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Nutritional differences between conventional, high quality, and organic milk: focus on sterols, tocopherols, and bioactive fatty acids

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Interpretive Summary

This study focuses on nutritional differences between conventional, high quality (D.M. 185/1991), and organic (in compliance with European Regulations) milk. Of particular interest in cow's milk was the presence of phytosterols, which are considered to be nutraceutical molecules, and lanosterol, a molecule with reported pharmacological action. The results highlight that current product categories and labels have a minimal effect on the tocopherols, sterol and fatty acid profile of commercial cow's milk. Furthermore, pasteurization process did not affect either nutritional characteristic or bioactive sterols and tocopherols content in the milk.

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 which may have functional actions. The aim of this study was to investigate the nutritional differences between three types of milk from a dairy plant: conventional, high quality (D.M. 185/1991), and organic (in compliance with European Regulations), focusing on minor components such as sterols of animal and plant origin (phytosterols), tocopherols, and bioactive fatty acids. Cholesterol ranged from 271.37 mg/100g of fat in conventional milk to 278.76 mg/100g of fat in organic milk. Lanosterol was the main minor animal sterol in cow's milk (ranging from 3.41 to 4.37 mg/100g 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/100g 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/100g of fat in high quality milk. The second most present phytosterol was beta-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 that there were no significant differences in gross, and sterol composition between the three types of milk. However, the only significant difference found was in the fatty acid profile, with a higher omega-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's milk.

Key Words: milk sterols, phytosterols, lanosterol, beta-sitosterol.

INTRODUCTION

Milk has being an important part of the human diet for thousands of years. In Europe, there are different types of bovine milk available on the market, with conventional and organic milk being the main types, which are produced in accordance with specific regulations (EC 852-853/2004, 848/2018, and 889/2008). On the Italian market, there is also a product category referred to as "high quality milk" (D.M. 185/91). 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. Additionally, 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, hormones, and minimizes the use of veterinary drugs. The cattle must also have access to pasture whenever possible, with at least 60% of the dry matter of the feeding ration being roughage, fresh or dried fodder, or silage (Reg. CE 848/2018). 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 (Rodríguez-Bermúdez et al., 2020; Manuelian et al., 2020). The nutritional value of cow's 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, polyunsaturated fatty acids, 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 to cholesterol (González Larena et al., 2011). Phytosterols have also become of interest as 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, anti-cancer, 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 between three 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 involved three different types of milk produced by the dairy plant: conventional, high quality and organic. For each product type, raw and pasteurized (75 °C for 15 seconds) milk samples were taken weekly within a month period for a total of 24 samples. Gross, mineral, fatty acid and sterol composition and tocopherols were evaluated in duplicate for each milk sample

Gross, mineral and fatty acid composition

The milk was transported to the laboratory in refrigerated tanks at 4°C. For each fresh milk sample, the following chemical analyses were carried out according to AOAC methods (2004): dry matter, total fat, total protein, ash, phosphorus by the colorimetric method, and Ca, Mg, K, Na, Zn by atomic absorption spectrophotometry.

Fat was extracted by the Rose-Gottlieb method. Methyl esters of fatty acids for gas chromatographic analysis were prepared using methanol sodium methoxide according to Christie (AOAC, 2004). The gas chromatography analysis of the milk was carried out as described in our previous work (Altomonte et al., 2019).

Sterol profile, alpha and gamma tocopherol analysis

Lipids and liposoluble compounds were extracted according to ISO 14156 (ISO-IDF, 2001), and were saponified with a solution of methanol potassium hydroxide (ISO 18252, 2006). The unsaponifiable fraction was extracted with hexane as described by ISO 18252, 2006 and Cervinkova et al. (2016).

The alpha-tocopherol, gamma- 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 (L = 30 m, ID = 0.25 mm, FT = 0.25 μm) (Phenomenex, Torrance, CA, USA) and FID detector. The carrier gas, helium, circulated at 1 mL/min in the constant flow mode. A split/split less injector in the split mode was used (split ratio, 1:10). The injector and detector were set at 270 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-alpha-cholestane used as internal standard as described by ISO 18252, 2006.

