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Sterol, tocopherol, and bioactive fatty acid differences between conventional, high-quality, and organic cow milk

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ABSTRACT

Milk contains several components that are important for human nutrition and health. To date, studies on organic and conventional milk have focused on their gross composition and fatty acid content, but little attention has been paid to the differences between other minor components, such as sterols and vitamins that may have functional actions. The aim of this study was to investigate the nutritional differences among 3 types of milk from a dairy plant: conventional, high-quality, and organic (in compliance with European regulations) milk, focusing on minor components such as sterols of animal and plant origin (phytosterols), tocopherols, and bioactive fatty acids. Cholesterol ranged from 271.37 mg/100 g of fat in conventional milk to 278.76 mg/100 g of fat in organic milk. Lanosterol was the main minor animal sterol in cow milk (ranging from 3.41 to 4.37 mg/100 g of fat), followed by desmosterol. The amount of total plant sterols in the analyzed milk ranged from 4.43 mg/100 g of fat in organic to 4.71 mg/100 g of fat in high-quality milk. Brassicasterol was the main sterol of plant origin which varied from 2.6 mg/100 g of fat in conventional and organic milk, to 2.93 mg/100 g of fat in high-quality milk. The second most present phytosterol was β-sitosterol, which ranged from 0.86 mg/100 g of fat in conventional to 0.97 mg/100 g of fat in high-quality, and organic milk. The results of the study showed no significant differences in gross and sterol composition between the 3 types of milk. However, the only significant difference found was in the fatty acid profile, with a higher n-3 content found in high-quality milk than in conventional and organic milk. These findings suggest that the investigated product categories and labels have minimal effect on the sterol and fatty acid profile of commercial cow milk.

Key words: milk sterols, phytosterols, lanosterol, β-sitosterol

INTRODUCTION

Milk has been an important part of the human diet for thousands of years. In Europe, different types of bovine milk are available on the market, with conventional and organic milk being the main types, which are produced in accordance with specific regulations (EC, 2004a,b,c, 2018). On the Italian market, there is also a product category referred to as "high-quality milk" (Italian Ministry of Health, 1991). According to regulations, high-quality milk must have certain nutritional parameters, such as a fat content of at least 3.50% and a protein content of at least 32 g/L in raw milk. In addition, the whey protein fraction, which is susceptible to heat treatment, must be at least 15.5% of the total protein content of pasteurized milk. Organic milk comes from animals that are fed using organic feed. The "organic" label ensures a production process without the use of synthetic fertilizers, pesticides, and hormones, and minimal use of veterinary drugs. The cattle must also have access to pasture whenever possible, with at least 60% of the DM of the feeding ration being roughage, fresh or dried fodder, or silage (EC, 2018). In general, consumers believe that organic food is healthier and of better quality than conventional food, likely due to its association with better environmental performance, animal welfare, and health (Manuelian et al., 2022; Rodríguez-Bermúdez et al., 2020). The nutritional value of cow milk is determined by many dietary components and functional compounds that are beneficial for well-being and health or for reducing the risk of disease (Diplock et al., 1999). Some functional components of milk include vitamins, PUFA, and minor sterols. Plant sterols (or phytosterols), such as β-sitosterol, campesterol, and stigmasterol, may also be present in milk, derived from the animal's diet (Martini et al., 2021b). Plant sterols are natural components of plants and perform many essential functions within the plant cells, similar to those that cholesterol performs in animal cells. However, phytosterols have a lower intestinal absorption rate compared with cholesterol (González Larena et al., 2011). Phytosterols have also become of interest as

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they have been associated with reducing cardiovascular risks (Fassbender et al., 2008; Gylling et al., 2014) and treating childhood dyslipidemia (Ribas et al., 2017). Additionally, anticancer, anti-atherosclerotic, anti-inflammatory, and antioxidant properties of plant sterols have been reported (Katan et al., 2003; Tapiero et al., 2003). To the best of our knowledge, there are only a few studies that have focused on the content of phytosterols in bovine milk (Duong et al., 2019; Fauquant et al. 2007), especially in commercial milk. To date, studies on organic and conventional milk have mainly focused on gross composition and fatty acids (Schwendel et al., 2015), with little attention to differences between other minor components such as sterols and vitamins, which may have functional actions. The aim of this study is to investigate the nutritional differences among 3 types of milk from a dairy plant—conventional, high-quality, and organic milk—focusing on minor components such as sterols of animal and plant origin (phytosterols), tocopherols, and bioactive fatty acids.

