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Differences in Sheep Milk Characteristics Focusing on Fatty Acid Profile Between Conventional and Organic Farming System

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Abstract

The aim of this study was to investigate potential variations in chemical composition, coagulation traits and Fatty Acid (FA) composition of sheep milk from organic (ORG) and Conventional (CON) dairy farms located in three regions of West Greece. Bulk milk was sampled twice per month from March to May over 2 years from organic (n=25) and conventional (n=15) dairy sheep farms. Milk samples were analyzed for their coagulation properties (r, K_{20}, a_{30}), chemical composition (fat, protein and lactose) and mineral content (Ca, Mg, K, Na). Milk fatty acids profile was also determined. Significant differences were found in protein content, total solids (TS) and solids-not- fat (SNF) contents as well as mineral (Ca, Mg and Na) content between organic and conventional milk. Total solids (TS) solids-not-fat (SNF) and protein contents were significantly higher ($P<0.001$) in organic (ORG) milk with respect to conventional milk. A higher content of Ca, Mg and Na found in organic milk in comparison to conventional ones. Regarding milk coagulation traits, the value of curd firmness a30 parameter was found significantly higher ($P<0.001$) and K_{20} parameter was found significantly lower ($P<0.01$) in organic milk in comparison with conventional ones, so more favorable from a technological point of view. Organic milk had significantly higher proportion of PUFA ($P<0.001$), MUFA ($P<0.001$) and CLA ($P<0.001$). The calculate Atherogenicity Index (AI) result more favorable in organic milk. Principal Components Analysis (PCA) of FA profile, showed a good discrimination between organic and conventional milk samples.

Keywords: Conventional; Fatty Acids Profile; Organic; Sheep Milk

Abbreviations

ADF	:	Acid Detergent Fiber
AI	:	Atherogenicity Index
FA	:	Fatty Acid
LSM	:	Least Square Means
NDF	:	Neutral Detergent Fiber
NS	:	Not Significant
PCA	:	Principal Components Analysis
RMSE	:	Root Mean Square Errors
SNF	:	Solids-Not- Fat
TS	:	Total Solids

Introduction

According to the European Commission Regulation 834/07, which replaced Reg. 2092/91, organic production is a holistic system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes (EC, 2007). In the organic livestock production system, particular attention is given to the access of animals to pasture, roughage dry matter intake has to make at least 60% total dry matter consumed and feeds have to be produced mostly with organic methods. The use of GMOs and the synthetic veterinary medicines are prohibited, and the choice of breeds should take account of their capacity to adapt to local conditions. A respectable number of consumers trust the organic products and are willing to pay a higher price, compared to conventional ones, since they believe that they are produced in a more environmentally friendly manner with better animal welfare [1]. However, the EU legislation not always forces farmers to respect these moral values and differentiate clearly from conventional methods of production [2].

The organic market has seen double digit growth in the latest decades, both in terms of the European market and the area of organically managed agricultural land - which now represents 5.7% of agricultural area in the EU-28 (2.4% in Europe). Dairy products hold a market share of between 5 and 10% in Austria, Germany and the Netherlands. Milk alone can reach even higher shares - 15.7% in Austria [3]. Milk and dairy products are with no doubt the most studied. There is very little information available regarding any essential differences in gross composition or other parameters of technological interests in milk from organic or conventional farms. It is difficult to establish a clear comparison between organic and conventional quality of products due to the great variation within the production methods.

Milk composition is determined by many factors among them, milk FA composition, including breed [4], species [5], lactation period [6], seasonal changes [7-9], geographical location [10-12], access to fresh herbage [5,10,13], silage type [14,15], oil supplementation [16,17] and farm management practices (organic vs. conventional) as it is related, mostly, to differences in the period of access to fresh herbage and the feeding systems in general (roughage to concentrates ratio, milk yield etc.) [9,18,19]. Many authors compared organic and conventional milk and reported significantly higher content of PUFA in organic bovine [4,6,10], goat [9,18], sheep [20] and buffalo milk [21]. The same authors, also, reported higher CLA content in organic milk, with the exception of Ellis et al. who found no difference between them [4]. Other authors concluded that retail milk of the two systems is of equal quality [22].

In recent years, much scientific interest has been allocated to the health benefits through the consumption of animal products, especially milk and meat from ruminants, since these are a major source of poly and mono-unsaturated fatty acids (PUFA and MUFA) [23]. Particularly, n-3 and n-6 PUFA, proved to induce positive effects on human health, such as protection against coronary and cardiovascular disease [24], prevention of cancer [25], antiatherogenic, antiobesity function [26] and other diseases. Some researchers associate the low n-6/n-3 ratio (1-4:1) with better health outcomes, since a more balanced n-6/n-3 ratio provide better elongation of α-linoleic acid to eicosapentaenoic acid [27]. Among PUFA, Conjugated Linolenic Acid (CLA), C18:2 cis-9 trans-11 isomer, has received the highest attention of researchers recently, since it appears to have many positive health effects, namely, modulating the lipid metabolism, lowering cancer and coronary heart risk [28] and having anti-diabetes properties [29]. Ruminant milk and meat are the best source of dietary CLA [30] therefore, their implementation on human diet might improve one's health. Nevertheless, since there is a lack of clinical and epidemiologic studies, and results are mostly related to animal studies, strong evidence is still needed by further research. The multifactorial aspect of those diseases and the way these factors interact, is an additional reason for them have not yet fully understood [29,31].