Statistical analysis

The results of the milk composition were analyzed by ANOVA using JMP software (JMP®, 2007, SAS, Cary, North Carolina, USA).

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 three 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 852 and 853/2004) and for high quality milk (minimum 3.2% protein and minimum 3.5% fat) (D.M.185/199). 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 (Rodriguez et al., 2001; Gaucheron, 2005) and did not shown 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 three commercial milk types (Table 2), however there was a tendency for the alpha tocopherol content to decrease from the conventional to high quality and organic milk. The alpha tocopherol content in this study was within the range previously described for bovine milk (Marino and Schadt, 2016) (1.08-2.11 mg/100 g of fat). In addition, the gamma tocopherol was similar to the findings of Gessner et al. (2015) in bovine milk (0.29-0.49 mg/100g of fat) and slightly lower compared to the results of Marino and Schadt (2016) (0.7-2.3 mg/100g of fat).

Although several studies have examined the cholesterol content of cow's 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 beta-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/100g of fat (corresponding to 10.28 mg/ml of milk) in conventional milk to 278.76 mg/100 g of fat (corresponding to 10.64 mg/ 100 ml of milk) in organic milk. These results are in agreement 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 are in agreement 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's milk (ranging from 3.41 to 4.37 mg/ 100g 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).

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 (around 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, Newmark, & Reddy, 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, Villareal, & Yang, 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 /100g 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's 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., 2021 a,b). Phytosterols in milk and dairy products have a cholesterol-lowering effect, 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 LDL cholesterol, in a dose-dependent manner (Bresson et al., 2008). Phytosterols are commonly used as a functional ingredient to fortify dairy products. Due to their ability to reach the brain, the physiological role of plant sterols in the central nervous system has been investigated (Rui et al., 2017). All the identified plant sterols have been found in varying amounts in butter and cream from cow's milk (Ebadnezhad et al., 2020; Nemati et al., 2022). In our study, brassicasterol was the main sterol of plant origin which varied from 2.6 mg / 100g of fat in conventional and organic milk, to 2.93 mg /100g of fat in high quality milk. The second most present phytosterol was beta-sitosterol, which ranged from 0.86 mg /100g of fat in conventional to 0.97 mg /100 g of fat in high quality and organic milk.

The fatty acid profile of the three types of milk was in agreement with the literature on cow milk reviewed by Markiewicz-Keszycka 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 vs. conventional (Manuelian et al., 2022). The significant differences were in the levels of C15:1, C16:1 c7, C18:0, C18:3 n3, C20:1, and C20:5. In particular, C18:3 n3 and C20:5 ($P \leq 0.01$), and total omega-3 ($P \leq 0.05$) were higher in the high-quality milk than in the conventional and organic milk. No difference in omega 3 content was found between conventional and organic milk, unlike findings reported by other authors (Manuelian et al 2022; Stergiadis et al., 2022). However, C18:3 n3 tended to be higher in organic than conventional milk, even though the difference was not statistically significant. The beneficial activity of C18:3 n3 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) in order to achieve a plasma cholesterol control effect (EFSA, 2017; Ilha et al., 2020); additionally, its anti-inflammatory potential has also been studied (Ren and Chung, 2007). The difference found in the omega 3 content also affected the omega 6/omega 3 ratio, which was significantly lower in high-quality milk at 3.33 ($P \leq 0.05$) compared to conventional and organic milk (3.82 and 3.83 respectively). The lower omega 6/omega 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-Keszycka 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 (2019) 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 alpha and gamma tocopherol content of the milk.

CONCLUSIONS

This study investigate the nutritional differences between three 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's 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. The 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 omega-3. These results suggest that current product categories and labels of cow's milk have minimal differences in the sterol and fatty acid profile.

