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# Assessment of Microbiological Quality and Physicochemical Parameters of Fruhe Made by Ovine and Goat Milk: A Sardinian (Italy) Cheese

Marco A. Murgia, Pietrino Deiana, Anna Nudda, Fabio Correddu , Luigi Montanari and Nicoletta P. Mangia \*

Dipartimento di Agraria, University of Sassari, 07100 Sassari, Italy; mamurgia@uniss.it (M.A.M.); pdeiana@uniss.it (P.D.); anudda@uniss.it (A.N.); fcorreddu@uniss.it (F.C.); lmontanari@uniss.it (L.M.)

\* Correspondence: nmangia@uniss.it; Tel.: +39-079-229287

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**Abstract:** Fruhe (Casu axedu) is a fresh cheese, traditionally manufactured in Sardinia (Italy) from sheep or goat milk, characterized by a compact coagulum obtained from raw or heat-treated whole milk. The objective of this study was to investigate the microbiological quality and physicochemical parameters of the sheep and goat Fruhe types of cheese at 21 days of cold storage. Chemical analyses showed that all Fruhe cheese samples were characterized by a pH below 4.4 and a variable content of total solid (22.75–21.06 g/100 g) proteins (5.4–10 g/100 g) and fat (3.9–15.7 g/100 g). The average residual lactose content was 2.6 g/100 g, while lactic acid content was 1.8 g/100 g. Microbial analyses revealed a high number of Lactic Acid Bacteria for both thermophilic and mesophilic streptococci (9 log CFU/g), and no pathogenic bacteria were found. The content of Free Amino Acids and Free Fatty acids point out that a good activity of rennet and microbial enzymes occurred, although Fruhe cheese is not subject to a ripening process. The present research reports the microbiological and nutritional characteristics of the sheep and goat Fruhe cheese that could represent the basis for further investigations, needful to improve its nutritional quality and to preserve its peculiarities.

**Keywords:** Fruhe; fermented curd; Lactic Acid bacteria; fermentation; safety; free amino acid; GABA; free fatty acid

## 1. Introduction

In Italy, approximately 4.7 million dairy ewes are reared, mainly located in the islands of Sardinia, where the agricultural economy is primarily based on dairy sheep farming [1]. On this island, 3 million animals produce about 65.6% of the total Italian sheep milk. Almost all milk is processed into cheeses to produce 90% of three Protected Designation of Origin (PDO) Italian sheep cheeses [2]. Only a small amount of milk is used to produce fresh dairy products with short shelf-life. Fruhe is an ancient traditional fresh cheese made on Sardinia island (Italy) by sheep and/or goat milk, consumed for centuries and very popular for its appealing and fresh flavor. In fact, traditionally, the Fruhe cheese is made in the months of June–August, with milk of late lactation when the milk composition and the environmental high temperatures are not suitable to produce medium and long-ripened cheeses. Fruhe cheese (Casu axedu) is produced from raw milk, heated to a temperature of 36–38 °C and coagulated with lamb or kid rennet paste. Normally, is not added a specific starter culture but a portion of whey from the previous processing is added [3]. Coagulation, at room temperature, occurred until a veil of serum is separated from the clot. After breaking the clot into 4–7 cm slices, the Fruhe cheese is stored at about 4–5 °C (always immersed in the whey) and consumed within 4–5 days.

The growing demand of consumers of fresh cheeses, especially during summer seasons, in areas with a large tourist influx, is favoring the production through the application of semi-industrial

technologies. In fact, in order to comply with the microbiological parameters imposed by the European legislation [4] and guarantee the safety of the product, as well as prolonging its shelf-life, some technological parameters have been applied such as the heat treatment of milk and the packaging of the product [5]. Nonetheless, its production process is not standardized. Currently, the Fruhe cheese available on the market differs mainly in the type of milk (sheep and/or goat) and the type of starter culture. In fact, although Fruhe cheese is still widely produced with the addition of natural starter such as whey, it is possible to find in the market fermented Fruhe cheese produced with the addition of commercial starter. This practice facilitates the management of the production process but, on the other hand, causes loss of traditional and typical characteristics of the product. The characterization of the Fruhe cheese could represent the basis for further investigation of various aspects such as the use of an autochthonous starter culture, which is needed to standardize the safety and obtain a 'denomination of origin' [6,7]. The objective of this study was to investigate the microbiological and nutritional characteristics of the sheep and goat Fruhe cheese types.