MATERIALS AND METHODS

Sampling

The study was carried out in January 2022 and involved milk produced by one of the main dairy plants located in Tuscany (central Italy). The dairy plant collected a total of about 50,000 kg of milk per day. About 30,000 kg of milk per day were designated as drinking milk; the remaining milk was processed into dairy products such as cheese and mozzarella. The investigation focused on high-quality, conventional, and organic whole milks. High-quality milk came from 10 farms; organic and conventional milks came from 8 and 9 farms, respectively. The animals were fed an average forage-to-concentrate ratio of 60:40 for high-quality, 52:48 for conventional, and 71:29 for organic farms. In organic farms, the animals had access to pasture according to the European Union regulations; however, during the sampling period, the contribution of pasture as a food source was low due to climatic conditions.

Milk arrived at the milk processing plant by compartmentalized, refrigerated milk container trucks at 4°C to 6°C. Whole milk underwent to filtration and in-line pasteurization (75°C for 15 s), and milk fat was not standardized. Representative samples of raw and pasteurized whole milk were taken for each product type (high-quality, conventional, and organic milk), from 6 different silos. Each production line had 2 dedicated silos of about 20,000 kg. Raw and pasteurized milk belong each from the same batch; processing and samplings were carried out on the same day. The silos were completely emptied every day and washed with acid and alkaline solutions. All the milks were taken once a week within a month period for a total of 24 samples. All the samples were collected in plastic bottles transported on ice boxes from the dairy plant to the laboratory at 4°C for the analysis of gross, mineral, fatty acid, and sterol composition, and tocopherols, which were evaluated in duplicate for each milk sample.

Gross, Mineral, and Fatty Acid Composition

Each fresh milk sample was analyzed within 24 h from the time of collection, for the following chemical analyses: DM, total fat, total protein, and ash. The analyses were carried out according to AOAC International (2004) methods. In brief, DM and ash were gravimetrically determined by oven-drying at 110°C and by combustion in a muffle furnace at 550°C. Fat was weighted after extraction according to ISO (2001; standard 14156), starting from 2-mL aliquot of milk and using a solution of ethanol, 25% to 30% ammonia solution, and diethyl ether. The fat extracted was stored a −20°C in dark vials for the following analyses. Methyl esters of fatty acids for GC analysis were prepared starting from the previously extracted fat using methanol sodium methoxide according to AOAC methods (AOAC International, 2004). The GC analysis of the milk was carried out as described in our previous work (Altomonte et al., 2019).

Protein determinations were made by the Kjeldahl method. Factor 6.38 was used for the conversion of nitrogen to CP.

Aliquots of each sample were frozen for subsequent mineral analysis. Mineral analysis was carried out after digestion with HNO_3 and $HClO_4$. Calcium, magnesium, potassium, and zinc were quantified by an atomic absorption spectrophotometer (Shimadzu AA-7000), while phosphorus was quantified using a colorimetric method (AOAC International, 2000) at 450 nm by UV-Vis spectrophotometer (Jasco V530).

Sterol Profile, α- and γ-Tocopherol Analysis

Lipids and liposoluble compounds previously extracted according to ISO (2021; standard 14156) were saponified with a solution of methanol potassium hydroxide (standard 18252; ISO, 2006). The unsaponifiable fraction was extracted with hexane as described by ISO (2006; standard 18252) and Cervinkova et al. (2016).

