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of the International Dairy Federation



Scientific Posters Presented at the IDF 5th International Symposium on the Challenge to Sheep and Goats Milk Sectors

18-20 April 2007, Alghero, Italy



ITALIA



Istituto Zootecnico e
Caseario per la Sardegna

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Special Issue of the International Dairy Federation

0801/ Part 4

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Disclaimer:

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The Challenge to Sheep and Goats Milk Sectors

Foreword

This issue of the Bulletin contains the Scientific Posters presented at the IDF 5th International Symposium on the Challenge to Sheep and Goats Milk Sectors, which took place from 18 to 20 April 2007, in Alghero, Italy.

About 300 delegates, including a significant number of postgraduate students from 30 countries, attended the event. 24 main lectures (which are being published in the peer reviewed journal "Small Ruminant Research") were presented, and 163 posters exhibited.

The Symposium, held under the auspices of the IDF, was the fifth in a series, presenting latest findings in the research area related to the sheep and goats dairy sector. The overall objective of the Symposium was to provide comprehensive insight into the most recent knowledge including latest research findings on husbandry and milk production, technology, chemistry, physics, microbiology, nutrition etc, without losing sight of the significance of markets and appropriate policies. The core themes were related to the sheep and goat milk, processing and product, characteristics of the product and market and perspectives.

Several aspects were presented and discussed over a three-day programme. In the field of raw milk, genetic, analytical and quality aspects were approached, particularly to enhance the nutritional and beneficial effects for human health. New process treatments for this sector were presented while particular interest was given to functional products. Nutritional and health components together with the analytical and sensorial aspects were presented in the session dedicated to the characteristics of the products. Finally market aspects focused on the valorisation of the traditional and "artisanal" cheeses.

The high number of delegates that attended the Symposium demonstrate that the sheep and goat sector is one of the most dynamic and increasingly stimulates interest in the world.

The IDF is most grateful to the Istituto Zootecnico e Caseario per la Sardegna and the Italian National Committee of the IDF for organizing and hosting the event, and more particularly to Dr A. Pirisi and Dr G. Piredda, as well as to the members of the Programme and Organizing Committees and to all authors for their valuable contribution to the work of IDF.

Christian Robert

June 2008

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Posters Session III. Characteristics of the Products

III-P124: Nutritional Aspects of Lipid Fraction in Bacterial and Mould Artisanal Ripened Cheeses Marketed in Spain: Free, Esterified Fatty Acids and Triacylglycerol Profiles

L. Alonso¹, J. Fontecha²

Summary

A comparative study of the lipid fraction, esterified fatty acids, free fatty acids (FFA) and triglyceride profile of a variety of artisanal bacterial and mould ripened cheeses made in Asturias (Spain) were undergone. Levels of FFA were slight in bacterial ripened cheeses due mainly to the action of the bacterial lipases except for goats milk cheeses which contain pregastric esterases in the rennet preparation. However, mould ripened cheeses experienced higher FFA levels mainly in Gamonedo and Cabrales (cow, goat and ewe's milk) cheeses due to the action of the *Penicillium roqueforti*. Esterified fatty acids between bacterial and mould ripened cheeses are statistically significant for C10 ($P < 0.05$) only for cow/goat cheeses. The highest differences between the two groups of cheeses were for the free fatty acids C4, C6, C10, C10:1, C12, C14, C14:1, C16, C18, C18:1 and C18:2 ($P < 0.05$) and C8, C15 ($P < 0.01$) and for the triglycerides C28, C34, C36, C48, C52 and C54 ($P < 0.05$) and C42 ($P < 0.01$).

1. Introduction

Today there is considerable interest regarding the relationship of environmental to health. Diet is particular interest because it is one aspect of our environment that can be modified in an attempt to improve our quality of life. In this context, the lipid fraction of cheese represent one of the main components for nutritional aspects and for the development of characteristic flavours. During cheese ripening the triglycerides, which represent the main lipidic component of the milk, undergo enzymatic hydrolysis into free fatty acids (FFA) and small quantities of mono and diglycerides. In major Blue cheese varieties *Penicillium roqueforti* is the primary driver of lipolysis. In bacterial ripened cheese lipolysis is carried out by the lipases produced by the microbial flora of milk and the lipase present in rennet and rennet paste.

Asturias is a region located Northwest Spain, this region is an important area in production and consumption of a variety of artisanal cheese varieties manufactured with cows, goats and ewes milk ripened by lactic acid bacteria or moulds (Alonso et al.1987). This paper presents information about nutritional aspects of the lipid fraction (esterified fatty acids, free fatty acids and triglyceride compositions) of different types of commercial artisanal bacterial and mould ripened cheeses made in Asturias, Spain.

2. Material and methods

Cheese samples. Three separate batches of different types of commercial bacterial and mould artisanal ripened cheeses manufactured in Asturias, Northern Spain were studied.

Milk fat analysis. Fats for the determination of free fatty acids and esterified fatty acids were extracted by the method of Martín-Hernández et al. (1988). Fat samples for triglyceride analysis were analysis as described Alonso (1993). Statistical analysis of the results was by the analysis of variance (Anova) by the software programme Excel 5.0.

3. Results and discussion

The values of short chain fatty acids (C4-C8) showed differences between bacterial and blue

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cheeses. The lowest content of butyric acid were found in La Peña, La Chivita and Cabrales cheeses with values of 2.43, 2.05 and 1.38%, respectively. These lower levels could be due to the fact that these cheeses are made with goats milk which has a lower content of butyric acid than cows milk. Differences in the levels of caprylic acid were found in cheeses manufactured exclusively with goats milk; thus, La Chivita and La Peña cheeses contained of 2.71 and 3.07 % respectively. The most pronounced differences in the medium chain fatty acids (C10-C14) were observed in the capric acid content of the cheeses made with goats milk, La Chivita and La Peña (10.12 and 10.46 %). The contents of free short chain fatty acids (C4-C8) varied slightly in each variety of bacterial ripened cheeses, but the major differences were observed in mould ripened cheeses. For the butyric acid the highest content for the blue cheeses Gamonedo and Cabrales with 1658 and 2585 mg/Kg respectively. The medium chain free fatty acids (C10-C14), showed differences even between bacterial ripened cheeses, the major differences was for capric acid for cheeses made with goats milk La Peña and La Chivita (1170 and 1702 mg/Kg) although the high value for this acid (C10) correspond for Gamonedo and Cabrales blue cheeses (1732 and 5039 mg/kg).

Total free fatty acid showed some difference in the bacterial ripened cheeses, the high value was for the goats cheese La Chivita with 22109 mg/Kg. This result is similar to that reported by De la Fuente, et al. (1993) in Majorero cheese who found a value of 20794 mg/Kg. The high value found for La Chivita goat cheese could be due to the type of semiartisanal rennet used which contains pregastric esterases with a high enzymatic activity in the triglyceride hydrolysis. The higher contents for the total FFA were for Cabrales and Gamonedo cheeses (31772 and 78567 mg/Kg). The total FFA found for Cabrales and Gamonedo are similar to those reported by Alonso et al. (1987). For the individual short chain triglycerides (C24-C34), La Chivita, La Peña and Cabrales cheeses have the highest contents for the C30 and C32 triglycerides due the fact that these cheeses are manufactured with goats milk and consequently the percentage of capric acid esterified into glycerol is higher than in cheeses made with cows milk.

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III-P125: Breed Effect on Raw Milk Goat Cheese Quality

S. Alvarez¹, P. Méndez¹, N. Darmanin¹, H.R. Briggs¹, M. Fresno¹

Summary

Two homogeneous groups of Canarian goats (Majorero and Palmero) were used. Both groups were fed the same diet. Basic physicochemical characteristics, fatty acids and sensorial profile were determined in cheeses at three stages of ripening. Cheeses made with Majorero milk presented better values for fat and protein parameters but less moisture content and pH. Genotype and ripening time had an important effect on fatty acid profile, with C10, C14, C16 and C18:1 as more important quantitative acids. Both breed and ripening affected nearly all sensorial parameters. Fresh cheeses made with Majorera goat milk presented higher roughness, firmness and friability values but were less elastic and soluble. Hard Palmero goat cheeses were more elastic and rough although firmness, friability and solubility values were lower.

1. Introduction

PDO cheeses are linked to the geographical area where they are produced. In the Canary Islands (Spain) there are two PDO cheeses: Majorero and Palmero, despite other reasons as the different island where they are made, cheese - making techniques, goat feeding, etc., are made with milk from two different local goat breeds: Majorera and Palmera goats. Majorero cheese is a cylindrical fat cheese (1 to 6 kg) with natural rind or rubbed with olive oil, paprika or "gofio", Canarian flour made with toasted cereal. Palmero cheese is an uncooked, pressed cheese (0.75 to 15 kg) that can be smoked with almond shells, dried prickly pears and Canarian pine wood and needles. Both cheeses are consumed at different ripening times.

In this paper was analyzed the effect of breed on cheese's sensory and chemical quality.

2. Material and methods

Two homogeneous groups of Canarian goats were used (Majorero and Palmero). Both groups were fed the same diet, the most commonly used feeding regime in Canary Islands, based principally on concentrated foodstuffs (concentrate for milk production, maize, barley) complemented with dehydrated alfalfa and cereal straw. Basic physicochemical characteristics, fatty acids and sensorial profile (texture, odour, flavour and taste) were determined in cheeses at three stages of ripening: fresh (2d), semi-hard (30d) and hard cheeses (60d). Statistical methodologies were made with SPSS 11.0.

3. Results and discussion

Cheeses made with Majorero milk presented better values for fat and protein parameters but less moisture content and pH (Table 1).

Analysing the organoleptic profile (Table 1), both breed and ripening had an important effect on sensorial characteristics affecting nearly all parameters. Fresh cheeses made with Majorera goat milk presented higher roughness, firmness and friability values but were less elastic and soluble. Hard Palmero goat cheeses were more elastic and rough although firmness, friability and solubility values were lower. Odour and flavour intensity was higher in ripened (semi-hard and hard) Majorero goat cheeses but favourable for Palmero cheeses in fresh ones. The odour and aroma intensity increased during ripening for both Majorera and Palmera milk cheeses.

As it was shown in other Italian goat breeds (Pizzillo et al, 2005) genotype had an important effect on fatty acid profile (Table 2). Medium chain fatty acid composition (C6-C14) on the cheese fat was affected except C14. Palmero cheese presented higher values for C6-C10 fatty acids; this fraction provides a specific goat's milk flavour that is sought after in this type of cheeses. Ripening time had also a significant effect on free fatty acid profile affecting eleven of

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the eighteen acids. The concentrations of oleic, linoleic, linolenic and conjugated linoleic acids increased up to 30 days and decreased between 30 and 60 days. These fatty acids are potentially involved as positive predisposing factors in the health of human consumers (Williams, 2000) being slightly higher in Majorero cheeses.