## 2. Materials and Methods

### 2.1. Sampling

Fruhe cheese samples produced with sheep (FS) and goat (FG) milk were collected from six Sardinian cheese making plants. In each plant, the cheese production was carried out in duplicate with variable technological parameters. All Fruhe packaged samples, were collected in duplicate and transported to the laboratory (Microbiology unit, Department of Agriculture, University of Sassari), and maintained at the temperature of 4 °C for 21 days (the average shelf life of the Fruhe cheese in the market) before analyses. A total of 24 samples were collected. For each sample, microbiological and physicochemical analysis were determined in triplicate.

### 2.2. Physicochemical Analyses

Physicochemical analyses were conducted by the following international standard methods: dry matter [8]; fat [9]; proteins, total nitrogen [10]  $\times$  6.38; lactose and lactic acid were also determined as previously described [11]; pH values, was measured with a pH meter (Crison Instruments SA, Barcelona, Spain); water activity (aw) was determined using the AW LAB SET H (Novasina, Lachen, Switzerland).

### 2.3. Microbiological Analysis

Fruhe cheese samples (10 mg) were homogenized in 90 mL of sterile Ringer's solution (Oxoid, Milan, Italy) for 2 min in a Stomacher Lab Blender 80 (PBI, Milan, Italy). Aliquots (1 mL) were 10-fold diluted in Ringer's solution and plated on the specific media used to quantify different microbial groups: Total microbial count, on Plate Count Agar (Oxoid, Milan, Italy) at 37 °C for 48 h; enumeration of presumptive mesophilic and thermophilic lactococci on M17 agar (Oxoid) at 22 °C and 45 °C respectively for 48 h in anaerobic condition (Gas-pack system, Oxoid); lactobacilli on Man Rogosa Sharpe (MRS, Oxoid) at 22 °C and 45 °C respectively for 48 h in anaerobic condition (Gas-pack system, Oxoid) [12]; staphylococci were enumerated on Baird Parker agar (BPA, Oxoid) supplemented with Egg Yolk Tellurite Emulsion (Oxoid) after incubation at 37 °C for 48h; presumptive colonies of coagulase-positive staphylococci were assayed for coagulase activity using the Staphylase test (Oxoid, Italy); total and faecal coliforms, Brilliant Green Bile Broth (Oxoid), 37 and 44 °C, respectively for 48 h (Most Probable Number method); yeasts on Yeast Peptone Dextrose Agar (YPDA; Oxoid) supplemented with chloramphenicol (150 mg/L) at 25 °C for 72–96 h; whilst staphylococci were enumerated on Baird Parker agar medium) supplemented with Egg Yolk Tellurite Emulsion (Oxoid, Milan, Italy) after incubation at 37 °C for 48 h. Viable counts were expressed as log colony forming units (log CFU/gram of sample  $\pm$  standard deviations) [13]. In addition, the presence or absence of

pathogenic bacteria was determined: *Salmonella* spp. using a Salmonella Rapid Test (Oxoid, Milan, Italy) and *Listeria* spp. using immunological method (Oxoid Listeria Rapid Test).

#### 2.4. Free Amino Acids (FAA) and Free Fatty Acids (FFA) Analysis

The extraction, identification and quantification of free amino acids (FAA) and free fatty acids (FFA) were carried out as detailed previously [12]. FAA were analyzed by an HP 1050 HPLC with an HP 1046A fluorescence detector (Hewlett-Packard Co., Wilmington, DE, USA) and HP Chemstation Rev. A.06.03 software (Hewlett-Packard Co.) [12]. FFA were extracted and determined by gas chromatography using an HP 5890 series II gas chromatograph (Hewlett-Packard Co. Wilmington, DE, USA), with auto-sampler and flame ionization detector. Data acquisition was carried out using HP Chemstation Rev. A.06.03 software (Hewlett-Packard Co.) [12].

#### 2.5. Statistical Analysis

Microbial data, the FFA and FAA contents were submitted to a descriptive statistic, in order to characterize the sheep and goat Fruhe cheese samples. One-way ANOVA was applied to physical and chemical parameters to evidence the differences between sheep and goat samples. Analysis was performed using MINITAB® software (Version 16.1.0, Minitab, State College, PA, USA).

### 3. Results and Discussion

Fruhe cheese is a traditional product whose peculiarity is due to the fact that the curd is never separated from the whey. The whole fermentation process, the biochemical change and storage take place in the whey. This aspect involves a continuous “exchange” of both microorganisms and metabolites between the whey and the curd/cheese.