The α-tocopherol, γ-tocopherol, and sterol profiles were simultaneously determined by a Perkin Elmer, Clarus 480 gas chromatograph, equipped with a fused-silica capillary column Zebron ZB-5MSi (length = 30 m, i.d. = 0.25 mm, film thickness = 0.25 μm; Phe-

Table 1. Chemical composition in conventional, high-quality, and organic cow milk

Item	Unit	Milk type			RMSE[1]	*P*-value
		Conventional	High quality	Organic		
DM	g/100 g of milk	13.23	13.26	13.58	0.60	0.49
Fat		3.79	3.82	3.82	0.30	0.97
Protein		3.31	3.36	3.33	0.13	0.69
Ash		0.66	0.68	0.67	0.03	0.67
Ca	mg/L	1,219.16	1,149.81	1,104.14	166.79	0.45
P		974.99	957.06	911.19	28.19	0.24
Mg		119.13	115.12	115.07	6.37	0.37
K		1,482.14	1,483.82	1,392.12	39.79	0.34
Na		392.90	341.70	492.87	177.22	0.28
Zn		5.08	5.04	4.89	0.54	0.78

[1]RMSE = root mean square error.

nomenex, Torrance, CA) and flame ionization detector. The carrier gas, helium, circulated at 1 mL/min in the constant-flow mode. A split/splitless injector in the split mode was used (split ratio, 1:10). The injector and detector were set at 270°C and 300°C, respectively. Compounds were identified by comparing the GC retention times with those for the pure standards analyzed under the same conditions and were quantified by reference to the 5-α-cholestane used as internal standard as described by ISO (2006; standard 18252).

Statistical Analysis

The results of the milk composition were analyzed by ANOVA using JMP software (JMP 2007, SAS Institute Inc., Cary, NC). The model contained the fixed effects of the type of milk and the heat treatment. The effect of the sampling time and the interaction between the type of milk and the heat treatment were not significant and were excluded from the statistical model. The significance of the differences between means was evaluated by Student's *t*-test considering $P \leq 0.05$ as the significance level.

RESULTS AND DISCUSSION

Table 1 shows the results of the chemical composition analysis of the 3 types of milk investigated. All of the commercial milk types had fat and protein percentages that were higher than the minimum requirements for conventional and organic whole milk (minimum 2.8% protein and minimum 3.2% fat; EC, 2004a,b) and for high-quality milk (minimum 3.2% protein and minimum 3.5% fat; Italian Ministry of Health, 1991). In a previous study, Müller and Sauerwein (2010) reported that the fat content was similar in organic and conventional milk, while the protein content was slightly lower in organic milk.

The mineral content was in line with other studies (Rodríguez Rodríguez et al., 2001; Gaucheron, 2005) and did not show differences between organic, conventional, and high-quality milk.

The literature on the nutritional differences between organic and conventional milk is conflicting (Schwendel et al., 2015). No significant differences were shown in the tocopherol content and sterol profile of the 3 commercial milk types (Table 2); however, there was a tendency for the α-tocopherol content to decrease from the conventional to high-quality and organic milk. The α-tocopherol content in this study was within the range previously described for bovine milk (0.8–2.18 mg/100 g of fat; Cichosz et al., 2017). In addition, the γ-tocopherol was similar to the findings of Gessner et al. (2015) in bovine milk (0.29–0.49 mg/100 g of fat) and lower compared with the results of Marino and Schadt (2016) (0.7–2.3 mg/100 g of fat).

Vitamin E is found in fat globule membranes, and its concentrations are much higher in butter than in milk. The vitamin E content of milk is determined by tocopherol levels in feed, as well as by the breed and the season (Marino and Schadt, 2016; Cichosz et al., 2017).