Table 1: Effects of ripening and breed on physicochemical and sensorial characteristics

	breed(b)		ripening time(r)			RSD	Level of significance		
	Majorera	Palmera	2d	30d	60d		b	r	Effect(bxr)
	LSM								
pH	5.77	6.15	6.51 ^c	5.25 ^a	6.12 ^b	0.12	0.001	0.001	0.001
Moisture (%)	42.54	44.95	47.50 ^c	44.67 ^b	39.07 ^a	0.68	0.001	0.001	0.001
Protein (%)	22.01	20.67	19.58 ^a	22.27 ^b	22.18 ^b	0.32	0.005	0.001	0.066
Fat (%)	26.77	23.64	20.21 ^a	24.82 ^b	30.58 ^c	0.81	0.001	0.001	0.001
Fat (% TS)	46.27	42.72	38.56 ^a	44.76 ^b	50.16 ^c	0.91	0.001	0.001	0.001
Roughness	1.67	1.68	1.26 ^a	1.88 ^b	1.88 ^b	0.60	0.854	0.001	0.001
Moisture	3.33	2.69	4.75 ^c	2.51 ^b	1.77 ^a	0.21	0.001	0.001	0.001
Elasticity	3.65	2.70	5.50 ^b	2.02 ^a	2.00 ^a	0.28	0.001	0.001	0.001
Firmness	2.52	3.70	3.01 ^b	2.13 ^a	4.20 ^c	0.19	0.001	0.001	0.001
Friability	2.67	3.31	2.45 ^b	2.00 ^a	4.53 ^c	0.21	0.001	0.001	0.001
Adhesivity	2.32	2.87	1.96 ^a	2.55 ^b	3.28 ^c	0.11	0.001	0.001	0.713
Solubility	4.17	3.47	3.21 ^a	5.59 ^b	3.78 ^a	0.26	0.457	0.001	0.001
Granulosity	1.39	2.22	1.88 ^b	1.28 ^a	2.25 ^b	0.11	0.001	0.001	0.064
Sweetness	0.00	0.17	0.25 ^b	0.00 ^a	0.00 ^a	0.04	0.004	0.001	0.001
Bitterness	0.00	1.46	0.00 ^a	0.52 ^b	1.66 ^c	0.20	0.001	0.001	0.001
Acidity	2.33	2.70	2.13 ^a	3.08 ^b	2.33 ^a	0.09	0.002	0.001	0.124
Pungent	0.17	1.46	0.00 ^a	0.52 ^b	1.92 ^c	0.21	0.001	0.001	0.001
Astringent	0.33	0.68	0.25 ^a	0.52 ^{ab}	0.74 ^b	0.08	0.001	0.001	0.001
Residual taste	3.89	3.71	1.86 ^a	4.61 ^b	4.94 ^b	0.25	0.206	0.001	0.001
Taste persistence	4.11	3.74	2.42 ^a	4.46 ^b	4.90 ^b	0.22	0.040	0.001	0.001
Odour intensity	3.14	3.79	2.21 ^a	3.67 ^b	4.51 ^c	0.17	0.001	0.001	0.001
Flavour intensity	3.08	4.00	2.13 ^a	3.71 ^b	4.79 ^c	0.20	0.001	0.001	0.001

LSM: Least square mean.

RSD: Residual standard deviation.

^{a-c} Within a row, means marked with different superscripts differ significantly ($p < 0.05$).

Table 2: . Effects of ripening and breed on fatty acid composition

	breed(b)		ripening time(r)			RSD	Level of significance		
	Majorera	Palmera	2d	30d	60d		b	r	Effect(bxr)
	LSM								
C4	0.64	0.74	0.69 ^{ab}	0.59 ^a	0.80 ^b	0.04	0.118	0.036	0.036
C6	2.34	2.58	2.43 ^{ab}	2.25 ^a	2.69 ^b	0.06	0.014	0.003	0.124
C8	3.19	3.60	3.42 ^b	3.16 ^a	3.60 ^b	0.06	0.001	0.001	0.080
C10	12.94	13.58	13.31 ^b	12.51 ^a	13.95 ^b	0.18	0.017	0.001	0.034
C10:1	0.45	0.46	0.44	0.45	0.47	0.01	0.632	0.290	0.027
C12	6.55	6.10	6.15	6.22	6.61	0.09	0.002	0.050	0.029
C14	11.56	11.57	11.52	11.47	11.70	0.07	0.963	0.324	0.066
C15	1.10	0.79	0.90 ^a	1.03 ^b	0.91 ^a	0.03	0.001	0.003	0.947
C16	32.72	32.85	32.83	32.45	33.08	0.16	0.633	0.197	0.004
C16:1	1.17	1.20	1.19	1.14	1.23	0.04	0.691	0.569	0.237
C18	5.19	6.02	5.60	6.09	5.13	0.19	0.013	0.056	0.025
C18:1	18.03	17.54	17.88 ^b	18.82 ^b	16.65 ^a	0.25	0.140	0.001	0.003
C18:2	3.11	2.27	2.72 ^{ab}	2.84 ^b	2.51 ^a	0.10	0.001	0.045	0.007
C18:3	0.36	0.30	0.35 ^b	0.35 ^b	0.28 ^a	0.01	0.009	0.006	0.110
CLA	0.57	0.35	0.51 ^b	0.53 ^b	0.35 ^a	0.03	0.001	0.001	0.018
C20	0.02	0.02	0.02	0.03	0.01	0.01	0.797	0.512	0.661
C20:4	0.08	0.05	0.06	0.08	0.05	0.01	0.174	0.649	0.173

LSM: Least square mean.

RSD: Residual standard deviation.

^{a-c} Within a row, means marked with different superscripts differ significantly ($p < 0.05$).

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III-P126: Level of Vitamins A and E and Cholesterol in Milk and Cheese from Goats Fed with Different Feeding Systems

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Summary

The aim of the experiment was to determine the effect of different feeding systems on the level of vitamins E, and A and cholesterol in goat milk and cheese. An experiment was carried out with twenty-four Sarda goats in early-lactation divided in three homogeneous groups. Two groups, supplemented with 300 g head⁻¹ day⁻¹ of commercial concentrate were allowed to browse for 22 hrs daily on 2 plots characterised by a different cover proportion of woody and herbaceous species. The third group was stall fed. DM intake was measured daily by measuring offer and correspondingorts in the stall-fed group, whereas in browsing animals it was estimated by direct observation of biting. The diet eaten by the browsing goats was simulated by hand plucking the vegetation. In three occasions the milk of each experimental group was collected and processed into cheese. Cheese was ripened at 24 hours and 2 months.

The contents in milk and cheese of vitamin E and A as well as cholesterol were determined by chromatographic method (HPLC). The results showed higher contents of vitamins E and A and lower content of cholesterol in browsing groups'dairy products as compared to those obtained by the stall-fed control ($P < 0.05$). This confirms the higher quality of dairy products obtained from grazing animals.

1. Introduction

Milk fat-soluble components such as vitamin A and vitamin E are strictly related to diet composition. In particular as reported by Morand-Fehr et al. (2007) their contents are higher in milk of goats reared in grazing system than indoors likewise the results from cows. Vitamin E plays a role particularly important in preventing lipid and cholesterol oxidation, whereas vitamin A is essential for the development and good functioning of the immune system. Cholesterol belongs to milk sterol fraction and it is of nutritional interest because high levels of cholesterol in plasma are associated with an increase risk of cardiovascular disease. Moreover as reported by Pizzoferrato et al. (2000), products from cholesterol oxidation are involved in some activities physiologically deleterious and this effect could be reduced by an higher daily intake of antioxidants likewise vitamin E (Rimn et al., 1993). The aim of this work was to investigate the influence of different feeding systems on the level of Vitamin A, E and cholesterol.

2. Material and methods

Twenty-four Sarda goats in early-lactation were divided in three homogeneous groups on the basis of DIM (71 ± 2 , means \pm SE), milk yield (1320 ± 71 ml), LW (47 ± 1.5 kg) and BCS (2.6 ± 0.03). Two groups, supplemented with 300 g head⁻¹ day⁻¹ of commercial concentrate were allowed to browse for 22 hrs daily on 2 plots characterised by a different cover proportion of woody and herbaceous species. These proportions were 90 and 10% in one plot (Low herbage cover - LH), 70 and 30% in the second plot (High herbage cover - HH). The third group (Control - C) was stall-fed with hay (1.2 kg head⁻¹ day⁻¹, 50% alfalfa and 50% ryegrass) and a commercial concentrate (0.6 kg head⁻¹ day⁻¹). In the stall-fed group DM intake was measured daily (as group average), whereas in browsing animals it was estimated by direct observation of biting (Kababya et al., 1998). The diet eaten by the browsing goats was simulated by hand plucking the vegetation. Body weight and BCS were recorded once a month whereas milk yield and milk composition fortnightly. In three occasions the milk of each experimental group was collected and processed into cheese. Cheese was ripened at 24 hours and 2 months. Samples from

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different cheese were freeze-dried and analyzed to determine the vitamins and cholesterol as reported by Manzi et al. (1996). The effect of feeding system on vitamin A, E and cholesterol contents in milk and cheese was evaluated using the analysis of variance.

3. Results and discussion

Feeding system influenced significantly the levels of vitamin E and vitamin A. In particular goat grazing in scrubland showed higher level of vitamin A (+25%) and vitamin E (+400%) in milk with respect to stall-fed goats (table 1) in line with the results by Pizzoferrato et al. (2000). Vitamin A content was also higher in LH than HH ($P < 0.05$). Cholesterol content of milk was higher (+25%) in the milk of indoor-fed group than in milk of pasture groups as already found by Pizzoferrato et al. (2000). These results were confirmed in cheese either at 24 hrs or 60 days ripening stage. The recovery percentage in cheese was very low for vitamin A with a decreasing trend from milk to 60 d ripened cheese in all treatments, in particular in stall fed group (-18% in 60 d cheese with respect to milk). Vitamin E and cholesterol on the contrary showed a good recovery with the same level found in milk and in cheese, except for 60 d ripened cheese in the stall-fed group.