Because of the different organic management in each area that should different effect on milk characteristics is important that the research on organic milk production should be carried out in each country independently. The goat and sheep dairy sector is a valuable piece of Greece's national economy, producing high quality dairy products, especially, traditional cheeses like feta, graviera etc. The extensive mountainous areas of the Greek country provide good opportunities for livestock farmers to make the transition to organic management, a change that could improve their income and help them create a sustainable local economy, preserve natural habitats and autochthonous species and prevent the land degradation that often comes with overgrazing or undergrazing [32]. Ronchi & Nardone report that the small ruminant's organic livestock systems of the Mediterranean areas may have the potential to make a positive contribution towards a better quality and sustainability of the agro-landscape and particularly soil and water quality conservation [33].

Although there are a number of studies, which have investigated the differences in cow's milk FA profile between organic and conventional production systems, there is few study on dairy sheep which have reported comparison of milk yield and composition from organic and conventional dairy sheep under controlled feeding regimen [1,9,15]. In the absence of data in differences of chemical and technological characteristic of organic sheep milk, the aim of this study was to compare chemical composition, milk coagulation properties and fatty acids profile of milk obtained from organic and conventional dairy farms located in the same area Aitoloakarnania region, West ofGreece).

Materials and Methods

Experimental Design

Over two lactation periods, bulk-tank milk samples were collected during springtime (from March to May, 14 samplings altogether) from 25 organic dairy-sheep farms located in 3 regions of Aitoloakarnania province of Greece, each situated at different altitude. At the same period, sheep milk was also collected from 15 conventional dairy-sheep farms (5 for each region respectively). Conventional farms were selected to have similar herd characteristics. Herd size was very variable from 60 to 150 milking sheep in organic and conventional farms. The average milk yield is similar in conventional farms with respect to organic farms, with low variability among farms.

Milk samples were collected from the area of: Region 1, (R1) 9 farms, with <200 m altitude; Region 2, (R2) 9 farms, with 350-750 m altitude; and Region 3, (R3) 7 farms, with 750-1000 m altitude. The organic dairy farms were certified according to the EU Regulation 834/2007, by the Institute of organic products, BIO Hellas, authorized by the Hellenic Ministry of Rural Development and Food. The samples collected were classified according to month of sampling (March, April, and May), lactation period (year) and geographical location. Diet composition of sheep in the organic as well as the conventional farms was assessed through appropriate questionnaires, completed with the aid of the researchers, together with production data. Annual requirements of animals in feed Dry Matter (DM) and Energy (Mj NEL/year) were calculated according to NRC (2007) and the respective amounts covered through grazing and purchased feeds were subsequently calculated. Animals in the organic system consumed on annual basis rough grazings, alfalfa (*Medicago sativa*) hay, wheat (*Triticum vulgare*) straw and concentrates (maize, wheat and barley grains). The average farm was covering 14.5% of DM and 12.5% of energy annual requirements through purchased roughages (hay and straw), as well as 11.5% of DM and 19.0% of energy annual requirements through purchased concentrates. Therefore, it was estimated that the remaining requirements of organic farms were covered through rough pasture grazing (i.e. 74% and 68.5% of of DM and energy requirements respectively). Moreover, conventionally farmed sheep consumed alfalfa hay, wheat straw and a commercial mixture thus covering 48% of DM and 32% of energy requirements through rough grazings.

Milk Analyses

The chemical composition of milk samples (fat, protein, lactose, total solids, solids non-fat) was analyzed using Milkoscan FT 6000 (Foss Electric Co., Denmark). Macroelements (Ca, Mg, Na and K) were measured using atomic absorption spectrometric (AAS) method according to ISO and IDF standards [34]. Milk coagulation properties: rennet coagulation time (r, min), curd firming time (k_{20}, min) and curd firmness (a_{30}, mm) were measured by a Formagraph 11700 (Foss Electric Co., Denmark). Rennet coagulation time is the time from the addition of rennet to the beginning of milk coagulation, curd firming time is defined as the time needed until the curd is firm enough to be cut and curd firmness is the width of the curd 30 min after the addition of rennet [35].

