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PROCESSAMENTO DE LEITE FLAVORIZADO COM ALTO TEOR PROTEICO POR AQUECIMENTO ÔHMICO

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Tese apresentada ao Programa de Pós-Graduação em Medicina Veterinária da Universidade Federal Fluminense, como requisito parcial para obtenção do título de Doutor em Medicina Veterinária.

Área de concentração: Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal.

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RESUMO

A utilização do Aquecimento Ôhmico (AO) (5.22V/cm, OH6; 6.96V/cm, OH8; 8.70V/cm, OH10; e 10.43V/ cm, OH12, 60Hz) no processamento de leite flavorizado sabor baunilha com alto teor proteico foi investigado. O processamento por OH liberou importantes compostos voláteis como acetaldeído e α-felandreno. A aplicação de forças intermediárias de campo elétrico (8.70V/cm) resultou na formação de um peptídeo β-caseína com atividade inibitória da ECA e compostos voláteis importantes (ácido acético, 2-pentanona e 3-metil 1-butanol), enquanto mantendo a concentração de ácido butírico e ácidos graxos monoinsaturados e poliinsaturados ácidos (MUFA e PUFA) em níveis mais semelhantes a amostra controle. Além disso, todas as amostras apresentaram teores de proteína superiores a 12 g/100 mL, consistindo em produtos com alto teor de proteína. AO gerou menor gasto energético e inativação microbiana mais significativa de bolores e leveduras, mesófilos totais e psicotrópicos. Além disso, AO em forças de campo elétrico mais baixas, principalmente 6.96V/cm, melhorou atividades antidiabéticas, antioxidantes e anti-hipertensivas e propriedades reológicas, e resultou em menores conteúdos de hidroximetilfurfural e maior índice de nitrogênio de proteína de soro de leite. Com relação avaliação sensorial pelo método *Free Comment*, a amostra controle foi ligeiramente a moderadamente associado negativamente com “sabor ácido”, “sabor de leite fresco”, “suavidade”, “sabor doce”, “sabor de baunilha”, “aroma de baunilha”, “viscoso” e “cor branca”. Por outro lado, o processamento de OH com campos elétricos mais intensos (8.70V/cm e 10.43V/ cm) produziu bebidas lácteas aromatizadas fortemente associadas aos descritores de leite “in natura” (“aroma de leite fresco” e “sabor de leite fresco”). Além disso, os produtos foram caracterizados pelos descritores “homogêneo”, “aroma doce”, “sabor doce”, “aroma de baunilha”, “cor branca”, “sabor de baunilha” e “suavidade”. Paralelamente, campos elétricos menos intensos (5.22V/cm e 6.96V/cm) produziram amostras mais associadas ao sabor amargo, viscosidade e presença de grumos. O sabor doce e o sabor do leite fresco foram os impulsionadores do gosto. De maneira geral, o AO é uma tecnologia promissora para o processamento de leite flavorizado com alto teor proteico.

Palavras-chave: Leite flavorizado, aquecimento ôhmico, tecnologia emergente, alto teor proteico, compostos bioativos, *free comment*.

ABSTRACT

Ohmic Heating (OH) (5.22V/cm, OH6; 6.96V/cm, OH8; 8.70V/cm, OH10; and 10.43V/cm, OH12, 60Hz) in high protein vanilla flavored milk processing was investigated. OH processing released important volatile compounds such as acetaldehyde and α-phelandrene. The application of intermediate electric field strengths (8.70V/cm) resulted in the formation of a β-casein peptide with ACE inhibitory activity and important volatile compounds (acetic acid, 2-pentanone and 3-methyl 1-butanol), while maintaining the concentration of butyric acid and monounsaturated fatty acids and polyunsaturated acids (MUFA and PUFA) at levels more similar to the control sample. In addition, all samples had protein contents greater than 12 g/100 mL, consisting of products with high protein content. OH generated lower energy expenditure and more significant microbial inactivation of molds and yeasts, total mesophiles, and psychotropics. Furthermore, OH at lower electric field strengths, mainly 6.96V/cm, improved antidiabetic, antioxidant, and antihypertensive activities and rheological properties, resulting in lower hydroxymethylfurfural contents and higher nitrogen content of whey protein. Regarding sensory evaluation by the Free Comment method, the control sample was slight to moderately negatively associated with "acid taste", "fresh milk taste", "smoothness", "sweet taste", "vanilla flavor", "vanilla aroma". ”, “viscous” and “white color”. On the other hand, the processing of OH with more intense electric fields (8.70V/cm and 10.43V/cm) produced flavored dairy beverages strongly associated with the descriptors of “in nature” milk (“fresh milk aroma” and “fresh milk flavor”). fresh"). In addition, the products were characterized by the descriptors "homogeneous", "sweet aroma", "sweet flavor", "vanilla aroma", "white color", "vanilla flavor" and "softness". At the same time, less intense electric fields (5.22V/cm and processing high-protein flavored milk.

Keywords: Flavored milk, ohmic heating, emerging technology, high protein content, bioactive compounds, free comment.

SUMÁRIO

NOTA DO AUTOR: a seção 3 DESENVOLVIMENTO, pp.15-92 encontram-se no idioma (inglês) e formatação dos periódicos científicos/revistas técnicas para os quais foram submetidos/publicados e pp. 93-95 encontram-se no idioma (português) e formatação do artigo técnico.

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1 INTRODUÇÃO

A tendência global por uma alimentação mais saudável vêm refletindo diretamente nas formulações de novos produtos. Dessa forma, as indústrias buscam cada vez mais alternativas para atender essa nova demanda, produzindo alimentos com maior apelo funcional e também utilizando tecnologias que sejam capazes de gerar menos perdas nutricionais para o alimento.

O leite e seus derivados estão entre os produtos alimentícios mais consumidos pela população brasileira, incluindo bebidas lácteas, iogurte, leites fermentados, queijos, entre outros. Além desses, o leite flavorizado possui como característica ser um produto com alta aceitação sensorial, processamento viável e aplicável a diversas plantas industriais, além de ser uma matriz favorável na incorporação de ingredientes funcionais. Em paralelo, bebidas com elevado teor proteico possuem grande potencial de mercado e comumente encontrados nos grupos de produtos lácteos.

O Aquecimento Ôhmico (AO) é uma das tecnologias emergentes que vem sendo estudada com maior frequência nos últimos anos. É uma tecnologia que converte a energia elétrica em energia térmica, e a forma de dissipação do calor na matriz alimentícia é mais uniforme e rápida e sem gerar gradientes de temperatura no produto. Outro grande benefício do AO é o fato de ser uma tecnologia que não causa prejuízos ao meio ambiente por não necessitar de queima de materiais, por exemplo, e principalmente quando utilizado fontes energéticas renováveis.

O AO se mostra eficaz também no que se refere a eliminação de microrganismos deteriorantes e patogênicos. Sua ação nas bactérias é devido a formação de poros na parede celular, o que gera uma perda de compostos importantes para a célula e posterior lise. Essa tecnologia se mostra eficaz também na eliminação de esporos bacterianos, características essas que fazem o produto apresentar estabilidade microbiológica durante seu prazo comercial.

Nesse contexto, este estudo realizou o processamento de leite flavorizado com alto teor proteico por Aquecimento Ôhmico, avaliando parâmetros físico-químicos, reológicos, funcionais, bioativos, microbiológicos, e sensoriais.

2 REVISÃO DE LITERATURA

2.1 TECNOLOGIAS EMERGENTES

Tecnologias emergentes podem ser definidas como tecnologia que estão sendo ou que serão desenvolvidos nos próximos anos, incluindo robóticas avançadas, biotecnologias, novos equipamentos com mais agilidade e melhor resultado de processamento, e que visam a melhoria do serviço bem como para o meio ambiente (SUN, 2014). Possuem como principais objetivos a redução de consumo de energia e o decréscimo de custos de produção, proporcionando sustentabilidade da cadeia produtiva, além de oferecer um produto de melhor qualidade e validade comercial (JAMBRAK et al., 2019). Esses objetivos são confirmados em pesquisa com profissionais do setor de alimentos de diferentes ramos nos Estados Unidos e Canadá, onde 94, 92 e 91% dos entrevistados afirmaram que as maiores razões para comercialização de tecnologias emergentes são melhor qualidade e valor agregado do produto, segurança e aumento da validade comercial, respectivamente (JERMANN et al., 2015).

No processamento de alimentos, existem diversas tecnologias que são consideradas emergentes, como por exemplo, aquecimento ôhmico, plasma frio, alta pressão hidrostática, micro-ondas, e ultravioleta. Tecnologias essas que visam o aprimoramento do processamento de alimentos, com diversos benefícios nutricionais, sensoriais, econômicos, e ambientais. Por se tratar de novas tecnologias, segundo Jermann et al. (2015), algumas barreiras ainda são limitantes para implementação dessas tecnologias, como o alto custo de instalação, falta de conhecimento sobre todas as variáveis de processo, bem como sua interação com as diferentes matrizes alimentícias.

As tecnologias emergentes já se mostram alternativas futuras positivas para o processamento de alimentos e podem ser divididas em tecnologias térmicas e não térmicas. O aquecimento ôhmico é um exemplo de tecnologia térmica, sendo capaz de gerar um aquecimento rápido e uniforme no produto, minimizando a formação de compostos aromáticos indesejáveis (*off-flavour)* e evitando perdas nutricionais no alimento (CAPPATO *et al*., 2017), em particular em produtos lácteos, que são muito susceptíveis a essas alterações.

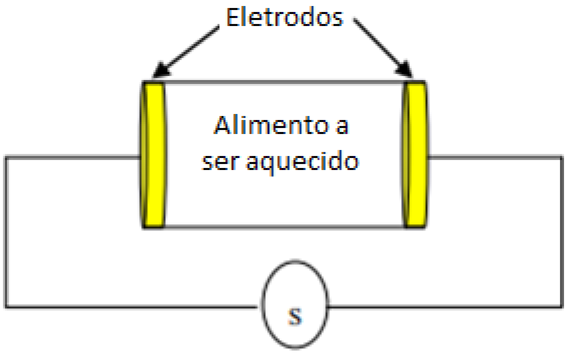
2.2 EQUIPAMENTO DE AQUECIMENTO OHMICO E CONDIÇÕES DE PROCESSO

Alguns termos são importantes para o processo de aquecimento ôhmico, tais como: condutividade elétrica, formato da onda e frequência, voltagem, e potência dissipada - efeito Joule, em que a resistência elétrica resulta em uma força na direção oposta ao movimento da carga elétrica. Essa força origina a realização de transferência de energia no sistema (ICIER, 2012; SILVA, SANTOS e SILVA, 2017).

O aquecimento ôhmico tem este nome devido à Lei de Ohm, representada pela equação 1.0:

$$V = R \times I$$

Onde V = voltagem, R = resistência elétrica e I = corrente elétrica.



Fonte: Adaptado de Sakr e Liu, 2014.
Figura 1: Esquema geral do aparelho ôhmico.

O campo elétrico é o fator que mais possui influência no aquecimento do material. Ele é definido pela corrente, potência e tensão aplicada no processo, considerando a distância entre os eletrodos, logo, uma dependência quadrática (ICIER, 2012). A área e a forma do eletrodo, bem como a característica do alimento são fatores que podem influenciar o campo elétrico formado (pode ser expresso por V/cm),

Em um modelo de aquecimento ôhmico, o controle do processo se torna indispensável, e para isso é importante controlar parâmetros como medição de temperatura, possíveis formações de pontos quentes ou “frios”, distribuição correta da condutividade elétrica, recipiente em que o alimento ficará durante o processo, entre outros fatores (JAEGER *et al*., 2016). Ou seja, a configuração do equipamento se torna importante, evitando assim uma não uniforme distribuição de temperatura e consequente erro no processo. Além disso, pode ser considerado um modelo de geração de calor interna, ou seja, não dependendo de trocas de calor durante o processo.

Na América Latina, o aquecimento se mostra como uma tecnologia promissora e representa por 19% das pesquisas realizadas na região (VILLANUEVA-RODRÍGUEZ, HERNÁNDEZ-HERNÁNDEZ E VILLANUEVA-RODRÍGUEZ, 2018).

2.3 COMPOSTOS BIOATIVOS

Os compostos bioativos vêm ganhando maior atenção nos últimos anos graças a seu potencial de reduzir o risco de incidência de diversas doenças, como por exemplo, hipertensão, câncer, obesidade, e diabetes.

As proteínas ganham destaques quando o assunto são os compostos bioativos, fato que se deve a sua importância não apenas tecnológica e nutricional (HSU et al., 2018), mas também nas funções biológicas (ISSHIKI et al., 2018) e funcionais (MIRALLES et al., 2017).

Esses compostos apresentam alta sensibilidade a algumas condições durante o processamento e estocagem do alimento, como a luz, baixo pH, oxigênio, e calor, podendo reduzir sua eficácia (CHAI et al., 2018; GLEESON, RYAN E BRAYDEN, 2016), o que mostra a importância de se conhecer o processo, bem como suas consequências que são geradas ao alimento. Diversos estudos têm relatado sobre peptídeos bioativos em produtos lácteos, bem como seus benefícios para a promoção da saúde (BASILICATA *et al*., 2017; CAPPATO *et al*., 2018b).

2.4 LEITE FLAVORIZADO

Leite flavorizado é um produto lácteo de alto consumo, atrás apenas do leite UHT. Tal popularidade se deve a sua grande variedade sensorial vista no mercado, sendo muitas vezes uma alternativa para o consumo de refrigerantes. . Além disso, é possível veicular nutrientes através da fortificação, e também dos benefícios dos probióticos,, sendo um produto com bastante apelo nutricional e saudável (ARAB et al., 2019;) Em termos tecnológicos, são derivados interessantes para indústria uma vez se considerado seu baixo custo de matéria prima (de modo geral necessitando apenas do leite e agente de sabor), e alto rendimento de produção (HOLKAR et al., 2019).

Como resultado, essas bebidas são tradicionalmente direcionadas a crianças e estão incluídas nos planos de refeições escolares em vários países, por exemplo, nos EUA e na Índia (TETRAPAK, 2020). Um interesse crescente em alimentos nutritivos e saudáveis, juntamente com a mudança de hábitos alimentares, estão fortalecendo a demanda global de leite com sabor (TETRAPAK, 2020) que pode representar uma solução nutricional para consumidores quem uma vida movimentada, representando uma refeição que pode ser consumida em curto espaço de tempo.

De fato, o mercado mundial de leite favorizado apresentou crescimento de 4,5% durante 2011-2018, registrando um quantitativo de vendas em 22 bilhões de litros em 2018 e sendo projetado um crescimento de 68.8 bilhões de dólares até 2024 (MARC, 2020). Isso deve a vários fatores como o fato de ser uma bebida pronta para beber que combina com o estilo de vida em movimento das pessoas, além poder ser comercializado em grande variedade de sabores que servem como atração para os jovens consumidores; e possuir em sua formulação nutrientes adequados para pessoas que levam uma vida saudável. Pesquisa recente conduzida no Brasil, Estados Unidos, China, Espanha e Alemanha, mostra que quase um quarto dos adultos (24%) e 41% dos seus filhos relatam o consumo diário de leite flavorizado; no Brasil e nos EUA, o produto é o preferido entre aqueles que consomem lácteos diariamente. Entre adultos, o consumo do produto está relacionado ao paladar em 55% dos casos e à saúde dos ossos em 41% dos casos (DSM, 2020).

2.5 PRODUTOS LÁCTEOS COM ALTO TEOR PROTEICO

O consumo de produtos lácteos contendo teor de proteína acima do teor encontrado nos produtos lácteos tradicionais está com constante crescimento, visto seus inúmeros benefícios à saúde como ganho de massa magra, ação imunomoduladora, ação anti-hipertensiva, dentre outras, podendo ser consumido por grande parcela da população que inclui desde crianças até atletas e idosos. A escolha destes produtos proporciona maior saciedade, praticidade, auxílio na perda de peso e substitutos de outras fontes proteicas (LASIK et al., 2016; PATEL, 2006).

Do ponto de vista tecnológico os produtos lácteos aparecem como matriz alimentícia ideal para ser serem enriquecidos com proteínas, na medida em que tecnologias de membranas, em especial a ultrafiltrarão, foram capazes de viabilizar a produção em larga escala ingredientes como concentrados e isolados proteicos de soro que podem ser utilizados formulação de produtos (BORGES et al., 2001). Proteínas lácteas possuem propriedades funcionais importantes quando usadas como ingredientes em alimentos, isso se dá pelo fato de sua alta solubilidade, capacidade de absorção de água, e capacidade de gelatinização e emulsificação (WEN-QIONG et al., 2019), além de reduzirem custos de produção e custos com matéria prima (BOŽANIĆ et al., 2014).

A adição de proteínas do soro em derivados lácteos visando o aumento do teor de proteínas e padronização, é capaz de influenciar diretamente nas características sensoriais do produto, e é uma prática comumente realizada pelas indústrias. Uma vez adicionada no produto, as proteínas tem a capacidade de modificar atributos sensoriais importantes, como viscosidade, cremosidade e suavidade. Pode-se considerar ainda, que o aumento do teor proteico visa amenizar perdas sensoriais de produtos reduzidos de gordura (LESME et al., 2020). Considerando aspectos regulatórios, um produto alimentício para ser considerado “de alto valor proteico”, deverá conter no mínimo 12g de proteína por porção em uma dieta de 2000 Kcal/dia (BRASIL, 2012).

Contudo, existe um custo adicional no valor de produtos lácteos com alto teor de proteína e isso deve ser levado em conta para garantir o êxito no lançamento no mercado, ainda que o consumidor esteja disposto a pagar mais um produto diferenciado, com elevado valor nutricional. Ressalta-se ainda que, embora o fornecimento de uma grande quantidade de proteína para o organismo possa ser benéfica à saúde, seu excesso pode causar problemas ao sistema renal, por sobrecarregar os rins. Do mesmo modo, produtos com grandes quantidades de proteína devem ser evitados por indivíduos que apresentam quadros crônicos de doenças renais.

Algumas questões parecem ainda necessitar de mais estudos, principalmente quanto a iogurtes e bebidas lácteas, a fim de se entender sobre como as condições de processamento afetam na reologia, estrutura e propriedades sensoriais. Adicionalmente, se torna necessário melhor esclarecimento no que diz respeito a legislação de produtos com alto teor proteico. O mercado de produtos lácteos com alto valor proteico já é uma realidade, devido ao aumento de práticas de vida saudável e preocupação com a alimentação, o que faz com que esses produtos tenham grande destaque e potencial de mercado.

3 DESENVOLVIMENTO

3.1 ARTIGO 1

Referenciar como: ROCHA, R.S. SILVA, R.; RAMOS, G.L.P.; CABRAL, L.A.; PIMENTEL, T.C.; CAMPELO P.H.; ZACARCHENCO, P.B.; FREITAS, M.Q.; ESMERINO, E.A.; SILVA, M.C. CRUZ, A.G. Ohmic heating treatment in high-protein vanilla flavored milk: Quality, processing factors, and biological activity. *Food Research International*, v.161, 111827, 2022.