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Table 1 Chemical composition in conventional, high quality and organic cow's milk

		Conventional	High quality	Organic		
	units	mean			RMSE[1]	*P*
Dry matter	g/100g of milk	13.23	13.26	13.58	0.605	0.488
Fat		3.79	3.82	3.82	0.303	0.975
Protein		3.31	3.36	3.33	0.134	0.690
Ash		0.66	0.68	0.67	0.032	0.668
Ca	mg/L	1219.16	1149.81	1104.14	166.79	0.447
P		974.99	957.06	911.19	28.19	0.241
Mg		119.13	115.12	115.07	6.369	0.383
K		1482.14	1483.82	1392.12	39.79	0.337
Na		392.90	341.70	492.87	177.218	0.280
Zn		5.08	5.04	4.89	0.537	0.779

[1]RMSE: Root mean square error

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

		Conventional	High quality	Organic		
	units	means			RMSE[1]	*P*
alpha-Tocopherol	% of unsaponifiable fraction	0.34	0.30	0.28	0.232	0.850
gamma- Tocopherol		0.15	0.13	0.15	0.103	0.922
Sterols of animal origin (total)		97.86	97.94	98.08	0.427	0.606
Cholesterol		96.41	96.51	96.45	0.720	0.960
Lanosterol		1.22	1.41	1.33	0.182	0.157
Desmosterol		0.22	0.14	0.18	0.286	0.540
Phytosterols (total)		1.65	1.63	1.49	0.219	0.345
beta-Sitosterol		0.40	0.35	0.30	0.211	0.664
Campesterol		0.25	0.19	0.22	0.097	0.535
Brassicasterol		0.94	1.03	0.91	0.138	0.228
Stigmasterol		0.05	0.06	0.06	0.040	0.908
alpha-Tocopherol	mg /100 g of fat	1.11	1.04	0.87	1.084	0.915
gamma -Tocopherol		0.47	0.40	0.50	0.407	0.885
Sterols of animal origin (total)		275.51	278.15	283.86	104.232	0.988
Cholesterol		271.37	273.81	278.76	101.641	0.990
Lanosterol		3.41	4.37	3.97	1.482	0.474
Desmosterol		0.73	0.54	0.68	1.329	0.881
Phytosterols (total)		4.63	4.71	4.43	2.000	0.963
beta-Sitosterol		0.86	0.97	0.97	0.613	0.936
Campesterol		0.69	0.61	0.67	0.418	0.922
Brassicasterol		2.60	2.93	2.60	0.994	0.750
Stigmasterol		0.17	0.21	0.19	0.174	0.899

[1]RMSE: Root mean square error

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

		Conventional	High quality	Organic		
	units	means			RMSE[1]	*P*
C4:0	g /100 g of fat	2.23	1.85	2.20	0.479	0.241
C6:0		1.97	1.83	1.95	0.169	0.253
C8:0		1.40	1.30	1.39	0.138	0.291
C10:0		3.50	3.32	3.48	0.298	0.418
C11:0		0.10	0.11	0.10	0.015	0.114
C12:0		4.06	3.92	4.07	0.300	0.545
C13:0		0.16	0.17	0.14	0.023	0.111
C14:0		12.19	12.15	12.14	0.418	0.971
C14:1		1.41	1.39	1.35	0.149	0.708
C15:0		1.35	1.47	1.33	0.131	0.101
C15:1		0.24 A	0.21 B	0.23 A	0.015	0.005
C16:0		34.69	39.31	35.00	4.349	0.092
C16:1 cis 7		0.18 a	0.12 b	0.17 ab	0.042	0.045
C16:1 cis 9		1.86	1.99	1.65	0.261	0.070
C17:0		0.59	0.61	0.59	0.069	0.775
C17:1		0.33	0.25	0.25	0.086	0.119
C18:0		8.02 ab	7.12 b	8.46 a	0.874	0.025
C18:1 *trans-9*		0.21	0.33	0.29	0.189	0.495
C18:1 *trans-11*		0.52	0.48	0.47	0.292	0.933
C18:1 *cis*-9		20.64	18.11	20.62	3.646	0.314
C18:2 *trans*-9.12		0.15	0.27	0.18	0.102	0.085
C18:2 *cis*-9.12		2.16	2.12	2.24	0.271	0.720
C18:3 n3		0.39 B	0.52 A	0.44 B	0.053	0.001
C18:3 n6		0.06	0.06	0.06	0.053	0.998
C20:0		0.08	0.08	0.09	0.027	0.913
CLA *cis*-9. *trans*-11		0.25	0.25	0.26	0.047	0.805
C20:1		0.17 a	0.12 b	0.18 a	0.037	0.020
C21:0		0.005	0.005	0.007	0.004	0.597
C20:2		0.01	0.02	0.01	0.007	0.261
C20:3 n3		0.15	0.14	0.13	0.021	0.200
C20:3 n6		0.10	0.09	0.10	0.023	0.814
C22:0		0.04	0.03	0.04	0.010	0.462
C22:1		0.04	0.04	0.05	0.010	0.372
C20:4		0.003	0.004	0.002	0.003	0.409
C20:5n3		0.04 B	0.05A	0.04 B	0.008	0.006
C22:2		0.01	0.01	0.01	0.005	0.453
C23:0		0.01	0.01	0.01	0.006	0.879