#### 3.1. Physicochemical Analyses

All physical and chemical parameters determined in sheep (FS) and goat (FG) Fruhe cheese samples are reported in Table 1. All measurements did not differ much between FS and FG samples: total solid (TS), varied from 22.75 to 21.06; fat, from 10.18 to 9.29; proteins, from 7.68 to 6.70, in FS and FG, respectively. The average residual lactose content was 2.6 g/100 g and lactic acid content was 1.8%. The pH values were 4.38 and 4.32 in FS and FG, respectively.

**Table 1.** Physicochemical parameters of Fruhe cheese produced with sheep (FS) and goat milk (FG).

Parameters	FS		FG	
	Average	sd	Average	sd
Dry matter, (g/100 g)	22.75	1.19	21.06	5.61
Fat (g/100 g)	10.18	0.39	9.29	3.02
Proteins (g/100 g)	7.68	0.78	6.70	1.81
Lactose (g/100 g)	2.40	0.41	2.79	0.29
Acid lactic (%)	1.82	0.56	1.80	0.30
pH	4.38	0.10	4.32	0.07
Water activity	0.99	0.01	0.99	0.01

sd, standard deviation.

Overall, physicochemical results are similar between FS and FG and reflect the composition of sheep and goat to the Sarda population [14] that is normally used for Fruhe cheese making.

The higher residue lactose content found in FS could favor post acidification processes and compromise the shelf life of the product [15]; moreover, it could make Fruhe cheese unsuitable for lactose-intolerant consumers. On the other hand, this could be an opportunity to use a probiotic as a starter, considering the documented health benefit of lactose in the presence of beneficial probiotic microorganisms [16,17]. The acidity values support the safety of the product despite the relatively

high water activity values (0.98–0.99) in both Fruhe cheese samples as pH values lower than 4.5 are in line with food safety regulations [4].

### 3.2. Microbiological Analysis

Microbiological analyses are reported in Table 2. Fruhe cheese produced from both sheep's and goat's milk showed a total microbial count between 7 and 8 log CFU/g with higher values in sheep than goat Fruhe samples. Similar results on sheep milk Fruhe and sheep milk mixed with goat milk Fruhe were reported in a previous research [5], but higher than those reported on Cameros cheese, a fresh cheese produced by goat milk [18].

**Table 2.** Microbial counts of Fruhe cheese produced with sheep (FS) and goat milk (FG).

Microbial Groups	FS		FG	
	log CFU/g	sd	log CFU/g	sd
Total Microbial Count * (PCA, 37 °C)	8.77	1.30	7.41	0.62
Thermophilic lactobacilli * (MRS, 45 °C)	8.23	1.91	5.78	0.30
Mesophilic lactobacilli * (MRS, 22 °C)	7.79	1.91	7.78	0.18
Thermophilic streptococci * (M17, 45 °C)	9.1	1.48	8.14	0.63
Mesophilic lactococci * (M17, 22 °C)	9.1	1.48	9.76	0.24
Coagulase negative staphylococci	5.86	1.66	6.37	2.01
Yeasts	4.34	1.41	5.15	
** Total coliforms	23		1100	
** Fecal coliforms	<3		<3	
<i>Salmonella</i>	absent		absent	
<i>Listeria</i>	absent		absent	

\*, in brackets are reported the medium (PCA, plate count agar; MRS, Man Rogosa Sharpe; M17) and the incubation temperature; \*\*, Most Probable Number methods; sd, standard deviation.

Overall, a high number of live Lactic Acid Bacteria (LAB) has been enumerated in the FS especially where both thermophilic and mesophilic streptococci reached the value of 9 log CFU/g. Lactobacilli were found to be less numerous than cocci even if they remained in concentrations of about 7–8 log CFU/g. Similar results were obtained in FG, except for thermophilic lactobacilli, whose concentration was found to be 5.78 log CFU/g.

It is well known that the microorganisms that characterize the cheese are derived from different sources such as milk, culture starter, as well as from the cheese-making environment.

As required for all cheeses at short maturation time (<2 months), Fruhe cheese production needs milk pasteurization; this heat treatment favors the development of strictly thermophilic LAB but also of mesophilic lactobacilli able to grow at 44 °C [19]. Thermophilic LAB can also be derived from the whey starter used in the production of Fruhe cheese, in view of the fact that other authors [20,21] revealed the predominance of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* in Giorddu, a sheep fermented milk by using whey from Fruhe cheese.