Table 1: Levels of vitamin A, vitamin E and cholesterol in milk and cheese from goats reared with different feeding system

		mg/100g fat	Vit. A	Vit. E	Cholesterol
Milk	C	"	$0.82^a \pm 0.07$	$0.68^a \pm 0.07$	$407^a \pm 30.65$
	HH	"	$0.97^{ab} \pm 0.20$	$3.66^b \pm 0.44$	$314^b \pm 46.63$
	LH	"	$1.09^b \pm 0.06$	$3.52^b \pm 0.20$	$294^b \pm 10.34$
Cheese 24 h	C	"	$0.68^a \pm 0.07$	$0.58^a \pm 0.06$	$394^a \pm 5.09$
	HH	"	$0.92^b \pm 0.05$	$3.74^b \pm 0.32$	$316^b \pm 9.97$
	LH	"	$1.06^c \pm 0.09$	$3.76^b \pm 0.42$	$309^b \pm 16.23$
Cheese 60 days	C	"	$0.66^a \pm 0.02$	$0.65^a \pm 0.05$	$390^a \pm 15.51$
	HH	"	$0.86^b \pm 0.03$	$3.74^b \pm 0.37$	$320^b \pm 12.44$
	LH	"	$1.01^c \pm 0.04$	$3.76^b \pm 0.37$	$304^b \pm 8.14$

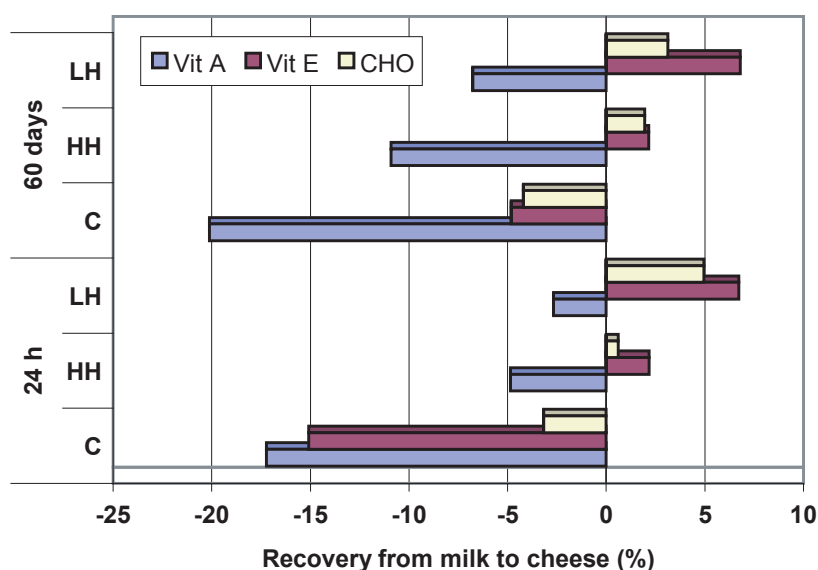


Figure 1. Recovery of vitamins and cholesterol from milk to cheese.

4. Conclusion

These preliminary results suggest that the grazing feeding regimen positively influences the vitamin A and vitamin E levels in milk and cheese while bringing about a lowering of cholesterol level. The efficiency of transfer ratio (from milk to cheese) was negative mainly in stall-feeding system. In conclusion, pasture based farming system results in goat dairy products rich in healthy micro-components for human nutrition. Feeding regimens based on a high proportion of browse (LH) seem beneficial for raising vitamin A level in goat dairy products.

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III-P127: Dynamics of Microbial Ecosystem During the Production of Calenzana, a Corsican Raw Milk Cheese

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Summary

The aim of this work was to study the dynamics of *Calenzana* microbial ecosystem. Microbial counts showed an important decrease of lactic acid bacteria populations during ripening. The high salt concentration of the cheese could explain the decrease of lactic acid bacteria populations during ripening. Presumptive salt-tolerant bacteria and yeasts and moulds remained constant during 4 months and decreased at the end of ripening. This decrease could originate from the removing of the surface microflora made by the cheese maker during ripening. Molecular methods were used to identify cheeses bacteria at the species level. TTGE method showed that *Lactococcus lactis* ssp. *lactis* is the dominant subspecies during the whole process of *Calenzana* cheese. *Lactococcus raffinolactis* were also frequently identified. Ewe cheeses differed from goat ones in the presence of *Leuconostoc mesenteroides* and *Leuconostoc citreum*. DDGE method showed the presence of numerous surface bacteria, e.g. *Brevibacterium linens*, *Corynebacterium* and *Arthrobacter* ssp. Associated with bacterial counts, the molecular approach proved to be very useful to characterize the dynamics of the bacterial population in raw milk cheese.

1. Introduction

Calenzana is a corsican soft cheese produced in the area of Balagne, at the north west of the island, from raw goat or ewe milk, according to artisanal process. Calenzana cheese process specificity relies on a high level of salting and ripening process which occurs in a closed wooden chest with periodical rind care. There are 2 varieties of cheese: the semi-ripened one called *primaticciu* (4 months) and the ripened one called *vecchjiu* (8 months). The aim of this work consisted in studying dynamics of cheese microbial ecosystem.

2. Material and methods

The work was carried out on two farms. The first one produce goat cheese, the second one ewe cheese. Cheese making was made according to traditional process, without starter adjunct. Four cheese making batches were studied on each farm. Microbial ecosystem was studied by means of (i) microbial counts (ii) molecular methods in order to identify cheeses bacteria at the species level. Temporal temperature gradient gel electrophoresis (TTGE) [1] was performed for the low-G+C-content genomes, denaturing gradient gel electrophoresis (DGGE) [2] for medium and high-G+C-content genomes. The Gel Compar software (Applied-Maths, Belgium) was used to analyze TTGE and DGGE gels.

Total solids and NaCl contents were determined. Analysis were carried out on milk and cheese at different technological steps: cheese after presalting (AP), cheese after salting (AS), cheese after 2 months of ripening, semi-ripened cheese (SR), cheese after 6 months of ripening and ripened cheese (RC). Sampling was performed using the standard methods [3].

3. Results and discussion

Microbial population changes

Among lactic acid bacteria, only presumptive lactococci (Figure1) and mesophilic lactobacilli (Figure 2) reached 10^9 cfu.g⁻¹ during the first technological steps (AP and AS steps). These bacteria constituted the dominant flora during the early stages of the process. Microbial counts showed an important decrease of lactic acid bacteria populations during ripening (from about

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10^9 to 10^4 - 10^6 cfu.g⁻¹ for presumptive lactococci). The populations of salt-tolerant bacteria and yeasts and moulds remained quite constant (about 10^4 - 10^6 cfu.g⁻¹) during 4 months (from AP to SR step) and decreased at the end of ripening. On Venaco, a corsican soft smear cheese, the populations of salt-tolerant bacteria and yeasts and moulds were higher (10^7 - 10^8 cfu.g⁻¹) [4]. This difference is due to the removing of the surface microflora made by the cheese maker during Calenzana ripening.

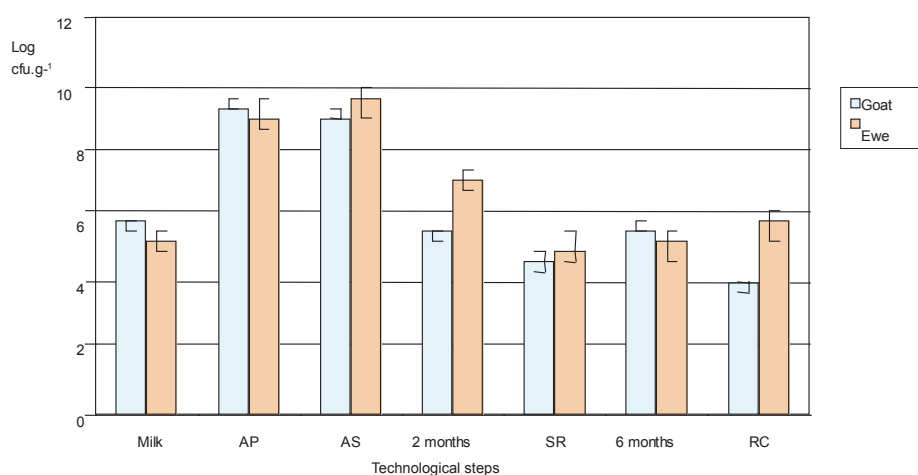


Figure 1. Presumptive lactococci counts (M 17). Mean and standard deviation.

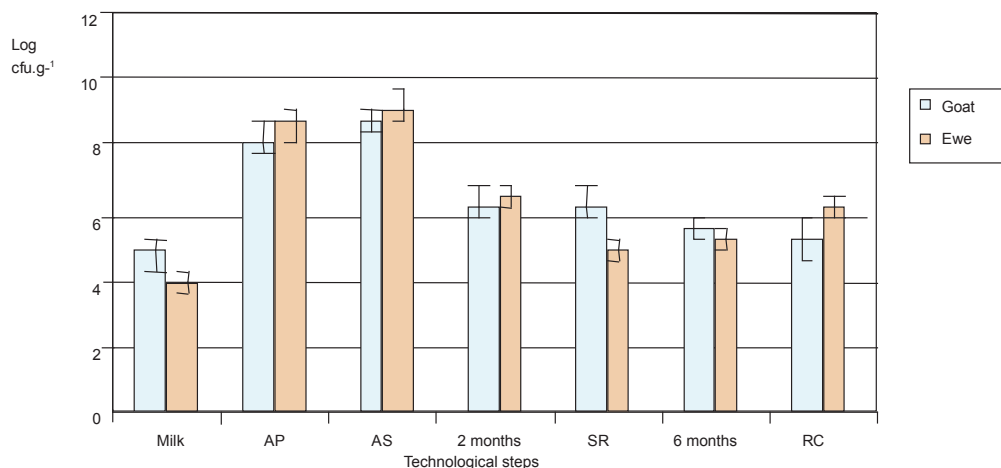


Figure 2. Presumptive mesophilic lactobacilli (MRS). Mean and standard deviation.

Microbial dynamics at the species level

Ewe and goat cheeses common features

TTGE method (Figure 3) showed that *Lactococcus lactis* ssp. *lactis* is the dominant subspecies during the whole process of Calenzana cheese. A band corresponding to *Lactococcus raffinolactis*/*S. equorum* was also frequently identified. *Lactobacillus plantarum* was absent in milk and not found in cheese before 2 months while *Lb. acidophilus* was already present in milk. Thus, *Lactobacillus plantarum* could come from cheese dairy environment while *Lb. acidophilus* was part of indigenous milk microflora.

Several species of negative coagulase staphylococci were identified.

DDGE method (Figure 4) showed the presence of numerous surface bacteria, more particularly in ewe cheese, e. g. *Brevibacterium linens*, *Corynebacterium* and *Arthrobacter* ssp. and some Gram negative bacteria.

Differences according to milk origin

Ewe cheeses differed from goat ones in the presence of *Leuconostocs*. *L. mesenteroides* was present from ewe milk until RC step and never identified in goat samples, *Leuconostoc citreum* was identified in 16 ewe cheese samples and in 4 goat ones. *Lb. acidophilus* was present in the 4 samples of goat milk but in only one of ewe milk. *Ec faecium* group (*Ec. faecium*, *Ec. durans* and *Ec. hirae*) was more frequently found in goat milk than in ewe one. *Staphylococcus aureus* was detected in 2 batches of goat milk and cheese at AP and AS steps, while no pathogenic bacteria were found in ewe cheese. The difference upon leuconostocs according to the milk origin could be due to the fact that ewe cheese seems to suit the nutritional and physiological needs of leuconostoc better than goat one (higher citrate content in ewe milk).