Although several studies have examined the cholesterol content of cow milk, there is limited information in the literature on the minor animal sterols, such as lanosterol and desmosterol that have been found in cow, goat, buffalo, camel, sheep, and donkey milk fat (Dhankhar et al., 2020; Martini et al., 2021a,b). Even fewer studies have examined the natural presence of phytosterols such as β-sitosterol, stigmasterol, and campesterol in milk (Duong et al., 2019; Martini et al., 2021a,b). As expected, cholesterol was the main sterol in all the types of milk analyzed, accounting for more than 96% of the total sterols. In particular, cholesterol ranged from 271.37 mg/100 g of fat (corresponding to 10.28 mg/100 mL of milk) in conventional milk to

Table 2. Sterol profile and tocopherol content in conventional, high-quality, and organic cow milk

Item	Unit	Milk type			RMSE[1]	*P*-value
		Conventional	High quality	Organic		
α-Tocopherol	% of unsaponifiable fraction	0.34	0.30	0.28	0.23	0.85
γ-Tocopherol		0.15	0.13	0.15	0.10	0.92
Sterols of animal origin (total)		97.86	97.94	98.08	0.43	0.61
Cholesterol		96.41	96.51	96.45	0.72	0.96
Lanosterol		1.22	1.41	1.33	0.18	0.16
Desmosterol		0.22	0.14	0.18	0.29	0.54
Phytosterols (total)		1.65	1.63	1.49	0.29	0.34
β-Sitosterol		0.40	0.35	0.30	0.21	0.66
Campesterol		0.25	0.19	0.22	0.10	0.53
Brassicasterol		0.94	1.03	0.91	0.14	0.23
Stigmasterol		0.05	0.06	0.06	0.04	0.91
α-Tocopherol	mg/100 g of fat	1.11	1.04	0.87	1.08	0.91
γ-Tocopherol		0.47	0.40	0.50	0.41	0.88
Sterols of animal origin (total)		275.51	278.15	283.86	104.23	0.99
Cholesterol		271.37	273.81	278.76	101.64	0.99
Lanosterol		3.41	4.37	3.97	1.48	0.47
Desmosterol		0.73	0.54	0.68	1.33	0.88
Phytosterols (total)		4.63	4.71	4.43	2.00	0.96
β-Sitosterol		0.86	0.97	0.97	0.61	0.94
Campesterol		0.69	0.61	0.67	0.42	0.92
Brassicasterol		2.60	2.93	2.60	0.99	0.75
Stigmasterol		0.17	0.21	0.19	0.17	0.90

[1]RMSE = root mean square error.

278.76 mg/100 g of fat (corresponding to 10.64 mg/100 mL of milk) in organic milk. These results agree with other studies (Fauquant et al., 2007; Tranchida et al., 2013), and the values we found were similar to the findings by Do et al. (2018) in milk from the same species (275.63 mg/100 g of fat). The percentages of minor animal sterols agree with the findings by Tranchida et al. (2013) who also reported that desmosterol and lanosterol were 0.22% and 1.22% of the animal sterols in butter from bovine milk. In our study, lanosterol was the main minor animal sterol in cow milk (ranging from 3.41 to 4.37 mg/100 g of fat, corresponding to 0.13 and 0.17 mg/100 mL of milk), within the range detected by Duong et al. (2019) (0.02–0.56 mg/100 mL of milk).

Desmosterol levels ranged from 0.54 to 0.73 mg/100g of fat, which is consistent with the results found by Dhankhar et al. (2020) in milk from the same species (about 0.60 mg/100 g of fat). Milk minor animal sterols are intermediate products formed during the biosynthetic pathway of cholesterol. Lanosterol has been shown to have beneficial effects, such as preventing colon cancer in experimental models (Rao et al., 2002). Lanosterol has also been used in experiments to therapeutically reverse cataracts in dogs (Zhao et al., 2015); the studies on lanosterol have bring to the development a pharmacological principle used for dogs and cats. Additionally, desmosterol has potential antiviral properties, as it has been found to improve membrane damage caused by the hepatitis C virus in vitro (Costello et al., 2016).

The amount of total plant sterols in the analyzed milk ranged from 4.43 mg/100 g of fat in organic to 4.71 mg/100 g of fat in high-quality milk (corresponding to 0.17 and 0.18 mg/100 mL of milk, respectively). Total plant sterols were slightly higher than the findings of Duong et al. (2019), who reported values lower than 0.12 mg/100 mL in cow milk. Higher amounts of phytosterols were detected in our previous studies on sheep and donkey milk (9.89 and 13 mg/100 g of fat, respectively; Martini et al., 2021a,b). Phytosterols are structurally very similar to cholesterol except that they contain some substitutions at the C24 position on the sterol side chain. Phytosterols have a variety of pharmacological functions such as cholesterol-lowering, anti-inflammatory, and antioxidant properties along with a role in the prevention of coronary heart disease. A cause-and-effect relationship has been established between the consumption of plant sterols and the lowering of low-density lipoprotein cholesterol, in a dose-dependent manner (Bresson et al., 2008). It is generally assumed that cholesterol reduction results directly from inhibition of cholesterol absorption through displacement of cholesterol from micelle.

