RURAL SUSTAINABILITY RESEARCH
52(347), 2024
ISSN – 2256-0939

Received: 27 July 2024 Revised: 20 September 2024 Accepted: 21 November 2024

Analysis of Fatty Acids and Antibacterial Whey Proteins in Organic and Conventional Milk: Potential Influence on Lactic Acid Bacteria Growth

***Stefanija Sabunevica[1], Vitalijs Radenkovs[2, 3], Kristīne Majore[1], Jeļena Zagorska[1]**
Latvia University of Life Sciences and Technologies, 2 Liela street, Jelgava, Latvia
[2]Research Laboratory of Biotechnology, Division of Smart Technologies, Latvia University of Life Sciences and Technologies, 22 Rigas street, Jelgava, Latvia
[3]Institute of Horticulture, 1 Graudu street, Ceriņi, Krimūnu parish, Dobeles county, Latvia

Abstract. Organic fermented milk products are an area of interest combining functional properties and sustainable practices. Limited information is available regarding the specific components of organic milk that may impact the growth of lactic acid bacteria. This study emphasised the differences in bioactive compounds between organic and conventional milk and their potential influence on lactic acid bacteria growth. Analysis of organic (n = 15) and conventional (n = 15) farm milk using GC-MS revealed differences in fatty acid (FA) concentrations, mainly mono-, polyunsaturated fatty acids, and conjugated linoleic acid. Individual FA, such as stearic, linoleic, and oleic acids, were up to 33.6%, 31.0%, and 25.23% higher in conventional milk. Detection of the whey proteins using the enzyme-linked immunosorbent assay (ELISA) showed lysozyme content was up to 40.6% higher in conventional (22.04 μg L^{-1}) than in organic (15.68 μg L^{-1}) milk. Conversely, lactoferrin content was 20.4% higher in the organic (45.27 μg L^{-1}) than in conventional (36.04 μg L^{-1}). No difference in the content of immunoglobulin A was found. The higher concentrations of lysozyme, mono- and polyunsaturated fatty acids in conventional milk could enhance a higher inhibitory activity against lactic acid bacteria compared to organic milk.
Key words: organic; conventional; bioactive compounds; lysozyme; lactoferrin; immunoglobulin A.

Introduction

Lactic acid bacteria (LAB) are a natural part of the raw milk microbiome, and are widely recognised for their significance in the production of fermented dairy products as starter cultures and probiotics (Reuben *et al*., 2020). During the fermentation process, the LAB growth is intricately linked to the composition of the substrate, which in the case of milk is in turn influenced by various factors such as environmental conditions and individual cow characteristics, season, breed, health, and feeding practices (Hayek *et al*., 2019; Schwendel *et al*., 2015). The organic system is linked to enhanced animal welfare, which is reflected in the milk's microbiota and technological quality (Gomes *et al*., 2020; Wagner *et al*., 2021). In addition to being a significant source of macronutrients in nutrition (fats, carbohydrates, and proteins), milk also provides biologically active compounds. Apart from vitamins, oligosaccharides and polar lipids, these compounds include antimicrobial whey proteins and fatty acids, which play physiological and biochemical roles in humans and possess the potential to influence starter cultures and other LAB in milk fermentation process, exerting growth-promoting or growth-inhibiting effects (Borgonovi *et al*., 2022; Breza-Boruta *et al*., 2022; Casillas-Vargas *et al*., 2021).

The production of fermented products using organic milk is of great interest as it combines the benefits of health-promoting functional food with sustainable practices (Brodziak *et al*., 2018; Florence *et al*., 2012; Grodkowski *et al*., 2023; Lambotte *et al*., 2023). However, organic farming certification entails a transition period and necessitates changes in farm management practices. Therefore, definitive research is required to better understand the potential benefits of organic dairy, including nutritional value, microbiological criteria, and the factors affecting the LAB growth (Linehan *et al*., 2024). Previous

* Corresponding Author's email:
stefanija.sabunevica@gmail.com

DOI: 10.2478/plua-2024-0017

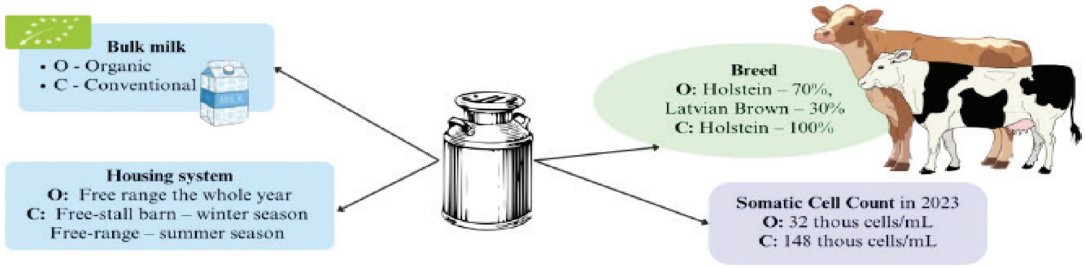

Figure 1. Characteristics of the herds involved in the study.

comparative studies have revealed differences between oligosaccharides, whey proteins, fatty acids, minerals, and vitamins in organic and conventional bovine milk (Borgonovi *et al*., 2022; Breza-Boruta *et al*., 2022; Kuczyńska *et al*., 2012; Manuelian *et al*., 2022; *Róin et al*., 2023). Despite these recent comparisons between management systems, there remains a significant knowledge gap regarding the compounds present in organic milk and their role in the production of fermented products, specifically factors affecting the growth of lactic acid bacteria (Florence *et al*., 2014; Sabunevica & Zagorska, 2023). This study aims to discern the composition of whey proteins and fatty acids, in organic and conventional bovine milk and to emphasise their role in the growth of lactic acid bacteria.

Materials and Methods

Farms

This study involved two farms in the north-eastern region of Latvia, situated about 1 km apart – one was certified as organic, and the other was conventional (Figure 1). The conventional farm had a herd of 40 Holstein cows, with half of them in lactation during the study. These cows were fed hay, silage, and concentrate supplements daily. Meanwhile, the organic farm had a total of 17 cows of the Latvian Brown and Holstein breeds, with ten of them in lactation. The organic cows were provided with hay, silage, and occasional garden fruit or potatoes. Both groups of cows were given salt blocks as well. The organic cows had outdoor access throughout the year and a roofed shelter in the field, whereas the conventional cows were kept indoors during the study period. Milking was carried out daily direct into the pipeline on both farms with the cows being given wheat flour before the milking process. All feed, except for the concentrate supplements and salt blocks, was produced locally at the farms.

Sampling

Bulk milk samples were systematically collected from January 2023 to May 2023 on each of the respective farms. Samples were collected at the same time every two weeks from the milk tank, where the milk was constantly stirred. Samples were collected in disposable, sealable 300 mL plastic storage bags. The collection of milk samples involved the opening of the milk tank lid, and the use of a sterilised cup to collect it. A total of 30 bulk samples were collected, comprised of 15 organic and 15 conventional milk samples. The samples were transported in a refrigerated container at 4 ± 1 °C to the Latvia University of Life Sciences and Technologies in Jelgava, Latvia. Upon arrival, the milk was stirred for 10 seconds to ensure homogeneous sampling and was divided into smaller aliquots of 50 mL sterile plastic screw-top containers. Subsequently, the aliquots were frozen using the shock freezer and stored at temperatures below −18 ± 1 °C for a maximum duration of 30 days until further analysis. The chemical composition, whey proteins and carbohydrates were analysed at the Food Institute, Faculty of Agriculture and Food Technology, and the fatty acids were detected at the Research Laboratory of Biotechnology, Division of Smart Technologies, Latvia University of Life Sciences and Technologies.