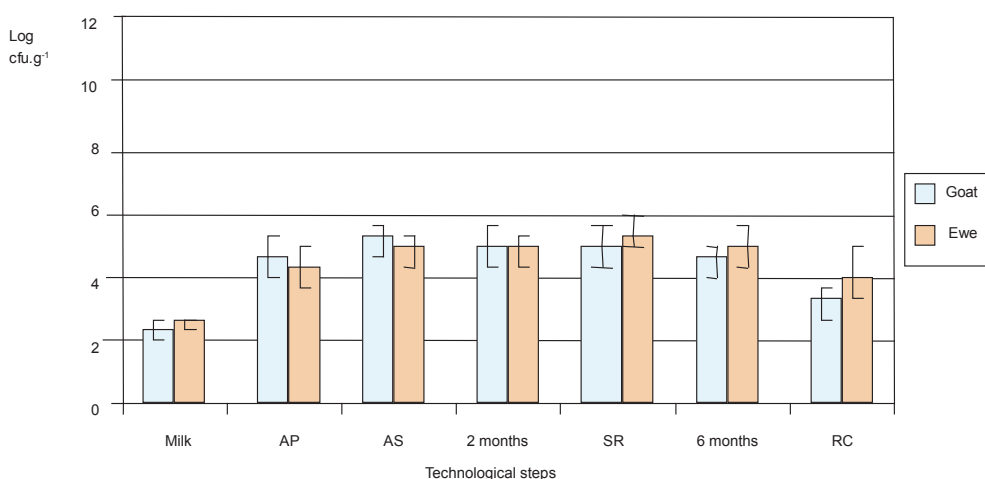


Figure 3. Presumptive salt tolerant bacteria counts (MSA). Mean and standard deviation.

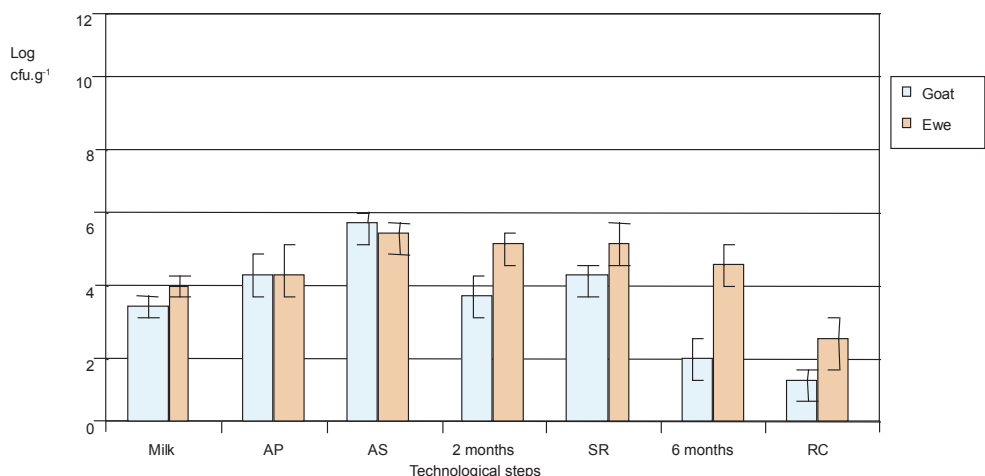


Figure 4. Presumptive yeasts and moulds counts (YGC). Mean and standard deviation.

Relationships between physicochemical characteristics and microbial change

The high salt concentration of the cheese (mean of 2.43 g.100 g⁻¹ and 3.44 g.100 g⁻¹ in goat and ewe old ripened cheeses, respectively) could explain the decrease of lactic acid bacteria populations during ripening. Lactic acid bacteria metabolism depends on NaCl/M ratio [5]. In Calenzana, this ratio being often over 6 %, this metabolism is lowered. Moreover, surface microflora being periodically removed, the ripening process is very slow.

TTGE evidenced that lactococci are present until the end of ripening while bacterial counts showed an important decrease of this species. This phenomenon can be due to stress conditions (high level of NaCl), which can transform a part of the lactococci population in viable but non cultivable cells or intact dead cells which are detected by TTGE.

4. Conclusion

These results show the diversity of the indigenous microflora of the Calenzana cheese, from milk to old-ripened cheese.

Microbial counts and molecular approach appear to be complementary in the study of the dynamics of a raw milk cheese microbial ecosystem. Microbial counts show the quantitative evolution of the micro-organisms while TTGE and DGGE are fast and efficient methods to study microbial dynamics at the species or subspecies level. The combination of the 2 approaches allows to better know the relationships between milk origin, process and microbial ecosystem.

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III-P128: Effect of Sheep Breed on Milk and Cheese Characteristics

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Summary

The aim of this research was to evaluate the effect of three sheep breeds (Gentile di Puglia, Altamura and Comisana) on the chemical composition, nutritional and aromatic properties of milk and cheese, and on the sensory properties of *Canestrato pugliese* cheese. Animals were reared all together at pasture and received equal quantities of supplement indoor. Three cheese-makings (*Canestrato pugliese*-type) for each breed were carried out for three consecutive days. The breed affected the chemical and organoleptic properties of the milk and cheeses. Fatty acid profile showed that Altamura breed was well discriminated from Gentile di Puglia and Comisana breed, which showed similar features.

1. Introduction

The dairy products characteristics depend on a large number of factors linked to animal management systems, climatic conditions, feeding, stage of lactation, etc. [4]. Breed seems to affect dairy products aromatic and sensory quality [6]. A previous study highlighted that native sheep breeds milk produced cheeses with different sensory and rheological characteristics (colour, taste, smell, flavour, friability and granulosity) [2, 4]. The aim of this study was comparing, in milk and cheese, chemical composition, fatty acid profile, aromatic properties and sensory properties of *Canestrato pugliese* cheese produced from three breeds of sheep: Gentile di Puglia (GP), Altamura (A) and Comisana (C) breed.

2. Material and methods

The experiment was carried out in winter at CRA of Foggia. All animal were fed on native pasture, also concentrate was offered in two equal meals at milking. Three cheese-makings (*Canestrato pugliese*) for each breed were carried out for three consecutive days. Milk and cheese samples were analyzed for chemical composition, fatty acid (FA) content and Volatile Organic Compounds (VOC). For each breed, chemical composition of milk and cheese was measured, on nine samples, according to standard methods. FA content in milk and cheese samples was assessed by gaschromatography analysis. Desaturation Index (DI) was estimated and VOC content was assessed by multiple dynamic headspace extraction and GC-MS [1]. Cheese's sensory profile was detected by ten panellists. Each attribute was evaluated on a 0-9 point graduated scale. Data were processed by GLM and the means were compared by LSD test. Sensory data were normalised before being submitted to ANOVA repeated measures procedures.

3. Results and discussion

Milk's chemical composition was affected by sheep breed (Table 1). GP and A breeds showed a higher fat content than C breed. Milk protein and ash were higher in GP than A and C breed. A similar pattern was observed in the *Canestrato pugliese* cheese. Our results confirm previous data obtained for the same sheep breeds [2, 5]. The sheep's genetic type affected milk and cheese FA profile and both showed a similar trend (Table 1). In milk and cheese, A breed showed a higher content of monounsaturated FA (MUFA) and total trans FA compared to other breeds. A significant lower level of polyunsaturated FA (PUFA), omega-3 and CLA was found in A breed,

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compared to C and GP breeds. As regards omega-6, slight differences between breeds were detected. No differences were found as for saturated FA (SFA). The DI was higher in A than in C and GP breed (31.1 vs 29.6 and 29.5; $P < 0.01$), thus suggesting possible differences in mammary desaturase activity. Milk and cheese VOC profile (Table 1) was affected by breed. In the milk and cheese from GP breed, ketones and acids excluded, a higher content of alcohols, aldehydes, terpenes, sesquiterpenes and aromatic hydrocarbons than A and C breed was detected. Previous studies [3] reported that within the same breed, large differences in cheese texture and taste may be linked to differences in the polymorphism of casein. Moreover breed affected some parameters of cheese sensory properties (Fig. 1). GP and A cheeses showed a higher values of "pecorino", "bitter" and "spicy" taste than C breed.

Table 1: Effect of breed (E.B.) on pH and chemical composition, fatty acid content (% FAME) and Volatile Organic Compound (u.a.) in milk and *Canestrato pugliese* cheese

Parameters	Milk				E.B.	Cheese				E.B.
	GP	A	C	SE		GP	A	C	SE	
pH	6.70	6.54	6.64	0.09	ns	5.40 ^a	5.12 ^b	5.07 ^b	0.02	***
Dry Matter %	19.85 ^a	20.32 ^a	18.38 ^b	0.43	*	62.56	62.88	62.78	0.56	ns
Fat %	8.13 ^a	8.10 ^a	7.13 ^b	0.06	***	24.87	25.29	24.67	0.59	ns
Protein %	7.07 ^a	6.78 ^b	6.32 ^c	0.09	**	22.4 ^a	19.15 ^b	20.28 ^{ba}	0.78	*
Ash %	1.04 ^a	0.99 ^b	0.98 ^b	0.01	*	6.40 ^a	5.74 ^b	6.14 ^a	0.12	***
<i>Fatty Acids</i>										
SFA	70.51	69.54	70.27	0.49	ns	71.01	69.86	71.65	0.48	&
MUFA	24.22 ^b	25.69 ^a	24.53 ^{ab}	0.41	*	23.87 ^b	25.74 ^a	23.08 ^b	0.4	**
PUFA	5.27 ^b	4.78 ^a	5.44 ^b	0.12	**	5.32 ^b	4.64 ^a	5.42 ^b	0.12	***
ω -3	1.09 ^b	0.88 ^a	1.14 ^b	0.02	***	1.09 ^b	0.84 ^a	1.08 ^b	0.02	***
ω -6	2.05	1.83	2.04	0.07	&	2.12 ^b	1.87 ^a	2.19 ^b	0.08	*
Total <i>Trans</i>	2.23 ^a	2.30 ^a	2.14 ^b	0.03	**	2.28 ^a	2.21 ^a	1.91 ^b	0.11	*
CLA	0.81 ^b	0.74 ^a	0.85 ^b	0.02	**	0.81 ^b	0.70 ^a	0.83 ^b	0.02	**
<i>VOC</i>										
Alcohols	428.7 ^a	319.6 ^b	336.1 ^{ba}	32.9	ns	893.3 ^a	291.1 ^b	224.3 ^b	47.22	***
Aldehydes	184.8 ^a	130.6 ^b	109.6 ^b	4.19	***	165.0 ^{ba}	111.9 ^b	228.3 ^b	24.64	*
Ketones	23.89	17.42	25.83	4.35	ns	68.26 ^a	16.33 ^b	33.69 ^b	8.58	*
Terpenes	260.2 ^a	232.4 ^a	193.6 ^b	9.46	***	521.8 ^a	142.9 ^b	180.1 ^b	17.72	***
Sesquiterpenes	5.65 ^a	3.03 ^b	2.76 ^b	0.29	***	21.70 ^a	12.00 ^b	18.42 ^{ba}	1.87	*
Acids	14.87 ^a	9.82 ^b	16.07 ^a	1.66	*	990.5	628.8	1109.7	164.7	ns
A. hydrocarbons	255.3 ^a	174.7 ^c	235.0 ^b	1.96	***	473.8 ^a	163.2 ^b	263.9 ^b	53.44	*

GP = Gentile di Puglia; A = Altamura; C = Comisana breed. Means within row with different superscripts differ at $P < 0.05$; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, & $P = 0.06$ and ns = not significant