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

Abstract

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

Keywords: high protein, functional foods, emerging technologies, microbial inactivation.

Industrial relevance

Ohmic heating can be applied in the processing of flavored milk with high protein content, generating more significant microbial inactivation and better physicochemical, technological, and functional parameters than conventional pasteurization. This study contributes to expanding the use of ohmic heating in the dairy industry, including products with high protein content.

1. Introduction

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

Milk and derivatives offer many essential nutrients for maintaining health and preventing chronic diseases (Voutilainen et al., 2022). Therefore, several dairy products have been available in the market, such as flavored milk and dairy beverages, fermented products, and cheeses. Dairy proteins, mainly whey proteins, have health benefits such as lean mass gain, immunomodulatory and anti-hypertensive actions, and antioxidant properties (Lasik et al., 2016). In addition, they may show important technological properties when used as a food ingredient (Wen-Qiong et al., 2019; Lesme et al., 2020). Drinks with high protein content, such as flavored milk, have excellent market potential and have been among the most consumed dairy products by people seeking better eating habits (Cornall, 2021).

Emerging technologies are gaining more and more prominence due to their various advantages compared to conventional heat treatment, such as more remarkable nutritional and sensory preservation and greater sustainability (Ribeiro et al., 2022). For example, ohmic heating (OH) is an emerging thermal technology consisting of an electrical current passing through a food product (Waziiroh et al., 2022). It shows several benefits, such as rapid and uniform generation of heating in the product, minimal nutritional losses, and lower formation of undesirable aromas and flavors (Cappato et al., 2017). In addition, it is a technology that, compared to conventional ones, causes minor damage to the environment when coming from clean energy sources (Balthazar et al., 2022).

OH has been utilized in different dairy products, such as cheese (Rocha et al., 2020), dairy beverages (Ferreira et al., 2019), infant formula (Pires et al., 2021), whey drinks (Pereira et al., 2020; Cappato et al., 2018) and dairy dessert (Kuriya et al., 2020). However, the application of OH to high-protein liquid dairy products has not been reported. OH may change the protein's functional properties and structure, with a consequent impact on the functional and technological properties of the products. The electric field strength has been considered the most critical process parameter to be evaluated (Waziiroh et al., 2022). Therefore, this study assessed the impact of OH treatment at different electric field strengths on the physicochemical, microbiological, technological, and functional properties of high-protein vanilla-flavored milk compared to conventional heat treatment.

2. Material and Methods

2.1 High-protein flavored milk processing

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

2.2 OH and conventional heat treatment

Six formulations were prepared: raw product (CONTROL, no conventional heat treatment or OH), conventional pasteurization (72 ºC/15 s; PAST), ohmic heating at 5.22 V/cm (OH6), ohmic heating at 6.96 V/cm (OH8), ohmic heating at 8.70 V/cm (OH10), and ohmic heating at 10.43 V/cm (OH12). The frequency used in the OH process was 60 Hz. Conventional heat treatment took place in a water bath (Marconi®). In ohmic heating, the system was constituted by a voltage generator (Variac®), a container with dimensions of 15.5 cm in length and 11.5 cm in width, two 316 steel electrodes, and the amperage, time and temperature markers. Process parameters were recorded and stored for energy parameters calculations during OH.

2.3 Energy consumption and processing parameters

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

$$\sigma = IL / AV \quad (1)$$

$$E = VIt \quad (2)$$

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

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

$$HG = \sigma * |\Delta V|^2 ut \quad (3)$$

$$VHG = \sigma * |\Delta V|^2 \quad (4)$$

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

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

$$E = V/d \quad (5)$$

Where d is the distance between the electrodes.

2.4 Gross composition

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

2.5 Color measurements

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

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

$$C* = \sqrt{a*^2 + b*^2} \quad (6)$$

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

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

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

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

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

$$YI = \frac{142.86b*}{L*} \quad (9)$$

2.6 Microbiological analysis

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

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

$$\gamma = log10\,(N0) - log10\,(Nf) \quad (10)$$

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

2.7 Rheology

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

$$\sigma=\sigma_0+k\gamma^n \quad (11)$$

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

2.8 Thermal load indicators

The hydroxymethylfurfural (HMF) content was measured using a spectrophotometric analysis and acidified medium. At the same time, the whey protein nitrogen index (WPNI) was measured using a turbidimetric analysis (Neves et al., 2016).

2.9 Bioactive compounds

The antioxidant capacity of the flavored milks was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the methodology described by Cappato et al. (2017) and Amaral et al. (2018). The anti-hypertensive activity was evaluated by the inhibitory activity of angiotensin-converting enzyme I (ACE), according to Konrad et al. (2014). The antidiabetic activity was determined through the inhibition of α-amylase and α-glucosidase, according to Lavelli et al. (2016).

2.10 Statistical analysis

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

3. Results and discussion

3.1 OH and conventional processing performances

Figure 1 presents the heating performance of flavored milk subjected to OH and conventional pasteurization. Formulations OH12 and PAST took shorter times (4.16 and 4.58 min, respectively) to achieve the processing temperature than OH6, OH8, and OH10 (20, 12, and 6 min, respectively). In addition, the increase in the electric fields (OH12: 10.43V/cm and OH10: 8.70V/cm) resulted in shorter processing times, in the same proportion that heat generation increased (10.8mV (OH12) and 5.3mV (OH10), respectively). These results suggest that OH is a viable alternative to conventional pasteurization due to the reduced processing times, depending on the electric field strength used. Other authors also verified that the increase in the electric field strength resulted in a shorter processing time in different dairy matrices (Kuriya et al., 2020; Pires et al., 2021; Bathazar et al., 2022).

Electrical conductivity increased in all samples processed by OH (Figure 2A). At the beginning of processing, all formulations showed similar values, ranging from 0.31 (OH6) to 0.41 S/m (OH12, $p > 0.05$). Electrical conductivity was also similar at the end of the process (0.80 and 0.79 S/m, $p > 0.05$). Electrical conductivity is a crucial factor as it directly affects the heating rate. Furthermore, it depends on the food composition: the more significant the mobility of the electrical charge, the more conductive the food will be (Fadavi & Salari, 2019). These results suggest that OH did not change the gross composition of the flavored milks.

Table 1 presents operational parameters related to OH processing and conventional pasteurization. The energy consumption in all OH treatments was lower than in conventional heating (Figure 3). The energy expenditure of samples submitted to OH ranged from 121.3 KJ (OH10) to 166.9 KJ (OH8). Authors suggest that the greater the electric field intensity is applied, the lower the energy expenditure due to the shorter processing time (Balthazar et al., 2022; Silva et al., 2020). This statement partly follows the present study; however, in this study, OH8 showed the highest energy consumption

(166.9 KJ), followed by OH 12 (155.4 KJ), among samples processed by OH. This event could be related to applying higher electrical energy necessary to promote the voltage, as described by Al-Hilphy et al. (2020). The results are essential to demonstrate that other factors can alter energy expenditure during processing by OH. OH processing also consumed much less energy than conventional pasteurization (Figure 3), following further research (Balthazar et al., 2022; Ghnimi et al., 2021; Pires et al., 2021; Silva et al., 2020).

The heat generation of the OH treatments increased in parallel with the applied electric field strength (Figure 2B). As a result, OH6 had the lowest heat generation, which justifies the longer time to reach 72 °C (Figure 1) and the less energy consumption (134.1 KJ). The same metric was observed in the volumetric heat, in which there was a gradual increase according to the greater electric field strength (Figure 2C). Therefore, a shorter process time results from the higher heating capacity of milk beverages generated by higher electric field strength (Hashemi et al., 2019).

The results demonstrate that PAST showed a shorter processing time together with OH12. Ohmic heating is effective because the heat is dissipated more uniformly than in conventional heating, and the possibility of using clean energy sources, with no problems for the environment (Balthazar et al., 2022; Rocha et al., 2020). Despite the fast-processing time, PAST was the sample with the highest energy consumption (503.5 KJ), emphasizing the advantage of using OH, regardless of the electric field strength. PAST used liquefied petroleum gas, a mix of propane and butane, which, when burned, contributes to the greenhouse effect. Thus, it is harmful to the environment (Murshed et al., 2021).

3.2 Gross composition

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

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

3.3 Color measurement

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

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

3.4 Microbial inactivation

The results related to microbial inactivation are shown in Figures 4 and 5. Compared to conventional pasteurization, the OH treatments generated lower microbial counts for all microbial groups at the end of the storage period (Figure 4). For example, on day 28 of storage, AMB and APB counts were 5.27 and 5.91 log CFU/mL, respectively, for the PAST sample, while the OH samples generated counts between 3.26 and 3.75 log CFU/mL for these microbial groups. At the same time, the count of molds and yeasts in the sample treated by pasteurization was 5.27 log CFU/mL, while in the OH samples, the counts were between 3.27 and 3.69 log CFU/mL. Similar values were observed when considering the behavior of lactic acid bacteria.

In all evaluated microbial groups, there was a more significant log reduction (γ) in the OH samples than in the conventional pasteurized sample (Figure 5). Overall, OH8 showed slightly higher log reductions than other intensities' ohmic treatments. The behavior of APB stands out in this case, wherein in conventional pasteurization, there was an inactivation close to zero (0.04 log CFU/mL). At the same time, in the OH treatments, the reduction varied from 0.87 to 1.04 log CFU/mL.

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

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

3.5 Rheology

Figure 6 shows the samples' shear stress and viscosity profiles obtained from the shear rate sweep (0.01-100 s^{-1}). The data showed an excellent adjustment to the Power-law model ($0.9781<R^2<0.9940$). The estimated rheological parameters are shown in Table 4. The conventionally heated sample (PAST) exhibited the highest shear stress and viscosity profiles, numerically expressed by the highest consistency index (0.9985 $Pa.s^n$), implying a well-structured protein network with low mobility. On the other hand, ohmically-heated samples (OH) have lower consistency indexes (0.0144-0.4585 $Pa.s^n$, $p < 0.05$).

In recent years, whey protein has been widely used as an ingredient in food products, such as high-protein dairy beverages, mainly due to the high biological value and unique technological and functional properties of β-lactoglobulin, such as emulsifying, gelation, and foaming abilities. Protein gelation occurs mainly after a driving force, such as heat, resulting in the unfolding of the native protein, aggregation, and formation of a three-dimensional network (Pereira et al., 2016). The network structure is influenced by the balance between attractive and repulsive forces among denatured protein molecules due to denaturation and aggregation mechanisms (Rodrigues et al., 2015). In this context, the pronounced differences in the network mobility observed between conventional heating and OH can be mainly attributed to the different patterns of protein unfolding, conformation, interactions, and aggregation (Sereechantarerk et al., 2021; Rodrigues et al., 2020). Conventional heating increased gel consistency due to a complete denaturation and denser protein aggregation. Otherwise, OH may induce more specific changes in proteins mainly due to thermal and non-thermal effects combined. The thermal effects are attributed to direct, rapid, and volumetric heating. In contrast, the non-thermal effects have been associated with changes in the polarity due to electric field alternation, resulting in alterations in molecular dipole orientation and molecular motions and rearrangements and, consequently, conformational changes in secondary and tertiary structures (Ferreira et al., 2021; Rodrigues et al., 2020). As the low viscosity characterizes flavored milks, the maintenance of lower consistency indices in OH-treated samples (more similar to CONTROL) is desired.

In addition, according to the nonlinear regression, all samples were characterized as non-Newtonian fluids with flow behavior indexes lower than one ($0.290 \leq n \leq 0.852$), indicating a shear-thinning behavior, where an increase in shear rate is followed by a decrease in viscosity due to the tendency of macromolecules to align towards the flow (Figure 6B). Significant differences were observed for all samples, especially among the ohmically-heated beverages. It can be noticed that an increase in electric field strength has caused a decrease in the flow behavior index (up to OH10). In contrast, the consistency index has followed the opposite trend increasing with the electric field intensity (Table 4). The main hypothesis for this behavior is that the rapid heating and electric field following OH promoted a lower degree of denaturation and aggregation, consequently leading to a weaker and thinner protein network structure (Sereechantarerk et al., 2021; Rodrigues et al., 2015). However, the ohmically-treated sample at 12 V/cm has shown quite different values of consistency (0.0452 Pa s^n) and flow behavior indexes

(0.6612), indicating that a critical value of electric field strength might be achieved during the ohmic heating treatment progression.

3.6 Indicators of thermal load

HMF is a Maillard reaction byproduct commonly used to indicate severe heating, and its consumption should be avoided. In Figure 7, it is possible to verify that the HMF content was close to zero in the control sample, which did not undergo any treatment. The sample treated by conventional heating showed the highest value of HMF (12.56 µmol/L), differing significantly ($p < 0.05$) from the samples treated by OH. The OH samples did not show significant differences (5.12 to 5.78 µmol/L; $p > 0.05$).

The WPNI value represents the denaturation of whey proteins as a function of processing (Ribeiro et al., 2021). Figure 7 shows that the highest value is associated with the control sample (10.24 mg WPN/mL), where there was no processing. The conventional treatment generated the lowest WPNI value (2.30 mg WPN/mL), indicating that pasteurization was the treatment where the greatest denaturation occurred. Among the OH treatments, the lower electric fields (OH6 and OH8) showed greater preservation of whey proteins ($p < 0.05$) when compared to treatments with higher electric fields (OH10 and OH12). Similar results were obtained in a study evaluating the treatment of infant milk formulas by OH (Pires et al., 2021). Other emerging technologies applied to dairy products, such as cold plasma (Ribeiro et al., 2021), also showed similar results to this study, indicating that these technologies are promising in lower production of undesirable substances, such as HMF, and more excellent preservation of whey proteins.

3.7 Bioactive compounds

The results related to bioactive compounds are shown in Figure 8. Processing by OH generated higher values ($p < 0.05$) for ACE inhibitory activity, DPPH, and α-glucosidase and α-amylase inhibitions compared with the product treated by conventional pasteurization and the control product. Furthermore, in all parameters evaluated, treatments with the lowest electric fields (OH6 and OH8) showed better results than treatments with higher electric fields (OH10 and OH12; $p < 0.05$), indicating that OH processing using a lower intensity electric field is capable of promoting greater preservation of the evaluated compounds.

The higher values of bioactive compounds can be related to the more significant number of bioactive peptides generated by ohmic heating, a result of the kinetics of milk proteins, which seems to be improved in emerging technologies, as indicated in recent studies (Cappato et al., 2018; Kuryia et al., 2020; Oliveira et al., 2022). However, the higher electric fields used in this study (OH10 and OH12) indicate a lower formation of bioactive compounds related to antioxidant, anticancer, and anti-diabetes activity, emphasizing the importance of evaluating processing parameters to optimize food processing.

4. Conclusion

This study was the first to evaluate the impact of OH treatment on the properties of flavored milk with high-protein content. All samples showed higher than 12 g/100 mL of protein, consisting of high protein content products. OH generated lower energy expenditure and more significant microbial inactivation of lactic acid bacteria, molds and yeasts, total mesophiles, and psychotropics. Furthermore, OH at lower electric field strengths, mainly OH8, resulted in improved antidiabetic, antioxidant, and anti-hypertensive activities and rheological properties, lower hydroxymethylfurfural contents, and higher whey protein nitrogen index. The results suggest that OH is a viable alternative in the processing of flavored milk with high protein content, given its ability to process in a short time and low energy expenditure, and the preservation of nutrients essential for maintaining health without compromising microbiological and technological properties.

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ANEXO

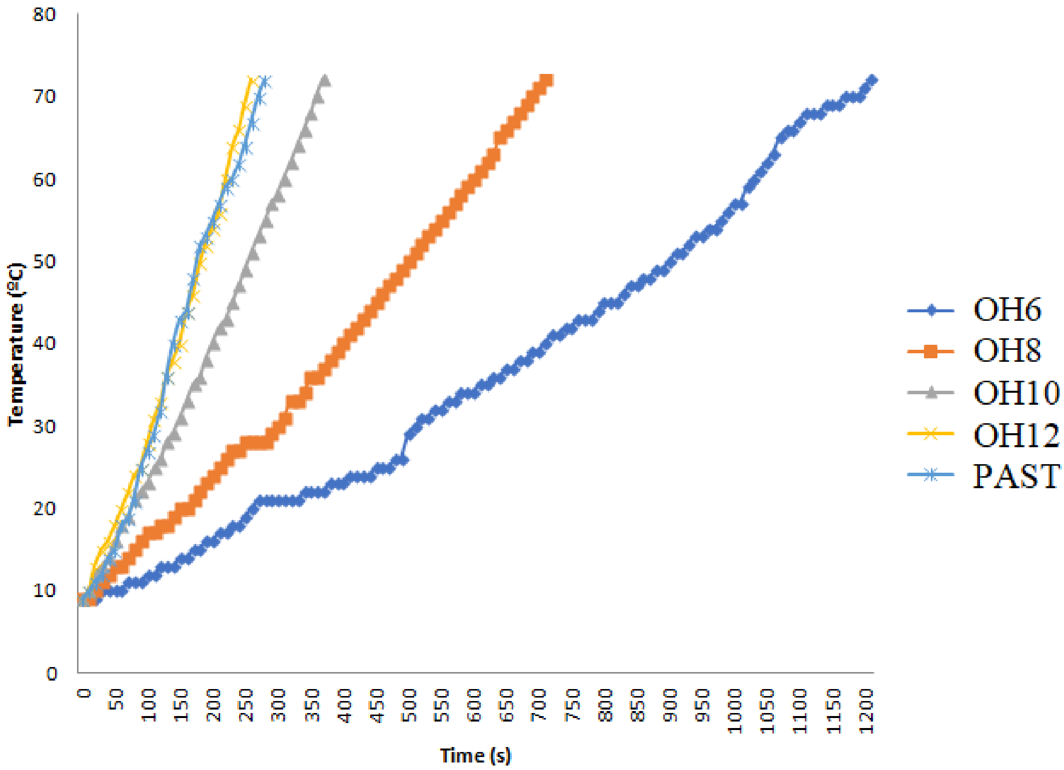

Figure 1. Behavior of the time/temperature binomial of samples during ohmic heating processing and conventional treatment. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively. PAST: Conventional pasteurization.