Analysis of milk components

Composition determination

After thawing overnight in a refrigerator at 4 ± 1 °C, the raw milk samples underwent analysis to determine the concentrations of total protein, fat, and lactose in whole milk. The analysis was conducted in accordance with the ISO 21543:2020 method (International Organisation for Standardisation, 2020) using a MilkoScan (Milkoscan™ Mars, Foss Analytical, Hillerød, Denmark) instrument.

Sample preparation for the whey protein determination

The modified approach of Ozturkoglu-Budak *et al*. was used to extract whey proteins (Ozturkoglu-Budak, 2018). Initially, raw milk samples were defrosted overnight in a refrigerator at a temperature of 4 ± 1 °C. Next, 4 mL of milk samples underwent centrifugation at a speed of 6000 rpm for 20 minutes to separate and eliminate fats, cell debris, and other components that are not soluble in water. The milk was heated to 35±1 °C, and chymosin CHY-MAX®M 1000 (Chr. Hansen Holding, Denmark) 1.0% solution was added to induce the casein coagulation. Following this, the sample was subjected to centrifugation once more at a speed of 6000 rpm for 20 minutes to precipitate the casein. Eventually, the liquid underwent filtration using a 1 μm pore filter (Watman No.1). The resulting whey was utilised for the quantitative analysis.

Lysozyme, lactoferrin and IgA quantification

The lysozyme concentration was determined using a commercially available Lysozyme Detection kit (LY0100, Sigma Aldrich, USA), while the lactoferrin concentration was measured using an ab274406 kit (Abcam, China), and the Immunoglobulin A concentration was assessed using KA2028 kit (Abnova, Taiwan). The studies were conducted by following the manufacturers' instructions, which included the preparation of reagents and samples. The lysozyme activity findings were analysed by measuring spectrophotometric readings at a wavelength of 450 nm using glass cuvettes with a volume of 3 mL. The instrument used for the measurements was the Varian Cary 1E UV/Visible spectrophotometer. The concentration of lactoferrin and IgA in the samples was determined using spectrophotometric wavelength readings, using a 96-well plate reader MicroBeta2® Microplate Counter (MicroBeta2® Microplate Counter, USA). In order to do quantitative analysis, calibration curves were created to determine the amounts of selected whey proteins in the samples.

Sample preparation for the carbohydrate determination

After raw milk samples were thawed overnight in a refrigerator at a temperature of 4 ± 1 °C, the 250 μL of the sample was treated with 750 μL of 0.05% (w/v) H_2SO_4 (for acidification), followed by centrifugation at 13,000 rpm for 10 minutes to remove cell debris and other compounds that are not soluble in water. After centrifugation, the fat was extracted and the resultant solution was further analysed.

Determination of carbohydrate profile

The quantification of carbohydrates was done using High-Performance Liquid Chromatography (HPLC) Shimadzu, equipped with a refractive index detector RID-10A (Shimadzu Corporation, Tokyo, Japan). Samples were quantified according to lactose, glucose, and galactose HPLC-grade external analytical standards (>99% purity; Sigma-Aldrich, MO, USA). Analysis was done using an Alltech NH_2 column (4.6×250 mm; 5 μm; Grace™, Columbia, MD, USA). The measurement conditions were as follows: the column temperature was maintained at 50 °C, the mobile phase was 0.05 M H_2SO_4 with isocratic elution at a flow rate of 0.5 mL min^{-1}, and the sample injection volume was 10 μL.

Preparation of lipid fraction by alkaline-assisted hydrolysis with subsequent liquid–liquid extraction

The isolation of lipophilic fraction from milk was done according to the procedure reported by Ruska *et al*. with minor modifications (Ruska *et al*., 2023). In brief, duplicate 5.0 ± 0.1 g samples were weighed in 50 mL reagent bottles and 30 mL of freshly prepared potassium hydroxide solution 10% (w/v) in 80% MeOH (MeOH:H_2O ratio 80:20 v/v) was added to each sample. The resulting mixture was incubated in a water bath "TW8" (Julabo®, Saalbach-Hinterglemm, Germany) at 65 °C for 3 hours. After hydrolysis, the cleavage of bonds present in the potassium salts of fatty acids (FAs) was done by decreasing the pH to 2.0 ± 0.2 using 3.2 mL 6 M HCl solution. The extraction of FAs was performed by liquid-liquid phase separation using n-hexane as a sole solvent. After hydrolysis, the samples were cooled to ambient temperature (22 ± 1 °C) and quantitatively transferred to 50 mL conical centrifuge tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany). Following that, 10 mL of n-hexane was added to each tube, followed by vortex-mixing for 1 minute. Finally, the layers were separated by centrifugation at 4500 rpm for 10 minutes in a "2-16KC" centrifuge (Sigma, Osterode near Harz, Germany). The top n-hexane layer was decanted and collected. The extraction procedure was repeated three times. The resulting lipophilic fraction (30 mL) was dried using a "Laborota 4002" rotary evaporator (Heidolph, Swabia, Germany) at 65 °C, and the dry residues were then reconstituted in 2 mL of pyridine. Before GC-MS analysis, the samples were filtered through a polytetrafluoroethylene hydrophobic (PTFE) membrane filter with a pore size of 0.45 μm.

Preparation of fatty acids for GC-MS analysis

The TMPAH reagent was used as a methylation agent of the functional groups to obtain volatile fatty acid methyl ester (FAMEs) derivatives. The methylation procedure was performed according to the methodology by Ruska *et al*. (Ruska *et al*., 2023).

The GC Conditions for FAMEs Analysis

The analysis of FAMEs was performed using a "Clarus 600" system PerkinElmer, Inc. (Waltham, MA, USA) coupled with a quadrupole "Clarus 600 C" mass-selective detector (Waltham, MA, USA). The conditions were adopted from Ruska *et al*. (Ruska *et al*., 2023).

Data analysis

For all traits, the differences between variables in organic and conventional milk and the significance of management system, production time (months) and the interaction effect were indicated by a two-way analysis of variance (ANOVA), setting the significance alpha <0.05. Before analysis, the normal distribution was determined using the Shapiro-Wilk test. Pairwise comparisons for management system and month of the study were done using Tukey's post hoc test. Statistical processing and the visualisation of the data were conducted in RStudio (R Core Team, R version 4.4.1).

Results and Discussion

Chemical composition

The data presented in Table 1 illustrate milk composition from certified organic and conventional dairy farms, with the results being expressed as mean ± standard deviation. The findings indicate both milks exhibited similar total fat, protein, and carbohydrate contents throughout the study period, with no significant differences observed between the two groups (Table 1).

During food processing, particularly, in the context of fermentation, the metabolic activity of LAB plays a significant role in the quality and nutritional aspect of dairy products (Sodini *et al*., 2002). Understanding the growth of LAB requires a comprehensive analysis of fat, protein, and carbohydrate concentrations as they are the main compounds in the metabolic pathways – proteolysis, glycolysis, and lipolysis in bacterial cells (Wang *et al*., 2021).

In organic milk, the fat content ranged from a low of 3.32 g 100 mL^{-1} in April to a high of 5.29 g 100 mL^{-1} in January (Figure 2a). Likewise, the protein content in organic milk was lowest at 2.63 g 100 mL^{-1} in March and the highest at 3.52 g 100 mL^{-1} in January (Figure 2b), while the lactose content ranged from a low of 4.26 g 100 mL^{-1} in January and to a highest of 4.71 g 100 mL^{-1} in February (Figure 2c). The observed values are comparable to those previously reported for both organic and conventional milk (Martini *et al*., 2023). Overall, it is well established that the total fat, lactose, and protein content in organic and conventional milk typically do not differ significantly (Linehan *et al*., 2024). However, certain studies have reported contrasting results, indicating conventional

Table 1

Chemical composition of organic and conventional milk

Component	Organic (n=15)	Conventional (n=15)	Organic	Conventional	*p*-value
	Content, g 100 mL^{-1}		CV, %		
Total protein	3.11 ± 0.41	2.91 ± 0.37	13	13	0.224
Total fat	4.05 ± 0.60	3.89 ± 0.39	15	10	0.519
Lactose	4.62 ± 0.54	4.49 ± 0.33	12	7	0.693
Galactose	0.008 ± 0.001	0.005 ± 0.001	10	11	0.588
Dry matter	12.45 ± 0.89	12.13 ± 0.55	7	8	0.309

Data is given as mean values ± standard deviation. Abbreviation: CV = coefficient of variation.