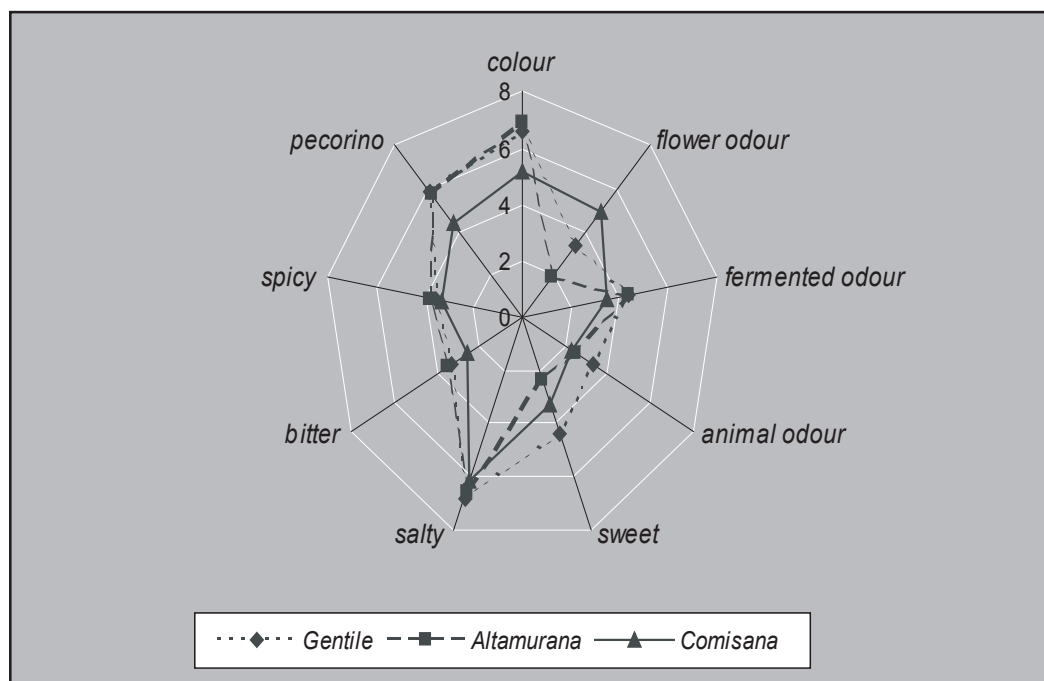


Figure 1. Effect of breed on sensory profile of *Canestrato pugliese* cheese.

4. Conclusion

Results indicate that chemical composition, FA profile, VOC compounds and sensory properties of milk and *Canestrato pugliese* cheese vary according to sheep breed. GP and A breeds produce milk and cheese having characteristics and nutritional and sensory properties distinguishable from others breed.

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III-P129: Nutritional and Aromatic Characteristics of “Carmasciano” and “Bagnolese” Cheese

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Summary

Carmasciano (C) and Bagnolese (B) sheep cheeses are produced in a small area of Campania region (Southern Italy) with traditional techniques. The distinctiveness of Carmasciano cheese is given by mephitic exhalations (sulfur) present in the production area. The aim of this study was comparing the chemical composition, nutritional, aromatic properties and sensory properties of C and B cheese. Results showed that nutritional, aromatic and sensorial profiles were different in the two cheeses analyzed, although having the same geographical production areas.

1. Introduction

The link between milk production conditions (feeding systems, breeds, floristic composition of grazing, etc.), natural environment (soil, climate) and product characteristics plays a fundamental role as for typical cheese [5, 4]. The characterization and maintenance of biodiversity is crucial for cheese in order to best reflect the typicity and diversity of their territory of origin [9]. The aim of this study was comparing the chemical composition, nutritional, aromatic properties and sensory properties of Carmasciano (C) and Bagnolese (B) cheeses produced from sheep milk. The two sheep cheeses were produced in a small area in Campania region (Southern Italy) with traditional techniques. The distinctiveness of Carmasciano cheese is given by mephitic exhalations (sulfur) present in the production area.

2. Material and methods

Our research was carried out in spring and involved twelve farms for each cheese. For each farm, three cheeses were collected and all cheeses were ripened, for six months, in the same ripening room. Cheese samples were analyzed as for chemical composition, sulfur content, FFA and FAME (fatty acids methyl esters), VOC (Volatile organic Compounds) and sensory properties. FA content in milk and cheese samples were assessed, as reported previously [3]. VOC content in milk and cheese was assessed by multiple dynamic headspace extraction and GC-MS [1]. The cheese sensory profile was detected by ten panelists. Each attribute was evaluated on a 0-9 point graduated scale. Data on chemical composition, FA, FAME and VOC of milk and cheese were processed by GLM procedures [8], and the means were compared by LSD test. Sensory data were normalised [6] before undergoing statistical analysis, and then were submitted to ANOVA repeated measure procedures.

3. Results and discussion

Results showed that the chemical composition, on DM of C and B cheeses was similar. The level of sulfur detected in both cheeses was not different (7112 ppm vs. 7103 ppm), moreover higher values were found when it was compared to cheeses (3243 ppm) coming from different areas of production. Bagnolese cheese showed a higher content of butanoic (1.64 ± 0.3 vs. 0.80 ± 0.3 , $P \leq 0.05$) and hexanoic free fatty acids (3.29 ± 0.4 vs. 2.35 ± 0.4 , $P \leq 0.05$) than Carmasciano cheese. Fatty acid composition varied according to different cheeses. Carmasciano cheese showed a higher level of monounsaturated (26.84 ± 0.94 vs. 24.55 ± 0.94 , $P \leq 0.05$) than the other ones. In addition, the amount of polyunsaturated fatty acids showed a decrease in C cheese (8.62 ± 0.4 vs. 10.62 ± 0.4 , $P \leq 0.05$). Levels of CLA were higher in B than in C cheese (1.71 ± 0.15 vs. 1.28 ± 0.15 , $P \leq 0.05$). Ours results may be linked to feeding systems,

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with a different incidence of pasture on diets [3]. Data on volatiles compounds, as reported in table 1, showed a different aromatic profile in cheeses. A greater amount of aldehydes and aromatic hydrocarbons was detected in Carmasciano, compared to Bagnolese cheese; on the contrary alcohols, esters and terpenes contents were higher in B cheese. The most abundant aldehydes in B cheese were nonanal (95.43 ± 0.33 vs. 80.19 ± 0.33 $P \leq 0.001$) and decanal (26.10 ± 0.23 vs. 15.79 ± 0.23 $P \leq 0.001$). The level of 1-octanol was higher in B cheese than in other ones (360.51 ± 2.17 vs. 22.36 ± 2.17 $P \leq 0.001$). Terpenes contents were higher in Bagnolese more than in Carmasciano cheeses. A higher content of terpenes was detected in B than C cheese. Dairy product terpenes have recently attracted interest both because of their possible impact on cheese properties and as potential markers to detect, in milk and cheese, the presence of diversified forages in dairy cows' diet [7]. Many authors [2, 10] reported that terpene profiles were considered as the forage signature in animal products. The level of camphene (50.47 ± 0.34 vs. 21.80 ± 0.34 , $P \leq 0.001$) and nerol (21.18 ± 0.30 vs. 3.95 ± 0.30 , $P \leq 0.001$) was higher in cheese from B group than in other ones. The intensity of sensory attributes showed a significant difference. Bagnolese cheese was characterized by a higher intensity of herbaceous, fermented and pecorino odour and bitter and spicier taste compared to other cheeses.

Table 1: Chemical composition of Carmasciano and Bagnolese cheese (%/DM).

	Bagnolese (B)	Carmasciano (C)	S.E.	Significance
Alcohols	404.96	188.76	3.93	$P < 0.001$
Aldehydes	138.16	172.55	1.03	$P < 0.001$
Ketones	51.59	30.56	0.14	$P < 0.001$
Esters	170.48	154.32	0.46	$P < 0.001$
Terpenes	358.59	52.59	0.15	$P < 0.001$
Hydrocarbons	318.49	3073.93	15.09	$P < 0.001$

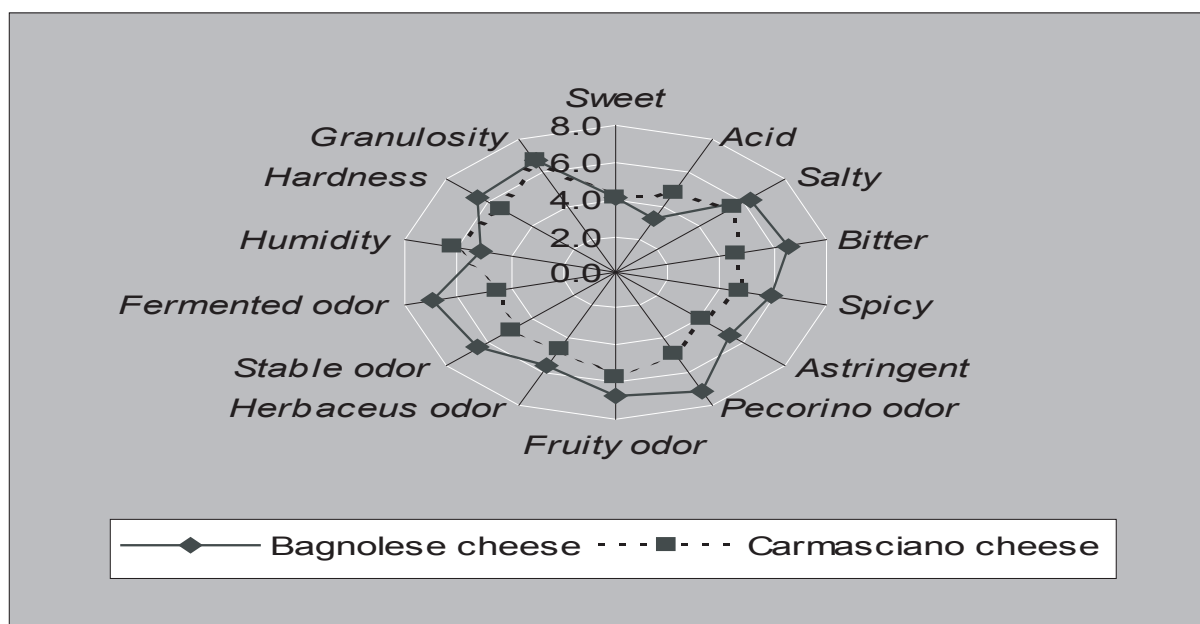


Figure 1. Sensory profile of Bagnolese and Carmasciano cheese

4. Conclusions

These results underline the role of management systems (feeding, environment) and the importance of cheese-making traditional techniques on the aromatic and nutritional value of typical Italian cheeses.