Mesophilic lactobacilli were determined in both Fruhe cheese types at the same concentration (7.7 log CFU/g). This microbial group is generally associated to raw milk microbiota and can persist during long-ripening cheese [22]. Selected mesophilic lactobacilli belonging to *L. casei/paracasei* group, recently reclassified as *Lactocaseibacillus casei/Lactocaseibacillus paracasei* [23], were reported as suitable to make sheep and goat fermented milk with potential functional traits [24–26].

Overall, the high concentration of LAB in both Fruhe type and their metabolic activity has allowed manufacturers to guarantee the microbiological safety of the analysed samples. In fact, pathogenic bacteria such as coagulase positive staphylococci, *Listeria* and *Salmonella* were absent, probably inhibited by the low acidity of the cheese (pH = 4.5). While, the presence of spoilage microorganisms such as coagulase negative staphylococci (CNS), total coliforms and yeasts, especially in FG, suggests that, during the cheese making and packaging, process cross-contamination may have occurred, highlighting an inadequate implementation of good hygiene practices.

However, coagulase negative staphylococci (CNS) were present in high concentration (6 log CFU/g). Although CNS are generally recognized as technological bacteria, able to contribute to the ripening of fermented meat products [27] and to counteract the development of spoilage/pathogenic microorganisms through the production of bacteriocin-like metabolites, the production of staphylococcal enterotoxins synthesis cannot be excluded [28].

The number of yeasts detected was on average about 4–5 log CFU/g, a value higher than those in ripened cheese at 1 days of ripening [29]. The presence of yeasts in limited numbers can be useful for the aroma of the product but, if present in a large number, could be responsible for product spoilage during packaging due to the excessive production of gas (e.g., carbon dioxide) [30].

### 3.3. Free Amino Acids (FAA) and Free Fatty Acids (FFA) Content

Free amino acid and free fatty acids contents represent an indicator of the proteolysis and lipolysis process. Table 3 reports a descriptive statistic of the free amino acid content in sheep and goat Fruhe cheese.

**Table 3.** Descriptive statistics of free amino acid content (mg/100 g of cheese) in Fruhe cheese produced with sheep (FS) and goat milk (FG).

Free Amino Acids (FAA)	FS					FG				
	mean	sd	min	max	AA/TAA <sup>1</sup>	mean	sd	min	max	AA/TAA <sup>1</sup>
<i>Essential amino acids</i>										
Leucine	1.72	1.1	0.6	3.6	6.64	1.73	2.2	0.5	6.2	6.41
Lysine	1.58	0.61	0.9	2.6	6.13	1.72	0.79	0.4	2.8	6.35
Phenylalanine	1.5	0.55	0.9	2.4	5.8	1.35	0.99	0.6	3.2	4.99
Valine	1.5	0.65	0.8	2.7	5.8	3.12	3.6	0.8	10	11.53
Histidine	1.2	0.42	0.8	1.9	4.64	1.07	0.34	0.7	1.6	3.95
Isoleucine	0.78	0.34	0.4	1.3	3.02	0.74	0.49	0.4	1.6	2.74
Threonine	0.77	0.33	0.2	1.1	2.97	0.82	0.33	0.4	1.4	3.02
Methionine	0.2	0.0	0.2	0.2	0.77	0.7	0.0	0.7	0.7	2.59
<i>Non-essential amino acids</i>										
Glutamic ac.	3.78	3.82	1.3	10.5	14.62	2.62	1.68	0.5	5	9.69
Glycine	2.97	0.86	1.9	4	11.48	3.85	0.77	2.7	4.9	14.24
γ-aminobutyric ac (GABA)	2.88	1.17	1.3	4.5	11.14	3.12	2.07	1.8	7.3	11.53
Alanine	2.15	1.4	1.1	4	8.32	1.85	0.8	1.2	3.4	6.84
Tyrosine	1.2	0.24	0.8	1.5	4.64	1.48	0.72	0.8	2.7	5.48
Aspartic acid	1.12	0.41	0.7	1.6	4.32	1.18	0.49	0.6	1.9	4.38
Proline	1.08	0.38	0.4	1.4	4.19	1.07	0.46	0.4	1.6	3.97
Ornithine	0.98	0.39	0.5	1.5	3.8	0.85	0.31	0.3	1.2	3.14
Arginine	0.9	0.17	0.7	1	3.48	1.4		1.4	1.4	5.18
Asparagine	0.86	0.25	0.6	1.1	3.33	0.7	0.29	0.4	1.1	2.59
Serine	0.6	0.19	0.3	0.8	2.32	0.6	0.15	0.4	0.8	2.22
Glutamine	0.43	0.15	0.3	0.6	1.68	0.2		0.2	0.2	0.74
Total FAA	25.85	8.1				27.03	10.63			

<sup>1</sup> AA/TAA = percentage of individual amino acid on the total amino acid content.