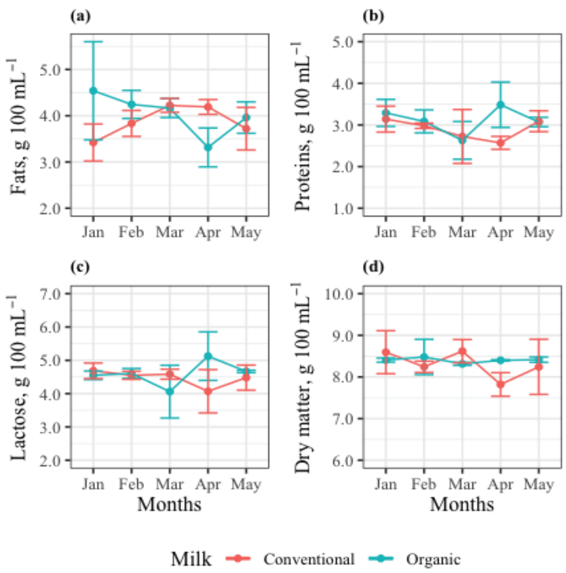

Figure 2. Changes in the chemical composition in the studied organic and conventional farm milk by month: (a) Fats; (b) Proteins; (c) Lactose; (d) Dry matter.

milk may have higher lactose and protein content compared to organic, while the fat content was reported to be higher in organic milk in other studies (Kouřimská *et al*., 2014; Manuelian *et al*., 2022; Róin *et al*., 2023).

Compared to the other microelements recent research evaluating the carbohydrates in milk and fermented dairy products is limited. Monitoring the glucose, galactose, and lactose levels can provide valuable insights into the growth of LAB in organic milk (Delgado-Fernández *et al*., 2019). Lactic acid bacteria primarily metabolise lactose to glucose and galactose further to lactic acid (Iskandar *et al*., 2019). Notably, the β-galactosidase catalyses lactose, leading the glucose metabolism to pyruvate via the Embden–Meyerhoff pathway, while galactose accumulates in the growth medium (Costa *et al*., 2016). The observed values are in line with the previously reported findings for conventional milk (Ohlsson *et al*., 2017). While monosaccharides such as glucose and galactose are present in low concentrations in milk, it is noteworthy that the glucose concentration in both milk groups was below the detection limit. The variability in the composition of carbohydrates in raw milk could be attributed to the metabolic activity of the raw milk microbiome, which has been observed to differ between organic and conventional farming (Gomes *et al*., 2020; Reuben *et al*., 2020).

Fatty acids

This study revealed the variability of the FA profile and the significant differences in the concentrations of individual fatty acids (FAs) and FA groups between

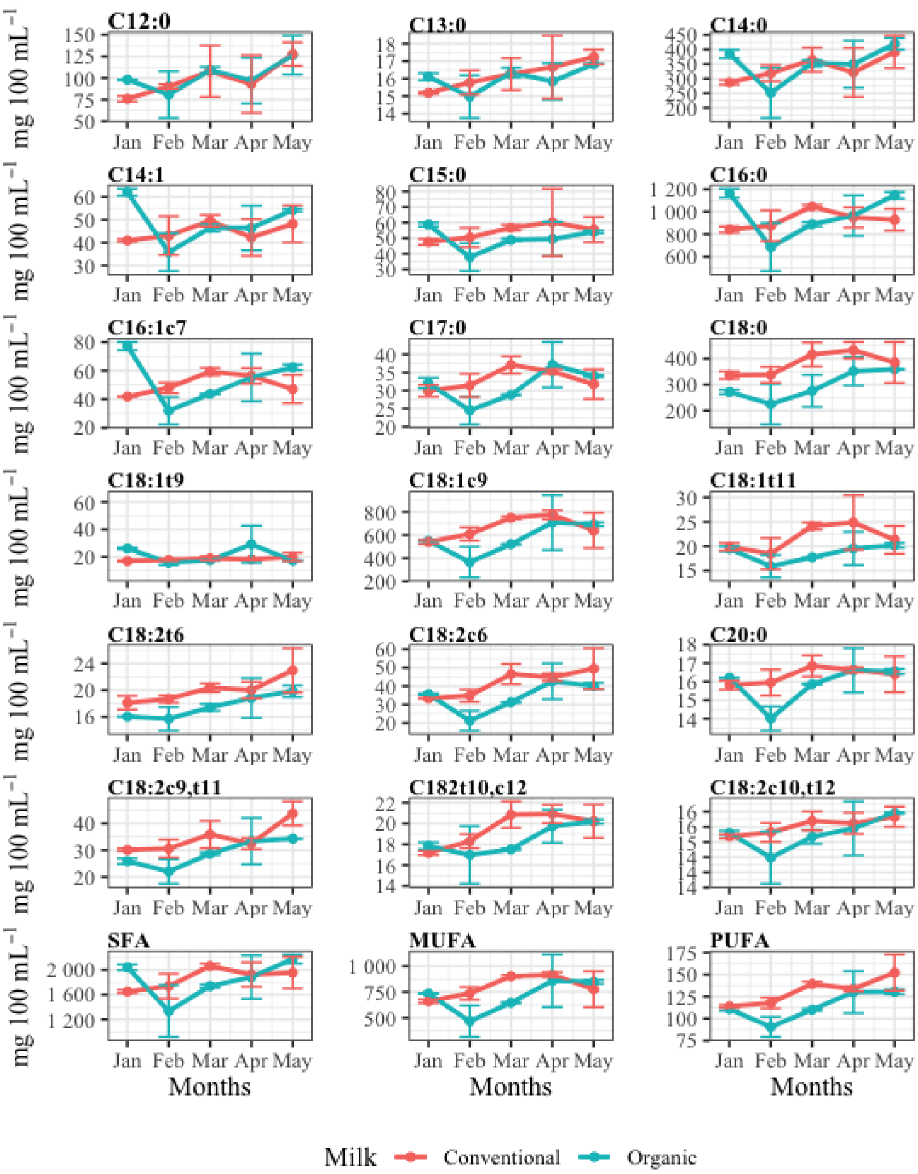

Figure 3. Fatty acid concentration changes by month in studied organic and conventional farm milk.

organic and conventional farms (Table 2). The observed values of individual FAs and FA groups are in line with those previously reported (Florence *et al*., 2012; Liu *et al*., 2020; Manuelian *et al*., 2022; Martini *et al*., 2023). Notably, the majority of the individual FAs were influenced by the management system as well as by the production month. The period from February to April affected the profile of FAs in organic milk the most, while conventional milk showed less variability (Figure 3). The observations suggest that the main differences in the FA profile between conventional and organic milk were related to a higher saturation of PUFA and MUFA in conventional milk. This finding contradicts commonly reported higher PUFA in organic milk (Collomb *et al*., 2008; Kucevic *et al*., 2016) . Similar to the obtained results, several authors also reported higher MUFA in conventional milk, with SFA concentrations remaining unaffected (Ellis *et al*., 2006; Givens & Lovegrove, 2016). On the contrary Martini *et al*. showed no difference in MUFA, PUFA, and SFA between the systems (Martini *et al*., 2023). The obtained results are possibly associated with the variations in the feed composition and regimen in management practices, e.g. periods of outdoor grazing, as the balance of fatty acids in the feed directly impacts the FA profile in milk (Butler *et al*., 2008; Róin *et al*., 2023).