Phytosterols are commonly used as a functional ingredient to fortify dairy products. Although phytosterol levels in mammalian tissue are normally low due primarily to poor absorption from the intestine and faster excretion from liver compared with cholesterol, they have been found to reach the brain (Rui et al., 2017). All the identified plant sterols have been found

Table 3. Fatty acid profile and fatty acid classes in conventional, high-quality, and organic cow milk

Item, g/100 g of fat	Milk type			RMSE[1]	*P*-value
	Conventional	High quality	Organic		
C4:0	2.23	1.85	2.20	0.48	0.24
C6:0	1.97	1.83	1.95	0.17	0.25
C8:0	1.40	1.30	1.39	0.14	0.29
C10:0	3.50	3.32	3.48	0.30	0.42
C11:0	0.10	0.11	0.10	0.01	0.11
C12:0	4.06	3.92	4.07	0.30	0.54
C13:0	0.16	0.17	0.14	0.02	0.11
C14:0	12.19	12.15	12.14	0.42	0.97
C14:1	1.41	1.39	1.35	0.15	0.71
C15:0	1.35	1.47	1.33	0.13	0.10
C15:1	0.24^{A}	0.21^{B}	0.23^{A}	0.01	0.005
C16:0	34.69	39.31	35.00	4.35	0.09
C16:1 *cis*-7	0.18^{a}	0.12^{b}	0.17^{ab}	0.04	0.04
C16:1 *cis*-9	1.86	1.99	1.65	0.26	0.07
C17:0	0.59	0.61	0.59	0.07	0.77
C17:1	0.33	0.25	0.25	0.09	0.12
C18:0	8.02^{ab}	7.12^{b}	8.46^{a}	0.87	0.02
C18:1 *trans*-9	0.21	0.33	0.29	0.19	0.49
C18:1 *trans*-11	0.52	0.48	0.47	0.29	0.93
C18:1 *cis*-9	20.64	18.11	20.62	3.65	0.31
C18:2 *trans*-9,12	0.15	0.27	0.18	0.10	0.08
C18:2 *cis*-9,12	2.16	2.12	2.24	0.27	0.72
C18:3n-3	0.39^{B}	0.52^{A}	0.44^{B}	0.05	0.001
C18:3n-6	0.06	0.06	0.06	0.05	1.00
C20:0	0.08	0.08	0.09	0.03	0.91
CLA *cis*-9,*trans*-11	0.25	0.25	0.26	0.05	0.80
C20:1	0.17^{a}	0.12^{b}	0.18^{a}	0.04	0.02
C21:0	0.005	0.005	0.007	0.00	0.60
C20:2	0.01	0.02	0.01	0.01	0.26
C20:3n-3	0.15	0.14	0.13	0.02	0.20
C20:3n-6	0.10	0.09	0.10	0.02	0.81
C22:0	0.04	0.03	0.04	0.01	0.46
C22:1	0.04	0.04	0.05	0.01	0.37
C20:4	0.003	0.004	0.002	0.00	0.41
C20:5n-3	0.04^{B}	0.05^{A}	0.04^{B}	0.01	0.006
C22:2	0.01	0.01	0.01	0.00	0.45
C23:0	0.01	0.01	0.01	0.01	0.88
C24:0	0.02	0.02	0.02	0.01	0.47
C24:1	0.02	0.02	0.02	0.01	0.70
C22:5	0.08	0.09	0.08	0.01	0.55
C22:6	0.02	0.01	0.01	0.01	0.07
SCFA[2] ($\leq$C10)	9.11	8.30	9.02	0.91	0.18
MCFA[3] ($\geq$C11 $\leq$ C17)	57.68	61.72	57.19	4.62	0.14
LCFA[4] ($\geq$C18)	33.21	29.99	33.79	4.05	0.17
SFA	70.97	73.36	71.05	4.19	0.46
MUFA	25.63	23.06	25.43	4.00	0.39
PUFA	3.40	3.58	3.51	0.37	0.62
UFA/SFA	0.41	0.36	0.42	0.08	0.41
n-3	0.65^{b}	0.77^{a}	0.67^{b}	0.07	0.01
n-6	2.48	2.55	2.58	0.28	0.78
n-6/n-3	3.82^{a}	3.33^{b}	3.83^{a}	0.32	0.01