For analysis of fatty acid composition, milk fat extraction was based on the Rose-Gottlieb gravimetric method described by IDF [36]. The Fatty Acid Methyl Esters (FAMEs) were prepared by base-catalyzed methanolysis (KOH in methanol) in accordance with the IDF procedure [37]. FAMES were quantified using a gas chromatograph GC 17 (Shimadzu Corporation, Kyoto, Japan) equipped with a SP 2340 capillary column (60m x 250 μm i.d., 0.25 μm film thickness; Supelco, Bellefonte PA, USA). The injection volume was 1μL (split 1:50). The flow rate of carrier gas (Helium) was $1mLmin^{-1}$, the injection temperature was 250˚C and the detector temperature was 270˚C. The oven temperature was set at 45˚C for 5 min, then increased at the rate of 5˚C min^{-1} to 150˚C and maintained for 5 min, with a final increase to 220˚C at the rate of 7˚C min^{-1} held for 20 min. The identification of the FAME peaks was performed by comparing the retention times of the FAME standards [38]. FAME Mix Supelco 37 Components, CLA (Octadecadienoic acid, conjugated, methyl ester, >99%) were purchased from Sigma–Aldrich. A GC Solution software (Shimadzu Corporation, Kyoto, Japan) was used for integration of the peaks. Identification of fatty acids was further confirmed by comparing the retention index with previously analyzed data at our laboratory both by GC-MS and GC. The individual FA content was expressed as weight percentage ($g\text{-}100g^{-1}$ of the total FA).

For the milk FA composition, the following equations were used for the appropriate calculations: Saturated Fatty Acids (SFA)

SFA=C4:0+C6:0+C8:0+C10:0+C12:0+C14:0+C16:0+C18:0 (1)

Mono-Unsaturated Fatty Acids (MUFA)

MUFA=C14:1+C16:1+C18:1 (2)

Poly-Unsaturated Fatty Acids (PUFA)

PUFA=C18:2+CLA+C18:3 (3)

The Atherogenicity Index (AI) was defined as:

AI= (C12:0+4 X C14:0+C16:0)/ (PUFA+MUFA) (4)

As proposed by Ulbricht & Southgate [39].

Statistical Analysis

Milk chemical composition, coagulation properties and FA composition were analyzed using the statistical software JMP 10.0 (SAS Institute Inc., Cary, NC, USA). For the comparison of the farming system, organic milk data were classified to two groups according to the lactation period (first lactation period, ORG1 and second lactation period, ORG2), while the conventional group (CON) the mean value of both lactation periods were used, since differences between the lactation periods were not significant (data not shown). All groups were compared in pairs (ORG1 vs CON, ORG2 vs CON, ORG1 vs ORG2) using one-way ANOVA and Tukey's HSD test. In those cases, where data showed unequal variances shown by Bartlett and Levene tests or abnormal distribution by -Shapiro-Wilk W test, the nonparametric Kruskal-Wallis test was used. Principal components analysis (PCA) was carried out on fatty acids composition using the SPSS 15.0 for Windows software (SPSS Inc., Chicago, IL, USA). The effects of lactation period month of sampling and geographical location were evaluated within the organic group, using a multi- factorial model of the generic form:

$$Y_{ijk}=\mu+\alpha_i+\beta_j+\gamma_k+(\alpha\beta)_{ij}+(\alpha\gamma)_{ik}+(\beta_\gamma)_{jk}+(\alpha\beta\gamma)_{ijk}+\varepsilon_{ijk} \quad (5)$$

Where Y_{ijk}=the observed variable, μ=the overall mean, α_i=lactation period effect (used as a grouping factor), β_j=month effect, γ_k=region effect, $(\alpha\beta)_{ij}$, $(\alpha\gamma)_{ik}$, $(\beta\gamma)_{jk}$, $(\alpha\beta\gamma)_{ijk}$= the interaction between the respective factors and εijk=residual error. The levels of significance for all tests were set to P<0.05, P<0.01 and P<0.001.

Results and Discussion

Effect of The Farming System

Chemical composition, coagulation properties and mineral content of milk from organic (ORG1 and ORG2) and conventional (CON) farms, during the trial, are shown in Table 1. Total solids (TS) and solids-not-fat (SNF) contents were significantly higher in organic than conventional milk (ORG1 vs. CON, P<0.01 and ORG2 vs. CON, P<0.05). Organic groups were also richer in their protein content than the conventional group (P<0.001). Significant difference was observed for lactose content between ORG2 and CON (P<0.05), as well as, within the organic group (ORG1 vs. ORG2, P<0.05), while the fat content did not differ compared with the corresponding ORG and CON. Few comparative studies have been published between organic and conventional sheep milk. Tsiplakou, et al. reported higher fat and TS content for conventional sheep milk, compared to the organic one, contrary to the results of this study [20]. According to these authors, the lower fat content can be attributed to the lower Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) of the animal's diet. Literature data on sheep milk composition report a range of 6.82-7.90% fat, 5.59-6.20% protein, 4.90% lactose, 12% SNF and 18.10% TS [23,40]. In a different study, Tsiplakou, et al. analyzed the milk composition of 4 sheep breeds (Awassi, Lacaune, Friesland, Chios), where sheep grazed on pasture during springtime and reported that sheep milk comprised of 6.90-7.20% fat, 5.50-5.90% protein, 5.00-5.10% lactose and 11.10-11.80% SNF [3]. These results appear similar values (except lactose) to those of the organic milk composition of the present study. Comparative studies for other types of milk report inconsistent results (higher, lower or insignificant differences) concerning the protein content of organic cow milk and fat content in organic cow and goat milk [6,9,18].

