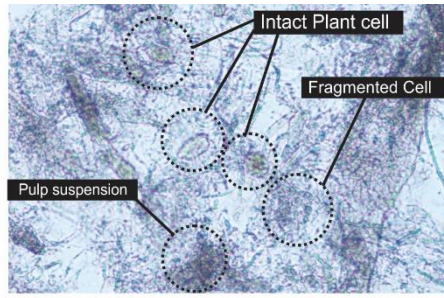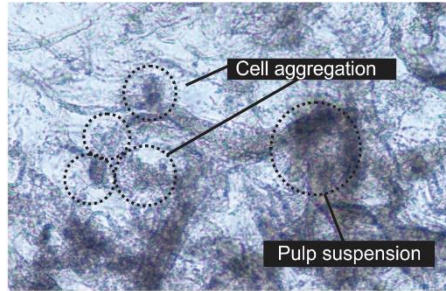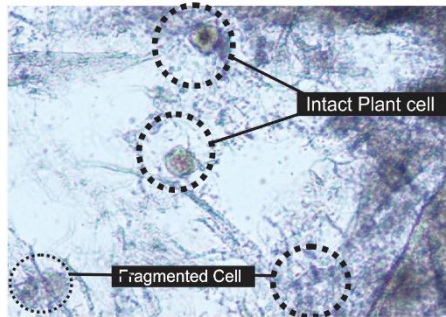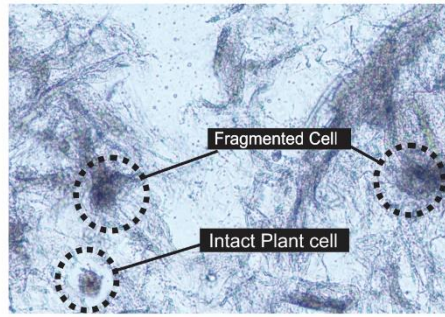
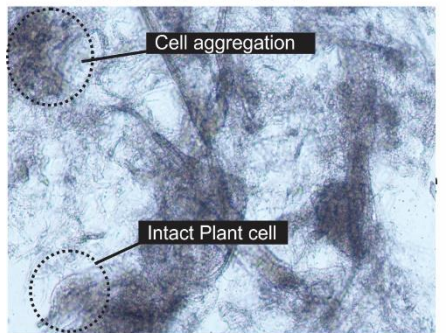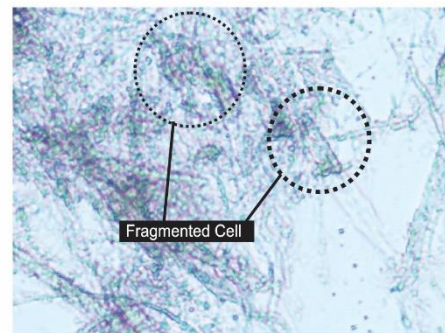**Figure 5.** Microstructures of Whey-raspberry flavored drink during Ohmic Heating and conventional processing (optical microscopy, 10× objective).

4.6 Time domain nuclear magnetic resonance (TDN-MR)

The TDN-MR (Figure 6) shows two different regions, where the first $T_{(2.1)}$ comprehend 0.1 -100ms and represent the protons and water molecules that may be associated to sugar molecules structure linked by hydrogen bond. The second domain $T_{(2.2)}$, 100-700ms represents the hydrogen and free water molecules that are related to the larger relaxation time. In liquid samples, the $T_{(2.2)}$ is driven by the system viscosity being this variable inversely proportional. Among the OH treated samples (Figure 6), the

