International Dairy Journal 146 (2023) 105733

Contents lists available at ScienceDirect

International Dairy Journal

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

Effect of ohmic heating in bioactive peptides, volatile compounds, and fatty acid profile in a high-protein vanilla flavoured milk drink

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ARTICLE INFO

Article history:
Received 15 March 2023
Received in revised form
25 May 2023
Accepted 8 June 2023
Available online 17 June 2023

ABSTRACT

High-protein vanilla flavoured milk was processed using ohmic heating (OH) at 60 Hz and 60, 80, 100 and 120 V (5.22, 6.96, 8.70 and 10.43 V cm^{-1}, respectively), coded as OH60, OH80, OH100, and OH120, respectively. Peptide, volatile compounds, and fatty acids profiles were compared with untreated and pasteurised samples. Pasteurisation or OH resulted in 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 desirable 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 desirable volatile compounds (acetic acid, 2-pentanone, and 3-methyl-1-butanol), while maintaining butyric acid concentration and monounsaturated and polyunsaturated fatty acid levels more similar to the untreated product. The results suggest that OH, mainly at 10.43 V cm^{-1}, is a promising technology in the development of milk drinks.

1. Introduction

Flavoured milk is a dairy product characterised by low viscosity, simple formulation, high versatility, and good sensory acceptance. The obligatory ingredients are milk and one more flavouring agent; however, some optional ingredients can be used, such as thickeners and stabilisers (Bisig & Kelly, 2022). Flavoured 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, Cassettari, McConnell, Kim, & Petocz, 2019). Flavoured milk has excellent market potential and high-protein options (flavoured milk drinks) could have better nutritional properties (Rocha et al., 2022).

Emerging technologies have been increasingly used recently (Rocha, Cavalcanti, 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 flavours in products than conventional heat treatments (Kuriya et al., 2020).

OH has already been applied to dairy products with characteristics similar to that of flavoured 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 flavoured milk are still scarce in the literature. In our previous study, we observed an increase in the biological activity of flavoured milk drink subjected to OH, mainly in anti-diabetic, antihypertensive, and antioxidant activities (Rocha et al., 2022). However, the impact of OH on the peptide profile, volatile compounds, and fatty acid 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.,

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https://doi.org/10.1016/j.idairyj.2023.105733
0958-6946/

2020), we hypothesise 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 [60 V (5.22 V cm^{-1}), 80 V (6.96 V cm^{-1}), 100 V (8.70 V cm^{-1}), and 120 V (10.43 V cm^{-1}), at 60 Hz, corresponding to the following codes: OH60, OH80, OH100, and OH120, respectively] on flavoured milk drink 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 flavoured milk drink processing

The methodology for producing flavoured milk drink 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 flavour, and 3% sucrose (União, São Paulo). The WPI concentration was selected, aiming for a final product with 12 g 100 mL^{-1} 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, 15 s), rapidly cooled (32 °C), and stored at refrigerated temperature (4 °C) until analysis (day 1). Six flavoured milk drink formulations were prepared: RAW (untreated), PAST (heat-treated), OH60 (60 V, 5.22 V cm^{-1}), OH80 (80 V, 6.96 V cm^{-1}), OH100 (100 V, 8.70 V cm^{-1}), and OH120 (120 V, 10.43 V cm^{-1}). 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^{-1}. 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 ionisation and desorption (MALDI), and a time-of-flight mass analyser (TOF). The equipment used was an autoflex® maX MALDI mass spectrometer (Bruker Daltonics) 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* 0.7–3.5 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 α-, β-, and κ-casein proteins and the major whey proteins, β-lactoglobulin (BLG), α-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, Aprea, Betta, Fogliano, and Gasperi (2020) was followed, with minor modifications as described below: the lyophilised 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 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. 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 × 0.25 mm × 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^{-1} until a temperature of 150 °C, followed by an increment of 5 °C min^{-1} until a temperature of 250 °C. Interface temperature: 250 °C, ionisation 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 by GC-FID

2.4.1. Samples

Samples of freeze-dried flavoured milk drink were weighed on an analytical scale (1.0000 ± 0.0001 g) and submitted to the lipid extraction process using the Bligh-Dyer method (Bligh & Dyer, 1959) modified as described below. After weighing the samples, 5.0 mL 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. 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 derivatised using a 14% BF3 methanolic solution, as described by Joseph and Ackman (1992).