Traits	LSM			RMSE	Contrasts		
Chemical composition	CON	ORG1	ORG2		ORG1 vs ORG2	ORG1 vs CON	ORG2 vs CON
Fat, %	6.49	6.83	6.95	0.57	NS	NS	NS
Protein, %	5.40	5.82	5.97	0.32	NS	***	***
[a]Lactose, %	4.67	4.69	4.44	0.15	*	NS	*
[a]SNF, %	10.94	11.48	11.44	0.47	NS	**	*
TS, %	17.43	18.30	18.34	0.85	NS	**	*
Rheological properties							
r, min	14.86	13.90	13.54	4.19	NS	**	**
[a]k_{20}, min	4.55	3.26	3.60	1.27	NS	***	***
a_{30}, mm	28.07	41.37	33.31	6.35	**	***	NS
Mineral content, mg/ 100g							
Ca	233.13	281.21	297.80	30.75	NS	*	**

+Mg	18.88	24.90	23.09	1.75	NS	**	***
+Na	56.32	111.09	100.00	14.50	NS	**	***
K	129.08	151.84	184.80	31.64	NS	NS	**
NS: Not Significant, *: P<0.05, **: P<0.01, ***: P<0.001 a: non-parametric Kruskal-Wallis test was used (Compares rank sums)							

Table 1: Least Square Means (LSM), Root Mean Square Errors (RMSE) and contrasts of chemical composition, rheological properties and mineral content of milk obtained from organic (ORG1, ORG2) and conventional (CON) farms.

Milk coagulation traits, were significantly different (P<0.001) on a30, which was higher in ORG1 and ORG2 against CON. Moreover, K20 (curd firming type) was significantly (P<0.01) lower, in organic milk compared to the conventional, therefore being more favorable from a technological point of view. Studies regarding coagulation properties of organic milk are scarce. In a study comparing farm management systems in a mountainous area of Italy [18], only K20 parameter was found significantly lower in organic than conventional bovine milk (4.01 vs. 5.13 min, P<0.05). Coagulation properties of sheep milk are affected by its chemical properties, including pH, larger casein micelle, more calcium per casein weight, and other mineral concentrations in milk, which causes differences in coagulation time, coagulation rate, curd firmness and amount of rennet needed. Larger casein micelles, more casein, calcium and fat content of sheep milk compared with goat and cow milk, contribute to its better cheese making properties, quality and cheese yield [23]. As organic milk showed higher protein and TS content compared to the conventional milk, this could be associated to the significantly better curd firmness (a30). Further research is necessary preferably with through different analytical methods, to clarify and showcase any possible differences in coagulation traits and technological properties of milk between different farm management systems.

Macroelements (Ca, Mg, Na, and K) were found in significantly higher content in the organic groups compared to the conventional one. Mg and Na contents were found higher in ORG2 group (ORG2 vs. CON, P<0.001) and in ORG1 group (ORG1 vs. CON, P<0.01) than the respective mineral contents of CON group. The same was observed for the Ca content in organic milk, which was much higher compared with conventional milk. Similar findings were reported for the mineral contents in other studies comparing farming systems [40] while Montello, found insignificant differences between the two systems in contrast to this study [18].

The mean percentages of each FA found in both organic and conventional milk are shown in Table 2. The statistical analysis showed significant differences between the fatty acid profiles of two groups. Significant differences were observed between organic and conventional milk about MUFA, PUFA contents and long chain fatty acids (LCFA, C14-C18), while for the short (SCFA) and medium chain (MCFA) fatty acids (C4-C12) no significant differences were observed, with the exception of caproic acid (C6:0). Organic milk had significantly lower content of SFA (P<0.001). Particularly, for the PUFA linoleic acid (C18:2 n-6) and α-linolenic acid (C18:3 n-3) were found higher (P<0.001 and P<0.05 respectively) in the organic ORG2 vs CON milk, while CLA content was significantly higher (P<0.001) in organic than conventional milk, in both comparisons. From human's health point of view, in the last decades, lauric acid (C12), myristic acid (C14) and palmitic acid (C16:0) have been indicated as the main fatty acids responsible for increasing plasma total and LDL cholesterol concentrations, while PUFA and MUFA are considered having a preventive and protective role against the cardiovascular disease [41]. Several authors have reported higher PUFA and lower SFA contents in milk from organic farms when compared to conventional ones [6,18,20], while Tsiplakou, et al. [20] also reported a higher CLA content in organic sheep milk (1.3 % vs. 1.1% of total FA). The ranging value of CLA in sheep milk as reported by Park, et al. is 0.56-0.97% of total FA, while in the present study, the CLA content of both organic and conventional sheep milk was found close to the above mentioned values [23].