The available literature indicates both saturated and unsaturated FAs exhibit antibacterial activity against a variety of Gram-positive and Gram-negative bacteria (Casillas-Vargas *et al*., 2021; Coates *et al*., 2023). The mechanisms through which FAs affect LAB growth in raw milk – bacteriostatic, bactericidal, or growth-stimulating - involve several interacting factors, such as FA type, FA concentration, bacterial strain, and the composition of the growth medium (Jenkins & Courtney, 2003). Additionally, the higher ratio between unsaturated and saturated FAs in a medium may play a protective role in the bacterial cell membrane and its metabolic activity (Beal *et al*., 2001). In general, long-chain FAs with a higher degree of unsaturation tend to have a stronger inhibitory effect than saturated FAs with the same carbon chain length (Desbois & Smith, 2010). The obtained results showed significantly higher sums of MUFA and PUFA in conventional milk, with corresponding concentrations almost 20% higher than in organic milk. Therefore, due to the higher concentrations of total unsaturated FA in conventional milk, it could be assumed that this milk may exert a higher inhibitory effect, while the observed similarity in total SFA concentrations in organic and conventional milk may contribute to a similar impact on LAB growth. On the contrary, the findings by Florence *et al*. suggest that due to the higher concentration of PUFA, organic milk has a favourable effect on *Lactobacillus bulgaricus* growth in yoghurt (Florence *et al*., 2012). Nevertheless, the role of individual FAs, rather than groups must be considered. In particular, Kankaanpää, *et al*. (2001) demonstrated the bactericidal activity of individual PUFA on LAB in MRS broth. Linoleic acid (C18:2t6) at a concentration of 4 mg 100 g^{-1} shows inhibitory activity against *Lactobacillus casei* and *Lactobacillus bulgaricus,* but does not affect *Lactobacillus GG* (Kankaanpää *et al*., 2001)). The obtained results in both organic and conventional milk exceed this concentration more than threefold. In another study, Partanen *et al*. showed that *Lactobacillus bulgaricus* require unsaturated FA, and that oleic acid (C18:1c9) is an essential growth factor (Partanen *et al*., 2001). Both – linoleic and oleic acids were significantly higher in in conventional milk. Additionally, within the MUFA, vaccenic acid (C18:1 trans 11), and linolelaidic (C18:2t6) among the PUFA, as well as pentadecanoic (C15:0), stearic (C18:0), and eicosanoic (C20:0) acids among the identified SFA, were higher in conventional milk. It has been noted, that the stereochemistry of the double bonds also plays a role in the antibacterial activity of FAs, with the *cis*- configuration being more inhibitory than the *trans*- configuration. Those, Jenkins & Courtney tested conjugated linoleic acid (CLA) isomers and showed their inhibitory effect on five *Lactobacillus* strains (*Lactobacillus zeae* ATCC 393*, Lactobacillus helveticus* L386, *Lactobacillus acidophilus* NCFM (NCK 56), *Lactobacillus reuteri* ATCC 55739 and ATCC 23272), noting the concentration-dependent and medium-dependent effect of this FA. The authors explained the differential effect by the presence of protective proteins in milk, but not in MRS broth (Jenkins & Courtney, 2003). The obtained results of CLA, represented by three isomers (C18:2c9t11, C18:2t10c12, C18:2c10t12 – CLA), were significantly higher in conventional milk. In contrast, Ellis *et al*., reported no significant difference between organic and conventional milk in terms of the conjugated linoleic or vaccenic FAs (Ellis *et al*., 2006).

The present results suggest that specific compounds in conventional and organic milk may have inhibitory and growth-promoting activity against some LAB. Higher concentrations of MUFA along with individual PUFA, such as linoleic and conjugated linoleic acids in conventional milk may contribute to an enhanced inhibitory activity against LAB when compared to organic milk. However, for a better understanding of the antibacterial or growth-promoting effects of FA on desirable LAB strains in organic milk, further research must consider the presence, concentration of individual FA, and the ratio of saturated and unsaturated FA.

Antimicrobial whey proteins

Proteins that possess antimicrobial properties found in bovine milk, such as lysozyme, lactoferrin,

Table 2

Fatty acid profile in studied conventional and organic farm milk

Fatty acid	Farm						Factor		
	Organic	Conventional	Organic	Conven-tional	Organic	Conven-tional	Management system	Production time, month	Management system x month
	Concentration, mg 100 g^{-1}		Relative abundance, %		CV, %				
C12:0	96.19 ± 25.64	100.34 ± 24.94	3.86	3.57	32	22	n.s.	**	n.s.
C13:0	15.74 ± 1.09	16.29 ± 1.11	0.63	0.58	6	12	n.s.	**	n.s.
C14:0	326.43 ± 88.85	339.99 ± 57.24	13.09	12.09	12	12	n.s.	*	n.s.
C14:1	45.4 ± 11.26	45.05 ± 7.18	1.82	1.60	10	10	n.s.	n.s.	n.s.
C15:0	46.78 ± 10.56	54.38 ± 10.61	1.88	1.93	7	6	*	n.s.	n.s.
C16:0	898.7 ± 242.54	931.71 ± 111.28	36.04	33.13	7	5	n.s.	n.s.	n.s.
C16:1c7	48.68 ± 18.64	51.14 ± 7.76	1.95	1.82	14	7	n.s.	n.s.	n.s.
C17:0	30.34 ± 6.45	33.24 ± 3.63	1.22	1.18	19	5	n.s.	*	n.s.
C18:0	285.66 ± 79.20	381.77 ± 57.79	11.46	13.58	11	9	***	***	n.s.
C18:1t9	20.86 ± 8.55	18.44 ± 1.75	0.84	0.66	30	11	n.s.	n.s.	n.s.
C18:1c9	535.07 ± 199.74	670.08 ± 107.91	21.46	23.83	12	9	**	**	n.s.
C18:1t11	18.02 ± 2.73	21.58 ± 4.01	0.72	0.77	26	13	**	*	n.s.
C18:2t6	17.25 ± 2.37	20.08 ± 2.23	0.69	0.71	28	9	***	***	n.s.
C18:2c6	32.01 ± 10.75	41.94 ± 8.32	1.28	1.49	12	10	***	***	n.s.
C20:0	15.48 ± 1.37	16.34 ± 0.7	0.62	0.58	14	15	**	**	n.s.
C18:2c9,t11	27.74 ± 7.07	34.61 ± 6.08	1.11	1.23	28	9	*	***	n.s.
C18:2t10,c12	18.25 ± 2.21	19.61 ± 1.69	0.73	0.70	35	11	*	***	n.s.
C18:2c10,t12	15.1 ± 0.83	15.54 ± 0.36	0.61	0.55	33	9	*	**	n.s.
Σ SFA	1715.32 ± 437.22	1874.06 ± 218.58	68.79	66.64	16	8	n.s.	*	n.s.
Σ MUFA	668.03 ± 226.99	806.29 ± 120.22	26.79	28.67	3	3	*	**	n.s.
Σ PUFA	110.35 ± 21.73	131.79 ± 16.59	4.43	4.69	8	7	***	***	n.s.
Σ total	2493.70 ± 672.3	2812.14 ± 333.4	100.00	100.00	-	-	-	-	-
Σ CLA	61.09 ± 9.83	69.76 ± 7.03	2.45	2.48	16	8	***	***	n.s.

*Data is given as mean values ± standard deviation. Abbreviations: FA = Fatty acids; Σ SFA = sum of saturated FA; Σ MUFA = sum of monounsaturated FA; Σ PUFA = sum of polyunsaturated FA; Σ total = sum of PUFA, MUFA and SFA; Σ CLA = sum of conjugated linoleic acids; CV = coefficient of variation. Asterisks indicate statistically significant differences between columns: *p < 0.05; **p < 0.01; ***p < 0.001; n.s., not significant p > 0.05.*

and immunoglobulins, etc., account for 1–2% of total whey proteins. Despite their minor quantities, these proteins have multiple positive effects on human health and also play a role in the production of fermented dairy products and the extension of dairy shelf life (Dziuba & Dziuba, 2014). The concentrations of individual whey proteins in organic and conventional bovine milk are shown in Table 3.