[a,b]Within a row, means without a common superscript differ at $P < 0.05$.

[A,B]Within a row, means without a common superscript differ at $P < 0.01$.

[1]RMSE = root mean square error.

[2]SCFA = short-chain fatty acids.

[3]MCFA = medium-chain fatty acids.

[4]LCFA = long-chain fatty acids.

in varying amounts in butter and cream from cow milk (Ebadnezhad et al., 2021; Nemati et al., 2022). In our study, brassicasterol was the main sterol of plant origin, which varied from 2.6 mg/100 g of fat in conventional and organic milk, to 2.93 mg/100 g of fat in high-quality milk. The second most present phytosterol was

Table 4. Chemical composition of raw and pasteurized cow milk

Item	Unit	Milk		RMSE[1]	*P*-value
		Raw	Pasteurized		
DM	g/100 g	13.27	13.44	0.60	0.50
Fat		3.88	3.74	0.30	0.30
Protein		3.32	3.34	0.13	0.68
Ash		0.68	0.67	0.03	0.43
Ca	mg/L	1,193.00	1,122.41	37.37	0.29
P		959.04	935.91	23.06	0.20
Mg		115.68	117.20	6.37	0.58
K		1,488.82	1,516.56	28.42	0.22
Na		398.02	420.30	177.22	0.77
Zn		5.01	5.00	0.54	0.98

[1]RMSE = root mean square error.

β-sitosterol, which ranged from 0.86 mg/100 g of fat in conventional to 0.97 mg/100 g of fat in high-quality and organic milk.

Phytosterols in milk derive from feed as they are natural active ingredients in seeds, fruits, grains, vegetables, and legumes. So far researchers have mainly investigated how the feeding practices with phytosterols influence human and animal metabolism. Few published studies exist reporting influence of phytosterol from feed on the quality of the products of animal origin (Duong et al., 2019; Xiao, et al., 2023) and their transfer efficiency from feed into milk is not still unknown.

The fatty acid profile of the 3 types of milk agrees with the literature on cow milk reviewed by Markiewicz-Kęszycka et al. (2013). Only a few significant differences in the fatty acid profile (Table 3) were found, which is in agreement with previous studies on commercial milk, organic versus conventional (Manuelian et al., 2022). The significant differences were in the levels of C15:1, C16:1 *cis*-7, C18:0, C18:3n-3, C20:1, and C20:5.

Diet is one of the main factors that influence milk fat fatty acid profile; however, endogenous synthesis and effects of physiological factors have also reported (Chilliard et al., 2007; Benbrook et al., 2018). In par-