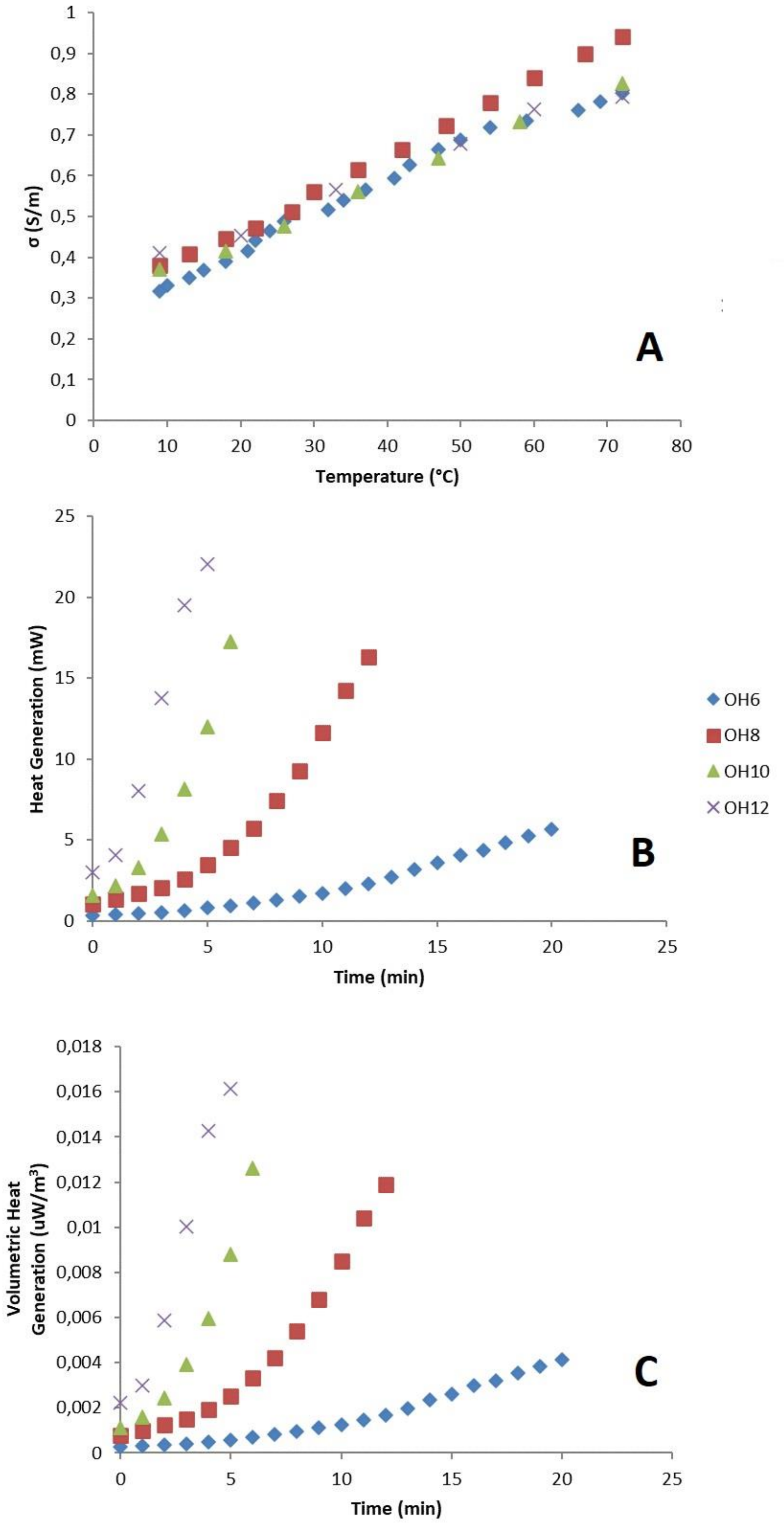

Figure 2. Operating parameters for OH processing of flavored milk from 9 to 72 °C. (A) electrical conductivity (σ); (B) heat generation; (C) volumetric heat generation. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

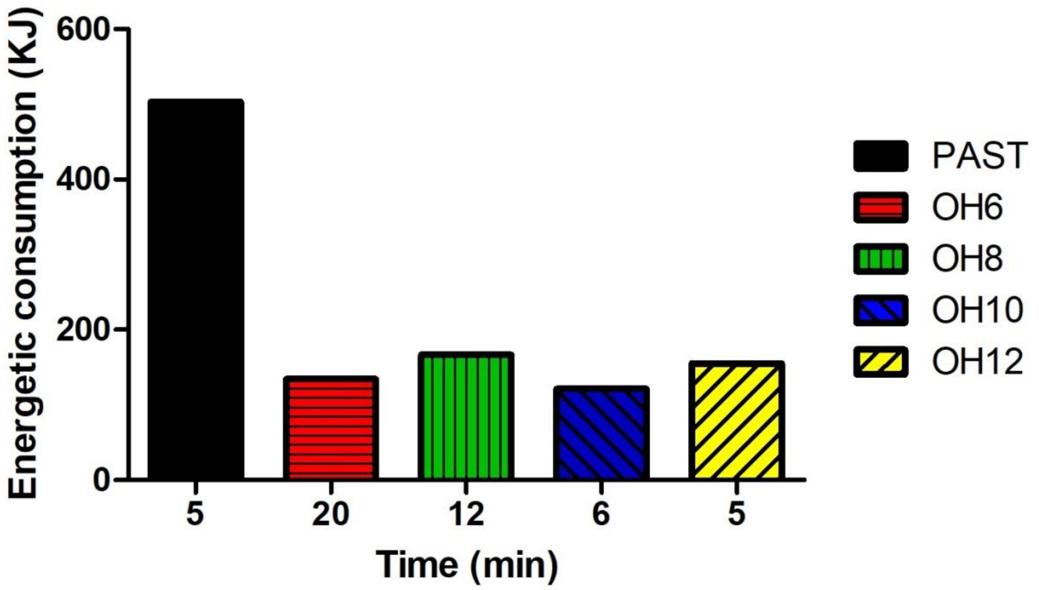

Figure 3. Comparison of energy consumption (KJ) between conventional heating (PAST) and ohmic heating. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

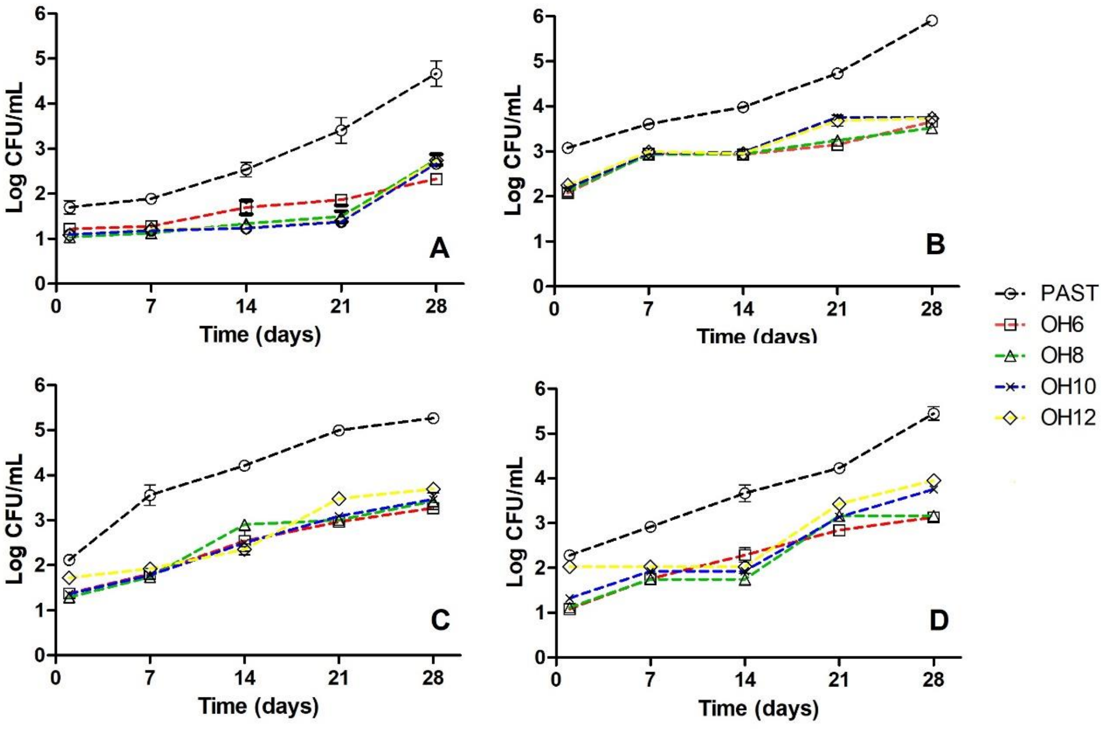

Figure 4. Microbial viability in the samples submitted to different treatments along the storage: (A): Aerobic Mesophilic Bacteria. (B): Aerobic Psichrotrophic Bacteria. (C): Molds and yeasts. (D) Lactic Acid Bacteria. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

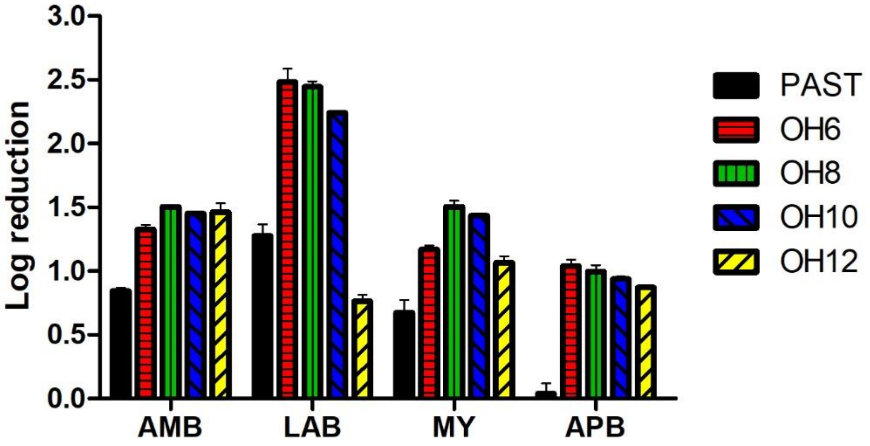

Figure 5. Microbial inactivation (γ) in treated samples compared to the control. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively. AMB: Aerobic Mesophilic Bacteria. APB: Aerobic Psichrotrophic Bacteria. MY: Molds and yeasts. LAB: Lactic Acid Bacteria.

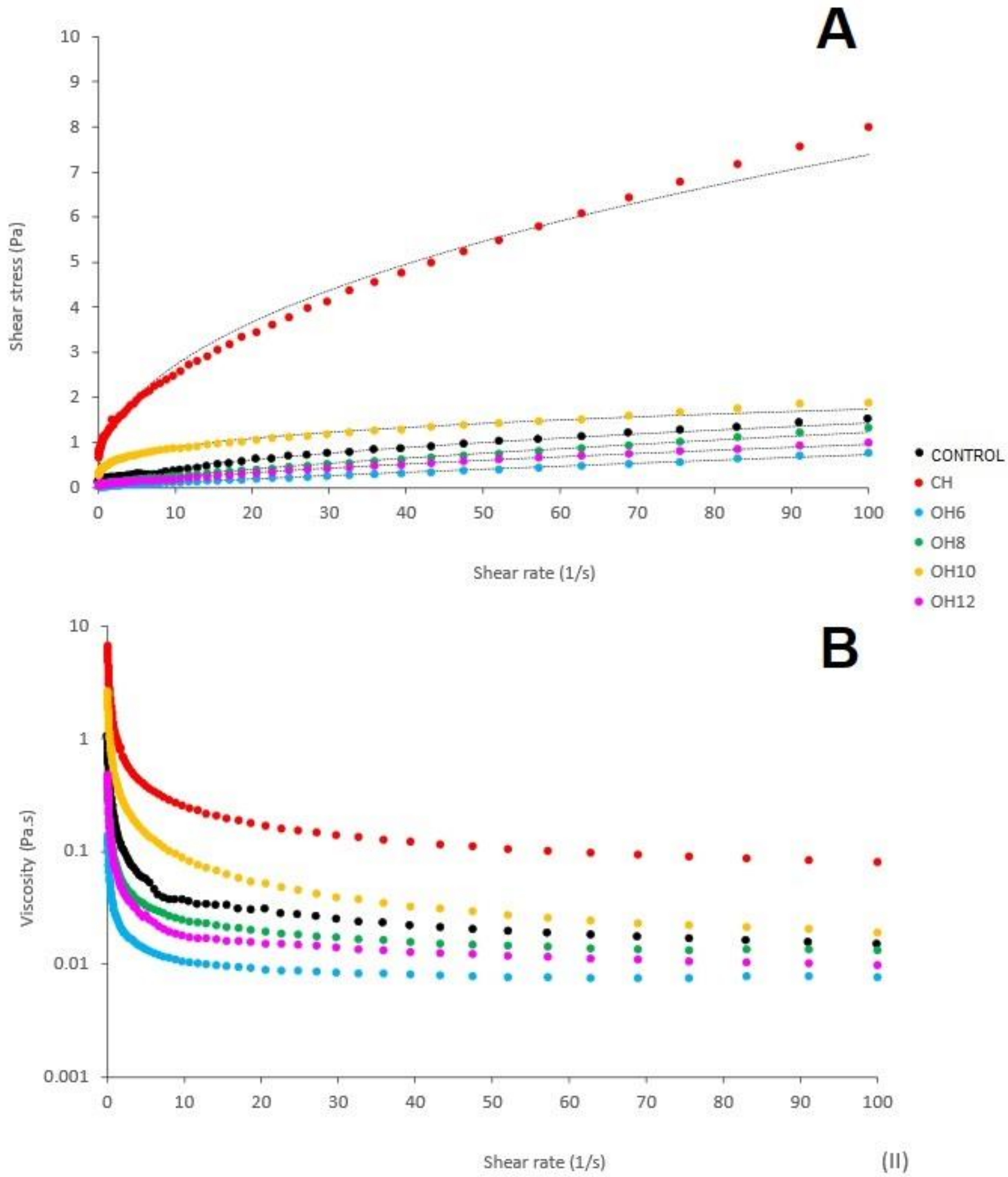

Figure 6. Flow curves (A) and viscosity (B) obtained from the shear stress sweep tests of the samples. Full symbols represent experimental data; dotted lines represent Power law model. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

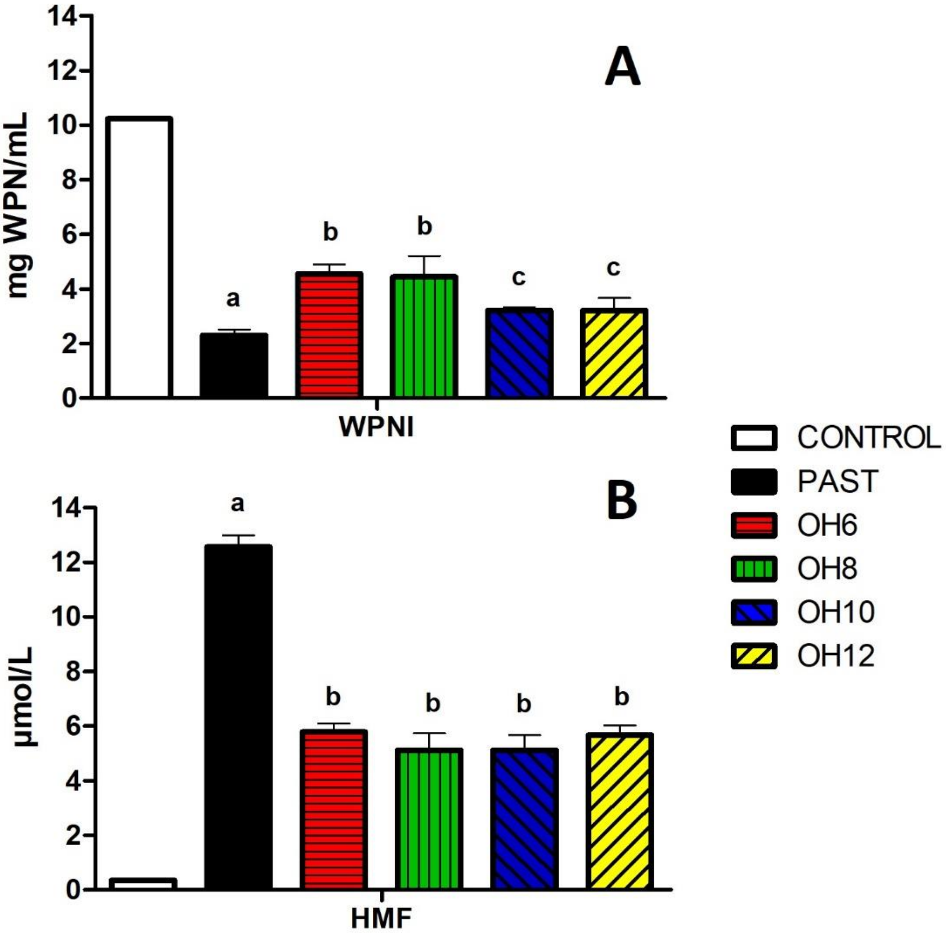

Figure 7. Indicators of thermal load of the samples. Different letters mean significant difference between treatments ($p < 0.05$). (A) - WPNI: Whey Protein Nitrogen Index. (B) - HMF: total hydroxymethylfurfural level. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

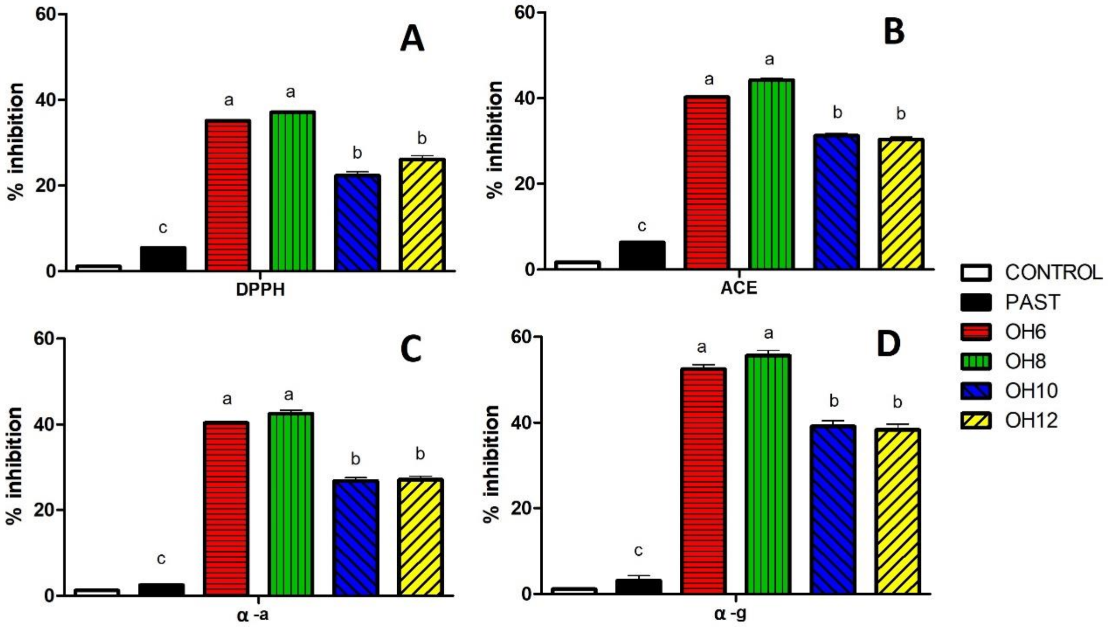

Figure 8. Functional activities of the samples. Different letters mean significant difference between treatments ($p < 0.05$). (A) - DPPH: 2,2-diphenyl-1-picrylhydrazyl. (B) - ACE: Angiotensin converting enzyme. (C) - α-a: α-amylase. (D) - α-g: α-glucosidase. PAST: Conventional pasteurization. OH6, OH8, OH10 and OH12: Ohmic heating at 5.22, 6.96, 8.70 and 10.43 V/cm, respectively.