Lysozyme

Lysozyme is a natural antimicrobial enzyme that catalyses the hydrolysis of the β-1,4-glycosidic linkages in the peptidoglycan layer, a major component of bacterial cell walls. Since LAB, like many other bacteria, contain peptidoglycan in their cell walls, they are susceptible to the action of lysozyme. Upon contact with LAB, lysozyme targets and cleaves the glycosidic bonds in the peptidoglycan layer, leading to the destabilisation and the lysis of the bacterial cell wall. This disruption compromises the structural integrity of the bacteria, inhibiting their ability to grow effectively (Azzolini *et al*., 2010; Khorshidian *et al*., 2022). However, due to the different mechanisms of lysozyme and the diversity of bacterial cell wall structures, LAB may have a certain sensitivity to lysozyme, and may

Table 3

Antimicrobial whey proteins in studied organic and conventional farm milk

Component	Farm				Factor		
	Organic	Conventional	Organic	Conven-tional	Manage-ment system	Production time, month	Management system x Month
	Concentration, µg L⁻¹		CV, %				
Lysozyme	15.68 ± 4.84	22.04 ± 5.32	31	24	*	n.s.	n.s.
Lactoferrin	45.27 ± 11.84	36.04 ± 12.56	26	35	n.s.	n.s.	n.s.
IgA	154.33 ± 30.54	149.84 ± 36.58	20	24	n.s.	n.s.	n.s.

*Data is given as mean values ± standard deviation. Abbreviation: CV = coefficient of variation. Asterisks indicate statistically significant differences between columns: *p < 0.05; n.s., not significant p > 0.05.*

vary according to the bacterial strain or species and the conditions in the medium (Benkerroum, 2008; Khorshidian *et al*., 2022; Masschalck *et al*., 2001). For instance, streptococci generally demonstrate more resistance to lysozyme than lactobacilli, while among lactobacilli, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* and *Lactobacillus fermentum* appear to be the species mostly affected by lysozyme (D'Incecco *et al*., 2016; Neviani *et al*., 1991). In addition, the sensitivity of lactobacilli to lysozyme is influenced by various parameters, such as growth phase, growth temperature, carbon sources and pH (Kozáková *et al*., 2005). As suggested by Masschalck *et al*., the inhibitory concentrations of lysozyme can be as low as 10 µg mL^{-1}, which is consistent with the lysozyme concentrations found in milk in this study (Masschalck *et al*., 2001).

The lysozyme concentrations, obtained in this study, ranged from 11.30 µg L^{-1} to 18.50 µg L^{-1} in organic milk, and from 14.60 µg L^{-1} to 29.50 µg L^{-1} in conventional milk (Figure 4a), showing significant differences between the two systems (Table 3). In contrast, Scharfen *et al*. demonstrated that the presence of 270 µg mL^{-1} lysozyme in goat's milk was associated with only a slight reduction in the number of LAB in the raw milk cheese (Scharfen *et al*., 2007). This disparity in effect may be attributed to differences in bacterial strains and the processing conditions. Lysozyme, an antimicrobial enzyme, typically maintains low concentration in the milk of healthy cows during lactation, as observed in this study. The obtained results are in line with earlier research on organic milk (Król *et al*., 2010; Kuczyńska *et al*., 2012). However, lysozyme's concentration can increase in case of mastitis, vary with cow breed and can change in response to the weather conditions (Carlsson *et al*., 1989; Król *et al*., 2010; Priyadarshini & Kansal, 2002).

Nevertheless, when milk is pasteurised before processing, lysozyme, being affected by thermal

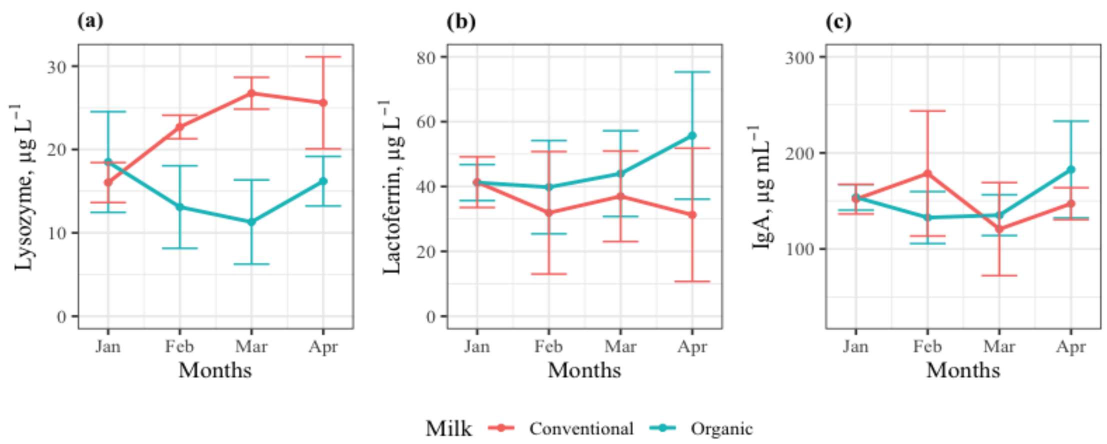

Figure 4. Changes by month in whey proteins in studied organic in conventional farm milk: (a) Lysozyme; (b) Lactoferrin; (c) Immunoglobulin A (IgA).

treatment, exhibits reduced or no inhibition activity against Gram-positive and Gram-negative bacteria (Masschalck *et al*., 2001; Scharfen *et al*., 2007). This indicates that the higher lysozyme concentration in conventional milk can negatively influence LAB growth in raw, but not in thermally treated milk. In raw milk, the release and activity of intracellular LAB enzymes involved in lactose hydrolysis or proteolysis may vary under the influence of lysozyme. Consequently, the concentration of valuable monosaccharides and nitrogen-containing compounds in the milk available for the growth of the starter cultures, which influence further dairy processes, is also altered (Ayivi *et al*., 2022). Furthermore, milk is a complex of different salts and other antimicrobial proteins such as lactoperoxidase, lactoferrin, immunoglobulins, N-acetyl-glucosaminase, etc., that affect the lysozyme's final activity (Benkerroum, 2008). Therefore, for a better understanding of the antibacterial effects of lysozyme on starter cultures in organic milk, the concentration and activity of lysozyme, in conjunction with other milk components, specific strains and dairy processes, must be taken into consideration.

Lactoferrin

Lactoferrin is an iron-binding glycoprotein, and by binding non-haem iron, which is essential for bacterial growth, lactoferrin causes bacteriostasis and has a direct bactericidal activity against various Gram-negative and Gram-positive bacteria (Bellamy *et al*., 1992; Cheng *et al*., 2008). The bacterial response to lactoferrin varies depending on the concentration and representatives of transferrin protein family, the bacterial strain, and the product's composition (Franco *et al*., 2010; Jugert *et al*., 2023; Kim *et al*., 2004). For instance, lactoferrin has been reported to partially inhibit the growth of *Streptococcus thermophilus*, but promote the growth of *Lactobacillus bulgaricus*, bifidobacteria, and other LAB strains in yoghurt (Franco *et al*., 2010; Kim *et al*., 2004). The obtained results indicate a slightly higher ($p>0.05$) lactoferrin concentration in organic than in conventional milk (Table 4). In both organic and conventional milk lactoferrin concentrations were not affected by the production month, ranging from a low of 39.80 µg L^{-1} in February to a high of 55.72 µg L^{-1} in April in organic milk (Figure 4b). These findings partly differ from previous studies, which reported threefold higher lactoferrin concentrations in organic milk compared to the obtained results, ranging from 123.8 µg L^{-1} to 334.99 µg L^{-1} (Brodziak *et al*., 2018; Kuczyńska *et al*., 2012). However, according to Cheng *et al*., lactoferrin concentration in milk from healthy lactating Holstein cows can have a wide range from 31.8 to 485.6 µg L^{-1}, depending on the yield and the lactation stage (Cheng *et al*., 2008). The observed variations in the concentration of lactoferrin in organic and conventional milk may lead to varying effects on the LAB in raw milk. However, in the context of the fermentation process, the role of lactoferrin on LAB growth in organic and conventional milk requires further research, as lactoferrin along with other whey protein fractions is sensitive to heat treatment (Goulding *et al*., 2021).