C24:0	0.02	0.02	0.02	0.009	0.468
C24:1	0.02	0.02	0.02	0.007	0.700
C22:5	0.08	0.09	0.08	0.015	0.551
C22:6	0.02	0.01	0.01	0.011	0.068
SCFA[2] (≤C10)	9.11	8.30	9.02	0.910	0.183
MCFA[3](≥C11≤C17)	57.68	61.72	57.19	4.616	0.137
LCFA[4](≥C18)	33.21	29.99	33.79	4.052	0.170
SFA[5]	70.97	73.36	71.05	4.194	0.458
MUFA[6]	25.63	23.06	25.43	3.998	0.387
PUFA[7]	3.40	3.58	3.51	0.367	0.619
UFA[8]/SFA	0.41	0.36	0.42	0.082	0.407
n3	0.65 b	0.77 a	0.67 b	0.072	0.012
n6	2.48	2.55	2.58	0.278	0.780
n6/n3	3.82a	3.33 b	3.83 a	0.323	0.010

[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; [5]SFA = saturated fatty acids; [6]MUFA = monounsaturated fatty acids; [7]PUFA = polyunsaturated fatty acids; [8]UFA = unsaturated fatty acids.

Table 4. Chemical composition of raw and pasteurized cow's milk

		Raw	Pasteurized		
	units	means		RMSE[1]	*P*
Dry matter	g /100 g	13.27	13.44	0.605	0.504
Fat		3.88	3.74	0.303	0.303
Protein		3.32	3.34	0.134	0.676
Ash		0.68	0.67	0.032	0.428
Ca	mg/L	1193.00	1122.41	37.369	0.2967
P		959.04	935.91	23.06	0.204
Mg		115.68	117.20	6.369	0.576
K		1488.82	1516.56	28.420	0.221
Na		398.02	420.30	177.218	0.768
Zn		5.01	5.00	0.537	0.984

RMSE[1]: Root mean square error

Table 5 - Sterol profile of raw and pasteurized cow's milk

		Raw	Pasteurized		
	units	means		RMSE[1]	*P*
alpha-Tocopherol	% of unsaponifiable fraction	0.32	0.29	0.232	0.804
gamma- Tocopherol		0.17	0.12	0.103	0.198
Sterols of animal origin (total)		97.90	98.02	0.427	0.532
Cholesterol		96.41	96.51	0.720	0.745
Lanosterol		1.32	1.32	0.182	0.997
Desmosterol		0.19	0.18	0.286	0.910
Phytosterols (total)		1.60	1.57	0.219	0.731
beta-Sitosterol		0.38	0.32	0.211	0.466
Campesterol		0.21	0.24	0.097	0.468
Brassicasterol		0.96	0.96	0.138	0.967
Stigmasterol		0.05	0.06	0.040	0.716
alpha-Tocopherol	mg /100 g of fat	1.04	0.97	0.232	0.874
gamma -Tocopherol		0.53	0.38	0.103	0.383
Sterols of animal origin (total)		270.45	287.90	0.427	0.695
Cholesterol		266.21	283.08	0.720	0.697
Lanosterol		3.67	4.16	0.182	0.452
Desmosterol		0.72	0.58	0.286	0.683
Phytosterols (total)		4.54	4.65	0.219	0.901
beta-Sitosterol		0.92	0.95	0.211	0.914
Campesterol		0.58	0.73	0.097	0.383
Brassicasterol		2.67	2.76	0.138	0.863
Stigmasterol		0.16	0.21	0.040	0.549