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III-P130: Determination of Proteolytic Pattern During Ripening of Pecorino Romano Cheese

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Summary

PDO *Pecorino Romano* is a semi-hard cheese made using whole ewe's milk and lamb rennet paste; after moulding the cheese was salted at 18°Beaumè, then dry salted another two times during ripening. Since peptidic composition at each ripening time can be an analytical parameter useful to trace the correct employed technology, nitrogen content and casein breakdown have been monitored in cheeses at different ripening age (24hours →8months). By PAGE at pH 8,6 analysis and immunoblotting with α_{s1} - and β -CN polyclonal antibodies of pH 4.6-insoluble nitrogen fraction of cheese, a more extent of α_{s1} -CN breakdown than β -CN resulted.

The 12%-TCA soluble nitrogen fraction of both 24h and 8 months old cheese samples, analysed by LC-ESI/MS, showed new formed peptides generating from α_{s1} -CN and β -CN degradation. α_{s1} f(1-23) and β_1 f(1-28) are lacking, but shorter derived peptides were found. On the basis of chemical and LC/MS results, it can conclude that proteolytic activity took place in the early stages of ripening, while the subsequent slowing down could be related to dry salted during ripening.

1. Introduction

PDO "Pecorino Romano" is a semi-hard cheese which obtained the EU label since 1996 (reg CEE n.1107).

The main technological steps of cheesemaking process, as reported in "Regulation Board", were: i) the thermisation of ewe bulk milk; ii) a natural starter as "scotta whey" added to milk; iii) lamb rennet paste as coagulant; iv) after moulding, the cheese was salted, by hand made sprinkling way. The "scotta whey" was a by-product of "Ricotta" production, after standing overnight at room temperature, so that an autochthon microflora, consisting mainly of *S. thermophilus*, *L. bulgaricus* and *L. lactis*, was selected. The synergic proteolytic activity of both, bacteric and rennet proteases, determined the typical sensorial profile of "Pecorino" cheese, such as texture and flavour. The reproducibility of these organoleptic qualities were depending on cheesemaking process standardisation and on the sequencing of enzymatic activity by which, peptides and aromatic molecules are produced. This metabolomic composition, depending on technological conditions of production process, could be used as molecular marker of cheese as well as molecular marker of process. To this aim the proteolysis of Pecorino PDO at two ripening stages (2 and 8 months) was determined by means of immunoelectrophoretic technique and LC/MS/MS analysis.

2. Material and methods

PDO Pecorino Romano samples at different ripening stages (1day, 1, 2, 3, 4, 5, 6, 7 and 8 months of ripening) were analyzed. The chemical analysis (NT, NS and NPN) was carried out in according to Rowland S.J. (1939). The preparation of cheese samples and immunoelectrophoretic analysis were carried out in according to Pirisi A. et al. (2007). The 120g L⁻¹ TCA soluble fractions were analyzed by LC/MS following Addeo et al. (1994).

3. Results and discussion

PAGE pH 8,6 and immunoblotting analysis

The profiles of the insoluble nitrogen fraction at pH 4,6 of PDO Pecorino Cheese samples at different ripening stages (24h →8 months) were shown in figure 1 after Blue comassie stain (A)

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and after immunodetection with specific polyclonal antibodies against α_{s1} -Cn (B) and β -Cn (C). The results showed that, on the basis of each casein composition, α_{s1} -Cn was the most hydrolysed fraction, since more quantitative differences and a lot of new molecular species than β -Cn counterpart were observed. In particular, the main new components from α_{s1} casein fraction, having the fastest mobility towards the anode, could be the α_{s1} -f(24-199) in analogy with the homologous bovine cheese counterpart (Addeo et al. 1995). This profile, compared with ovine casein *in vitro* hydrolysed with chymosin, showed new formed peptides (α_{s1} I) with a higher mobility than chymosin digest (Mauriello, work in progress). On the contrary, in the curd after 24h, the two components labelled *a* and *b* had the same mobility of *in vitro* hydrolysed. This meant that in ovine cheese the α_{s1} I could be derived from α_{s1} -f(24-199) by the action of milk endoproteases, primarily plasmin.

LC/ESI/MS analysis

The TCA 12% soluble nitrogen of "Pecorino" cheese at 2 and 8 months of ripening was analysed by means of LC/ESI/MS analysis. The results were displayed on the relative casein primary structure in figures 2 and 3.

Considering α_{s1} -Cn results, the highest number of derived peptides are located in the sequence 1-52. The expected α_{s1} f(1-23), originating by chymosin action was lacking, only very short fragments (the longest was 1-14) were obtained from its degradation. By comparison each other cheese at 2 and 8 months, the peptides number of 8 months old was decreasing, but not the size of fragments. In β -Cn it also can observe a similar result, firstly, no expected β f(1-28) was occurred, but shorter derived peptides were found. The number of peptides identified after 8 months of aging dramatically decreased. On the other hand, the NPN level strongly increased from 15% to 51% in the early stage of ripening (24h→2months), while a slight NPN increase, from 51% to 69% in 2 and 8 months ripened cheese respectively, was observed. This data could be due to an increase of free aminoacid amount.

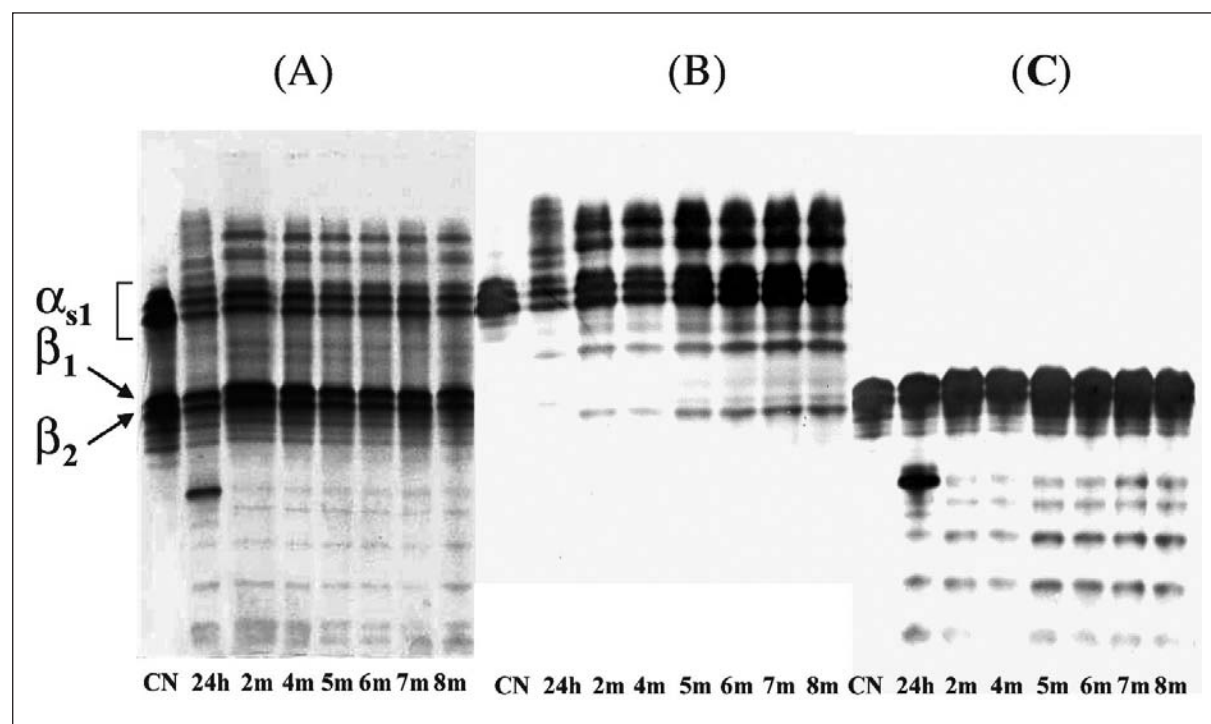


Figure 1. Polyacrylamide gel electrophoresis at pH 8,6 (A) and immunoblotting with specific polyclonal antibodies against α_{s1} -CN (B) and β -CN (C) of pH 4,6 insoluble fraction of Pecorino cheese samples at different stages of ripening (24h, 2, 4, 6, 7 and 8 months)

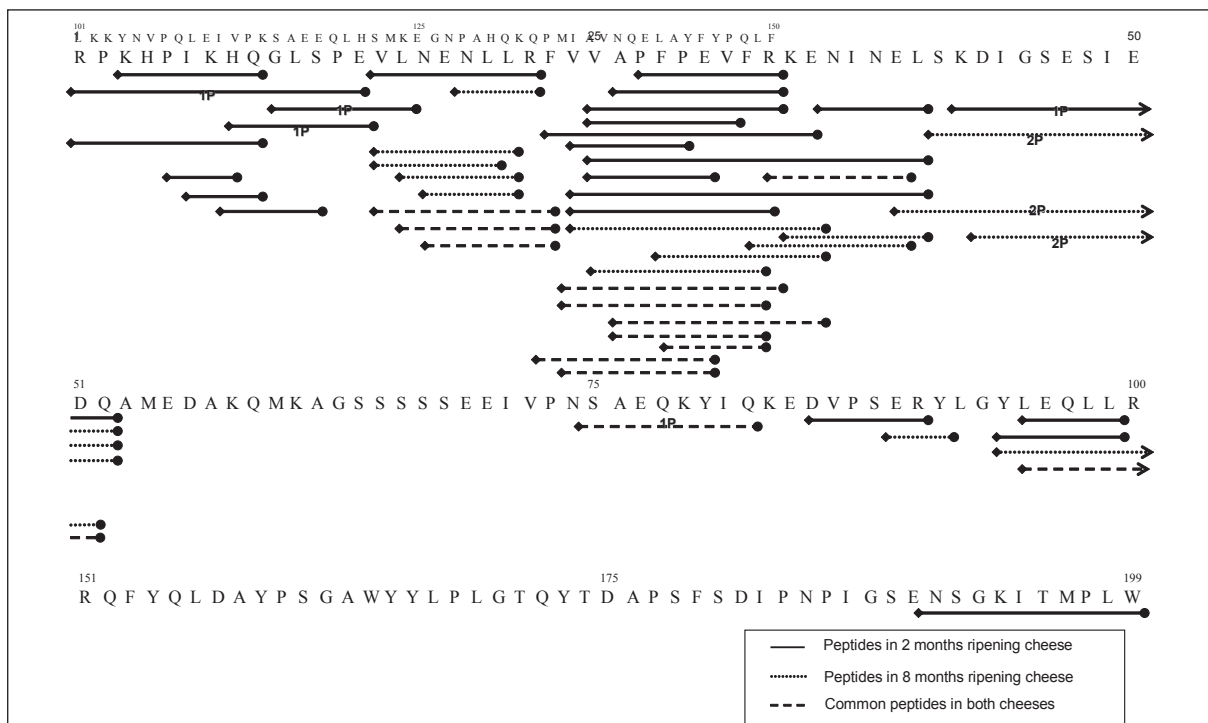


Figure 2. Primary structure of ovine α_{s1} -casein with peptides identified by LC/MS/MS analysis of TCA 12% soluble fraction.