2.4.2. Separation, identification, and quantification of fatty acid methyl esters by GC-FID

A Shimadzu gas chromatograph, model GC-2014, was equipped with an AOC-20i auto-injector and a flame ionisation detector (FID) for the fatty acid methyl ester (FAME) analyses. The analytes were

separated in a fused silica capillary column with Agilent DB-225-M polar stationary phase (30 m × 0.25 mm × 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

Table 1 presents the bioactive peptides identified by high-resolution mass spectrometry MALDI-TOF-MS. The mass spectra of the triplicate analyses of the bioactive peptides obtained by MALDI-TOF-MS for the RAW, PAST, OH60, OH80, OH100, and OH120 samples, can be found in Supplementary material Fig. S1.

The primary markers were observed between 820 and 2880 Da. Five of the peaks were related to β-casein, 2 to α_{S1}-casein, and 2 to α_{S2}-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).

Table 1
Bioactive peptides identified by high-resolution mass spectrometry MALDI-TOF-MS in the RAW, PAST, OH60, OH80, OH100, and OH120 freeze-dried high-protein flavoured milk drink and their biological activities for the peptides identified according to the literature.[a]

m/z	Protein	peptide	Segment	Bioactivity	Reference
RAW					
908	β-casein	KVLPVQK	184–191	Antioxidant	Rival et al. (2001), Tonolo et al. (2018)
1052	α_{S1}-casein	NONLLRFF	17–24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α_{S1}-casein	RPKHPIKHQ	16–24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160–169	ACE inhibitor	Adams et al. (2020)
1264	β-casein	EPVLGPVRGPFP	210–221	ACE inhibitor	Adams et al. (2020)
1717	α_{S2}-casein	TKKTKLTEEEKNRL	163–176	Antimicrobial	Sistla (2013)
PAST					
1052	α_{S1}-casein	NONLLRFF	17–24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α_{S1}-casein	RPKHPIKHQ	16–24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160–169	ACE inhibitor	Adams et al. (2020)
1251	α_{S2}-casein	YIPIQYVLSR	46–55	Opioid, C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba, Tani, and Yoshikawa (1989), Takahashi et al. (1997)
1717	α_{S2}-casein	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)
OH60					
908	β-casein	KVLPVQK	184–191	Antioxidant	Rival et al. (2001), Tonolo et al. (2018)
1052	α_{S1}-casein	NONLLRFF	17–24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α_{S1}-casein	RPKHPIKHQ	16–24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160–169	ACE inhibitor	Adams et al. (2020)
1251	α_{S2}-casein	YIPIQYVLSR	46–55	Opioid, C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α_{S2}-casein	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)
OH80					
1052	α_{S1}-casein	NONLLRFF	17–24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α_{S1}-casein	RPKHPIKHQ	16–24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160–169	ACE inhibitor	Adams et al. (2020)
1251	α_{S2}-casein	YIPIQYVLSR	46–55	Opioid, C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α_{S2}-casein	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)
OH100					
977	β-casein	RDMPIQAF	198–205	ACE inhibitor	Yamamoto et al. (1994)
1052	α_{S1}-casein	NONLLRFF	17–24	ACE inhibitor	Papadimitriou et al. (2007)
1140	α_{S1}-casein	RPKHPIKHQ	16–24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160–169	ACE inhibitor	Adams et al. (2020)
1251	α_{S2}-casein	YIPIQYVLSR	46–55	Opioid, C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α_{S2}-casein	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)
OH120					
1140	α_{S1}-casein	RPKHPIKHQ	16–24	ACE inhibitor	Saito et al. (2000)
1151	β-casein	HQPHQPLPPT	160–169	ACE inhibitor	Adams et al. (2020)
1251	α_{S2}-casein	YIPIQYVLSR	46–55	Opioid, C3a receptor agonist	Alvarez-Ordóñeza et al. (2013), Chiba et al. (1989), Takahashi et al. (1997)
1717	α_{S2}-casein	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)

[a] Abbreviations are: RAW, unheated sample; PAST, conventional pasteurisation sample; OH, ohmic heating; MS, mass spectrometry.

All flavoured milk drink samples showed the peptides α_{S1}-casein (f16–24, *m/z* 1140, RPKHPIKHQ) and β-casein (f160–169, *m/z* 1151, HQPHQPLPPT), related to the anti-hypertensive activity (ACE inhibitor) (Saito, Nakamura, Kitazawa, Kawai, & Itoh, 2000; Sawh et al., 2020). Furthermore, they presented the peptide α_{S2}-casein (f163–176, *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 pasteurisation or OH.