Immunoglobulin A (IgA)

The major immunoglobulin classes in bovine are IgG, IgA, and IgM. Bovine IgA occurs as secretory IgA in colostrum and milk. Colostrum is secreted during the first five days after birth, during which time immunoglobulins make up to 70–80% of total protein. After five to seven days, immunoglobulin concentrations decrease to only 1–2% of total protein in milk, and IgA concentrations range from 50 to 140 µg mL^{-1} (Mehra *et al*., 2006). The results obtained showed no significant difference in the IgA concentration between organic and conventional milk, and the production month did not have any impact (Table 3). In organic milk the IgA concentration ranged from 132.68 µg mL^{-1} in February to 182.68 µg mL^{-1} in April (Figure 4c). The resemblance in IgA concentration found in this study can be attributed to the similarity of the farms, such as management and environment, lactation stage and yield, as well as the number of lactations, and the cows' health status (Cheng *et al*., 2008; Costa *et al*., 2021; Gomes *et al*., 2011).

IgA is an anti-inflammatory antibody, that possesses a high pathogenic binding capacity and is therefore suggested for the functional food market (Kitano *et al*., 2020). Recent research shows that it has a variable influence on LAB and other bacteria, depending on the strains and concentration in the product (Bartkiene *et al*., 2020). For instance, bovine colostrum, which contains higher lactoferrin and IgA compared to milk, exhibits higher antibacterial activity against pathogens while improving the growth of LAB, including *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Renchinthand *et al*., 2007). Therefore, it can be assumed, that when the milk has a high concentration of these proteins it is more favourable and beneficial for the growth of LAB. Nonetheless, it is suggested that IgA is very sensitive to fermentation, emphasising the need to increase the stability of IgA in fermented products during the technological processes (Bartkiene *et al*., 2020).

The composition and quality of fermented dairy products and their potential health benefits are directly influenced by the inherent variations in raw milk composition. Bioactive compounds present in milk, such as PUFA, including CLA, and whey proteins, may have an effect on the lactic acid bacteria present in raw milk and those used in the fermentation process. In the context of the compositional disparities between organic and conventional milk, it has been noted that these differences are likely to be attributed to specific farm practices and production months. Limited research exists that specifically investigates organic milk as a growth substrate for starter cultures or other LAB compared to conventional milk, highlighting the necessity for further exploration in order to understand the potential influence of organic milk as a raw material for the production of fermented dairy products.

Conclusions

In comparing the bioactive compounds present in organic and conventional milk, notable disparities were observed between the two. Both organic and conventional milk demonstrated the presence of components with antimicrobial properties. It is plausible that the elevated concentrations of lysozyme as well as mono- and polyunsaturated fatty acids in conventional milk contribute to a more pronounced inhibitory effect on the lactic acid bacteria growth when compared to organic milk. Further investigation is necessary to fully understand the influence of these individual components and their interactions on the growth patterns of lactic acid bacteria in both organic and conventional milk.

References

Ayivi, R. D., Ibrahim, S. A., Krastanov, A., Somani, A., & Siddiqui, S. A. (2022). The impact of alternative nitrogen sources on the growth and viability of *Lactobacillus delbrueckii ssp. bulgaricus*. *Journal of Dairy Science*, 105(10), 7986–7997. DOI: 10.3168/jds.2022-21971

Azzolini, M., Tosi, E., Veneri, G., & Zapparoli, G. (2010). Evaluating the efficacy of lysozyme against lactic acid bacteria under different winemaking scenarios. *South African Journal of Enology and Viticulture*, 31(2), 99–105. DOI: 10.21548/31-2-1406

Bartkiene, E., Lele, V., Sakiene, V., Zavistanaviciute, P., Ruzauskas, M., Stankevicius, A., … Juodeikiene, G. (2020). Fermented, ultrasonicated, and dehydrated bovine colostrum: Changes in antimicrobial properties and immunoglobulin content. *Journal of Dairy Science*, 103(2), 1315–1323. DOI: 10.3168/jds.2019-16357

Beal, C., Fonseca, F., & Corrieu, G. (2001). Resistance to freezing and frozen storage of streptococcus thermophilus is related to membrane fatty acid composition. *Journal of Dairy Science*, 84(11), 2347–2356. DOI: 10.3168/jds.S0022-0302(01)74683-8

Bellamy, W., Takase, M., Yamauchi, K., Wakabayashi, H., Kawase, K., & Tomita, M. (1992). Identification of the bactericidal domain of lactoferrin. *Biochimica et Biophysica Acta (BBA)/ Protein Structure and Molecular*, 1121(1–2), 130–136. DOI: 10.1016/0167-4838(92)90346-F

Benkerroum, N. (2008). Antimicrobial activity of lysozyme with special relevance to milk. *African Journal of Biotechnology*, 7(25), 4856–4867.

Borgonovi, T. F., Virgolin, L. B., Janzantti, N. S., Casarotti, S. N., & Penna, A. L. B. (2022). Fruit bioactive compounds: Effect on lactic acid bacteria and on intestinal microbiota. *Food Research International*, 161(February). DOI: 10.1016/j.foodres.2022.111809

Breza-Boruta, B., Ligocka, A., & Bauza-kaszewska, J. (2022). Natural Bioactive Compounds in Organic and Conventional Fermented Foods. *Molecules*.

Brodziak, A., Król, J., Litwińczuk, Z., & Barłowska, J. (2018). Differences in bioactive protein and vitamin status of milk from certified organic and conventional farms. *International Journal of Dairy Technology*, 71(2), 321–332. DOI: 10.1111/1471-0307.12462

Butler, G., Nielsen, J. H., Slots, T., Seal, C., Eyre, M. D., Sanderson, R., & Leifert, C. (2008). Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: Seasonal variation. *Journal of the Science of Food and Agriculture*, 88(8), 1431–1441. DOI: 10.1002/jsfa.3235

Carlsson, Å., Björck, L., & Persson, K. (1989). Lactoferrin and Lysozyme in Milk During Acute Mastitis and Their Inhibitory Effect in Delvotest P. *Journal of Dairy Science*, 72(12), 3166–3175. DOI: 10.3168/jds.S0022-0302(89)79475-3

Casillas-Vargas, G., Ocasio-Malavé, C., Medina, S., Morales-Guzmán, C., Del Valle, R. G., Carballeira, N. M., & Sanabria-Ríos, D. J. (2021). Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Progress in Lipid Research*, 82, 1–28. DOI: 10.1016/j.plipres.2021.101093

Cheng, J. B., Wang, J. Q., Bu, D. P., Liu, G. L., Zhang, C. G., Wei, H. Y., … Wang, J. Z. (2008). Factors affecting the lactoferrin concentration in bovine milk. *Journal of Dairy Science*, 91(3), 970–976. DOI: 10.3168/jds.2007-0689

Collomb, M., Bisig, W., Bütikofer, U., Sieber, R., Bregy, M., & Etter, L. (2008). Fatty acid composition of mountain milk from Switzerland: Comparison of organic and integrated farming systems. *International Dairy Journal*, 18(10–11), 976–982. DOI: 10.1016/j.idairyj.2008.05.010