		LSM				Contrasts	
FA	CON	ORG1	ORG2	RMSE	ORG1 vs ORG2	ORG1 vs CON	ORG2 vs CON
C4:0	1.91	1.72	1.69	1.12	NS	NS	NS
C6:0	2.22	1.57	1.90	0.53	NS	**	NS
C8:0	2.61	2.38	2.38	0.53	NS	NS	NS
C10:0	8.64	8.12	8.02	1.69	NS	NS	NS
[+]C12:0	4.97	5.00	4.90	1.01	NS	NS	NS
C14:0	13.72	13.36	12.95	0.92	NS	NS	*
[a]C14:1	0.51	1.35	0.57	0.11	***	***	NS
C16:0	32.98	29.98	28.14	1.97	NS	***	***
[+]C16:1	1.45	0.61	1.82	0.56	***	***	*
C18:0	7.37	9.21	8.63	1.42	NS	***	NS
C18:1	20.38	24.06	24.75	2.23	NS	***	***
C18:2 ω-6	2.08	2.33	2.68	0.32	**	**	***
[a]C18:3 ω-3	0.59	0.64	0.33	0.32	**	NS	*
[a]CLA c9 t11	0.56	0.88	1.27	0.28	**	***	***
SFA	74.43	70.22	68.61	2.52	NS	***	***
MUFA	22.34	26.22	27.14	2.36	NS	**	***
PUFA	3.23	3.86	4.25	0.66	*	**	***
AI	3.66	2.98	2.72	0.38	NS	***	***
NS: Not Significant, *: P<0.05, **: P<0.01, ***: P<0.001 A: non-parametric Kruskal-Wallis test was used (Compares rank sums)							

Table 2: Least Square Means (LSM), Root Mean Square Errors (RMSE) and contrasts of Fatty Acid Profiles (FA, % of total FA) and Atherogenicity Index (AI) of milk obtained from organic (ORG1, ORG2) and conventional (CON) farms.

In this respect, the AI was calculated as proposed by Ulbricht & Southgate and defined above (eq. 4) [33]. A lower value of AI indicates a more beneficial fatty acid profile for the human health. In the present study, AI was significantly lower (P<0.001) in organic than conventional milk (2.98 and 2.72 vs 3.66 for ORG1 and ORG2 vs. CON respectively). The AI, has also been reported lower in organic than conventional milk by some authors [18, 20], while Tsiplakou, et al. reported an even lower value of AI than that of the present study (2.2 vs. 2.72) [20]. In grazing sheep milk, this index ranged from 1.71 to 0.81 when the season changed from winter to spring and *Chrysanthemum coronarium* was used as fresh forage. When 4 different forages were compared (*Chrysanthemum coronarium*, annual ryegrass, burr medic and sulla), AI ranged, in the spring time, from 0.81 for *Chrysanthemum coronarium* to 3.24 for sulla (*Hedysarum coronarium*) [14,15]. In the present study, AI's value is similar to the one given by Addis et al. for annual ryegrass (2.5). As it has already been mentioned, milk FA profile is affected mostly by the animal diet but also other factors such as breed, season etc. [14,15]. The results of this study, confirm that the more beneficial FA profile of organic than conventional sheep milk, observed, when compared the organic and conventional farming system in Greece, could be attributed to access of sheep to fresh grazing in the organic farming system, for a longer period of time (more hours a day and more days in a year) than in the conventional one.

The FA profile of both organic and conventional milk was analyzed by PCA in order to verify a possible discrimination of the farm management system of sheep milk. The loading plot is shown in Figure 1. The first component explained the 46.57% of FA variance and the second component the 20.46% of FA variance, giving a cumulative value of 67.02% of FA variance explained by the PCA analysis. There is a clear discrimination of SCFA (C4-C12) in the upper side of the plot, PUFA (with CLA and C18:2) in the right, central side of the plot, and also, LCFA with MUFA and C18:1 in the bottom right side of the plot. These groups indicate strong correlations among the FAs. For the discrimination of FA according to the farm management system, the score plot was produced using the varimax rotation method, as it is shown in Figure 2. Organic samples (ORG1 and ORG2, right side of the plot) are clearly distinguished from the conventional (CON, left side of the plot), confirming that PCA analysis provides a useful tool, to trace the origin of the farm management system of sheep milk. Similar results were found by Miotello, who analyzed the profile of fatty acids of cheeses produced by organic and conventional, using PCA and reported a good discrimination of organic cheeses, especially, those produced from milk during the summer and spring, while those produced during the winter were similar in their FA profile to the conventional cheeses [18]. According to the author, the differences were attributed to the higher concentration of n-3 FA, PUFA and CLA in organic milk produced during the grazing period (late spring and summer).