Table 1. Operating conditions of Ohmic and Conventional Processing

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

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

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

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

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

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

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

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

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

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

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

3.2 ARTIGO 2

Referenciar como: ROCHA, R.S.; MAHIEU, B.; FILHO, E.R.T.; ZACARCHENCO, P.B.; FREITAS, M.Q.; MÁRSICO, E.T.; PIMENTEL, T.C.; ESMERINO, E.A.; CRUZ, A.G. Free comment as a valuable approach to characterize and identify the drivers of liking of high-protein flavored milk drink submitted to ohmic heating. Food Research International, v.165, 112517, 2023.

Free comment as a valuable approach to characterize and identify the drivers of liking of high-protein flavored milk drink submitted to ohmic heating

Abstract

Flavored milk is a popular dairy product traditionally processed by pasteurization, which is a safe and robust process. Still, it can imply a greater energy expenditure and a more significant sensorial alteration. Ohmic heating (OH) has been proposed as an alternative to dairy processing, including flavored milk. However, its impact on sensory characteristics needs to be evidenced. This study used Free Comment, an underexplored methodology in sensory studies, to characterize five samples of high-protein vanilla-flavored milk: PAST (conventional pasteurization 72 ºC/15s); OH6 (ohmic heating at 5.22 V/cm); OH8 (ohmic heating at 6.96 V/cm); OH10 (ohmic heating at 8.70 V/cm), and OH12 (ohmic heating at 10.43 V/cm). Free Comment raised similar descriptors to those found in studies that used more consolidated descriptive methods. The employed statistical approach allowed to observe that pasteurization and OH treatment have different effects on the sensory profile of products, and the electrical field strength of PH also has a significant impact. PAST was slightly to moderately negatively associated with “acid taste,” “fresh milk taste,” “smoothness”, “sweet taste,” “vanilla flavor”, “vanilla aroma,” “viscous,” and “white color.” On the other hand, OH processing with more intense electric fields (OH10 and OH12) produced flavored milks strongly associated with the “in natura” milk descriptors (“fresh milk aroma,” and “fresh milk taste”). Furthermore, the products were characterized by the descriptors “homogeneous”, “sweet aro,””, “sweet taste," “vanilla aroma," “white color," “vanilla taste”, and “smoothness”. In parallel, less intense electric fields (OH6 and OH8) produced samples more associated with a bitter taste, viscosity, and lumps presence. Sweet taste and fresh milk taste were the drivers of liking. In conclusion, OH with more intense electric fields (OH10 and OH12) was promising in flavored milk processing. Furthermore, free comment was a valuable approach to characterize and identify the drivers of liking of high-protein flavored milk submitted to OH.

Keywords: dairy drink; ohmic heating; free comment; consumer test.

1. Introduction

The food sector is increasingly competitive, which motivates the food industries to become more attentive to innovation possibilities. In this way, great attention has been paid to developing new products and reformulating products already present in the portfolio to meet consumer expectations better. In front of a shelf full of possibilities, the current consumer tends to become increasingly informed when inspecting, judging, and choosing products, considering extrinsic and intrinsic characteristics.

The dairy sector has extensive competition in both national and international markets. Such competitiveness is manifested in the most diverse categories of products, e.g., cheeses, yogurts, flavored milk, and conventional milk. There are several focuses for innovations in dairy products, such as the addition of bioactive and functional ingredients (Adinepour et al., 2022), the use of raw materials with a natural and sustainable appeal (Schiano et al., 2021), the development of healthier food products (reduction of salt, sugar, and fats) (Antunes et al., 2021), and elaboration with emerging processing technologies (Ribeiro et al., 2022).

Flavored milk is a generally ready-to-drink beverage made with milk, sugar, flavorings, and, eventually, food coloring. Flavored milk is traditionally marketed as pasteurized products, kept under refrigeration, or as ultra-high temperature processed (UHT) products, which do not require refrigeration. Furthermore, flavored milk has already demonstrated good performance when elaborated with emerging processing technologies, such as pulsed electric field (Bermúdez-Aguirre et al., 2010) and ultrasound (Monteiro et al., 2018). In our previous study, we observed that flavored milk submitted to ohmic heating (OH) presented increased functional properties (anti-diabetic, anti-hypertensive, and antioxidant activities) and improved rheological characteristics (Rocha et al., 2022). Furthermore, the electrical field strength was a critical process parameter (Rocha et al., 2022).

The impact of emerging processing technologies on sensory characteristics has been the subject of investigations by researchers in sensory and consumer science (Cardello et al., 2007; Perrea et al., 2015; Priyadarshini et al., 2019). Generally, research is concerned with two main points: evaluating how technology affects the intrinsic sensory characteristics of products (appearance, aroma, flavor, and texture) (Silva et al., 2020) and investigating consumers' perception of the use of emerging technologies (dos Santos Rocha et al., 2022).

Free comment (FC) is a research technique initially widespread in socio-economics, psychology, sociology, epidemiology, and marketing (Lawrence et al., 2013). However, FC has gradually gained acceptance in food science (Fonseca et al., 2016; Lawrence et al., 2013; Mahieu, Visalli, Thomas, et al., 2020; Mahieu et al., 2022). The methodological structure of the FC resembles the projective technique "word association" (Esmerino, Tavares Filho, et al., 2017), requiring the participant to evaluate a product using their vocabulary freely, that is, without using scales or lists with pre-defined terms (Ares & Deliza, 2010).

The data from the FC are words, isolated or organized in small sentences. This way, they can be analyzed with qualitative research approaches (e.g., content analysis and thematic analysis) or quantitative analysis techniques. The quantitative methods will transform textual data into codes (Esmerino, Ferraz, et al., 2017) and later evaluate them with statistical techniques, such as Chi-square global and per cell (Lee et al., 2013; Rodrigues et al., 2021; Vidal et al., 2015), and Cochran's Q test (Mahieu, Visalli, & Schlich, 2020). However, Mahieu, Visalli, & Schlich (2020) postulated that using the traditional Chi-square (Pearson) and Cochran's Q test might be limited for FC data because the p-value (in Pearson's chi-square test) is only valid when the observations are independent, when no cell in the contingency table has a low count (>5), and when the table is not very sparse (Mahieu, Visalli, & Schlich, 2020). In the FC, the same consumer (evaluator) evaluates all samples of the set, which compromises the independence of the observations and, consequently, the validity of the classic Chi-square (Pearson).

Therefore, Mahieu et al. (2022) proposed a modified chi-square to analyze data from multiple responses, where the experimental units are the evaluations. In addition, the model includes a multiple-response dimensionality dependence test, a multiple-response correspondence analysis (MR-CA), and a hypergeometric multiple-response test to investigate which descriptors are significantly associated with which product.

However, the authors evaluated only cooked ham, and more studies are necessary to validate the methodology and the statistical approach.

This study used FC with the statistical analysis approach proposed by Mahieu et al. (2022) to characterize high-protein vanilla flavored milk processed by OH or pasteurization. Our aim is to validate the utilization of FC and the suggested statistical approach to describe samples submitted to emerging technologies and state the impact of OH on the sensory profile of the products. Furthermore, the drivers of liking and disliking for flavored milk were assessed.

2. Material and Methods

2.1 High-protein flavored milk processing

The flavored milk processing was published recently (Rocha et al., 2022). First, refrigerated raw milk (3% w/w fat, 3% w/w protein, Ateliê do Queijo, Casemiro de Abreu, Brazil) was added with 10% industrial whey protein isolate (WPI, 90% total protein, Sooro Renner Nutrição S/A, Paraná), 3% sucrose (União, São Paulo) and 0.5% w/w vanilla flavor. The concentration of WPI was defined to obtain a product with 12 g of protein per 100 mL, classifying it as a high-protein drink (BRASIL, 2012). The samples were then processed by conventional heat treatment or OH.

Five formulations were prepared: conventional pasteurization (72 ºC/15 s; PAST), OH at 5.22 V/cm (OH6), OH at 6.96 V/cm (OH8), OH at 8.70 V/cm (OH10), and OH at 10.43 V/cm (OH12). All samples submitted to OH followed the same binomial time/temperature that the PAST sample, and for both processes, the parameters were controlled by a clock and bookmarks to voltage, current, and/or temperature. In all samples, after reaching the temperature and waiting for 15 s, the samples were immediately immersed in a cold-water solution for rapid cooling. The electric field strength values result from calculating the voltage applied to the process with the distance between electrodes.

2.2 Free comment

The FC was performed following Mahieu et al. (2022). One hundred and ten consumers of flavored milks (aged 18-65, 65 male, 45 female, at least once a week) were invited to describe the sensory profiling of the samples in the following order: appearance (visual aspect), aroma, taste, and texture. For each sensory modality, the following instructions were given to the consumers:

- ✓ Appearance: “Please describe the appearance of this flavored milk.”
- ✓ Aroma: “Please describe the aroma of this flavored milk.”
- ✓ Taste: “Please describe the taste of this flavored milk.”
- ✓ Texture: “Please describe the texture of this flavored milk.”

After the FC task, the consumers rated the flavored milk samples' overall liking using an unstructured nine-point hedonic scale. The study was approved by the Ethical Committee of the Federal Institute of Rio de Janeiro (IFRJ), protocol number 51314321.0.0000.5268.

2.3 Statistical Analysis

2.3.1 New approach to analyzing FC data

This study used a data analysis methodology different from that traditionally used in studies based on citation frequency, for example, Check-all-that-apply (CATA) and FC. The data obtained with FC are, by nature, textual, unstructured, and derived from the free expression of consumers. Therefore, they can be analyzed with positivist approaches (from the quantitative research paradigm) or interpretivist approaches (from the qualitative research paradigm). In food science, quantitative approaches have mostly consolidated over qualitative ones. To use quantitative techniques on FC data, it is necessary to perform treatments on the open responses provided by consumers to make them structured data. Cleaning, lemmatization, exclusion of terms of low semantic value, and grouping of terms with similar meanings are the most used treatments (Anandarajan et al., 2019).

In this way, open-ended responses are systematically converted into some terms of most significant interest. For example, nouns and adjectives are the most used grammatical classes to investigate consumers’ perception of products in food science (Mahieu, Visalli, Thomas, et al., 2020). Thus, consumer terms are grouped into latent descriptors based on an ascending hierarchical classification.

Traditionally, adjectives and nouns extracted from consumer comments are tabulated in contingency tables. Such tables expose the “attribution” or “non-attribution” of each descriptor term, for each product, by each of the consumers who participated in the test. Thus, data can be analyzed by citation frequency or the absolute value of citation count. The dependence (or independence) between products (samples) and descriptors (terms cited by consumers), exposed in the contingency tables, is traditionally analyzed by Chi-square (Lee et al., 2013; Rodrigues et al., 2021; Vidal et al., 2015), while significant differences between proportions are routinely analyzed via Cochran's Q test (Stephen & Adruce, 2018). Therefore, it is customary to use correspondence analysis (CA) to generate a factor map that decomposes this dependence between the evaluated products and the descriptors. As a rule, the decomposition is carried out in axes of maximum dependence organized in descending order, adopting all axes (Bemfeito et al., 2021; Portela et al., 2022; Pramudya & Seo, 2018).

However, despite the traditional use of chi-square, some critical limitations related to using this technique in data from the FC and CATA were pointed out by Mahieu, Visalli, & Schlich (2020). That's because the p-value (in Pearson's chi-squared test) is only valid when the observations are independent, when no cell in the contingency table has a low count (>5) and when the table is not very sparse (Mahieu, Visalli, & Schlich, 2020). Furthermore, in studies with FC, the same consumer (evaluator) evaluates all the samples of the set, which compromises the independence of the observations.

Mahieu, Visalli, & Schlich (2020) proposed a dimensionality test using chi-square and Monte-Carlo simulation (simulations = 1000, α = 5%) to calculate valid p-values. Furthermore, the authors proposed a modified chi-square square framework dedicated to analyzing multiple-response data in which experimental units are the evaluations (Mahieu et al., 2021). To overcome the noise caused by including all axes in the CA factorial map plot, the authors proposed a step-by-step method to test the dependence of each CA axis. This way, only the significant axes can be considered in the analysis. The number of significant CA axes is determined using the Monte-Carlo dependence tests (simulations = 1000, α = 5%).

There is also the traditionalism of using chi-square per cell in the raw dataset, which ends up encompassing all axes, including those that are just noise. As this can lead to an over-interpretation of the data, Mahieu, Visalli, & Schlich (2020) also proposed using Fisher's exact test, not in the raw data, but in data from the inversion of the calculations used in the CA. Fisher's exact tests per cell with a one-sided greater alternative hypothesis can be conducted on the derived contingency table corresponding to significant axes to assess relations between products and descriptors.

In the usual CA, products are compared according to the ratio between the citations of each descriptor and the total number of citations of all descriptors combined. So, samples containing products with very different average citation rates will distort the graphical representation (Mahieu et al., 2021). MR-CA overcomes this limitation by scaling products according to the number of reviews rather than the number of citations received (Mahieu et al., 2021). Thus, the fact that some products receive more citations than others from the same sample set does not affect MR-CA as classical CA.

2.2.2 Statistical approach

The descriptions obtained by the FC task were cleaned, lemmatized, and filtered. Then, the descriptors with similar meanings were grouped by triangulation technique, i.e., three researchers independently grouped the terms. Furthermore, the results generated individually by the three researchers were discussed in a subsequent meeting to reach a consensus (Pontual et al., 2017). Finally, the sensory descriptors mentioned by at least 5% of the participants were considered for quantitative analysis to avoid losing a large amount of information and were cross-tabulated with the consumers and the products, indicating whether each descriptor was cited in the corresponding evaluation or not, in a similar way as CATA data.

To depict the sensory space, a MR-CA (Mahieu et al., 2021) was performed based on the descriptor citation proportions of each product. In addition, a confidence ellipse (α = 5%) was built based on bootstrap resampling of the consumers (1000 simulations).

The consumers were clustered using local Clustering around Latent Variables (CLV) (Vigneau & Qannari, 2003) with consolidation. The hierarchical tree was cut at the step of the maximum relative increase in the clustering criterion. This resulted in retaining two clusters of consumers. Individual consumer liking scores were then regressed against MR-CA axes to investigate which directions of the sensory space mainly drive liking and the differences between the two clusters.

For each pair of products and descriptor, a multiple-response hypergeometric test was performed with a two-sided alternative hypothesis ($\alpha = 5\%$) to investigate positive and negative associations between products and descriptors. Finally, for each of the two clusters of consumers, drivers of liking were investigated following the approach proposed by Mahieu et al. (2022), considering the panel and each cluster. Data analyses were performed using R 4.1.0 (R Core Team, 2020).

3. Results and Discussion

The FC data for the flavored milk samples processed by OH or pasteurization and containing high protein content resulted in the fourteen descriptors that can be seen in Table 1. The data analysis strategy employed was intended to find the primary product descriptors to investigate consumer perception, associations between products and descriptors to check the effect of treatment on samples, and then evaluate consumers' liking drives to verify which of the descriptors are most related to "liking" behavior of consumers.

Despite using a methodology based on the free expression of consumers instead of a closed list of pre-defined descriptors (according to the CATA), almost all the attributes raised in the FC were in agreement with those used in other studies that evaluated flavored milk (Guinard & Mazzucchelli, 1999; Oliveira et al., 2015; Oliveira & Deliza, 2021; Thompson et al., 2004). Only "white color," "acid taste," and "fresh milk aroma" were not found in the literature. This suggests an excellent ability of consumers to describe samples freely. In addition, much of the consumer vocabulary could be grouped into synonyms and latent descriptors.

The Bi-plot of the MR-CA (Figure 1) shows the positioning of the samples concerning the descriptors. As an indirect consequence, the Bi-plot presents a general aspect of the samples' sensory profile and the vectors' position related to the "liking" of consumers. Therefore, the position of samples and descriptors in the dimensions and among themselves (product x descriptor) is initially considered. The MR-CA Biplot allowed us to visualize the associations between samples and descriptors satisfactorily. However, the density of essential vectors in the second quadrant led to a deeper investigation of the association between samples and descriptors. Thus, the hypergeometric multiple response test was used to increase the clarity of the association between samples and descriptors (Figure 2).

In the inspection of the "liking" vectors presented in Figure 1, an antagonistic behavior of the two vectors that represent the two clusters of consumers is observed, as well as the predominance of consumers in cluster 1 and its consequent impact on the general vector "like" (panel). In this way, we can infer that the consumers were divided into two groups based on the acceptance of the products, with more consumers on cluster 1 (n=77).

The descriptors "viscous," "bitter taste," and "presence of lumps" were the most positively associated with the first dimension. Among the samples, OH8 and OH6 were the most associated with Dim1 and the descriptors mentioned above. The intensity of these associations between samples and descriptors can be confirmed with the multiple-response hypergeometric test (Figure 2). The low association of the "liking" vectors with the first dimension signals that OH6 and OH8 were the least accepted samples.

Still, in the first dimension, “homogeneous” and “sweet aroma” were more negatively associated, while “fresh milk aroma” showed a subtle negative association. Among the samples, mainly OH12 and, more subtly, OH10 showed a negative association with Dimension 1. Only the OH12 sample was related to all descriptors with a stronger negative association in Dimension 1 (Figure 2). The association of the “like” vectors of Cluster 1 and all consumers (panel) was also negative concerning Dimension 1. In this way, OH10 and OH12 were the most accepted samples considering dimension 1.

The second dimension was positively associated with the descriptors “sweet taste," “vanilla aroma," “white color," “yellow color," “fresh milk taste,” and “acid taste,” and more subtly with “vanilla taste”, and “smoothness”. Among the samples, only OH10 and OH12 were associated with Dim2, and the attributes positively related to it. The vectors representing the “liking” of consumers in cluster 1 and, more subtly, of total consumers (panel) also had a positive association with the second dimension. In this way, OH10 and OH12 were the most accepted samples considering dimension 2.

“Fresh milk aroma” and, more subtly, “sweet aroma” and “presence of lumps” were the attributes most negatively associated with the second dimension. Only “PAST” was more significantly associated with the second dimension among the samples, evidencing its different sensory profile compared to the samples treated by OH. PAST was also slightly to moderate negatively associated with “acid taste”, “fresh milk taste”, “smoothness”, “sweet taste”, “vanilla flavor”, “vanilla aroma”, “viscous”, and “white color”. The “liking” vector of the second cluster of consumers was negatively associated with the second dimension.Our results demonstrated that most of the consumers liked more the samples submitted to OH at stronger electrical fields (OH10 and OH12, n=77, cluster 1), but, there is also a number of consumers with preference for the PAST product (n=33, cluster 2), which is commonly found at the supermarkets. The samples’ positioning demonstrated consumers’ ability to discriminate between protein-rich flavored milk treated by pasteurization and by OH.