The heating process (pasteurisation and OH) changed the peptide profile. The β-casein peptide fraction (f210–221, *m/z* 1264, EPVLGPVRGPFP) was observed only in the sample without heat treatment (RAW), suggesting an impact of heating in this peptide. This peptide has been associated with ACE-inhibitory activity (Adams, Sawh, Green-Johnson, Jones Taggart, & Strap, 2020). On the contrary, the processed products (PAST, OH60, OH80, OH100, and OH120) presented the peptides α_{S2}-casein (f46–55, *m/z* 1251, YIPIQYVLSR) and the β-casein (f208–224, *m/z* 1881, YQEPVLGPVRGPFPIIV), which were not detected in the untreated product. These results demonstrate that the utilisation 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 (Birkemo, O'Sullivan, Ross, & Hill, 2009; Rojas-Ronquillo et al., 2012; Yamamoto, Atsuko, & Toshiaki, 1994).

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 (f184–191, *m/z* 908, KVLPVQK) from the untreated product. This peptide is related to antioxidant activities (Rival, Boeriu, & Wichers, 2001; Tonolo et al., 2018) and it was not identified in the other processed samples (PAST, OH80, OH100, and OH120). This result suggests that the low applied electric field strength in OH60 caused less impact on this peptide fraction. It has been reported that OH at high voltages and pasteurisation may promote protein decomposition, resulting in changes in the peptide profile (Ulug, Jahandideh, & Wu, 2021). OH80 sample showed a similar peptide profile to PAST, while OH100 resulted in the appearance of β-casein peptide (f198–205, *m/z* 977, RDMPIQAF), which has demonstrated ACE-inhibitory activity (Yamamoto et al., 1994). Finally, at high electric field strengths (OH120), the α_{S1}-casein (f17–24, *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 (Kuriya et al., 2020; Rocha, Silva, et al., 2020b, 2022; Silva et al., 2020). 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 (Pereira, Teixeira, & Vicente, 2011). In this way, some bonds in protein structure may break, resulting in a higher concentration of peptides (Alizadeh & Aliakbarlu, 2020). 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 volatile compounds identified in the samples of flavoured milk drinks with high protein content are described in Table 2. In all, 39 compounds were detected, 38 in the control sample (RAW), 26 in the product submitted to conventional pasteurisation (PAST), and 30, 28, 38, and 30 in samples subjected to OH (OH 60; OH 80, OH 100, and OH 120, respectively). Previous studies have identified 47 compounds in guava-flavoured whey beverages (Silveira et al., 2018) and 30 compounds in chocolate milk drinks (Coutinho et al., 2019).

Table 2
Mean results of MS gross area for the profile of volatile compounds present in high-protein flavoured milk drink samples obtained with different ohmic heating treatments.[a]

Volatile compound	RI	RAW	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

[a] Abbreviations are: RAW, unheated sample; PAST, conventional pasteurisation sample; OH, ohmic heating; RI, retention index; X, presence; –, absence.

Twenty-three compounds were detected in all flavoured milk drinks (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 flavoured milk drink and maintenance after processing (pasteurisation or OH). These volatile compounds are associated with typical (hexanal, 3-methylbutanal), and fruity and floral flavours (mainly esters) of milk and dairy products (Cheng, 2010; Ranadheera et al., 2019).

The heating process (pasteurisation 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 odour (Santamarina-García et al., 2023), while 1-pentanol is associated with alcoholic aroma (Ranadheera et al., 2019). Cyclohexanol has already been reported in dairy products from raw milk, contributing to their typical aroma and flavour (Calzada, del Olmo, Picon, & Nuñez, 2015).

However, OH utilisation resulted in the identification of acetaldehyde, with a progressive increase as the electric field strength increased (1089.0, 1159.4, 1656.4, 2220.2), 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 utilisation also resulted in the maintenance of α-phellandrene and loss of 2-propanone that is related to off-flavour 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, Rubino, Claps, Sepe, & Morone, 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 sulphur compounds, forms sulphur/eggy and cooked flavours (Vazquez-Landaverde, Torres, & Qian, 2006). It has been associated with boiled potatoes, cooked cabbage, and sulphur 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 sulphur-containing compounds. More prolonged exposure to high temperatures when using low electric field strengths may lead to greater oxidation of compounds (Whitt, Pranata, Carter, Barbano, & Drake, 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 flavour (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, Jomaa, Abd El-Wahed, & El-Seedi, 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 flavours (acetic acid, 2-pentanone, and 3-methyl-1-butanol). This formulation showed a higher acceptance by consumers and was characterised by the presence of vanilla, fresh milk, and sweet aromas (Rocha et al., 2023).