The total content in free amino acid (TFAA) was similar (25–27 mg/100 g) among the cheeses of the two species as well as the content of individuals FAA, probably due to the same ripening time of the two cheeses analyzed. The total FAA content is lower than values found in several cheeses at different ripening times [12,19], but if we take into account that I) Fruhe cheese is a fermented fresh curd- and II) LAB enzymes are mainly involved in the second half of the cheese ripening period [31], the FAA content highlights that a good proteolysis process occurred.

Overall, the most abundant non-essential FFA of both species were glutamic acid, glycine, γ-aminobutyric and alanine. The glutamic acid was the most abundant FAA in sheep whereas glycine was most abundant in goats' cheese. The analyzed samples showed a relative high concentration of g-aminobutyric acid (GABA) whose physiological functions are well-known; GABA is a central nervous system neurotransmitter that plays important physiological roles in humans [32]. It did not originate from casein, but accumulated as a metabolic product of microorganisms as reported previously [33]. In some Fruhe cheese samples, GABA was on average over 20% of TFAA, a value

higher than other cheeses with a different ripening period [34]. The amount of GABA was slightly higher in goats than sheep samples.

The large variability observed in the Fruhe cheese of both species, is likely related to differences in the composition of raw milk and/or the applied technological process and/or microorganisms present. Moreover, the starter composition can affect the different levels of individual FAA released, based on their enzyme system and the degree of autolysis in the cheese [35].

Lipolysis is another important biochemical event occurring during cheese ripening, and consequently, it has been more extensively studied in hard than in fresh cheese [36]. Lipolysis consists of the enzymatic cleavage of the triglycerides ester linkages between glycerol and fatty acids, producing glycerol, FFA, mono- and diglycerides. This process is very important for the quality and sensorial properties of cheese, considering that the released FFAs can be precursors of volatile compounds such as methyl ketones, secondary alcohols, esters and lactones that contribute to cheese flavor [31,37]. Table 4 reports a descriptive statistic of the free fatty acid content (FFA) in sheep and goat Fruhe cheese.

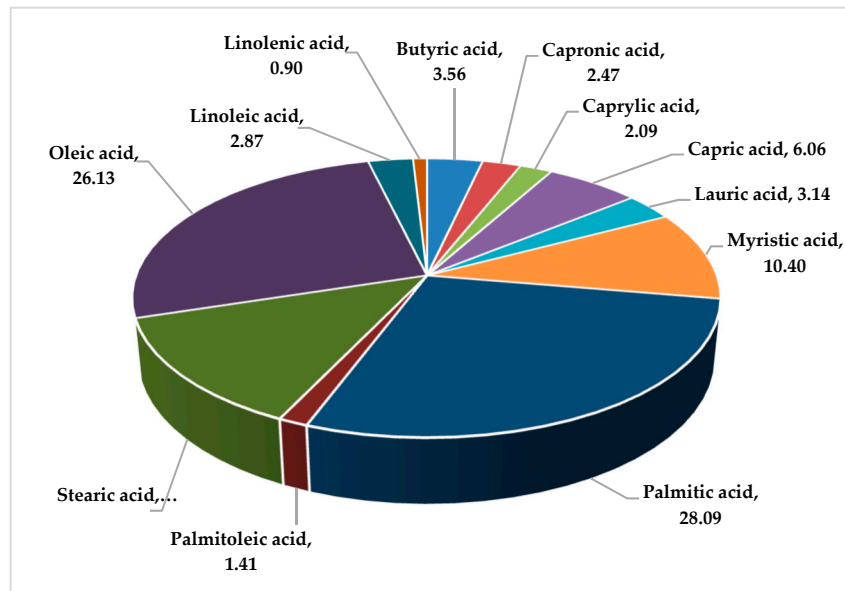
**Table 4.** Descriptive statistics of free fatty acid content (mg/100 g of cheese) in Fruhe cheese produced with sheep (FS) and goat milk (FG).