[1]RMSE: Root mean square error

Table 6- Fatty acid profile and fatty acid classes of raw and pasteurized cow's milk

		Raw	Pasteurized		
	units	means		RMSE[1]	*P*
C4:0	g /100 g of fat	2.02	2.16	0.479	0.494
C6:0		1.93	1.90	0.169	0.638
C8:0		1.38	1.35	0.138	0.624
C10:0		3.45	3.42	0.298	0.853
C11:0		0.11	0.10	0.015	0.834
C12:0		4.02	4.02	0.300	0.970
C13:0		0.15	0.16	0.023	0.251
C14:0		12.16	12.17	0.418	0.954
C14:1		1.37	1.40	0.149	0.650
C15:0		1.36	1.41	0.131	0.342
C15:1		0.23	0.23	0.015	0.452
C16:0		35.95	36.71	4.349	0.682
C16:1 cis 7		0.15	0.16	0.042	0.879
C16:1 cis 9		1.78	1.88	0.261	0.404
C17:0		0.59	0.61	0.069	0.588
C17:1		0.25	0.30	0.086	0.164
C18:0		8.08	7.65	0.874	0.255
C18:1 *trans-9*		0.27	0.28	0.189	0.831
C18:1 *trans-11*		0.49	0.48	0.292	0.933
C18:1 *cis*-9		19.97	19.61	3.646	0.819
C18:2 *trans*-9.12		0.21	0.19	0.102	0.779
C18:2 *cis*-9.12		2.18	2.17	0.271	0.891
C18:3 n3		0.45	0.46	0.053	0.654
C18:3 n6		0.06	0.06	0.053	0.923
C20:0		0.09	0.08	0.027	0.570
CLA *cis*-9. *trans*-11		0.26	0.25	0.047	0.627
C20:1		0.16	0.16	0.037	0.898
C21:0		0.01	0.01	0.004	0.594
C20:2		0.02	0.01	0.007	0.087
C20:3 n3		0.14	0.13	0.021	0.357
C20:3 n6		0.10	0.09	0.023	0.412
C22:0		0.04	0.03	0.01	0.713
C22:1		0.05	0.04	0.01	0.277
C20:4		0.00	0.00	0.003	0.804
C20:5 n3		0.04	0.05	0.008	0.302
C22:2		0.02A	0.01B	0.005	0.006
C23:0		0.01	0.01	0.006	0.946

C24:0	0.02	0.02	0.009	0.629
C24:1	0.016 b	0.026 a	0.007	0.047
C22:5	0.08	0.08	0.015	0.592
C22:6	0.02	0.01	0.011	0.070
SCFA[2] (≤C10)	8.78	8.84	0.91	0.889
MCFA[3](≥C11≤C17)	58.46	59.26	4.616	0.687
LCFA[4](≥C18)	32.75	31.91	4.052	0.625
SFA[5]	71.73	71.85	4.194	0.947
MUFA[6]	24.74	24.68	3.998	0.971
PUFA[7]	3.53	3.47	0.367	0.713
UFA[8]/SFA	0.40	0.40	0.082	0.986
n3	0.70	0.69	0.072	0.919
n6	2.55	2.52	0.278	0.772
n6/n3	3.68	3.65	0.323	0.838

[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; [5]SFA = saturated fatty acids; [6]MUFA = monounsaturated fatty acids; [7]PUFA = polyunsaturated fatty acids; [8]UFA = unsaturated fatty acids.