Among the samples treated by OH, there was a difference in the sensory profile according to the intensity of the electric field used in the processing. In OH, the electric field strength is closely related to the time the sample is subjected to heating; stronger electric fields allow the sample to reach the desired temperature in processing faster (Cappato et al., 2017; Silva et al., 2020). Theoretically, there is a hypothesis that exposing the food to shorter heating periods reduces the intensity of undesirable heat-promoted changes in the matrix (Coolbear et al., 2022).

The action of heat on milk proteins, e.g., the direct and indirect actions, favors the formation of peptides with a bitter taste, which is a concern for the dairy industry due to its negative impact on sensory quality (Fox et al., 2015). OH seeks to expose the product to shorter heating times, saving energy and mitigating the changes caused by heating. Samples OH6 and OH8 were the most associated with the descriptor “bitter taste,” surpassing even the traditional sample processed by pasteurization (PAST). In this way, the lower intensity of the electric field of these samples when compared to the others and, consequently, the longer time needed to reach the desired processing temperature were responsible for the bitter taste. The PAST sample was not associated with the descriptor “Bitter taste,” assuming neutrality values. The samples processed with more intense fields of OH10 and OH12 showed a negative association with the “Bitter Taste.” Silva et al. (2020) reported that low and medium-intensity electric fields might be associated with increased bitterness in the samples.

The results for “bitter taste” corroborated the initial hypothesis. Therefore, the hypothesis that heat promotes sensory changes in dairy products (Fox et al., 2015) can be used to formulate a derived hypothesis: products with less aggressive heat treatments preserve more of the natural characteristics of the milk matrix. Thus, the hypothesis suggests that such an effect could be reflected in the descriptors related to fresh milk. “Fresh milk taste” was more strongly associated with products treated with more intense fields OH10 and OH12, while OH6 was neutral and OH8 and PAST were negatively associated. Regarding the “Fresh milk aroma,” it can be observed that only OH12 and PAST were strongly associated.

The aroma and taste of vanilla also seem to have been affected by the treatment with higher intensity OH, being more associated with samples treated with stronger electric fields. The sweet aroma, according to fresh milk, was more associated with the OH12 and PAST samples. The analysis of aroma descriptors suggests that these seem more sensitive to the electric field intensity when compared to the analogous flavor descriptors. Acid taste, unlike bitter, does not necessarily represent a defect in dairy products. Thus, the simple association of the sample with such a descriptor does not allow apparent inferences.

Although the hypergeometric test is practical for the analyst to investigate its hypotheses regarding the product, it may not be sufficient to rank the most critical points for product improvement. Likewise, it is insufficient to reveal whether a descriptor is mentioned as a “quality” or a sensory “defect.” In this way, the analysis was performed for the product-liking drivers to clarify which attributes are of greater importance to consumers and avoid an excessive interpretation of the data by the analyst. So often, what interests trained analysts and tasters are irrelevant to the final consumer (Chen & Opara, 2013; Mendes da Silva et al., 2021).

Figure 3 highlights the distinct behavior of consumer clusters about liking drivers. For cluster 1 (Figure 3a), the descriptors “sweet taste” and “fresh milk taste” were identified as positive drivers related to consumer liking. However, when analyzing the “sweet taste,” only the OH8 sample showed a positive association, and only the PAST sample showed a negative association (Figure 2). Regarding the “fresh milk taste,” samples OH10 and OH12 showed a positive association, while OH8 and OH6 showed a negative association. The only driving of dislike was the “vanilla taste,” positively associated with the OH10 sample and negatively associated with the PAST and OH8 samples. For cluster 2, no drives were identified. In this way, it may be concluded that the impact of OH on sweet taste and the maintenance of fresh milk taste contributed to increase its acceptance by consumers.

Among the studied samples, OH12 presented a better balance in the attributes of interest to the analysts, for example, a low association with the bitter taste and the presence of lumps and a strong association with the flavor and aroma of fresh milk. Also, a good balance in terms of consumer “liking” drivers, except for “sweet taste,” which was below expectations. Therefore, OH12 proved to be the most promising sample among those studied, surpassing the pasteurized standard sample and the others treated with OH. However, the OH12 sample would need a brief correction of its sweet taste to better adhere to consumers’ preferred drivers.

4. Conclusion

This was the first study to use free comment as a method to describe the sensory profile of flavored milk submitted to ohmic heating and identify the drivers or liking and disliking. FC demonstrated to be a suitable methodology as consumers raised the descriptors commonly observed in studies with other sensory methodologies. The employed statistical approach allowed to observe that pasteurization and OH treatment have different effects on the sensory profile of products and the electrical field strength also has a significant impact. Ohmic heating processing with more intense electric fields produces flavored milk that is more associated with attributes of fresh milk and less associated with a bitter taste. At the same time, less intense electric fields tend to produce products that are more bitter, more viscous, and with a more significant presence of lumps.

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ANEXO

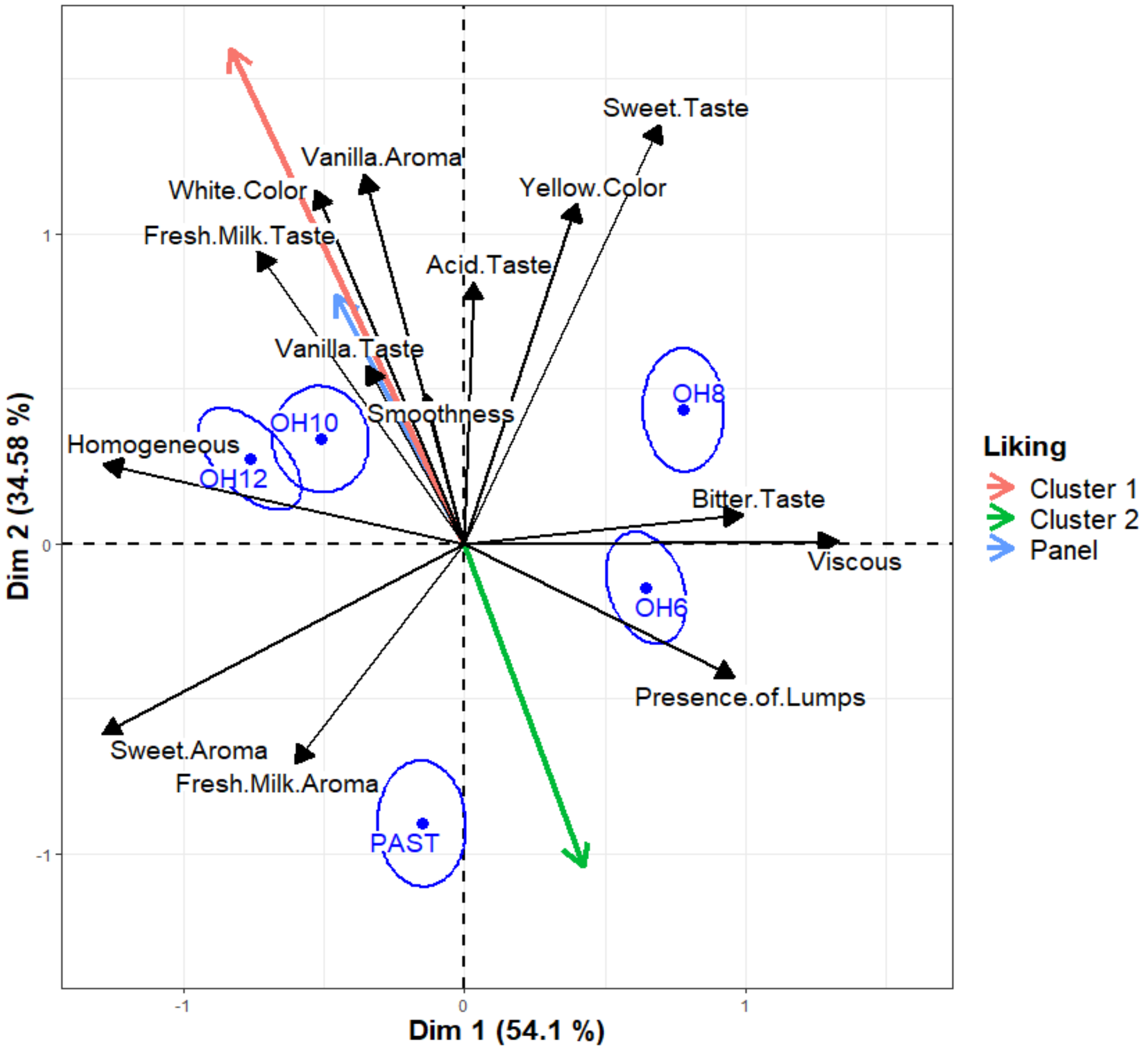

Figure 1. Biplot from multiple-response Correspondence Analysis, their confidence ellipse and the mean panel (Liking) and by segment (Cluster 1 and Cluster 2) liking scores (projected as supplementary variable)

	OH10	OH12	OH6	OH8	PAST
Acid.Taste	60.91	71.82	54.55	75.45	42.73
Bitter.Taste	50.00	40.91	73.64	85.45	57.27
Fresh.Milk.Aroma	59.09	72.73	62.73	34.55	75.45
Fresh.Milk.Taste	65.45	71.82	45.45	39.09	28.18
Homogeneous	62.73	66.36	19.09	30.00	47.27
Presence.of.Lumps	57.27	46.36	98.18	81.82	75.45
Smoothness	79.09	60.91	58.18	64.55	52.73
Sweet.Aroma	59.09	86.36	42.73	23.64	73.64
Sweet.Taste	46.36	38.18	49.09	66.36	13.64
Vanilla.Aroma	90.00	89.09	61.82	79.09	43.64
Vanilla.Taste	68.18	53.64	56.36	37.27	32.73
Viscous	22.73	33.64	77.27	64.55	37.27
White.Color	78.18	91.82	56.36	67.27	39.09
Yellow.Color	35.45	41.82	35.45	58.18	18.18

Figure 2. Sensory descriptors with positive (green cells) and negative (red cells) and neutral (white cells) associations with products according with multiple-response hypergeometric test (α = 5%).

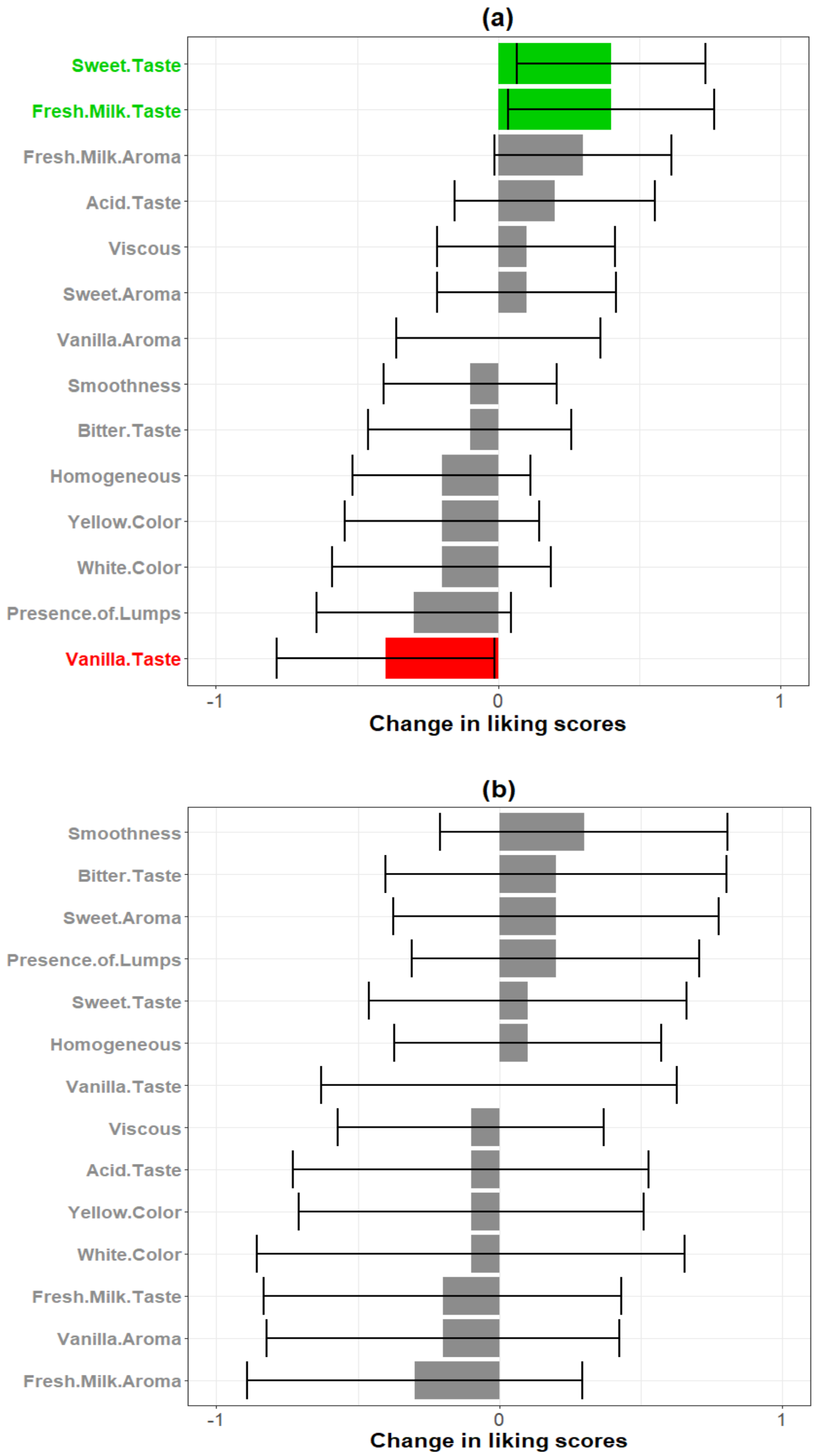

Figure 3. Regression loadings of each descriptor with their respective confidence intervals (α = 5%) for the two segments of consumers: (a) G1 (N = 77) and (b) G2 (N = 33).

Table 1. Sensory descriptors obtained with Free Comment task (n=120 consumers)

Appearance	*"Please describe the appearance of this flavored milk"*	White color Yellow color Presence of lumps Homogeneous
Aroma	*"Please describe the aroma of this flavored milk"*	Fresh milk aroma Sweet aroma Vanilla aroma
Taste	*"Please describe the taste of this flavored milk"*	Acid taste Bitter taste Fresh milk taste Sweet taste Vanilla taste
Texture	*"Please describe the texture of this flavored milk"*	Smoothness Viscous

3.3 ARTIGO 3

Submetido como: ROCHA, R.S.; OLIVEIRA, L.B.; MORAIS, S.T.B.; SOUTELINO, M.E.M.; SILVA, M.C.; FREITAS, M.Q.; UNIOR, S.B.; PIMENTEL, T.C.; ESMERINO, E.A.; CRUZ, A.G. Effect of ohmic heating in bioactive peptides, volatile compounds, and fatty acid profile in high protein vanilla flavored milk, Food Chemistry, 2023.

Effect of ohmic heating in bioactive peptides, volatile compounds, and fatty acid profile in high protein vanilla flavored milk

Abstract

High protein vanilla flavored milk was processed using Ohmic Heating (OH) under different processing conditions (5.22V/cm, OH6; 6.96V/cm, OH8; 8.70V/cm, OH10; 10.43V/ cm, OH12 – 60Hz) and evaluated for the peptide, volatile compounds, and fatty acids profiles compared with untreated and pasteurized samples. Pasteurization or OH resulted in the loss of a β-casein peptide fraction with ACE-inhibitory activity, volatile compounds (1-pentanol, cyclohexanol, isobutyl butyrate, and ethyl decanoate), and fatty acids. However, OH processing released important volatile compounds (acetaldehyde, α-phellandrene). The application of intermediate electric field strengths (OH100) resulted in forming of a β-casein peptide with ACE-inhibitory activity and important volatile compounds (acetic acid, 2-pentanone, and 3-methyl 1-butanol), while maintaining butyric acid concentration and monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) levels more similar to the untreated product. The results suggest that OH, mainly at 10.43V/cm is a promising technology in the development of flavored milk.

Keywords: dairy products; emerging technologies; bioactive compounds; fatty acid; volatile.

1. Introduction

Flavored milk is a dairy product characterized by low viscosity, simple formulation, high versatility, and good sensory acceptance. The obligatory ingredients are milk and one more flavoring agent; however, some optional ingredients can be used, such as thickeners and stabilizers (Bisig & Kelly, 2022). Flavored milk is associated with a healthy diet because it provides the same nutrients as milk, such as fat, protein, vitamins, and calcium. It is also an alternative for feeding children and the elderly (Fayet-Moore et al., 2019). Flavored milk has excellent market potential and is among the most consumed dairy products, and high-protein options could have better nutritional properties (Rocha et al., 2022).

Emerging technologies have been increasingly used recently (Rocha et al., 2020a). Among these technologies, Ohmic Heating (OH) consists of an electrical current passing through a food matrix, generating rapid and uniform heating, which could result in the maintenance of sensory and nutritional characteristics, and the more significant formation of bioactive compounds. In addition, it may be considered a clean technology when coming from renewable energy sources (Rocha et al., 2022). Finally, it may assist in forming small peptides with bioactive properties and easy digestibility, producing lower amounts of unpleasant aromas and flavors in products than conventional heat treatments (Kuriya et al., 2020).

OH has already been applied to dairy products with similar characteristics to flavored milk, such as acerola dairy beverage (Cappato et al., 2018) and raspberry dairy beverage (Ferreira et al., 2019). However, studies using OH in the processing of flavored milk are still scarce in the literature. In our previous study, we observed an increase in the biological activity of flavored milk subjected to OH, mainly in anti-diabetic, anti-hypertensive, and antioxidant activities (Rocha et al., 2022). However, the impact of OH on the peptide profile, volatile compounds, and fatty profile of food products with high protein content has not been evaluated so far.

Considering that OH may change the volatile compounds and directly influence the peptide and fatty acid profiles (Kuriya et al., 2020), we hypothesize that its application in a product rich in proteins could generate a higher concentration of peptides with potential health benefits and important volatile compounds and fatty acid profile. Therefore, this study evaluated the effect of OH at different electric field strengths (5.22V/cm, OH60; 6.96V/cm, OH80; 8.70V/cm, OH100; 10.43V/cm, OH120 – 60Hz) on flavored milk with high protein content compared to conventional heat treatment. In our previous study, processing parameters, formation of bioactive compounds, and energy expenditure were evaluated (Rocha et al., 2022). Thus, this work aims to evaluate the peptide profile, volatile compounds (VOCs), and fatty acid profiles (FAPs).