Table 5. Sterol profile of raw and pasteurized cow milk

Item	Unit	Milk		RMSE[1]	*P*-value
		Raw	Pasteurized		
α-Tocopherol	% of unsaponifiable fraction	0.32	0.29	0.23	0.80
γ-Tocopherol		0.17	0.12	0.10	0.20
Sterols of animal origin (total)		97.90	98.02	0.43	0.53
Cholesterol		96.41	96.51	0.72	0.74
Lanosterol		1.32	1.32	0.18	0.10
Desmosterol		0.19	0.18	0.29	0.91
Phytosterols (total)		1.60	1.57	0.22	0.73
β-Sitosterol		0.38	0.32	0.21	0.47
Campesterol		0.21	0.24	0.10	0.47
Brassicasterol		0.96	0.96	0.14	0.97
Stigmasterol		0.05	0.06	0.04	0.72
α-Tocopherol	mg/100 g of fat	1.04	0.97	0.23	0.87
γ-Tocopherol		0.53	0.38	0.10	0.38
Sterols of animal origin (total)		270.45	287.90	0.43	0.69
Cholesterol		266.21	283.08	0.72	0.70
Lanosterol		3.67	4.16	0.18	0.45
Desmosterol		0.72	0.58	0.29	0.68
Phytosterols (total)		4.54	4.65	0.22	0.90
β-Sitosterol		0.92	0.95	0.21	0.91
Campesterol		0.58	0.73	0.10	0.38
Brassicasterol		2.67	2.76	0.14	0.86
Stigmasterol		0.16	0.21	0.04	0.55

[1]RMSE = root mean square error.

Table 6. Fatty acid profile and fatty acid classes of raw and pasteurized cow milk

	Milk			
Item, g/100 g of fat	Raw	Pasteurized	RMSE[1]	*P*-value
C4:0	2.02	2.16	0.48	0.49
C6:0	1.93	1.90	0.17	0.64
C8:0	1.38	1.35	0.14	0.62
C10:0	3.45	3.42	0.30	0.85
C11:0	0.11	0.10	0.01	0.83
C12:0	4.02	4.02	0.30	0.97
C13:0	0.15	0.16	0.02	0.25
C14:0	12.16	12.17	0.42	0.95
C14:1	1.37	1.40	0.15	0.65
C15:0	1.36	1.41	0.13	0.34
C15:1	0.23	0.23	0.01	0.45
C16:0	35.95	36.71	4.35	0.68
C16:1 *cis*-7	0.15	0.16	0.04	0.88
C16:1 *cis*-9	1.78	1.88	0.26	0.40
C17:0	0.59	0.61	0.07	0.59
C17:1	0.25	0.30	0.09	0.16
C18:0	8.08	7.65	0.87	0.25
C18:1 *trans*-9	0.27	0.28	0.19	0.83
C18:1 *trans*-11	0.49	0.48	0.29	0.93
C18:1 *cis*-9	19.97	19.61	3.65	0.82
C18:2 *trans*-9,12	0.21	0.19	0.10	0.78
C18:2 *cis*-9,12	2.18	2.17	0.27	0.89
C18:3n-3	0.45	0.46	0.05	0.65
C18:3n-6	0.06	0.06	0.05	0.92
C20:0	0.09	0.08	0.03	0.57
CLA *cis*-9,*trans*-11	0.26	0.25	0.05	0.63
C20:1	0.16	0.16	0.04	0.90
C21:0	0.01	0.01	0.00	0.59
C20:2	0.02	0.01	0.00	0.09
C20:3n-3	0.14	0.13	0.02	0.36
C20:3n-6	0.10	0.09	0.02	0.41
C22:0	0.04	0.03	0.01	0.71
C22:1	0.05	0.04	0.01	0.28
C20:4	0.00	0.00	0.00	0.80
C20:5n-3	0.04	0.05	0.01	0.30
C22:2	0.02[A]	0.01[B]	0.00	0.006
C23:0	0.01	0.01	0.00	0.95
C24:0	0.02	0.02	0.01	0.63
C24:1	0.02[b]	0.03[a]	0.01	0.047
C22:5	0.08	0.08	0.01	0.59
C22:6	0.02	0.01	0.01	0.07
SCFA[2] (≤C10)	8.78	8.84	0.91	0.89
MCFA[3] (≥C11 ≤ C17)	58.46	59.26	4.62	0.68
LCFA[4] (≥C18)	32.75	31.91	4.05	0.62
SFA	71.73	71.85	4.19	0.96
MUFA	24.74	24.68	4.00	0.97
PUFA	3.53	3.47	0.37	0.71
UFA/SFA	0.40	0.40	0.08	0.99
n-3	0.70	0.69	0.07	0.92
n-6	2.55	2.52	0.28	0.77
n-6/n-3	3.68	3.65	0.32	0.84

[A,B]Within a row, means without a common superscript differ at $P < 0.01$.