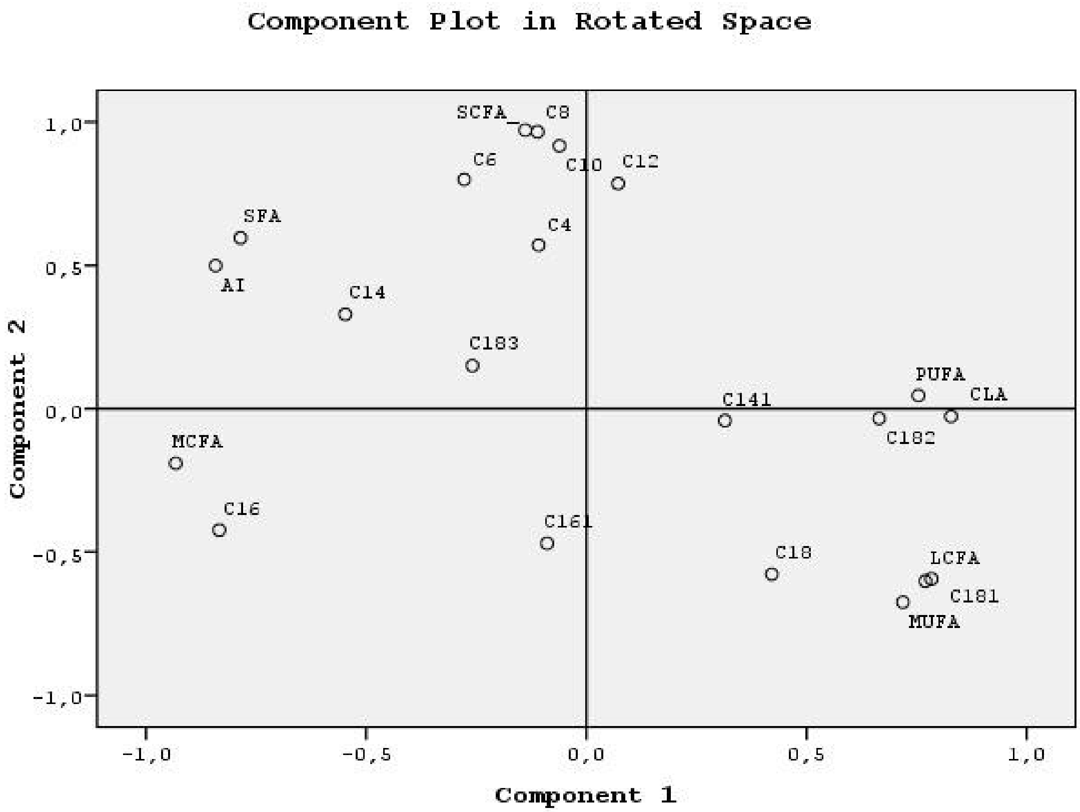

Figure 1: Loading plot of the FA profile of organic and conventional milk (Component 1, 46.57% of variance- Component 2, 20.46% of variance).

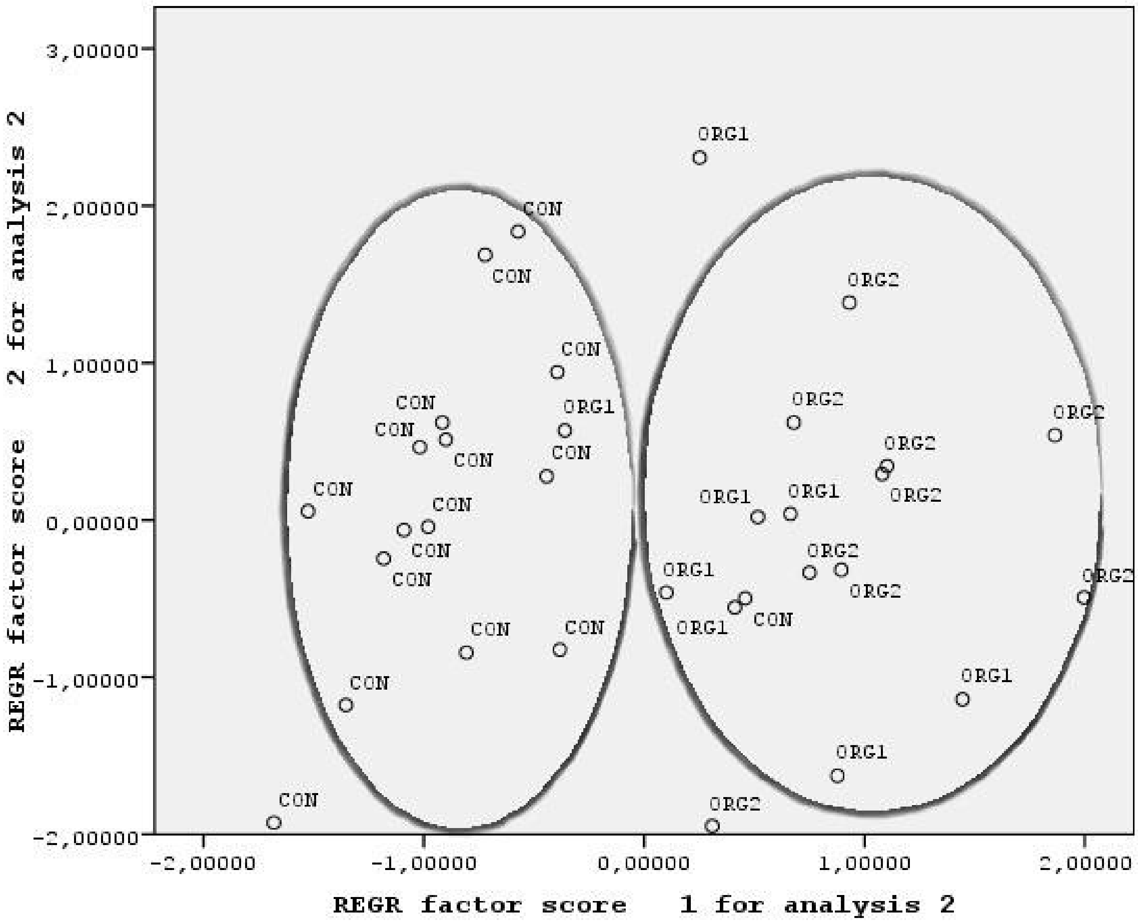

Figure 2: Score plot of milk FA profile obtained from organic (ORG1, ORG2) and conventional (CON) farms.