2. Material and Methods

2.1 High-protein flavored milk processing

The methodology of flavored milk with high protein content was previously published (Rocha et al., 2022). Refrigerated raw milk (3% w/w protein, 3% w/w fat, Ateliê do Queijo, Casemiro de Abreu, Brazil) was added with 10% industrial whey protein isolate (90% total protein, Sooro Renner Nutrição S/A, Paraná), 0.5% w/w vanilla flavor, and 3% sucrose (União, São Paulo). The WPI concentration was selected, aiming for a final product with 12 g/100 mL protein, considered a high-protein product by legislation (Brasil, 2012). The samples were then processed by conventional heat treatment or OH, following the same binome time/temperature (72°C/15s), rapidly cooled (32 ºC), and stored at refrigerated temperature (4 ºC) until analysis (day 1). Six flavored milk formulations were prepared: CRU (untreated), PAST (heat-treated), OH60 (5.22V/cm), OH80 (6.96V/cm), OH100 (8.70V/cm), and OH120 (10.43V/ cm). Electric field strength was calculated using the applied voltage and the distance between the two electrodes. The process was conducted at frequencies of 60 Hz.

2.2 Peptide profiles

2.2.1 Samples

Before analysis, the samples were mixed with the matrix solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile with 0.1% trifluoroacetic acid at a concentration of 10 mg/mL. Subsequently, 0.5 μL of this mixture was applied to a target plate suitable for MALDI (MTP 384, Bruker Daltonics, Bremen, Germany) and dried at room temperature.

2.2.2 Analysis by MALDI-TOF-MS

Bioactive peptide profiles were obtained using high-resolution mass spectrometry, matrix-assisted laser ionization and desorption (MALDI), and a time-of-flight mass analyzer (TOF). The equipment used was an autoflex® maX MALDI mass spectrometer

(Bruker Daltonics, Bremen, Germany) equipped with a 355 nmNd: YAG laser. Mass spectra were acquired in positive reflection mode using an accelerating voltage of 19 kV and a laser frequency of 1 kHz. The ion detection range was m/z 0.7 to 3.5 kDa. External calibration was performed using a standard mixture of peptides. Data were acquired using Flex Control software, and spectra were processed using Flex Analysis software (version 3.4, Bruker Daltonics). Searches were performed on the amino acid sequences of the alpha, beta, and kappa-casein proteins and the major whey proteins, beta-lactoglobulin (BLG), alpha-lactalbumin (ALA), and bovine serum albumin (BSA).

2.3 Volatile compounds

2.3.1 Samples

For the sample preparation for extraction of volatiles by solid phase microextraction (SPME), the methodology described by Bottiroli et al. (2021) was followed, with minor modifications as described below: the lyophilized samples were weighed adequately on an analytical scale (1.0000 ± 0.0001 g) in 60 mL glass vials equipped with a screw cap and PTFE/silicone septum suitable for SPME. After weighing, the samples were diluted with 7 mL of Milli-Q water (the same proportion for reconstitution powdered Ninho milk (Nestle)). The flask was closed and placed in a jacketed beaker, connected to a thermostatic bath at 40 ± 1 °C. The sample was stirred at 200 rpm with a magnetic stirrer and a magnetic stirring bar. The equilibration time was 10 min. After this period, the DVB-type SPME fiber 2 cm long /CAR/PDMS (carboxy/divinylbenzene/polydimethylsiloxane) (previously conditioned according to the manufacturer's instructions) was exposed to the headspace to capture the volatiles. The extraction time was 1 h. The fiber was immediately collected, and the captured volatiles were desorbed for 5 minutes in the gas chromatograph injector coupled to the mass spectrometer (GC-MS). A blank fiber was performed between each sample extraction to ensure the absence of peaks in the running blanks and ensure the quality of the SPME extraction procedures. All samples were injected in triplicates and the results shown are their averages.

2.3.2 Separation and identification of volatiles by GC-MS

A Shimadzu gas chromatograph, model GC-2014 plus, was coupled to a quadrupole mass spectrometric detector (MS) for the chromatographic analyses. The analytes extracted by SPME were desorbed in the chromatograph injector and then separated in an Agilent DB-5MS capillary column (30 m x 0.25 mm x 0.25 μm), under the following chromatographic conditions: injector at 250 °C operating in mode split-less for 1.0 min; helium carrier gas at a constant pressure of 57.3 kPa; oven temperature program starting at 35 °C (1 min), followed by an increment of 3 °C/min until a temperature of 150 °C, followed by an increment of 5 °C/min until a temperature of 250 °C. Interface temperature: 250 °C, ionization source EI +70 EV, m/z 35 to 350. A solution of n-alkanes (C8-C20) was injected into the GC-MS under the same conditions as the sample to obtain temperature-programmed retention rates of volatile compounds (Van Den Dool & Kratz, 1963). The identification of the analytes was carried out by comparing the retention indices (RI) and the mass spectra obtained for the sample with the mass and IR spectra from the literature (NIST, 2011 and WILEY7), with a similarity of at least 85 % for mass spectra, and maximum variation in RI of ± 10.

2.4 Fat content analysis and determination of Fatty Acid Methyl Esters (FAME) by GC-FID

2.4.1 Samples

Samples of freeze-dried flavored milk were weighed on an analytical scale (1.0000 ± 0.0001 g) and submitted to the lipid extraction process using the Bligh-Dyer method (1959) modified as described below. After weighing the samples, 5.0 mL of chloroform:methanol:water (1:2:0.8) containing 0.001% BHT (w/v) was added. In this proportion, the three solvents coexist as a homogeneous mixture, capable of extracting the lipids from the sample. Then, the samples were stirred with a vortex-type shaker (10 min). After stirring, another 1.4 mL of chloroform and 1.4 mL of an aqueous solution of sodium sulfate 1.5% (w/v) were added, which caused the total separation of the chloroform (containing the extracted lipids) from the mixture methanol:water. Afterward, the samples were vortexed again (5 min) and centrifuged at 3000 rpm for 5 min to promote separation between the aqueous phases. The extraction process was performed twice to ensure maximum lipid content recovery. Aliquots of the organic phase containing the lipids were collected, and the chloroform was eliminated using a rotary evaporator under vacuum and at a temperature of 39.5 °C.

The lipid fractions obtained after extraction were weighed to quantify the fat content and then derivatized using a 14% BF_3 methanolic solution, as described by Joseph & Ackman (1992). Triacylglycerols were converted into fatty acid methyl esters (FAME) in this step. After derivatization, the analytes were extracted with the aid of 1500 μL of isoctane and transferred to a 1.5 mL amber vial equipped with a screw cap and PTFE/silicone septum. Finally, the internal standard methyl tricosanoate at a concentration of 0.333 mg/mL was added to the derivatized samples.

2.4.2 Separation, identification, and quantification of FAME by GC-FID

A Shimadzu gas chromatograph, model GC-2014, was equipped with an AOC-20i auto-injector and a flame ionization detector (FID) for the FAME analyses. The analytes were separated in a fused silica capillary column with Agilent DB-225-M polar stationary phase (30 m x 0.25 mm x 0.25 μm) suitable for FAMES separation, under the following chromatographic conditions: injection volume 1 μL, injector at 240 °C operating in 1:130 split mode for 1.0 min; helium carrier gas at a constant pressure of 58.3 kPa; oven temperature program: starting at 100 °C (5 min), followed by an increase of 3 °C/min^{-1} up to a temperature of 230 °C (15 min); detector temperature: 240 °C. A standard solution of GLC-85 fatty acid methyl esters (Nu-Chek Prep. Inc., USA) was used to identify the analytes injected into the GC-FID under the same chromatographic conditions as the samples. For the quantification of the analytes, the standard internal method was used, as described by Visentainer (2012), while the semi-quantification (relative % area) of the FAME was obtained, as described by IOFI (2011).

3. Results and Discussion

3.1 Peptide profiles by MALDI-TOF-MS

Figure 1 shows the mass spectra of the triplicate analyses of the bioactive peptides obtained by MALDI-TOF-MS for the CRU, PAST, OH60, OH80, OH100, and OH120 samples. Table 1 presents the bioactive peptides identified by high-resolution mass spectrometry MALDI-TOF-MS.

The primary markers were observed between 820 and 2880 Da. Five of the peaks were related to β-casein, 2 to αS1-casein, and 2 to αS2-casein. Among all the peptides identified in the different treatments, eight bioactive peptides were detected based on the peptide sequencing and relating to previously published studies (Table 1).

All flavored milk samples showed the peptides αS1-casein (f 16-24, m/z 1140, RPKHPIKHQ) and β-casein (f 160-169, m/z 1151, HQPHQPLPPT), related to the anti-hypertensive activity (ACE inhibitor) (Saito et al., 2000, Sawh et al., 2020). Furthermore, they presented the peptide αS2-casein (f 163-176, m/z 1717, TKKTKLTEEEKNRL), which has demonstrated antimicrobial activity (Sistla, 2013). The results indicate that important bioactive peptides were presented in the untreated product and kept in the products after pasteurization or OH.

The heating process (pasteurization and OH) changed the peptide profile. The β-casein peptide fraction (f 210-221), m/z 1264, EPVLGPVRGPFP) was observed only in the sample without heat treatment (CRU), suggesting an impact of heating in this peptide. This peptide has been associated with ACE-inhibitory activity (Adams et al., 2020). On the contrary, the processed products (PAST, OH60, OH80, OH100, and OH120) presented the peptides αS2-casein (f 46-55, m/z 1251, YIPIQYVLSR) and the β-casein (f 208-224/ m/z 1881, YQEPVLGPVRGPFPIIV), which were not detected in the untreated product. These results demonstrate that the utilization of heating, regardless of the processing type, resulted in the formation of bioactive peptides with opioid and C3a receptor agonist activity and immunomodulatory, antithrombin, antimicrobial, and ACE inhibitory effects (Yamanot et al., 1994; Birkemo et al., 2009; Rojas-Ronquillo et al., 2012).

The effect of OH was dependent on the electric field strength. The application of low electric field strengths (OH60) resulted in the maintenance of the β-casein peptide (f 184-191, m/z 908, KVLPVQK) from the untreated product. This peptide is related to antioxidant activities (Rival et al., 2001, Tonolo et al., 2018), and it was not identified in the other processed samples (PAST, OH60, OH80, OH100, and OH120). OH80 sample showed a similar peptide profile to PAST, while OH100 resulted in the appearance of β-casein peptide (f 198-205, m/z 977, RDMPIQAF), which has demonstrated ACE-inhibitory activity (Yamamoto et al., 1994). Finally, at high electric field strengths (OH120), the αS1-casein (f 17-24, m/z 1052, NENLLRFF), with anti-hypertensive activity (Papadimitriou et al., 2007), was not identified.

OH in processing foods with antioxidant and anti-hypertensive potential has been reported in different dairy matrices (Rocha et al., 2022; Silva et al., 2020; Kuriya et al., 2020; Rocha et al., 2020b). The effect of OH on the formation of bioactive peptides can be related to its non-thermal effect, which promotes denaturation in specific parts of the protein, altering the denaturation and conformation of the initial structure when compared to conventional treatments. However, drastic conditions of OH may result in the loss of bioactive peptides (Cappato et al., 2018). Cappato et al. (2018) evaluated the use of OH in the peptide profile of dairy beverages. The results also followed a metric close to that found in this study, suggesting that processing in intermediate OH conditions can be beneficial in forming these compounds. Our results suggest that OH100 would be the most recommended sample considering the peptide profile, presenting 7 peptides with potential health effects.

3.2 Volatile compounds

The average results of the volatile compounds identified in the samples of flavored milk with high protein content are described in Table 2. In all, 39 compounds were detected, 38 in the control sample (CRU), 26 in the product submitted to conventional pasteurization (PAST), and 30, 28, 38, and 30 in samples subjected to OH (OH 60; OH 80, OH 100, and OH 120, respectively). The number of volatile compounds was low compared to that reported by Liu et al. (2022) when analyzing fermented milks (n=95). Volatile compounds originate from three metabolic pathways: lipolysis and subsequent free fatty acid (FFA) metabolism, metabolism of lactose, lactate, and residual citrate, and the process of proteolysis that results in the formation of peptides and free amino acids (FAA) (Santamarina-García et al., 2022). The manufacturing and maturation process of many dairy products, mainly cheeses and fermented milk, help in the formation of volatile compounds due to several reactions that occur from the action of microorganisms and enzymes, aligned with the fermentation and maturation time of these products (Zhang et al., 2022). Flavored milk is not added with microorganisms or subjected to fermentation or maturation, which explains the lower number of volatile compounds.

Twenty-three compounds were detected in all flavored milks (ethyl alcohol, hexane, 3-methylbutanal, 2-ethyl-1-butanol, octane, hexanal, ethyl butyrate, ethylbenzene, o-xylene, m-xylene, butyl acrylate, 1-propylbenzene, pseudocumene, β-pinene, o-ethyltoluene, limonene, 2-ethyl-1,4-dimethyl-benzene, 2-methyl-2- undecanethiol, 3-ethyl-o-xylene, nonaldehyde, capric acid, 2-propyl-1-heptanol, and tridecanol). The results suggest their presence in the untreated flavored milk and maintenance after processing (pasteurization or OH). These volatile compounds are associated with typical (hexanal, 3-methylbutanal), and fruity and floral flavors (mainly esters) of milk and dairy products (Cheng, 2010, Ranadheera et al., 2019).

The heating process (pasteurization and OH) resulted in the disappearance of 4 volatile compounds, 1-pentanol, cyclohexanol, isobutyl butyrate, and ethyl decanoate.

Extrinsic factors such as temperature can result in the degradation of specific volatile compounds in untreated products (Ma et al., 2023). Ethyl decanoate is commonly related to vanilla odor (Santamarina-García et al. 2022), while 1-pentanol with alcoholic aroma (Ranadheera et al., 2019). Cyclohexanol has already been reported in dairy products from raw milk, contributing to their typical aroma and flavor (Calzada et al., 2015).

However, OH utilization resulted in the identification of acetaldehyde, with a progressive increase as the electric field strength increased (1089.00, 1159.49, 1656.43, 2220.20), corresponding to OH60, OH80, OH100, and OH120, respectively. This compound is reported as one of the main ones responsible for the aroma in fermented dairy products and products submitted to some additional treatment (Liu et al., 2022). OH utilization also resulted in the maintenance of α-phellandrene and loss of 2-propanone. 2 propanone is related to off-flavor problems in dairy products (Silveira et al., 2018). Furthermore, α-phellandrene is related to citrus, green, mint, and resinous notes and may be derived from cow feed (Fedele et al., 2005). The progressive exposure to electric current (Table 2) may have resulted in a greater release of these compounds from the matrix.

The effect of OH was dependent on the electric field strength. The application of low electric field strengths (OH60) resulted in the presence of methanediol. Methanediol, similar to other sulfur compounds, forms sulfur/eggy and cooked flavors (Vazquez-Landaverde, Torres, Qian, 2006). It has been associated with boiled potato, cooked cabbage, and sulfur aromas in milk and dairy products (Cheng, 2010). This result may be linked to the fact that differences in the heating step and electrical exposure affect the formation of sulfur-containing compounds. More prolonged exposure to high temperatures when using low electric field strengths may lead to greater oxidation of compounds (Whitt et al., 2022).

The application of intermediate electric fields (OH100) resulted in the identification of acetic acid, 2-pentanone, and 3-methyl 1-butanol in the products. 3-methyl 1-butanol is a compound related to the aroma of fresh, buttery, and creamy dairy products, which can be beneficial in improving sensory characteristics (Ranadheera et al., 2019). It is a branched fatty acid commonly derived from extensive proteolysis (Gutiérrez-Peña et al., 2021), demonstrating the impact of OH on the proteins. At the same time, 2-pentanone is associated with sweet and fruit aromas, and acetic acid is related to an acidic flavor

(Ranadheera et al., 2019). Acetic acid may be originated from lactose degradation or oxidation of esters, ketones, or aldehydes (Gutiérrez-Peña et al., 2021), which may have been promoted by OH. Finally, isopentyl acetate was maintained at high electric field strengths (OH120). Esters, including isopentyl acetate, are associated with typical aromas in many herbs and have been linked to the predominant aromas in dairy products, such as kefir (Farag et al., 2020).

Our results suggest that OH100 would be the most recommended sample considering the volatile compounds profile, presenting a higher number of volatile compounds (n=38), including some with importance to milk aromas and flavors (acetic acid, 2-pentanone, and 3-methyl 1-butanol).

3.3 Fat content and Fatty Acid Methyl Esters (FAME)

Table 3 shows the results for the fat content present in each sample. Fat content in the OH120 treatment was similar to the untreated product ($p > 0.05$), while the other flavored milk samples showed lower fat content ($p < 0.05$). The results are directly related to the processing time and exposure of the product to heat. OH may promote higher structural preservation of the components present in the food, depending on the process parameters (Ferreira et al., 2019). In this sense, OH at higher electric field strengths (OH120) resulted in the maintenance of the fat content. The utilization of low electric field strengths (OH60) resulted in a similar impact to pasteurization (PAST), while the utilization of intermediate electric field strengths reduced the fat content (OH80 and OH100).

Table 4 shows the quantitative (mg of fatty acid methyl ester/gram of fat) and semi-quantitative (relative % area) results of methyl esters of fatty acids present in the samples. Figure 2 presents the fatty acids identified in the flavored milk samples and their chemical structure.

Fatty acids are classified according to the number of carbon atoms (C) in long-chain fatty acids (LCFA), medium-chain fatty acids (MCFA), and short-chain fatty acids (SCFA) or based on the presence of double bonds between carbon atoms in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Wang et al., 2022). With more than 400 different fatty acids (FA), milk fat is milk's most complex and highly variable component. It is mainly present in triglyceride (approximately

98%), the bond of three fatty acids in a glycerol molecule (Wilms et al., 2022).

Oleic acid (C18:1n-9 (cis)), palmitic acid (C16:0), and stearic acid (C18:0) were the fatty acids present in more significant amounts in flavored milk samples, with emphasis on the CRU sample that obtained higher values of these compounds compared to all other treatments (199.32 m/g, 147.84 m/g, 89.30m/g, respectively). These results are expected because these three are the most predominant FA found in milk fat and adipose tissue (Burch, Pineda, and Lock, 2021), in addition to the fact that flavored milk does not undergo any stage of fermentation, maturation, and hydrolysis of its components, with results close to those found in the in the untreated product.

Palmitic acid (C16:0) (PA) is the main saturated fatty acid (SFA), accounting for about 30% of the fatty acids in milk (Marangoni & Ghazani, 2021). In bovine milk, approximately 40% of PA is in the glycerol molecule's sn-2 position (β-palmitate). The position of PA in the glycerol structure is an important factor in digestion due to the action of pancreatic enzymes selectively acting on the sn-1 and sn-2 positions (Wilms et al., 2022). Therefore, many studies aim at animal supplementation with PA aiming at milk with a higher fat content, market, and industrial value (Landry et al., 2022).

Heating process (pasteurization or OH) and OH process parameters significantly impacted the fatty acid profile of the flavored milks. All processed products (PAST, OH60, OH80, OH100, and OH120) showed a lower content of SFA, MUFA and PUFA compared to raw milk ($p < 0.05$), demonstrating the impact of heating on these compounds. The effect was more pronounced for PAST and OH6, with the lowest observed concentration of the fatty acids ($p < 0.05$). On the other hand, higher maintenance of the fatty acid contents was observed with OH’s higher electric field strengths, mainly OH100 ($p < 0.05$).