Free Fatty Acid (FFA)		FS				FG			
		mean	Sd	min	max	mean	sd	min	max
Butyric acid	C4:0	10.5	1.8	8.6	12.8	9.7	2.2	7.0	13.6
Capronic acid	C6:0	5.4	1.9	2.7	8.6	8.7	2.6	5.1	13.1
Caprylic acid	C8:0	4.4	2.1	1.6	7.9	7.5	2.6	4.7	10.9
Capric acid	C10:0	12.3	6.2	4.1	23.3	22.6	9.4	10.8	34.2
Lauric acid	C12:0	7.9	3.0	4.4	13.3	10.0	2.7	6.9	13.0
Myristic acid	C14:0	30.3	7.6	20.4	42.2	28.8	7.0	18.6	40.0
Palmitic acid	C16:0	79.5	5.6	73.4	88.5	80.3	12.6	55.9	90.2
Palmitoleic acid	C16:1c9	4.5	0.7	3.7	5.4	3.4	1.5	2.1	5.9
Stearic acid	C18:0	33.7	10.2	20.6	48.2	40.9	9.2	26.2	53.9
Oleic acid	C18:1c9	74.6	9.4	61.9	85.6	74.0	13.8	47.5	85.8
Linoleic acid	C18:2n6	8.0	0.9	7.4	9.7	8.4	1.0	7.4	9.7
Linolenic acid	C18:3n3	2.7	0.6	1.9	3.5	2.4	0.3	1.9	2.8
Total FFA		273.9	23.6			295.6	43.2		

The FFA content (mean of 285 mg/100 g) and composition were very similar among the cheeses of the two species. TFFA content resulted lower than that observed in different ripened goat and sheep cheese [12,38]. Otherwise, the acid curd Xinotyri, a cheese similar to Fruhe, showed a FFA content lower than those obtained in this study [22]. As the Fruhe cheese process was carried out by using thermised milk and rennet without lipase enzyme (calf liquid rennet), the content of FFA is likely due to the activity of microbial lipase. Among individual FFA, the palmitic, oleic, and stearic acids revealed mean contents of 79.9, 74.3 and 37.3 mg/100 g, respectively. Together, these FFA represented about the 63% of the total FFA and, individually, the 28.09, 26.13 and 13.06 %, respectively (Figure 1).

Capric acid was the major short chain FFA determined in both species, although the results for FG showed a value almost double that of FS (12.3 and 22.6 mg/100 g respectively). The highest value of capric acid among short FFA was consistent with previous work on both sheep milk and dairy by-products [39]. Among the polyunsaturated FFA, linoleic (C18:2n6) and alpha-linolenic (C18:3n3) acid averaged 8.2 and 2.5 mg/100 g, respectively. In both Fruhe cheese types, the value of linoleic acid was shown to be higher than values observed in sheep and goat cheeses at different ripening times [40]. The presence of polyunsaturated FFA of n-3 family, with alpha-linolenic acid being the most abundant, is favorable to the nutritional value of the Fruhe product. The high concentration of these types of FA in small ruminant dairy products, usually higher than that found in cow milk [41], is of particular interest and deserves greater consumer attention. PUFA n3 are indeed considered beneficial for human health because have been associated with anti-inflammatory and immunomodulatory effects [42], are essential

for the normal growth and development, in particular in newborns, and play an important role in the prevention and treatment of cardiocirculatory diseases, hypertension diabetes, and arthritis [43]. In general, although the Fruhe is a fresh cheese, the observed results on both proteolytic and lipolytic processes suggest a good activity of rennet and microbial enzymes.



**Figure 1.** Free fatty acids (FFA) composition (% of total FFA) Fruhe cheese (mean of sheep and goat).

#### 4. Conclusions

This first study on the microbiological and physico-chemical characteristics of sheep and goat Fruhe cheese highlighted the microbiological quality and the potential nutritional value, which, if exploited, could allow a diversification of sheep and goat cheese productions. The use of microorganisms from sheep or raw goat milk and traditional products, selected and adequately managed in the cheesemaking, could allow the maintenance of biodiversity as well as the standardization of the technological process. The differences observed between the two species could be mainly explained by the composition of the raw milk used, although other factors such as the different technological parameters applied, the whey starter management and the environmental conditions of production could have influenced the final composition of the Fruhe cheese.

All these aspects must be taken into account to setup a production disciplinary and for a potential recognition of the designation of origin.

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