Effect of The Month, Lactation Period and Geographical Location

The effects of the month, lactation period and region on the chemical composition, coagulation properties and FA profile are shown in Table 3. Since the interaction among the three main factors could not be estimated, the complexity of the model was reduced, therefore the split-plot model was used, with factor A being the month, factor B the region and the lactation period defined as the group. No significant interactions were observed for most traits, with the exception of SFA, MUFA, where interactions were found between region and month (R x M, P<0.05) and lactation period and month (L x M, P<0.05). With the exception of SFA, MUFA, where interactions were found between region and month (R x M, P<0.05) and lactation period and month (L x M, P<0.05).

	LSM								RMSE				P value	
Traits	Month			Lactation period		[b]Region				Main factor			Interaction	
	March	April	May	1	2	R1	R2	R3		M	L	R	R x M	L x M
Fat, %	6.95	6.72	6.84	6.77	6.91	6.81	6.59	7.11	0.53	NS	NS	NS	NS	NS
Protein, %	6.09	5.95	5.72	5.77	6.07	5.97	5.89	5.89	0.39	NS	NS	NS	NS	NS
Lactose, %	4.51	4.65	4.58	4.69	4.47	4.55	4.56	4.62	0.15	NS	**	NS	NS	NS

SNF, %	11.65	11.75	11.17	11.40	11.65	11.53	11.48	11.56	0.53	NS	NS	NS	NS	NS
TS, %	18.60	18.35	18.00	18.16	18.47	18.31	18.01	18.63	0.93	NS	NS	NS	NS	NS
r, min	15.17	16.57	13.43	14.11	16.01	12.66	14.67	17.84	3.7	NS	NS	*	NS	NS
K_{20}, min	3.42	3.67	4.52	4.53	3.21	3.44	4.06	4.11	1.2	NS	*	NS	NS	NS
a_{30}, mm	39.92	35.85	36.24	40.92	33.74	40.48	36.38	35.14	6.26	NS	*	NS	NS	NS
C4:0	1.19	1.38	1.97	1.85	1.18	1.52	1.99	1.03	1.17	NS	NS	NS	NS	NS
C6:0	1.88	1.69	1.67	1.56	1.93	1.92	1.78	1.55	0.57	NS	NS	NS	NS	NS
C8:0	2.63	2.49	2.11	2.31	2.51	2.54	2.34	2.35	0.51	NS	NS	NS	NS	NS
C10:0	9.01	8.50	6.90	7.74	8.53	8.67	7.92	7.83	1.49	NS	NS	NS	NS	NS
C12:0	5.37	5.21	4.32	4.80	5.14	5.36	4.92	4.62	0.88	NS	NS	NS	NS	NS
C14:0	13.5	13.17	12.74	13.17	13.10	13.72	12.94	12.76	0.82	NS	NS	NS	NS	NS
C14:1	0.88	1.00	1.05	1.38	0.58	0.99	0.97	0.97	0.12	NS	***	NS	NS	NS
C16:0	28.19	28.08	29.42	29.46	27.66	28.22	28.99	28.47	1.85	NS	NS	NS	NS	NS
C16:1	0.93	1.38	1.30	0.62	1.78	1.19	1.14	1.28	0.3	NS	***	NS	NS	NS
C18:0	8.60	8.51	9.47	9.34	8.38	8.06	8.83	9.7	1.46	NS	NS	NS	NS	NS
C18:1	24.33	24.29	25.35	24.50	24.81	24.26	24.39	25.32	2.08	NS	NS	NS	NS	NS
C18:2 n-6	2.13	2.46	2.35	2.00	2.63	2.16	2.41	2.38	0.28	NS	***	NS	NS	NS
C18:3 n-3	0.34	0.60	0.44	0.60	0.32	0.47	0.46	0.46	0.33	NS	NS	NS	NS	NS
CLA c9 t1	0.93	1.30	0.94	0.83	1.29	0.95	1.05	1.17	0.34	NS	*	NS	NS	NS
SFA	70.87	68.96	68.60	69.80	69.15	70.38	69.18	68.87	1.95	NS	NS	NS	*	*
MUFA	25.75	26.67	27.70	26.76	26.65	26.05	26.90	27.18	1.84	NS	NS	NS	*	*
PUFA	3.38	4.36	3.71	3.44	4.19	3.57	3.93	3.95	0.68	NS	*	NS	NS	NS
AI	3.01	2.79	2.72	2.90	2.78	3.03	2.78	2.72	0.25	NS	NS	*	**	**
b: R1: <200 m, R2: 350-750 m, R3: 750-1000 m														

Table 3: Least Square Mean Values (LSM), Root Mean Square Error (RMSE), P value for main factors and their interactions for milk chemical composition, rheological properties and FA composition (% of total FA).