1000Hz-25V showed lesser relaxation time, which demonstrate to be slightly more viscous, on the other hand, the 10Hz-25V and 45V-60Hz, showed bigger relaxation time; therefore, less viscous. Cappato et al. (2018) found less relaxation time for all OH treated samples in a study with acerola whey drink, while the present study found more viscous behavior (less relaxation time) only for 1000Hz, 10Hz-25V and 80V-60Hz. More studies are need to better understand the effect of OH in liquid systems. However, TDN-MR appears to be an excellent method for a complex system to investigate minor components by setting only specific parts of the food matrix to be tested; therefore, it shows to be fast, non-destructive and selective, which makes this technique an alternative for food research (Papaemmanouil et al., 2015). This method can also provide important information to the industry when manufacturing dairy drinks since the sensory is directly affected by viscosity, which is related to the product's texture. Therefore, it plays an important role for the food industry, guaranteeing a rapid use during production line.
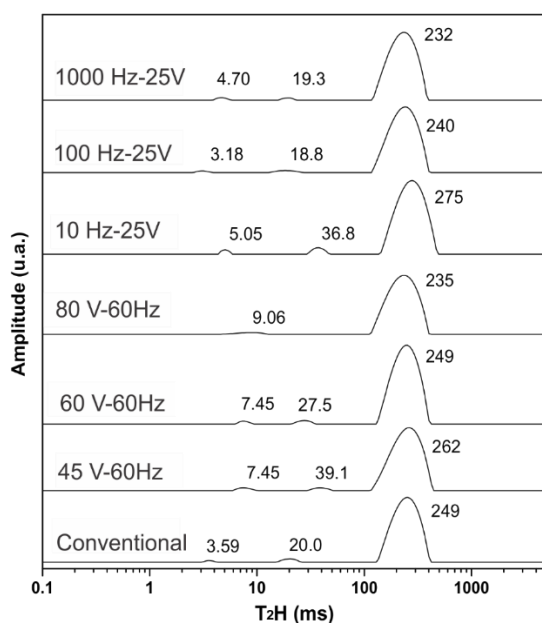

Figure 6. Distribution curves of transverse relaxation domains of TD-NMR of

Whey raspberry-flavored drink submitted to conventional processing and Ohmic Heating.

5 Conclusion

This study showed that the ohmic heating parameters for both frequency and voltage have an impact on, color, rheology particle size and microstructure, DSC and TDN-MR for raspberry-flavored whey drink. Depending on the OH configuration, the characteristic of the beverage can be established either for low or high viscosity, where the former implies in small particle size i.e. (all the voltage treated samples 45, 60, 80 V-60Hz) and the latter would suggest the use of 10Hz-25V and 1000Hz-25V to treat the raspberry-flavored whey drink at the industry scale. Further studies are needed to better understand other conditions, as well as, a sensory evaluation should be performed to study the consumer perception. Overall the findings can aid the industry to find the best parameters to set the OH equipment in manufacturing a desired final product.

6   Acknowledgments

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CONCLUSÕES GERAIS

O aquecimento ôhmico teve um impacto nas concentrações e na disponibilidade dos compostos bioativos na bebida láctea bor framboesa, com um aumento desses compostos, como demonstrado pelos ensaios de capacidade antioxidante (DPPH, FRAP e TPC), bem como uma maior inibição ACE. Um aumento de 10% da inibição anti-hiperglicêmica também foi observado, provavelmente devido à liberação de compostos favorecido pelo campo elétrico. As antocianinas tiveram uma resposta melhor dentro das amostras tratadas com voltagem, sem diferenças significativas quando comparadas ao tratamento convencional. Em contraste, os tratamentos que variam a frequência mostraram um efeito negativo sobre as antocianinas, diminuindo seus níveis. Mudanças no perfil de ácidos graxos foram observadas nas amostras tratadas com OH, com resultados menos favoráveis para os índices IA e IT e os níveis de DFA e HSFA, quando comparados ao tratamento convencional. Este estudo mostrou que os parâmetros de aquecimento ôhmico para a frequência e voltagem apresentam um impacto na cor, no tamanho das partículas de reologia e na microestrutura, DSC e TDN-MR para a bebida de soro com sabor de framboesa. Dependendo da configuração de OH, a característica da bebida pode ser estabelecida para baixa ou alta viscosidade, onde a primeira implica em tamanho de partícula pequeno, ou seja, todas as amostras tratadas com voltagem (45, 60, 80V-60Hz) e a segunda partículas maiores o que sugeriria o uso de 10Hz-25V e 1000Hz-25V para tratar a bebida láctea com sabor de framboesa em escala industrial. Mais estudos são necessários para melhor compreender outros parametros de processo (frequência e voltagem) e suas implicações na bebida , bem como, uma avaliação sensorial deve ser realizada para estudar a percepção do consumidor. Entretanto no geral, os resultados podem ajudar a indústria a encontrar os melhores parâmetros para definir o equipamento OH no tratamento do produto desejado.