Costa, A., Goi, A., Penasa, M., Nardino, G., Posenato, L., & De Marchi, M. (2021). Variation of immunoglobulins G, A, and M and bovine serum albumin concentration in Holstein cow colostrum. *Animal*, 15(7), 100299. DOI: 10.1016/j.animal.2021.100299

Costa, M. P. D., Frasao, S., B., Lima, B. R. C., Rodrigues, B. L., & Junior, C. A. C. (2016). Simultaneous analysis of carbohydrates and organic acids by HPLC-DAD-RI for monitoring goat's milk yogurts fermentation. *Talanta*, 152, 162–170. DOI: 10.1016/j.talanta.2016.01.061

D'Incecco, P., Gatti, M., Hogenboom, J. A., Bottari, B., Rosi, V., Neviani, E., & Pellegrino, L. (2016). Lysozyme affects the microbial catabolism of free arginine in raw-milk hard cheeses. *Food Microbiology*, 57, 16–22. DOI: 10.1016/j.fm.2015.11.020

Delgado-Fernández, P., Corzo, N., Olano, A., Hernández-Hernández, O., & Moreno, F. J. (2019). Effect of selected prebiotics on the growth of lactic acid bacteria and physicochemical properties of yoghurts. *International Dairy Journal*, 89, 77–85. DOI: 10.1016/j.idairyj.2018.09.003

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, 85(6), 1629–1642. DOI: 10.1007/s00253-009-2355-3

Dziuba, B., & Dziuba, M. (2014). Milk proteins-derived bioactive peptides in dairy products: Molecular, biological and methodological aspects. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 13(1), 5–25. DOI: 10.17306/j.afs.2014.1.1

Ellis, K. A., Innocent, G., Grove-White, D., Cripps, P., McLean, W. G., Howard, C. V., & Mihm, M. (2006). Comparing the fatty acid composition of organic and conventional milk. *Journal of Dairy Science*, 89(6), 1938–1950. DOI: 10.3168/jds.S0022-0302(06)72261-5

Florence, A. Béal, C., da Silva, R. C., & Oliveira, M. N. (2014). Survival of three *Bifidobacterium* animalis subsp. lactis strains is related to trans-vaccenic and α-linolenic acids contents in organic fermented milks. *Lwt*, 56(2), 290–295. DOI: 10.1016/j.lwt.2013.11.036

Florence, A. C. R., Béal, C., Silva, R. C., Bogsan, C. S. B., Pilleggi, A. L. O. S., Gioielli, L. A., & Oliveira, M. N. (2012). Fatty acid profile, trans-octadecenoic, α-linolenic and conjugated linoleic acid contents differing in certified organic and conventional probiotic fermented milks. *Food Chemistry*, 135(4), 2207–2214. DOI: 10.1016/j.foodchem.2012.07.026

Franco, I., Castillo, E., Pérez, M. D., Calvo, M., & Sánchez, L. (2010). Effect of bovine lactoferrin addition to milk in yogurt manufacturing. *Journal of Dairy Science*, 93(10), 4480–4489. DOI: 10.3168/jds.2009-3006

Givens, D. I., & Lovegrove, J. A. (2016). Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: A systematic literature review and meta- and redundancy analyses. *British Journal of Nutrition*, 116(1), 1–2. DOI: 10.1017/S0007114516001604

Gomes, S. I. F., van Bodegom, P. M., van Agtmaal, M., Soudzilovskaia, N. A., Bestman, M., Duijm, E., … van Eekeren, N. (2020). Microbiota in Dung and Milk Differ Between Organic and Conventional Dairy Farms. *Frontiers in Microbiology*, 11(July), 1–12. DOI: 10.3389/fmicb.2020.01746

Gomes, V., Madureira, K. M., Soriano, S., Libera, A. M. M. P. Della, Blagitz, M. G., & Benesi, F. J. (2011). Factors affecting immunoglobulin concentration in colostrum of healthy holstein cows immediately after delivery. *Pesquisa Veterinaria Brasileira*, 31(SUPPL. 1), 53–56. DOI: 10.1590/S0100-736X2011001300009

Goulding, D. A., O'Regan, J., Bovetto, L., O'Brien, N. M., & O'Mahony, J. A. (2021). Influence of thermal processing on the physicochemical properties of bovine lactoferrin. *International Dairy Journal*, 119, 105001. DOI: 10.1016/j.idairyj.2021.105001

Grodkowski, G., Gołębiewski, M., Slósarz, J., Grodkowska, K., Kostusiak, P., Sakowski, T., & Puppel, K. (2023). Organic Milk Production and Dairy Farming Constraints and Prospects under the Laws of the European Union. *Animals*, 13(9), 1–20. DOI: 10.3390/ani13091457

Hayek, S. A., Gyawali, R., Aljaloud, S. O., Krastanov, A., & Ibrahim, S. A. (2019). Cultivation media for lactic acid bacteria used in dairy products. *Journal of Dairy Research*, 86(4), 490–502. DOI: 10.1017/S002202991900075X

Iskandar, C. F., Cailliez-Grimal, C., Borges, F., & Revol-Junelles, A. M. (2019). Review of lactose and galactose metabolism in Lactic Acid Bacteria dedicated to expert genomic annotation. *Trends in Food Science and Technology*, 88(February), 121–132. DOI: 10.1016/j.tifs.2019.03.020

Jenkins, J. K., & Courtney, P. D. (2003). *Lactobacillus* growth and membrane composition in the presence of linoleic or conjugated linoleic acid. *Canadian Journal of Microbiology*, 49(1), 51–57. DOI: 10.1139/w03-003

Jugert, C. S., Didier, A., Plötz, M., & Jessberger, N. (2023). Strain-specific Antimicrobial Activity of Lactoferrin-based Food Supplements. *Journal of Food Protection*. DOI: 10.1016/j.jfp.2023.100153

Kankaanpää, P. E., Salminen, S. J., Isolauri, E., & Lee, Y. K. (2001). The influence of polyunsaturated fatty acids on probiotic growth and adhesion. *FEMS Microbiology Letters*, 194(2), 149–153. DOI: Pasi E. Kankaanpää, Seppo J. Salminen, Erika Isolauri, Yuan Kun Lee, The influence of polyunshttps://doi.org/10.1111/j.1574-6968.2001.tb09460.x

Khorshidian, N., Khanniri, E., Koushki, M. R., Sohrabvandi, S., & Yousefi, M. (2022). An Overview of Antimicrobial Activity of Lysozyme and Its Functionality in Cheese. *Frontiers in Nutrition*, 9(March). DOI: 10.3389/fnut.2022.833618

Kim, W. S., Ohashi, M., Tanaka, T., Kumura, H., Kim, G. Y., Kwon, I. K., … Shimazaki, K. I. (2004). Growth-promoting effects of lactoferrin on *L. acidophilus and Bifidobacterium spp*. *BioMetals*, 17(3), 279–283. DOI: 10.1023/B:BIOM.0000027705.57430.f1

Kitano, N., Isobe, N., Noda, J., & Takahashi, T. (2020). Concentration patterns of antibacterial factors and immunoglobulin A antibody in foremilk fractions of healthy cows. *Animal Science Journal*, 91(1), 1–5. DOI: 10.1111/asj.13372

Kouřimská, L., Legarová, V., Panovská, Z., & Pánek, J. (2014). Quality of cows' milk from organic and conventional farming. *Czech Journal of Food Sciences*, 32(4), 398–405. DOI: 10.17221/510/2012-cjfs

Kozáková, D., Holubová, J., Plocková, M., Chumchalová, J., & Čurda, L. (2005). Impedance measurement of growth of lactic acid bacteria in the presence of nisin and lysozyme. *European Food Research and Technology*, 221(6), 774–778. DOI: 10.1007/s00217-005-0026-x

Król, J., Litwińczuk, Z., Brodziak, A., & Barłowska, J. (2010). Lactoferrin, lysozyme and immunoglobulin G content in milk of four breeds of cows managed under intensive production system. *Polish Journal of Veterinary Sciences*, 13(2), 357–361.