The most significant interactions were observed for AI (R x M, L x M, P<0.01). The significant interaction of R x M for SFA and MUFA, is attributed to the significant difference for the month April between the milk samples of regions R1 and R3. In the April, SFA was found significantly higher and MUFA was found significantly lower in R1 milk samples than the respective R3 milk samples. Concerning AI, for the interaction of R x M, the significance is attributed not only to the differences between R1 and R3 milk samples, but also between R1 and R2 milk samples for the month April. Additional differences were observed between April for R1 and May for R3 milk samples. AI had its highest value in April for R1 (3.29) and lowest for R3 in the same month (2.41). Concerning AI and coagulation parameter r, the main factor, region, was also found important. These results indicate that the geographical location, with reference to the altitude, could be important for the quality of milk's FA composition. The few studies relating the effect of the altitude of geographical location to the FA composition of milk, reporting differences analogous to those observed between summer and winter milk FA profiles [10-12]. The same authors determined the composition of 44 summer milk samples from three geographical sites (lowlands, 600-650m-mountains, 900-1210m-highlands, 1275-2120m). They observed the largest increases as a function of altitude for the concentrations of CLA (0.87, 1.61 and 2.36 g 100g-1 respectively) and the C18:1 trans 10 and trans 11 (2.11, 3.66 and 5.10 g 100g-1). This was mostly related to the large botanical biodiversity, with more than 40 species, that constitute the highland pastures in comparison to hay meadows of the lowlands and the greater presence of certain pasture species with a high PUFA content, of the plant families Asteraceae, Apiaceae and Lamiaceae that had been positively correlated to the high PUFA content of milk [7-12]. In the present study, differences in PUFA, CLA or other individual FA among the three examined locations, were less obvious, maybe due to the smaller altitude differences than the ones examined by Collomb, et al. indicating smaller differences in the synthesis of their pasture. Further research is needed, to analyze the synthesis of pasture lands of the Greek provinces, a necessary step to verify the importance of the altitude of grazing areas and its effect on the produced milk's FA profile.

The significant interaction found between lactation period and month (L x M) for the SFA, MUFA and AI is attributed mostly to a year to year variations of pasture's availability and quality and to the maturity of the pasture in the late spring. The same observation was done for the lactation period alone, where significant differences were found for lactose ($P<0.01$), k20 ($P<0.05$), a30 ($P<0.05$) and individual FA C14:1 ($P<0.001$), C16:1 ($P<0.001$), C18:2 n-6 ($P<0.001$), CLA c9 t11 ($P<0.05$) and PUFA ($P<0.05$).

Butler, et al., (2011) also reported a significant effect of lactation period on PUFA ($P<0.001$), SFA ($P<0.05$), CLA c9, t11 ($P<0.01$), linoleic and α-linolenic acid ($P<0.001$). According to the authors, these significant differences in milk's FA composition between successive years, were attributed to the variations in the quality and availability of fresh and conserved forage due to the contrasting weather conditions between the two years of study (30% higher rainfall and 12% lower soil and air temperature, was recorded for the summer of the year with the lower concentrations of PUFA, CLA, linolenic and linoleic acid). Such conditions may affect animal's behavior, reducing its grazing intakes, resulting in the farmers' increasing usage of concentrate feeds [6].

Finally, no significant differences were observed base on the effect of month, since the period of months examined was restricted (March to May) and according to literature [6,7,10,18], the most significant differences are usually observed between the seasons (summer and winter) [42,43].

Conclusion

According to the present study data, organically produced sheep milk in Greece differed in quality compared to the conventional one. These quality traits are considered favourable for human health among them, the beneficial FA, with higher contents of PUFA, MUFA and CLA, and lower contents of SFA, as well as, a more beneficial AI (lower). Differences were clear for both organic groups (ORG1 and ORG2) compared with the conventional one, but differences were also observed between the organic groups. Organic milk, also, had higher protein, TS and mineral (Ca, Mg, Na, and K) contents, but did not differ significantly in rheological properties compared to the conventional milk. PCA analysis gave a good separation of organic and conventional samples providing a potentially useful tool for the traceability of the farm management system of sheep milk. With respect to the other group of main factors examined, the strongest effect was that of lactation period, for some of the individual FAs from the MUFA and PUFA groups, as well as, some less obvious differences in rheological properties and lactose. Some significant interactions were also observed between lactation period and month (L x M), and region and month (R x M) for MUFA, SFA and AI. The R x M interaction effect, could be attributed to differences in the altitude of the three regions indicating some important differences in the botanical biodiversity and as a result the quality of pastures affecting the FA profile of produced milk. This effect, related to the pasture quality and synthesis, needs to be further investigated in the future, since the Mediterranean areas are less favored and the protection of these semi-mountainous provinces of Greece is directly related to the income and the sustainability of the farmers. The organic farming and livestock system seems ideal for these areas, as many studies have shown. Another reason for this, is that organic dairy products, in many cases, seem to be of higher quality than the conventional ones.

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