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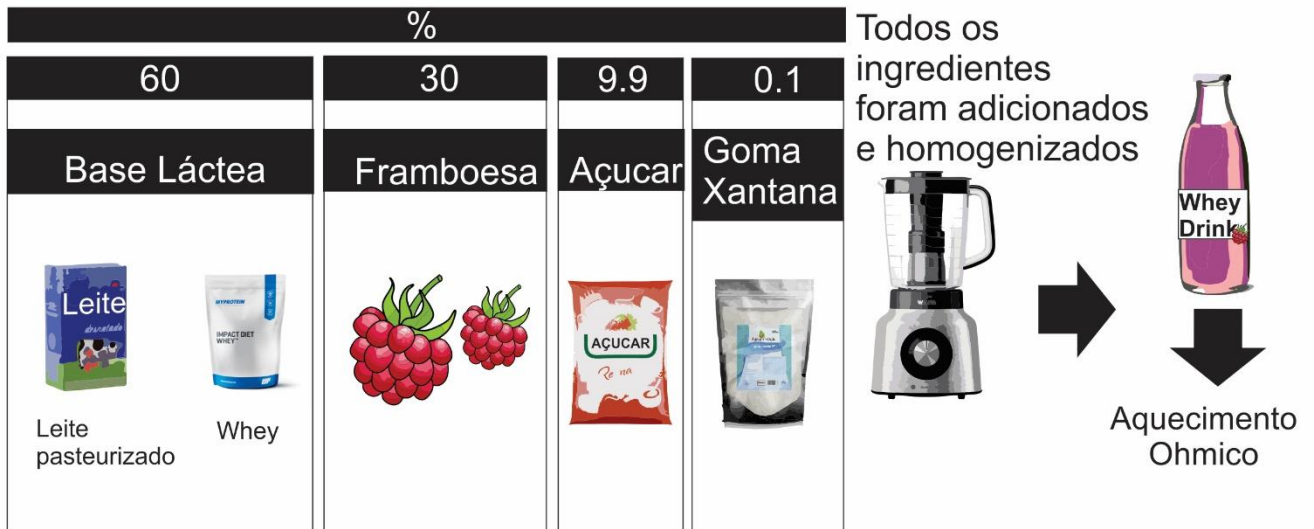
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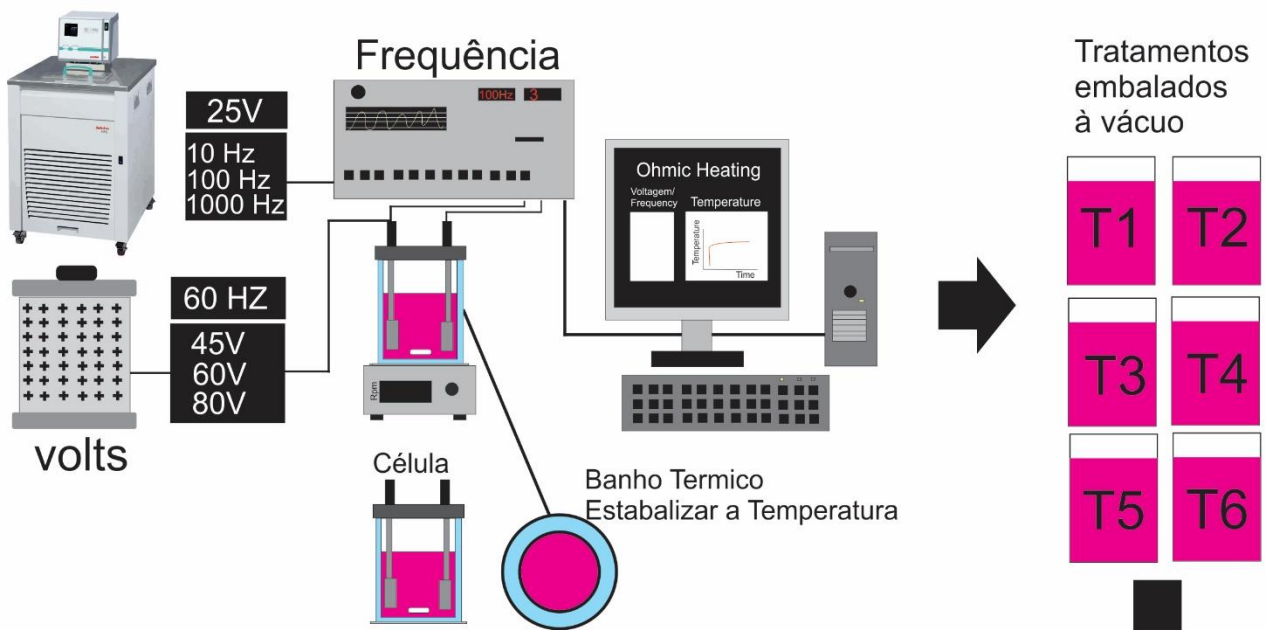
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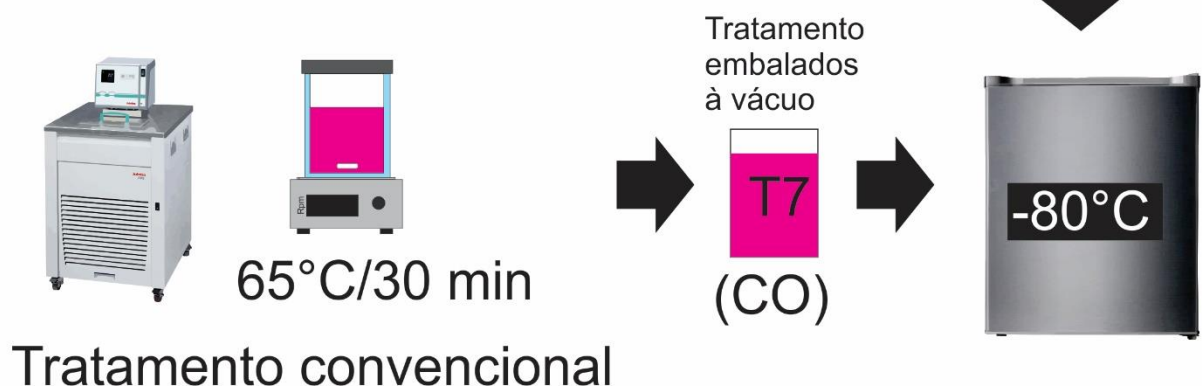
PREPARO DA BEBIDA

	%		
60	30	9.9	0.1
Base Láctea	Framboesa	Açucar	Goma Xantana

Leite pasteurizado   Whey

Todos os ingredientes foram adicionados e homogenizados

Whey Drink

Aquecimento Ohmico

AQUECIMENTO OHMICO

Frequência

25V

10 Hz
100 Hz
1000 Hz

100Hz 3

Ohmic Heating

Voltagem/Frequency   Temperature

Temperature

Time

Tratamentos embalados à vácuo

T1	T2
T3	T4
T5	T6

60 HZ

45V
60V
80V

volts

Célula

Banho Termico
Estabilizar a Temperatura

CONVENCIONAL

65°C/30 min

Tratamento convencional

Tratamento embalados à vácuo

T7
(CO)

-80°C