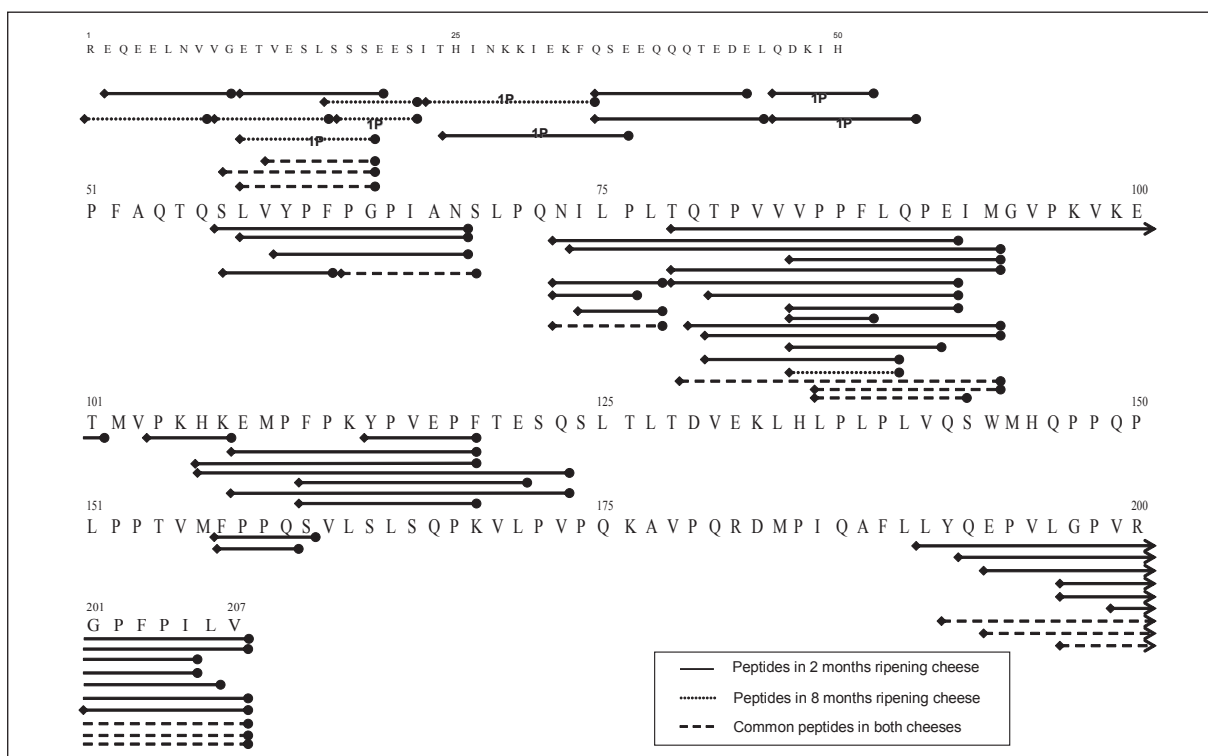


Figure 3. Primary structure of ovine β -casein with peptides identified by LC/MS/MS analysis of TCA 12% soluble fraction.

4. Conclusion

On the basis of these results we can conclude that the α_{s1} -Cn and β -Cn were the most sensitive fractions susceptible to enzymatic action. In addition, a strongly proteolytic phenomenon took place during the early stage of ripening (24h→2M) mainly, and then slowing down. So, a number of peptides decrease after 8 months of aging and NPN amounts exhibited a slight increase. This phenomena could be related to dry salted during ripening.

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III-P131: Improving Near Infrared Transmittance Spectroscopy (NIT) in the Determination of Moisture, Fat, Protein and NaCl in Pecorino Romano PDO Cheese

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Summary

The aim of the research was to develop and to validate a Near Infrared Transmittance (NIT) spectroscopy calibration for Pecorino Romano PDO cheese composition analysis. A calibration set of 75 samples was analysed for moisture, fat, protein and NaCl by the traditional reference methods and the NIT spectroscopy. Calibrations were developed by a Partial Least Squares regression. Standard Error of Calibration (SEC) and Squared Correlation Coefficient (R^2_c) were determined for moisture (SEC=0.17, $R^2_c=0.99$), fat (SEC=0.28, $R^2_c=0.95$), protein (SEC=0.39, $R^2_c=0.91$), and salt (SEC=0.08, $R^2_c=0.99$). To validate the calibration, a set of 25 samples was used, and Standard Error of Prediction (SEP) with coefficient of determination (R^2_v) were determined for moisture (SEP=0.21, $R^2_v=0.99$), fat (SEP=0.43, $R^2_v=0.95$), protein (SEP=0.38, $R^2_v=0.91$), and salt (SEP=0.12, $R^2_v=0.99$). These results show that a strong calibration model was obtained for all four components of Pecorino Romano PDO cheese and NIT spectroscopy should be recommended for industrial quality control.

1. Introduction

Near Infrared Transmittance (NIT) spectroscopy is a valuable tool, useful for quality control in the dairy industry. NIT analysis is more feasible, faster and less expensive than traditional chemical methods. It allows to determine several parameters simultaneously and does not require heavy skilled personnel [1, 2]. Nevertheless, NIT needs robust and reliable calibrations that have to be developed by means of enough varied samples, quite similar to the ones that will be analyzed in the future [3]. Calibrations developed by the instrument producers cover a wide range of dairy products, but mainly they are developed and experienced for the cow's products analysis. The aim of the research was to develop and to validate a NIT calibration for composition analysis of Pecorino Romano PDO, a sheep milk cheese.

2. Material and methods

A total of 100 Pecorino Romano PDO cheese samples were splitted in two sets of 75 e 25 samples, which were respectively used to develop NIT calibration and validation. Each sample was grated and splitted in two parts, which were respectively analyzed by means of reference methods and NIT. The reference analysis were carried out as follows: moisture was determined by thermobalance (Halogen Moisture Analyzer HB43, Mettler Toledo), fat according to FIL-IDF 5A: 1969, protein according to FIL-IDF 25: 1964, and NaCl according to FIL-IDF 88A: 1988. The NIT sample portion was splitted into three sub-parts and each was twice analysed. Therefore for each sample a total amount of six spectra were obtained. On the whole, 464 spectra were used for calibration and 150 spectra for validation.

Spectra were recorded in transmittance mode by a Foodscan Lab instrument (FOSS Electric A/S, Hillerød, Denmark) with a scanning range from 850 to 1050 nm and wavelength increments of 2 nm. Calibrations were developed by a Partial Least Squares (PLS) regression, using WinISI III software (ver.1.50e, Infrasoft International, LLC). To compare between the results obtained from the NIT and those measured with reference methods for a given component, the values of Standard Error of Calibration (SEC), coefficient of determination of calibration (R^2_c), Standard Error of Prediction (SEP), coefficient of determination of validation (R^2_v), were considered.

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3. Results and discussion

Table 1 shows statistics on sample sets used to develop and validate the calibrations. The amount of samples used (N), mean, standard deviation (S.D.), minimum (Min) and maximum (Max) value of sample range, standard error of calibration (SEC) or validation (SEP), and determination coefficient of calibration (R^2_c) or validation (R^2_v) are shown for each parameter.

Table 1: NIT calibration and validation sets statistics for Pecorino Romano Cheese PDO

	Calibration set							Validation set						
	N	Mean ^a	S.D. ^a	Min ^a	Max ^a	SEC	R^2_c	N	Mean ^a	S.D. ^a	Min ^a	Max ^a	SEP	R^2_v
Moisture	75	31.8	1.54	27.2	36.4	0.17	0.988	25	31.6	1.78	26.6	34.2	0.21	0.987
Fat	75	32.6	1.29	28.7	36.5	0.28	0.952	25	33.0	1.33	30.2	36.5	0.43	0.953
Protein	75	26.1	1.29	22.2	30.0	0.39	0.909	25	26.3	0.95	23.8	28.2	0.38	0.914
NaCl	75	5.7	0.95	2.9	8.5	0.08	0.994	25	5.5	0.87	3.6	8.3	0.12	0.986

^a Values expressed as g/100g.

Figure 1 shows the scatter-plot of comparison between reference and predicted values for moisture. Moisture calibration showed a SEC= 0.17 and a R^2_c = 0.988, while validation showed a SEP= 0.21 and R^2_v = 0.987.

Figure 2 shows the scatter-plot of comparison between reference and predicted values for fat. For fat the performances were in calibration SEC= 0.28 and R^2_c = 0.952, while in validation SEP= 0.43 and R^2_v = 0.953. Even if SEP appeared increased compared with SEC, R^2_c repeated the value obtained in calibration.

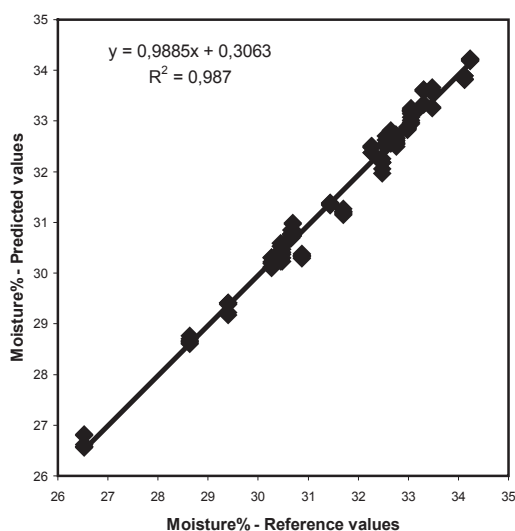


Figure 1. Comparison for Moisture% between reference and predicted values of validation sample set of Pecorino Romano PDO cheese.

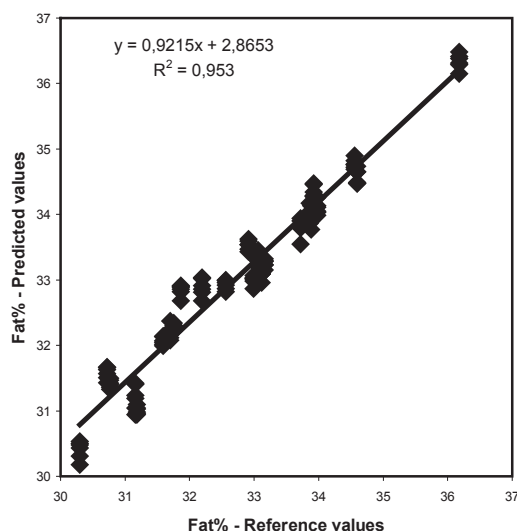


Figure 2. Comparison for Fat% between reference and predicted values of validation sample set of Pecorino Romano PDO cheese.

Figure 3 shows the scatter-plot of comparison between reference and predicted values for protein. For protein the calibration values showed a SEC= 0.39 and a R^2_c = 0.909. This values were very close to SEP= 0.38 and R^2_v = 0.914 obtained in validation.

Figure 4 shows the scatter-plot of comparison for NaCl between reference and predicted values. For NaCl the values in calibration SEC= 0.08 and R^2_c = 0.994, and in validation SEP= 0.12

and $R^2 = 0.986$ were obtained. These results showed that calibration and validation performances are very close.