[a,b]Within a row, means without a common superscript differ at $P < 0.05$.

[1]RMSE = root mean square error.

[2]SCFA = short-chain fatty acids.

[3]MCFA = medium-chain fatty acids.

[4]LCFA = long-chain fatty acids.

ticular, C18:3n-3 and C20:5 ($P \leq 0.01$), and total n-3 ($P \leq 0.05$) were higher in the high-quality milk than in the conventional and organic milk. No difference in n-3 content was found between conventional and organic milk, unlike findings reported by other authors (Manuelian et al. 2022; Stergiadis et al., 2019). Some studies have reported that lactating cows fed a legume plants-based diet produce milk with elevated levels of

long-chain n-3 fatty acids, mainly C18:3 and C20:5n -3 (Chilliard et al., 2007; Benbrook et al., 2018) that can be also linked a reduced rumen biohydrogenation (Dewhurst et al., 2003).

Similar to what was reported in the aforementioned studies, we hypothesized that our results could be linked to a low ruminal biohydrogenation of feed fatty acids, resulting in a higher transfer of n-3 fatty acids in milk. In fact, the diet of high-quality milk farms included higher percentages of leguminous forages (alfalfa, Egyptian clover) in the diet (20% of the dry substance of the ration vs. 16% of organic farms and 18% of conventional farms; data not shown).

The beneficial activity of C18:3n-3 fatty acid is recognized by the European Food Safety Authority, which has set adequate intake values in adults (0.5% of the energy level of the diet) to achieve a plasma cholesterol control effect (EFSA, 2017; Oliveira Godoy Ilha et al., 2020); in addition, its anti-inflammatory potential has also been studied (Ren and Chung, 2007). The difference found in the n-3 content also affected the n-6:n-3 ratio, which was significantly lower in high-quality milk at 3.33 ($P \leq 0.05$) compared with conventional and organic milk (3.82 and 3.83, respectively). The lower n-6:n-3 ratio in high-quality milk appears to be positive, as the World Health Organization and Food and Agriculture Organization Expert Committee recommends that the n-6:n-3 fatty acid ratio should be below 4. In fact, such a proportion has been linked to a considerable reduction (about 70%) in the number of deaths caused by cardiovascular diseases (Markiewicz-Kęszycka et al., 2013).

Regarding the effect of heat treatment, no significant differences were found for any of the parameters investigated (Tables 4, 5, and 6), with the exception of some minor fatty acids such as C22:2 ($P \leq 0.01$) and C24:1 ($P \leq 0.05$). The impact of pasteurization on the gross composition and lipid profile was also reported to be insignificant by Xu et al. (2020) in bovine milk and by Martini et al. (2018) in donkey milk. In addition, phytosterols have been shown to be quite resistant to heat treatment and milk processing in a study carried out by Martini et al. (2021b) on sheep milk. Lastly, although processing and manufacturing can negatively affect the tocopherol content of milk (Delgado et al., 2014; Martini et al., 2021b), pasteurization did not result in significant differences in the α- and γ-tocopherol content of the milk.

CONCLUSIONS

This study investigates the nutritional differences between 3 types of milk from a dairy plant and represents one of the few investigations focusing on minor components such as sterols of animal and plant origin (phytosterols) and tocopherols. Of particular interest in cow milk was presence of phytosterols, which are considered to be nutraceutical molecules, and lanosterol, a molecule with reported pharmacological action. Our findings indicate that the pasteurization process did not affect the content of bioactive sterols in the milk. There were not found differences on nutritional characteristic, tocopherols, sterol profile between the commercial milk types. Some differences were observed in the fatty acid profiles in particular high-quality milk had higher quantities of n-3. These results suggest that current product categories and labels of cow milk have minimal differences in the sterol and fatty acid profile. The few differences observed in the fatty acids profile seem mostly linked to the feed used in the cow rations. The effects of season on fatty acids and sterol profile as well as of geographical areas should be further investigated.

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