OH100 flavored milk showed maintenance of C4:0 similar to the untreated product ($p < 0.05$). Butyric acid (C4:0) is an important cellular mediator and regulator of intestinal cell functions, participating in the development of intestinal tissue, gene expression, reducing oxidative stress, and immune modulation (Bedford and Gong, 2018). In sensory terms, the presence of butyric acid has been reported as an essential factor in improving the sensory characteristics of yogurt (Huang et al., 2020) and also used alone in the production of cheese-flavored snacks (Menis-Henrique et al., 2019). OH100 sample also showed the highest MUFA and PUFA levels among the processed products ($p < 0.05$). PUFA fatty acids are essential since they must be inserted through the diet, as the body does not synthesize them. They actively maintain and promote health by preventing various diseases (Lee et al., 2023).

Ohmic heating has been constantly used to process dairy products with an evaluation of the fatty acid profile (Rocha et al., 2020b; Silva et al., 2020a). The results demonstrate that variations in electric field strength strongly influence the fatty acid profile. The OH 100 treatment was the one that presented the closest results to the untreated sample, mainly about FA of nutritional and sensory importance, in addition to PUFA values, thus being the most suitable treatment for use in the processing of flavored milk with high protein content.

4. Conclusion

This was the first study to evaluate the peptide, volatile compounds, and fatty acids profiles of a high protein vanilla flavored milk processed by OH or pasteurization. Heating process (pasteurization or OH) resulted in the loss of important compounds, such as a β-casein peptide fraction with ACE-inhibitory activity, volatile compounds (1-pentanol, cyclohexanol, isobutyl butyrate, and ethyl decanoate), and fatty acids. However, OH processing released important volatile compounds (acetaldehyde, α-phellandrene). The application of intermediate electric field strengths (OH100) is advisable as it resulted in forming of a β-casein peptide with ACE-inhibitory activity and important volatile compounds (acetic acid, 2-pentanone, and 3-methyl 1-butanol), while maintaining butyric acid concentration and MUFA and PUFA levels more similar to the untreated product. The results suggest that OH, mainly at 10.43V/cm, is a promising technology in the development of flavored milk.

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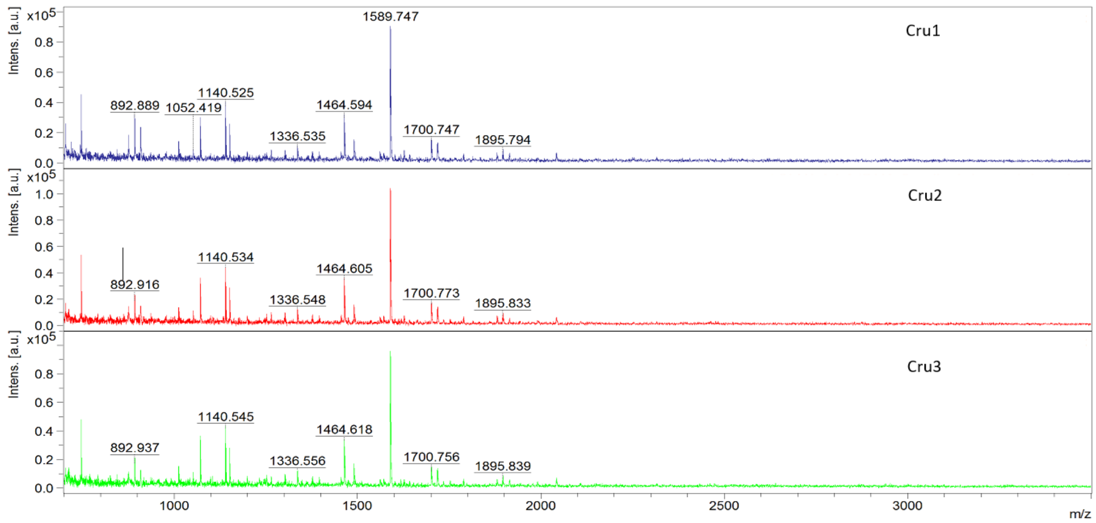
ANEXO



Intens. [a.u.]
x10^5
Cru1
1589.747
1140.525
892.889
1052.419
1464.594
1336.535
1700.747
1895.794
Cru2
1140.534
1464.605
892.916
1336.548
1700.773
1895.833
Cru3
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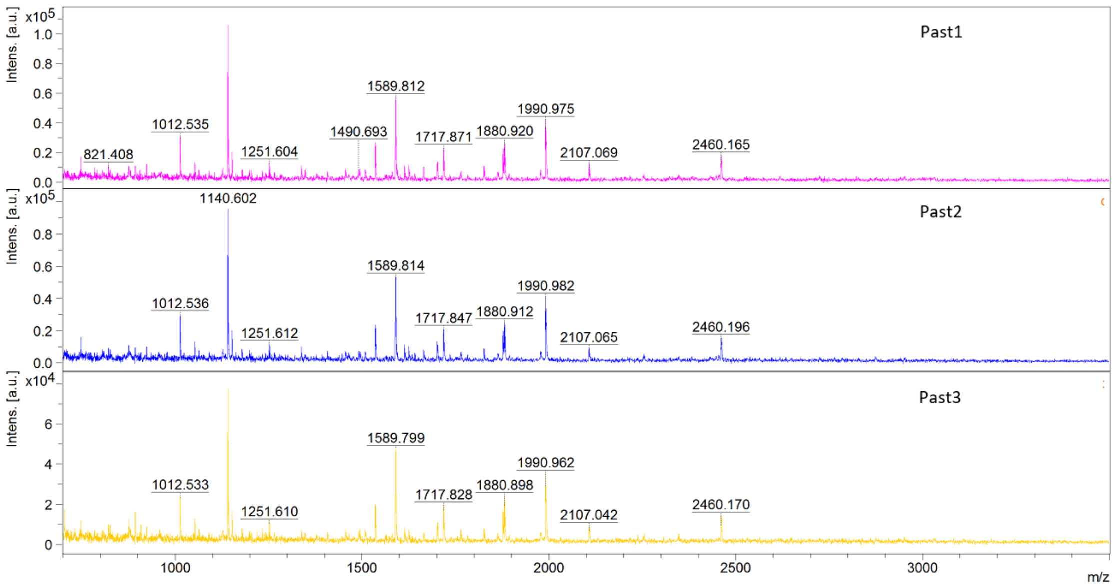
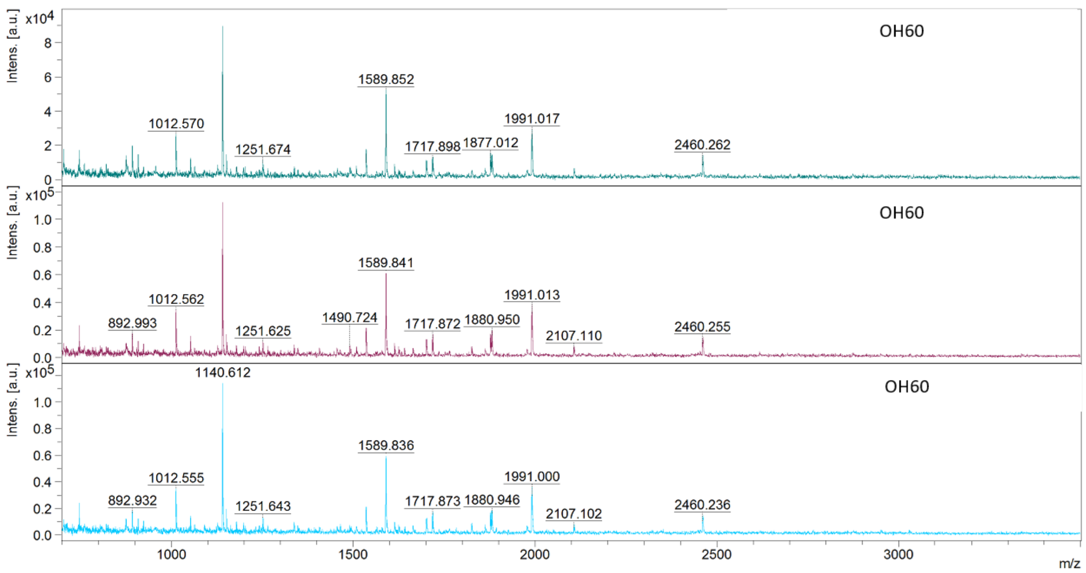
1464.618
892.937
1336.556
1700.756
1895.839
1000
1500
2000
2500
3000
m/z



Intens. [a.u.]
x10^5
Past1
1589.812
1990.975
1012.535
1490.693
1717.871
1880.920
821.408
1251.604
2107.069
2460.165
1140.602
Past2
1589.814
1990.982
1012.536
1717.847
1880.912
1251.612
2107.065
2460.196
x10^4
Past3
1589.799
1990.962
1012.533
1880.898
1717.828
1251.610
2107.042
2460.170
1000
1500
2000
2500
3000
m/z



Intens. [a.u.]
x10^4
OH60
1589.852
1991.017
1012.570
1251.674
1717.898
1877.012
2460.262
x10^5
OH60
1589.841
1012.562
1991.013
892.993
1490.724
1717.872
1880.950
1251.625
2107.110
2460.255
1140.612
OH60
1589.836
1012.555
1991.000
892.932
1717.873
1880.946
1251.643
2107.102
2460.236
1000
1500
2000
2500
3000
m/z

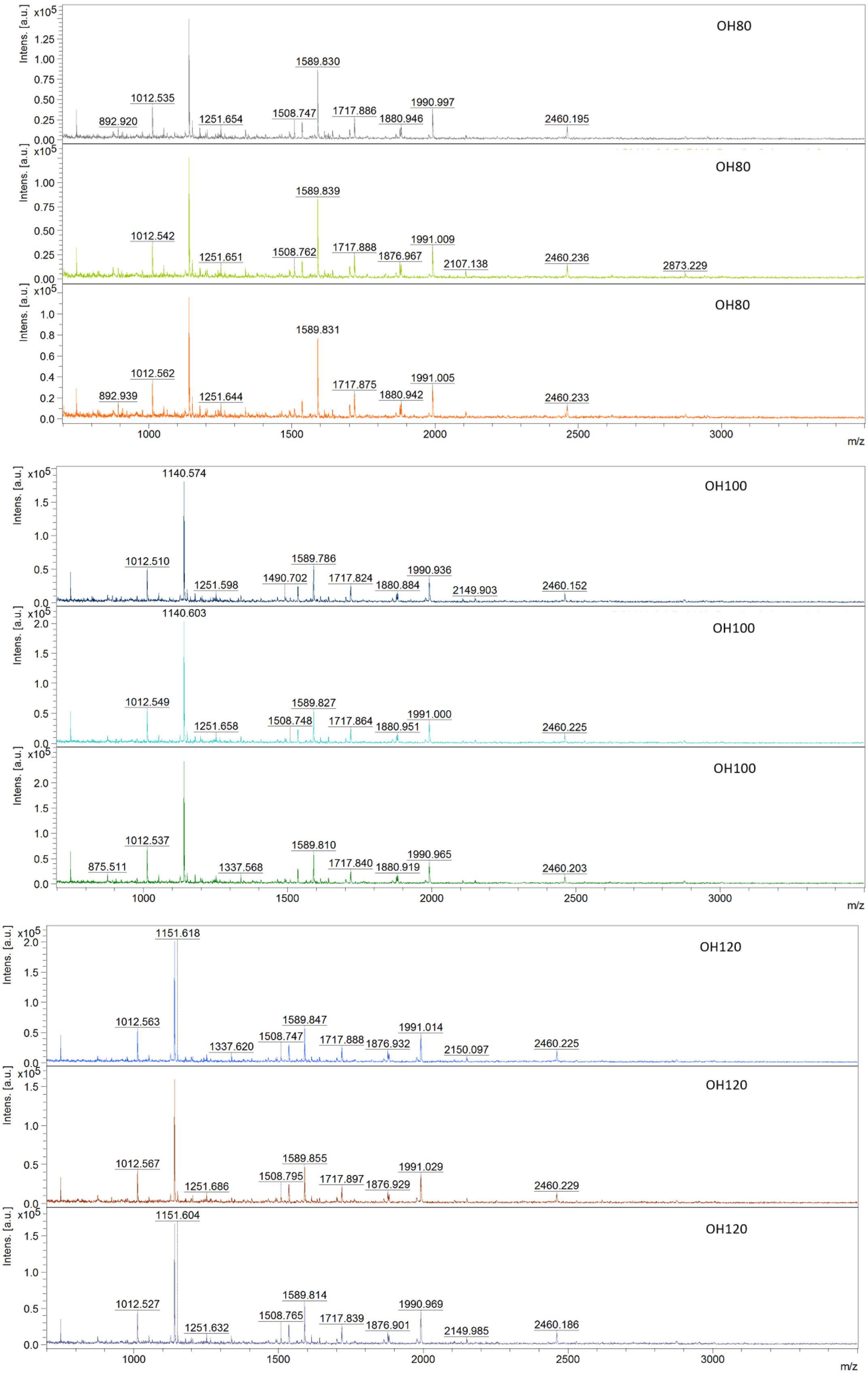


Figure 1. Mass spectra of triplicate analyzes of bioactive peptides obtained by MALDI-TOF-MS for CRU, PAST, OH 60, OH 80, OH 100, OH 120 samples.

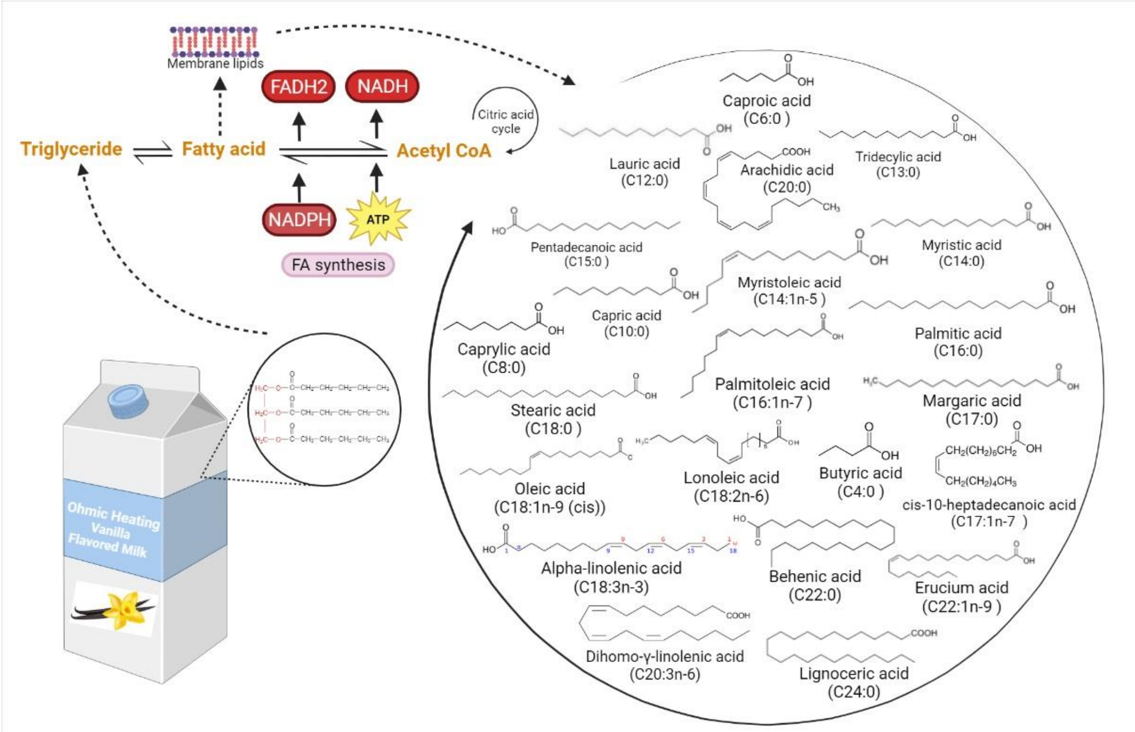


Figure 2. Simplified scheme and structure of fatty acids found in flavored milk samples.

Table 1. Bioactive peptides identified by high-resolution mass spectrometry MALDI-TOF-MS in the CRU freeze-dried high protein dairy drink exception, PAST, OH 60, OH 80, OH 100, OH 120 and their biological activities for the peptides identified according to the literature.

			CRU		
m/z	**Protein**	**peptide**	**Segment**	**Bioactivity**	**References**
908	β-casein	KVLPVQK	184 - 191	antioxidant	Rival et al. (2001), Tonolo et al. (2018)
1052	α-casein S1	NONLLRFF	17-24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α-casein S1	RPKHPIKHQ	16-24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160-169	ACE inhibitor	Adams & Sawh (2020)
1264	β-casein	EPVLGPVRGPFP	210-221	ACE inhibitor	Adams & Sawh (2020)
1717	α-casein S2	TKKTKLTEEEKNRL	163 - 176	antimicrobial	Sistla (2013)
			PAST		
m/z	**Protein**	**peptide**	**Segment**	**Bioactivity**	**References**
1052	α-casein S1	NONLLRFF	17-24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α-casein S1	RPKHPIKHQ	16-24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160-169	ACE inhibitor	Adams & Sawh (2020)
1251	α-casein S2	YIPIQYVLSR	46-55	Opioid , C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α-casein S2	TKKTKLTEEEKNRL	163 - 176	antimicrobial	Sistla (2013)
1881	β-casein	YQEPVLGPVRGPFPIIV	208-224	Immunomodulator , antithrombin, antimicrobial, ACE inhibitor	Yamamoto et al. (1994), Birkemo et al. (2009), Rojas- Ronquillo et al. (2012)
			OH 60		
m/z	**Protein**	**peptide**	**Segment**	**Bioactivity**	**References**
908	β-casein	KVLPVQK	184 - 191	antioxidant	Rival et al. (2001), Tonolo et al. (2018)
1052	α-casein S1	NONLLRFF	17-24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α-casein S1	RPKHPIKHQ	16-24	ACE inhibitor	Saito et al. (2000)

1151	β-casein	HQPHQPLPPT	160-169	ACE inhibitor	Adams & Sawh (2020)
1251	α-casein S2	YIPIQYVLSR	46-55	Opioid , C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α-casein S2	TKKTKLTEEEKNRL	163 - 176	antimicrobial	Sistla (2013)
1881	β-casein	YQEPVLGPVRGPFPIIV	208-224	Immunomodulator , antithrombin, antimicrobial, ACE inhibitor	Yamamoto et al. (1994), Birkemo et al. (2009), Rojas- Ronquillo et al. (2012

OH 80

m/z	Protein	peptide	Segment	Bioactivity	References
1052	α-casein S1	NONLLRFF	17-24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α-casein S1	RPKHPIKHQ	16-24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160-169	ACE inhibitor	Adams & Sawh (2020)
1251	α-casein S2	YIPIQYVLSR	46-55	Opioid , C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α-casein S2	TKKTKLTEEEKNRL	163 - 176	antimicrobial	Sistla (2013)
1881	β-casein	YQEPVLGPVRGPFPIIV	208-224	Immunomodulator , antithrombin, antimicrobial, ACE inhibitor	Yamamoto et al. (1994), Birkemo et al. (2009), Rojas- Ronquillo et al. (2012)

OH 100

m/z	Protein	peptide	Segment	Bioactivity	References
977	β-casein	RDMPIQAF	198-205	ACE inhibitor	Yamamoto et al. (1994)
1052	α-casein S1	NONLLRFF	17-24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α-casein S1	RPKHPIKHQ	16-24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160-169	ACE inhibitor	Adams & Sawh (2020)
1251	α-casein S2	YIPIQYVLSR	46-55	Opioid , C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α-casein S2	TKKTKLTEEEKNRL	163 - 176	antimicrobial	Sistla (2013)
1881	β-casein	YQEPVLGPVRGPFPIIV	208-224	Immunomodulator , antithrombin, antimicrobial, ACE inhibitor	Yamamoto et al. (1994), Birkemo et al. (2009), Rojas- Ronquillo et al. (2012

OH 120

m/z	**Protein**	**peptide**	**Segment**	**Bioactivity**	**References**
1140	α-casein S1	RPKHPIKHQ	16-24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160-169	ACE inhibitor	Adams & Sawh (2020)
1251	α-casein S2	YIPIQYVLSR	46-55	Opioid , C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α-casein S2	TKKTKLTEEEKNRL	163 - 176	antimicrobial	Sistla (2013)
1881	β-casein	YQEPVLGPVRGPFPIIV	208-224	Immunomodulator , antithrombin, antimicrobial, ACE inhibitor	Yamamoto et al. (1994), Birkemo et al. (2009), Rojas- Ronquillo et al. (2012

Table 2. Mean results of MS gross area for the profile of volatile compounds present in high protein dairy drink samples obtained with different ohmic heating treatments .