3.3. Fat content and fatty acid methyl esters

Fat content ranged from 11.9% ± 0.12 (OH80) to 17.0% ± 0.27 (OH120). The other samples had a fat content of 16.3% ± 0.40 (RAW), 13.9% ± 0.14 (PAST), 14.4% ± 0.14 (OH60), and 12.8% ± 0.13 (OH100). Fat content in the OH120 treatment was similar to the untreated product ($p > 0.05$), while the other flavoured milk drink 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 utilisation of low electric field strengths (OH60) resulted in a similar impact to pasteurisation (PAST), while the utilisation of intermediate electric field strengths reduced the fat content (OH80 and OH100). The effect of OH on fat content is scarcely studied, and most of the studies report only the effect on the fatty acid profile (Kuriya et al., 2020; Silva et al., 2020). In this way, more studies are necessary to evaluate the mechanism of OH action on the fat content.

Table 3 shows the quantitative (mg fatty acid methyl ester g^{-1} fat) and semi-quantitative (relative % area) results of the samples' methyl esters of fatty acids. Fig. 1 presents the fatty acids and their chemical structure identified in the flavoured milk drink samples.

Fatty acids are classified according to the number of carbon atoms (C) in long-chain fatty acids (LCFAs), medium-chain fatty acids (MCFAs), and short-chain fatty acids (SCFAs) or based on the presence of double bonds between carbon atoms in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) (Wang et al., 2022). With more than 400 different fatty acids (FAs), 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 flavoured milk drink samples, with emphasis on the RAW sample that obtained higher values of these compounds compared with all other treatments (199.3, 147.8, and 89.3 mg g^{-1}, respectively). These results are expected because these three are the most predominant FA found in milk fat and adipose tissue (Burch, Pineda, & Lock, 2021), in addition to the fact that flavoured milk does not undergo any stage of fermentation, maturation, and hydrolysis of its components, with results close to those found in the untreated product.

Palmitic acid (C16:0) (PA) is the main 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 (pasteurisation or OH) and OH process parameters significantly impacted the fatty acid profile of the flavoured milk drinks. All processed products (PAST, OH60, OH80, OH100, and OH120) showed a lower content of SFA, MUFA and PUFA compared with raw flavoured milk drink ($p < 0.05$), 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 flavoured milk drink 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

Table 3
Quantitative (mg fatty acid methyl ester g^{-1} fat) and semi-quantitative (relative area; %) results of fatty acid methyl esters present in high-protein flavoured milk drink samples obtained with different ohmic heating treatments.[a]

Fatty acid	RAW		PAST		OH60		OH80		OH100		OH120	
	(mg g^{-1})	Area (%)	(mg g^{-1})	Area (%)	(mg g^{-1})	Area (%)	(mg g^{-1})	Area (%)	(mg g^{-1})	Area (%)	(mg g^{-1})	Area (%)
C4:0 (butyric acid)	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 (caproic acid)	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 (caprylic acid)	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 (capric acid)	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 (lauric acid)	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 (tridecyl acid)	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 (myristic acid)	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 (myristoleic acid)	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 (pentadecanoic acid)	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 (palmitic acid)	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 (palmitoleic acid)	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 (margaric acid)	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 (stearic acid)	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 (linoleic acid)	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 (α -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 (arachidic acid)	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 (eicosenoic acid)	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 (eicosadienoic acid)	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 (behenic acid)	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 (erucius acid)	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 (lignoceric acid)	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	349.3^{a}	60.1	265.3^{e}	60.0	289.1^{d}	60.7	300.9^{c}	60.1	317.2^{b}	60.3	315.0^{b}	60.6
MUFA	212.4^{a}	37.9	162.8^{e}	38.1	171.8^{d}	37.5	183.2^{c}	04.3	192.4^{b}	37.9	189.9^{b}	37.9
PUFA	10.3^{a}	2.4	7.50^{c}	1.7	7.8^{c}	1.7	8.3^{b}	1.7	8.6^{b}	1.7	6.7^{d}	1.3
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