Kucevic, D., Trivunovic, S., Bogdanovic, V., Cobanovic, K., Jankovic, D., & Stanojevic, D. (2016). Composition of raw milk from conventional and organic dairy farming. *Biotechnology in Animal Husbandry*, 32(2), 133–143. DOI: 10.2298/bah1602133k

Kuczyńska, B., Puppel, K., Gołębiewski, M., Metera, E., Sakowski, T., & Słoniewski, K. (2012). Differences in whey protein content between cow's milk collected in late pasture and early indoor feeding season from conventional and organic farms in Poland. *Journal of the Science of Food and Agriculture*, 92(14), 2899–2904. DOI: 10.1002/jsfa.5663

Lambotte, M., De Cara, S., Brocas, C., & Bellassen, V. (2023). Organic farming offers promising mitigation potential in dairy systems without compromising economic performances. *Journal of Environmental Management*, 334(December 2022). DOI: 10.1016/j.jenvman.2023.117405

Linehan, K., Patangia, D. V., Ross, R. P., & Stanton, C. (2024). Production, Composition and Nutritional Properties of Organic Milk: A Critical Review. *Foods*, 13(4), 1–23. DOI: 10.3390/foods13040550

Liu, N., Pustjens, A. M., Erasmus, S. W., Yang, Y., Hettinga, K., & van Ruth, S. M. (2020). Dairy farming system markers: The correlation of forage and milk fatty acid profiles from organic, pasture and conventional systems in the Netherlands. *Food Chemistry*, 314(December 2019), 126153. DOI: 10.1016/j.foodchem.2019.126153

Manuelian, C. L., Vigolo, V., Burbi, S., Righi, F., Simoni, M., & De Marchi, M. (2022). Detailed comparison between organic and conventional milk from Holstein-Friesian dairy herds in Italy. *Journal of Dairy Science*, 105(7), 5561–5572. DOI: 10.3168/jds.2021-21465

Martini, M., Altomonte, I., Sodi, I., Vasylieva, Y., & Salari, F. (2023). Sterol, tocopherol, and bioactive fatty acid differences between conventional, high-quality, and organic cow milk. *Journal of Dairy Science*, 106(12), 8239–8248. DOI: 10.3168/jds.2023-23378

Masschalck, B., Van Houdt, R., Van Haver, E. G. R., & Michiels, W. C. (2001). Inactivation of gram-negative bacteria by lysozyme, denatured lysozyme, and lysozyme-derived peptides under high hydrostatic pressure. *Applied and Environmental Microbiology*, 67(1), 339–344. DOI: 10.1128/AEM.67.1.339-344.2001

Mehra, R., Marnila, P., & Korhonen, H. (2006). Milk immunoglobulins for health promotion. *International Dairy Journal*, 16(11), 1262–1271. DOI: 10.1016/j.idairyj.2006.06.003

Neviani, E., Carminati, D., Veaux, M., Hermier, J., & Giraffa, G. (1991). Characterization of Lactobacillus helveticus strains resistant to lysozyme . *Le Lait*, 71(1), 65–73. DOI: 10.1051/lait:199115

Ohlsson, J. A., Johansson, M., Hansson, H., Abrahamson, A., Byberg, L., Smedman, A., … Lundh, Å. (2017). Lactose, glucose and galactose content in milk, fermented milk and lactose-free milk products. *International Dairy Journal*, 73, 151–154. DOI: 10.1016/j.idairyj.2017.06.004

Ozturkoglu-Budak, S. (2018). Effect of different treatments on the stability of lysozyme, lactoferrin and β-lactoglobulin in donkey's milk. *International Journal of Dairy Technology*, 71(1), 36–45. DOI: 10.1111/1471-0307.12380

Partanen, L., Marttinen, N., & Alatossava, T. (2001). Fats and Fatty Acids as Growth Factors for Lactobacillus delbrueckii. *Systematic and Applied Microbiology*, 24(4), 500–506. DOI: 10.1078/0723-2020-00078

Priyadarshini, S., & Kansal, V. K. (2002). Lysozyme activity in buffalo milk: Effect of lactation period, parity, mastitis, season in India, pH and milk processing heat treatment. *Asian-Australasian Journal of Animal Sciences*, 15(6), 895–899. DOI: 10.5713/ajas.2002.895

Renchinthand, G., Bae, H. C., & Nam, M. S. (2007). Measurement of lactoferrin, IgA, IgG<inf>1</inf>, IgG<inf>2</inf>, antibacterial activity, and lactic acid bacterial growth in holstein colostrum. *Korean Journal for Food Science of Animal Resources*, 27(4), 522–530. DOI: 10.5851/kosfa.2007.27.4.522

Reuben, R. C., Roy, P. C., Sarkar, S. L., Rubayet Ul Alam, A. S. M., & Jahid, I. K. (2020). Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *Journal of Dairy Science*, 103(2), 1223–1237. DOI: 10.3168/jds.2019-17092

Róin, N. R., Lokuge, G. M. S., Fredsted, M. B., Sundekilde, U. K., Larsen, M. K., Larsen, L. B., & Poulsen, N. A. (2023). Variations in fatty acids, micronutrients and metabolites in discrete milk lines of Danish dairy milk. *International Dairy Journal*, 147. DOI: 10.1016/j.idairyj.2023.105786

Ruska, D., Radenkovs, V., Juhnevica-Radenkova, K., Rubene, D., Ciprovica, I., & Zagorska, J. (2023). The Impact of Biotechnologically Produced Lactobionic Acid in the Diet of Lactating Dairy Cows on Their Performance and Quality Traits of Milk. *Animals*, 13(5). DOI: 10.3390/ani13050815

Sabunevica, S., & Zagorska, J. (2023). Organic Milk as Medium for Lactic Acid Bacteria Growth: A Review. *Rural Sustainability Research*, 49(344), 73–86. DOI: 10.2478/plua-2023-0010

Scharfen, E. C., Mills, D. A., & Maga, E. A. (2007). Use of human lysozyme transgenic goat milk in cheese making: Effects on lactic acid bacteria performance. *Journal of Dairy Science*, 90(9), 4084–4091. DOI: 10.3168/jds.2006-808

Schwendel, B. H., Wester, T. J., Morel, P. C. H., Tavendale, M. H., Deadman, C., Shadbolt, N. M., & Otter, D. E. (2015). Invited review: Organic and conventionally produced milk-An evaluation of factors influencing milk composition. *Journal of Dairy Science*, 98(2), 721–746. DOI: 10.3168/jds.2014-8389

Sodini, I., Lucas, A., Oliveira, M. N., Remeuf, F., & Corrieu, G. (2002). Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing. *Journal of Dairy Science*, 85(10), 2479–2488. DOI: 10.3168/jds.S0022-0302(02)74330-0

Wagner, K., Brinkmann, J., Bergschmidt, A., Renziehausen, C., & March, S. (2021). The effects of farming systems (organic vs. conventional) on dairy cow welfare, based on the Welfare Quality® protocol. *Animal*, 15(8), 100301. DOI: 10.1016/j.animal.2021.100301

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., … Geng, W. (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. *Frontiers in Bioengineering and Biotechnology*, 9(May), 1–19. DOI: 10.3389/fbioe.2021.612285

Acknowledgement

The authors acknowledge the support approbation of antibacterial whey protein detection methods from the European Economic Area and Norway Grants (EEA/NO) programme "Research and Education" project "Investigation of antimicrobial properties of Latvia and Norway cow and goat milk", Nr. EEA-GRANT-207.

The authors acknowledge the support for the detection of fatty acids, antibacterial whey proteins in milk samples from the project "Strengthening the Institutional Capacity of LBTU for Excellence in Studies and Research", funded by the Recovery and Resilience Facility.