Volatile compound	RI	CRU	PAST	OH60	OH80	OH100	OH120
acetaldehyde	381	-	-	X	X	X	X
Ethyl alcohol	446	X	X	X	X	X	X
Methanethiol	464	X	-	X	-	X	-
2-Propanone	481	X	X	-	-	-	-
hexane	600	X	X	X	X	X	X
ethyl acetate _	605	X	-	-	X	-	X
Acetic Acid	646	X	-	-	-	X	-
2- Pentanone	653	X	-	X	-	X	X
3- Methylbutanal	655	X	X	X	X	X	X
3- Methyl -1-butanol	736	X	-	X	-	X	X
2- Ethyl -1-butanol	766	X	X	X	X	X	X
1- Pentanol	768	X	-	-	-	-	-
Octane	800	X	X	X	X	X	X
hexanal	802	X	X	X	X	X	X
ethyl butyrate _	805	X	X	X	X	X	X
Ethylbenzene	859	X	X	X	X	X	X
o -Xylene	867	X	X	X	X	X	X
isopentyl acetate	878	X	-	-	-	-	X
butyl ether	883	X	-	X	X	X	X
Cyclohexanol	885	X	-	-	-	-	-
m -Xylene	892	X	X	X	X	X	X
- butyl acrylate	900	X	X	X	X	X	X
Heptanal	904	X	X	X	-	X	X
Cumenian	924	X	X	-	X	X	X
α-Phelandrene	926	X	-	X	X	X	-
1- Propylbenzene	952	X	X	X	X	X	X
isobutyl butyrate _	958	X	-	-	-	-	-

Pseudocumene	969	X	X	X	X	X	X
β-pinene	975	X	X	X	X	X	X
o -Ethyltoluene	980	X	X	X	X	X	X
limonene	1029	X	X	X	X	X	X
2- Ethyl - 1,4 -dimethyl-benzene	1077	X	X	X	X	X	X
2- Methyl -2- undecanethiol	1080	X	X	X	X	X	X
3- Ethyl-o-xylene	1085	X	X	X	X	X	-
Nonaldehyde	1106	X	X	X	X	X	X
capric acid	1201	X	X	X	X	X	X
2- Propyl -1- heptanol	1208	X	X	X	X	X	X
tridecanol	1307	X	X	X	X	X	X
ethyl decanoate	1399	X	-	-	-	-	-
Total volatile organic compounds		38	26	30	28	32	30

*RI= retention index; X: presence , - : absence.

Table 3. Results of fat content by Bligh-Dyer present in samples of high protein dairy drink obtained with different ohmic heating treatments.

Sample	Percentage of fat in the sample (g/100 g)
CRU	16.3 ± 0.40[a]
PAST	13.9± 0.14[c]
OH 60	14.4 ± 0.14[c]
OH 80	11.9 ± 0.12[e]
OH 100	12.8 ± 0.13[d]
OH 120	17.0 ± 0.27[a]

Table 4. Quantitative (mg of fatty acid methyl ester/gram of fat) and semi-quantitative (relative area%) results of fatty acid methyl esters present in high protein dairy beverage samples obtained with different ohmic heating treatments.

Fatty acids	**CRU**		**PAST**		**OH60**		**OH80**		**OH100**		**OH120**	
	(mg/g)	Area %	(mg/g)	Area %	(mg/g)	Area %	(mg/g)	Area %	(mg/g)	Area %	(mg/g)	Area %
C4:0 (acid butyric)	11.78^{a}	1.49	8.97^{c}	1.50	10.97^{b}	1.70	10.90^{b}	1.61	12.01^{a}	1.69	11.99^{a}	1.71
C6:0 (acid caproic)	8.52^{a}	1.23	6.37^{d}	1.21	7.65^{c}	1.35	7.68^{c}	1.29	8.33^{b}	1.33	8.32^{b}	1.35
C8:0 (acid caprylic)	4.42^{a}	0.76	3.32^{e}	0.75	4.06^{d}	0.85	3.92^{d}	0.78	4.23^{c}	0.81	4.37^{b}	0.84
C10:0 (acid capric)	10.00^{a}	1.62	7.57^{d}	1.62	8.79^{c}	1.75	8.84^{c}	1.67	9.40^{b}	1.69	9.51^{b}	1.73
C12:0 (acid lauric)	12.03^{a}	2.01	9.16^{d}	2.02	11.13^{b}	2.28	10.64^{c}	2.08	11.15^{b}	2.07	11.89^{a}	2.24
C13:0 (acid tridecyl)	0.45^{a}	0.08	0.34^{c}	0.08	0.39^{b}	0.08	0.40^{a}	0.08	0.42^{a}	0.08	0.43^{a}	0.08
C14:0 (acid myristic)	49.09^{a}	8.42	37.46^{d}	8.45	41.28^{c}	8.67	42.90^{c}	8.57	44.99^{b}	8.55	44.77^{b}	8.62
C14:1n-5 (acid myristoleic)	4.87^{a}	0.84	3.77^{e}	0.86	4.15^{d}	0.88	4.37^{c}	0.88	4.55^{b}	0.87	4.51^{b}	0.88
C15:0 (acid pentadecanoic)	8.52^{a}	1.48	6.46^{f}	1.47	7.01^{e}	1.49	7.35^{d}	1.48	7.72^{b}	1.48	7.57^{c}	1.47
C16:0 (acid palmitic)	147.84^{a}	25.87	112.22^{e}	25.79	120.49^{d}	25.78	126.78^{c}	25.79	133.25^{b}	25.77	131.66^{b}	25.81
C16:1n-7 (acid palmitoleic)	6.74^{a}	1.19	5.15^{e}	1.19	5.52^{d}	1.19	5.83^{c}	1.20	6.10^{b}	1.19	5.96^{b}	1.18
C17:0 (acid margaric)	5.20^{a}	0.92	3.95^{e}	0.91	4.19^{d}	0.90	4.43^{c}	0.91	4.66^{b}	0.91	4.58^{b}	0.91
C17: 1n-7 (cis -10 - heptadecanoic acid)	0.97^{b}	0.17	0.83^{c}	0.19	0.95^{b}	0.21	1.03^{b}	0.21	1.11^{a}	0.22	1.11^{a}	0.22
C18:0 (acid stearic)	89.30^{a}	15.87	67.79^{d}	15.81	71.40^{d}	15.50	75.30^{c}	15.54	79.25^{b}	15.55	78.07^{b}	15.52
C18: 1n-9 (cis) (oleic acid)	199.32^{a}	35.67	152.39^{d}	35.79	160.62^{d}	11.35	171.31^{c}	35.61	179.53^{b}	35.47	177.25^{b}	35.48
C18:2n-6 (acid linoleic)	5.73^{a}	1.03	4.05^{d}	0.96	4.27^{c}	0.94	4.56^{b}	0.95	4.75^{b}	0.94	3.03^{e}	0.61
C18: 3n-3 (alpha-linolenic acid)	2.68^{a}	0.48	2.02^{c}	0.48	1.98^{c}	0.44	2.20^{b}	0.46	2.19^{b}	0.44	2.03^{c}	0.41
C20:0 (acid arachidic)	1.14^{a}	0.20	0.87^{d}	0.20	0.90^{d}	0.20	0.96^{c}	0.20	1.01^{b}	0.20	0.98^{c}	0.20
C20:1n-9 (acid eicosenoic)	0.17^{c}	0.03	0.37^{b}	0.09	0.31^{b}	0.07	0.34^{b}	0.07	0.78^{a}	0.16	0.71^{a}	0.14
C20:2n-6 (acid eicosadienoic)	0.16^{a}	0.03	0.12^{a}	0.03	0.14^{a}	0.03	0.13^{a}	0.03	0.15^{a}	0.03	0.15^{a}	0.03
C22:0 (acid behenic)	0.18^{a}	0.03	0.13^{a}	0.03	0.15^{a}	0.03	0.15^{a}	0.03	0.14^{a}	0.03	0.14^{a}	0.03
C22:1n-9 (acid erucius)	0.38^{a}	0.07	0.29^{c}	0.07	0.30^{c}	0.07	0.33^{b}	0.07	0.36^{a}	0.07	0.37^{a}	0.07
C20: 3n-3 (dihomo-α-linolenic acid)	0.33^{a}	0.06	0.27^{b}	0.06	0.29^{b}	0.07	0.30^{ab}	0.06	0.34^{a}	0.07	0.32^{a}	0.06
C20: 3n-6 (dihomo-γ-linolenic acid)	0.86^{a}	0.16	0.59^{c}	0.14	0.60^{c}	0.13	0.64^{bc}	0.14	0.63^{b}	0.13	0.62^{b}	0.13
C22:2n-6 (13,16-docosatrienoic acid)	0.61^{a}	0.11	0.45^{d}	0.11	0.52^{c}	0.12	0.54^{c}	0.12	0.56^{b}	0.11	0.55^{b}	0.11

C24:0 (acid lignoceric)	0.90[a]	0.16	0.71[c]	0.17	0.73[c]	0.16	0.70[c]	0.15	0.77[b]	0.15	0.80[b]	0.16
Total fatty acids												
SFA	349.37[a]	60.15	265.30[e]	60.03	289.13[d]	60.75	300.95[c]	60.19	317.29[b]	60.30	315.08[b]	60.67
MUFA	212.44[a]	37.97	162.80[e]	38.19	171.84[d]	37.52	183.21[c]	04.38	192.43[b]	37.98	189.90[b]	37.98
PUFA	10.37[a]	2.43	7.50[c]	1.78	7.80[c]	1.73	8.37[b]	1.76	8.61[b]	1.72	6.70[d]	1.36
n-3	3.01[a]	1.09	2.29[c]	0.55	2.27[c]	0.50	2.49[b]	0.53	2.54[b]	0.51	2.34[c]	0.48
n-6	7.36[a]	1.33	5.21[d]	1.24	5.53[c]	1.22	5.87[b]	1.23	6.07[b]	1.21	4.36[e]	0.88
PUFA/SFA	0.03	3.12	0.03	2.97	0.03	2.84	0.03	2.93	0.03	2.86	0.02	2.24

*SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

3.4 ARTIGO 4 (TÉCNICO)

PRODUTOS LÁCTEOS COM ALTO TEOR DE PROTEÍNA: CONSIDERAÇÕES TECNOLÓGICAS

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O aumento do número de consumidores preocupados com sua saúde e hábitos alimentares vêm refletindo diretamente nas indústrias, que buscam cada vez mais alternativas para atender essa nova demanda, produzindo alimentos com maior apelo nutricional e funcional. Leite e seus derivados estão entre os produtos alimentícios mais consumidos pela população brasileira, incluindo bebidas com elevado teor proteico, bebidas lácteas, produtos fermentados, queijos, entre outros.

As proteínas do leite, além dos benefícios nutricionais, também são bastante utilizadas pela indústria de alimentos na busca de melhorias de produtos processados, nos quesitos de palatabilidade, textura e rendimento. O consumo de produtos lácteos contendo alto teor de proteína está em constante crescimento, devido a seus inúmeros benefícios à saúde, como ganho de massa magra, ação imunomoduladora, ação anti-hipertensiva, dentre outras. Atualmente encontram-se no mercado diversos produtos lácteos com elevação do seu valor proteico, como iogurtes e bebidas lácteas.

O aumento do teor de proteínas produtos lácteos pode ocorrer antes da fermentação ou após a fermentação. Quando ocorrido antes do processo fermentativo, podem ser utilizados principalmente caseinatos e isolados proteicos de soro, sendo esses dois últimos as alternativas tecnológicas mais comuns, uma vez que apresentam concentração de proteína em torno de 90%. Outras alternativas é a adição de leite em pó e processos por membrana. Importante comentar que, para atender os aspectos regulatórios, um produto pode ser considerado “de alto valor proteico” quando contém, no mínimo, 12g de proteína por porção.

A adição de proteínas do soro em derivados lácteos é capaz de influenciar diretamente nas características sensoriais do produto e se constitui uma prática comumente realizada pelas indústrias. Uma vez adicionada no produto, as proteínas têm a capacidade de modificar atributos sensoriais importantes no iogurte, como viscosidade, cremosidade e suavidade. Pode-se considerar ainda, que o aumento do teor de proteína na formulação dos produtos visa minimizar perdas sensoriais em produtos reduzidos de gordura.

Contudo, existe um custo adicional no valor de produtos lácteos com alto teor de proteína e isso deve ser levado em conta para garantir o êxito no lançamento no mercado, ainda que o consumidor esteja disposto a pagar mais um produto diferenciado, com elevado valor nutricional. Ressalta-se ainda que, embora o fornecimento de uma grande quantidade de proteína para o organismo possa ser benéfica à saúde, seu excesso pode causar prejuízos ao sistema renal, por sobrecarregar os rins. Do mesmo modo, produtos com grandes quantidades de proteína devem ser evitados por indivíduos que apresentam quadros crônicos de doenças renais.

Algumas questões parecem ainda necessitar de mais estudos, principalmente quanto a iogurtes e bebidas lácteas, a fim de se entender sobre como as condições de processamento afetam na reologia, estrutura e propriedades sensoriais. Adicionalmente, se torna necessário melhor esclarecimento no que diz respeito a legislação de produtos com alto teor proteico.

O mercado de produtos lácteos 'proteinados' já é uma realidade, devido ao aumento de práticas de vida saudável e preocupação com a alimentação, o que faz com que esses produtos tenham grande destaque e potencial de mercado.

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4 CONSIDERAÇÕES FINAIS

O AO melhorou melhorou os parâmetros de qualidade do leite flavorizado com alto teor proteico, além de gerar menor gasto energético e níveis de hidroximetilfurfural (HMF), e melhor aceitação sensorial através de um método inovador e eficaz. De maneira geral, todos os campos elétricos utilizados neste estudo geraram benefícios em relação a amostra controle (pasteurização convencional). Esse estudo é inédito se tratando da utilização de AO para o processamento de um leite flavorizado com alto teor proteico, sendo uma importante contribuição para comunidade científica e tecnológica. Em conclusão, AO demonstrou ser uma tecnologia promissora para o desenvolvimento de leite flavorizado com alto teor proteico.

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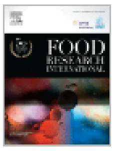
6 ANEXOS

6.1 COMPROVANTE DE PUBLICAÇÃO DO ARTIGO 1

ELSEVIER

Food Research International

Volume 161, November 2022, 111827



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

Ramon S. Rocha [a b], Ramon Silva [a b], Gustavo L.P. Ramos [a b], Louise A. Cabral [c], Tatiana C. Pimentel [d], Pedro H. Campelo [e], Patricia Blumer Zacarchenco [f], Mônica Q. Freitas [b], Erick.A. Esmerino [b], Marcia C. Silva [a], Adriano G. Cruz [a]

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6.2 COMPROVANTE DE PUBLICAÇÃO ARTIGO 2

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Food Research International

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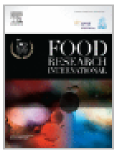

Free comment as a valuable approach to characterize and identify the drivers of liking of high-protein flavored milk drink submitted to ohmic heating

Ramon S. Rocha [a] [b], Benjamin Mahieu [c], Elson R. Tavares Filho [a], Patrícia B. Zacarchenco [d], Mônica Q. Freitas [b], Eliane T. Mársico [b], Tatiana C. Pimentel [e], Erick A. Esmerino [b], Adriano G. Cruz [a]

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6.3 COMPROVANTE DE SUBMISSÃO DO ARTIGO 3

Food Chemistry

Effect of ohmic heating in bioactive peptides, volatile compounds, and fatty acid profile in high protein vanilla flavored milk

--Manuscript Draft--

Manuscript Number:	FOODCHEM-D-23-00534
Article Type:	Research Article (max 7,500 words)
Keywords:	Dairy Products; Emerging technologies; bioactive compounds; fatty acid; Volatile
Corresponding Author:	Tatiana Colombo Pimentel, Ph.D Paranavaí, BRAZIL
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	Stanislau Junior
	Tatiana Colombo Pimentel, Ph.D
	Erick Esmerino
	Adriano Cruz
Abstract:	High protein vanilla flavored milk was processed using Ohmic Heating (OH) under different processing conditions (5.22V/cm, OH6; 6.96V/cm, OH8; 8.70V/cm, OH10; 10.43V/ cm, OH12 – 60Hz) and evaluated for the peptide, volatile compounds, and fatty acids profiles compared with untreated and pasteurized samples. Pasteurization or OH resulted in the loss of a β-casein peptide fraction with ACE-inhibitory activity, volatile compounds (1-pentanol, cyclohexanol, isobutyl butyrate, and ethyl decanoate), and fatty acids. However, OH processing released important volatile compounds (acetaldehyde, α-phellandrene). The application of intermediate electric field strengths (OH100) resulted in forming of a β-casein peptide with ACE-inhibitory activity and important volatile compounds (acetic acid, 2-pentanone, and 3-methyl 1-butanol), while maintaining butyric acid concentration and monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) levels more similar to the untreated product. The results suggest that OH, mainly at 10.43V/cm is a promising technology in the development of flavored milk.

6.4 ARTIGO PUBLICADO EM BLOG TÉCNICO

https://www.milkpoint.com.br/colunas/adriano-gomes-da-cruz/produtos-lacteos-com-alto-teor-de-proteina-consideracoes-tecnologicas-219071/

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