[a] Abbreviations are: RAW, unheated sample; PAST, conventional pasteurisation sample; OH, ohmic heating; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

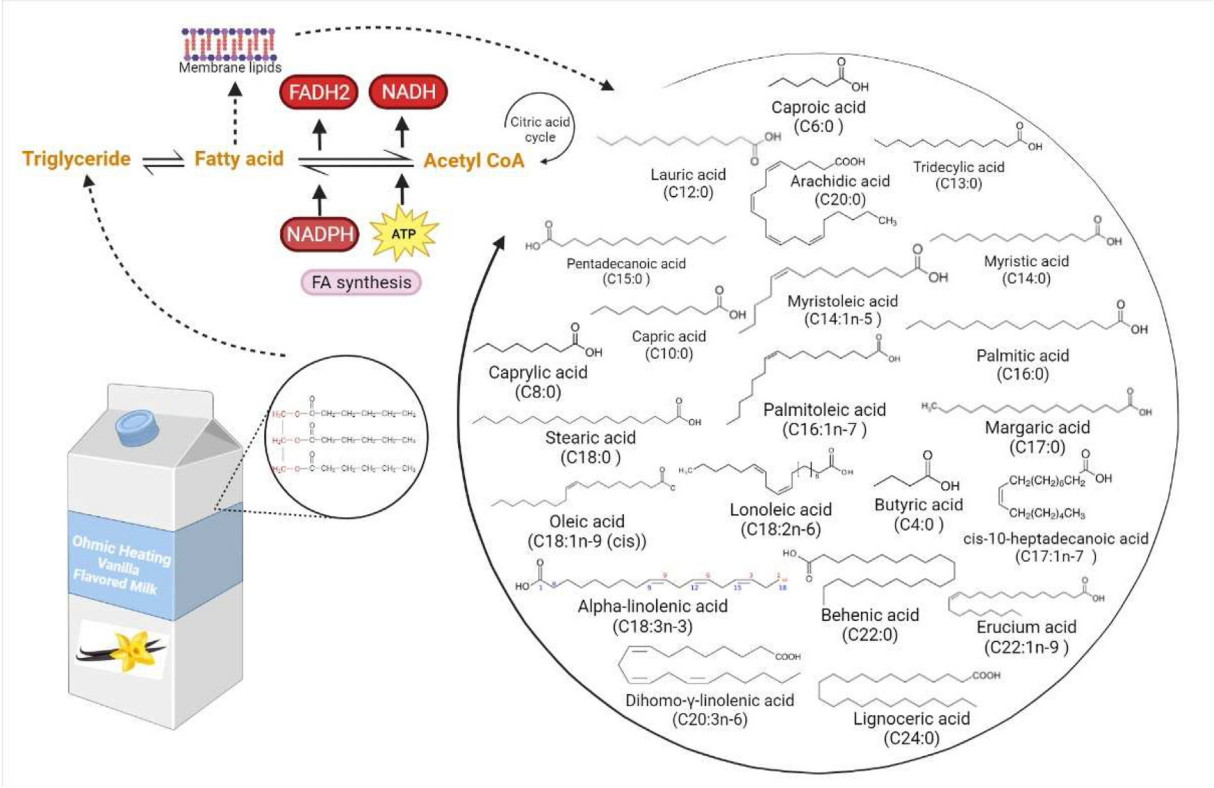

Fig. 1. Simplified scheme and structure of fatty acids found in flavoured milk drink samples.

functions, participating in the development of intestinal tissue, gene expression, reducing oxidative stress, and immune modulation (Bedford & 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). OH100 sample also showed the highest MUFA and PUFA levels among the processed products ($p < 0.05$). PUFAs are essential since they must be inserted through the diet, as the body does not synthesise them. They actively maintain and promote health by preventing various diseases (Lee, Oh, Jo, Cho, & Park, 2023).

Ohmic heating has been constantly used to process dairy products with an evaluation of the fatty acid profile (Rocha, Silva, et al., 2020b; Silva et al., 2020). 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 flavoured milk drink 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-flavoured milk drink processed by OH or pasteurisation. Heating process (pasteurisation 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 8.7 V cm^{-1}, is a promising technology in developing flavoured milk drink.

Declaration of competing interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data availability

Data will be made available on request.

Acknowledgements

The authors are grateful to IFRJ, USP, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) financing code 001, in Brazil, for their financial support and the productivity grants, and BioRender App (https://app.biorender.com) for figure creation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2023.105733.

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