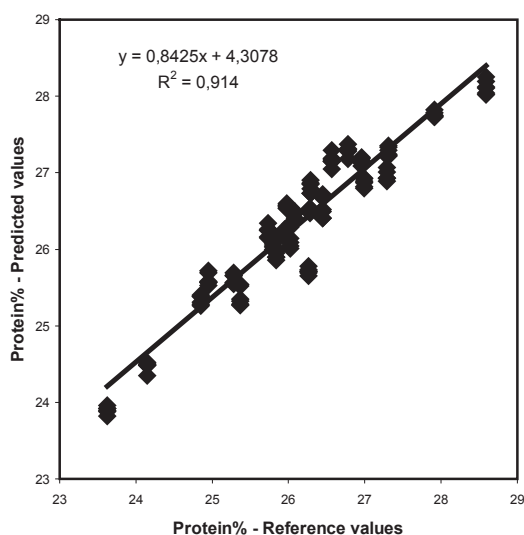


Figure 3. Comparison for Protein% between reference and predicted values of validation sample set of Pecorino Romano PDO cheese.

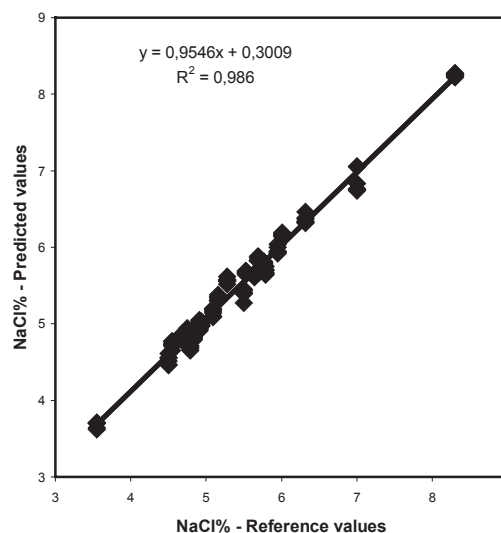


Figure 4. Comparison for NaCl% between reference and predicted values of validation sample set of Pecorino Romano PDO cheese.

Variability required for calibration had been assured by a calibration set enough wide, in agreement with Rodriguez-Otero et al. [4]. Moreover, the mean, S.D. and the range of the validation and calibration sets showed similarity, as required according to Mark et al. [3].

The calibrations performances were marked out by SEC values, ranging from between 0.08 to 0.39, and by a R^2_c always higher than 0.90. Validation SEP and R^2_v values give evidence of calibration effectiveness due to their similarity. R^2 values have to be considered excellent for all parameters since it was higher than 0.91, according to Karoui et al. [5].

Finally, performances of our calibration were comparable to those obtained by other authors with NIT for moisture and fat [6-8], whereas references about NIT calibration for protein and salt were not found.

4. Conclusion

According to these results, it could be asserted that NIT calibration obtained for Pecorino Romano PDO cheese was robust and reliable. Therefore it was suitable to use in the industrial quality control. By means of collaborative efforts, a specific NIT calibration for milk products was successfully developed inside an industrial laboratory. The lab analysis capacity was remarkably increased by means of the advantages of near-infrared spectroscopy.

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III-P132: *Listeria Monocytogenes* Contamination Sources in Sheep Cheese Processing Plants and Strains Virulence Genes Typing

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Summary

A survey on *Listeria monocytogenes* (*Lm*) contamination patterns was carried out in 10 sheep cheese processing plants, located in Sardinia (Italy). The samples were collected from the processing line during production. The samples were taken in cheese and ricotta processing area from food contact surfaces (raw milk filters, moulds, tables, ripening shelves and cheese and ricotta washing machine) and non food contact surfaces (floor drains or floors/walls). Pooled samples from rind surfaces of hard cheese and ripened ricotta were also taken.

Lm strains were isolated from 48 samples (18.8%) taken from 9 cheese factories (90%) and other *Listeria* spp strains were isolated from 39 samples (15.3%) from 10 cheese factories (100%). *Lm* was detected in raw milk filters (16.7%), cheese rinds (5.4%), ripened ricotta surfaces (18.2%), cheese moulds (20%), tables and shelves (9.8%), cheese washing machines (60%), ricotta washing machines (20%), drains or walls/floors surfaces (19.4%). *Lm* strains analysed for serotype with traditional and molecular method belonged to groups 1/2a (62,8%), 4/b (19,8%), 1/2b (14,0%) and 1/2c (3,5%). All *Lm* strains analysed for virulence genes *prfa*, *clp*, *inlA*, *inlB*, *inlC*, *hly*, *lisR*, *actA*₁, *actA*₂ were found as positive.

1. Introduction

Listeria monocytogenes (*Lm*) is the causative agent of Listeriosis in humans, which is associated to severe clinical signs as septicaemia, meningitis and abortion. Since Listeriosis is mainly a foodborne disease a relevant epidemiological role is ascribed to most frequently and heavily contaminate products [6]. In surveys carried out on milk and whey products *Lm* prevalence ranged from between 1,4 to 86% [3,10,14]. Among milk and whey sheep cheese produced in Sardinia, *Lm* was formerly detected on the surface of Pecorino Romano PDO rind and ripened ricotta cheese [4]. Actually, no data or evidence is available about a link between the contamination of these food products and human Listeriosis. Nevertheless, in the last years *Lm* contamination determined some troubles for the sheep cheese-making industry as it caused recalls of ripened ricotta cheese exported in USA [8]. In Pecorino Romano PDO and ripened ricotta cheese processing milk thermization and whey heating are involved, respectively. In these processes, the time-temperature combinations are useful to achieve at least 2-3 log reduction in *Lm* concentrations [9]. This performance criterion has to be regarded as safe to reduce *Lm* at the levels that could be usually detected in sheep raw milk. Tracing *Lm* post-process contaminations in these products gives evidence that it mainly occurred from the environment [11]. This study was carried out to detect *Lm* and *Listeria* spp niches and sources of contamination in sheep cheesemaking plants. *Lm* isolated strains were also characterized for serotypes and virulence genes profiles.

2. Material and methods

A survey on *Listeria monocytogenes* (*Lm*) contamination patterns was carried out in 10 sheep cheese-making plants, located in Sardinia (Italy). The samples were collected from the processing line during production. The samples were taken in Pecorino Romano PDO and ripened ricotta cheese processing area from food contact surfaces (raw milk filters, moulds, tables, ripening shelves and cheese and ricotta washing machine) and non food contact surfaces (floor drains or floors and walls). Pooled samples from rind surfaces of hard cheese and ripened ricotta were also taken. The sampling was done according to ISO 18593 by using a commercial kit, which

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included a sterile sponge moistened in neutralizing buffer and sterile gloves within a re-sealable polythene pouch (Qualicum scientific LTD, Ottawa, Canada). Each sample was obtained by sponge swabbing 3-4 non-delimited area in each environment or 3-4 surfaces of ricotta or cheese shapes. Each sample was enriched, and then plated to Oxford and ALOA agar according to the ISO 11290-1 method. From each plate up to three colonies showing characteristic features for *Listeria* genus were isolated and stored at -80°C . Strain confirmation test were performed according to the standard specifications. All isolated strains were identified by using apiListeria kit (bioMérieux, France). 84 out 195 strains were analysed for *iap* gene [2]. They were also tested for serotypes through traditional [13] and two different molecular methods [1,5]. These strains were also tested for the following genes encoding for virulence factors: *prfA*, *clpP*, *inlA*, *inlB*, *inlC*, *hly*, *lisR*, *actA*₁ and *actA*₂ [15].

3. Results and discussion

Lm strains were isolated from 9 out of 10 cheese factories. *Lm* average prevalence in samples was 21,7%, ranging from between 7,6% to 52,3% in each plant. Other *Listeria* spp were isolated in all plants (100%). Overall samples prevalence was 17,7%, ranging from between 3,8 and 34,5%. Table 1 shows the results on *Lm* and other *Listeria* spp prevalence in raw milk filters, in food contact and non-food contact surfaces, in the surface of Pecorino Romano PDO (5.4%) and ripened ricotta cheese (18.2%). *Lm* overall contamination rate in the samples through plant areas ranged from between 5,5 to 54,6%. The higher *Lm* prevalence was detected in samples taken from the salting area (21,7%) and washing room (54,6%). In 9 out 10 plants *Lm* was recovered in almost one of the sampling sites along the Pecorino Romano PDO processing line. The premises along the processing line of ripened ricotta cheese was less contaminated and a positive site was detected in only 4 out of 10 plants. From the samples 506 *Listeria* spp strains were isolated and they were identified as follows: *Listeria innocua* (257), *Lm* (195), *Listeria ivanovii* (43) e *Listeria welshimeri* (11). In 25 samples *Lm* was the only species isolated whether it was associated to other *Listeria* spp strains in 23 samples. In 39 samples were isolated only *Listeria* spp other than *Lm*. The *Lm* serovars distribute themselves as follows: 1/2a (63.1%), 4b (22.4%), 1/2b (10.6%), and 1/2c (3.9%). The most frequently isolated serotypes 1/2a and 4/b were respectively detected in 8 and 4 plants. The same serotype contaminated the products surface and the environment (food-contact and non food-contact surfaces). In the same dairy plant were isolated from 1 to 3 serotypes. *Iap* gene was detected in all *Lm* strains (84). All *Lm* strains analysed for virulence genes *prfA*, *clp*, *inlA*, *inlB*, *inlC*, *hly*, *lisR*, *actA*₁, *actA*₂ were found as positive.

Table 1: *Lm* and other *Listeria* spp average prevalence (%) in samples collected from ewe's cheesemaking plants

surface	samples	<i>Lm</i>		<i>Listeria</i> spp*	
	n	n	%	n	%
raw milk filter	12	2	16.7	5	41.7
cheese moulds	10	2	20.0	1	10.0
tables/shelve	41	4	9.8	4	9.8
cheese washing machine	20	12	60.0	5	25.0
ricotta washing machine	5	1	20.0	1	20.0
drains	108	21	19.4	21	19.4
hard cheese	37	2	5.4	1	2.7
ripened ricotta cheese	22	4	18.2	1	4.5

*= *Listeria* spp that differs from *L. monocytogenes*.

4. Conclusion

The results of our study show that *Lm* and *Listeria* spp were isolated in 90% and 100% of sheep cheese-making plants. As *Lm* is widespread in the plants premises, it was found that the surfaces of Pecorino Romano POD (5,4%) and ripened ricotta cheese (18.2%) were also contaminated. Our last results on tracking *Lm* in the sheep cheese-making located in Sardinia [11] give evidence that this micro-organism is not introduced by means of raw milk. This conclusion agrees with the results of the inactivation tests [9].

In these plants our results are helpful in developing control and monitoring procedures to reduce *Lm* contaminations in the plant environment. The most-higher prevalence was found in the washing and the brining areas. The cheese and ricotta washing machines were the most frequently found positive for *Lm* and *Listeria* spp and they are a relevant contamination source during processing [11]. The drains should be regarded as an effective indicator of *Lm* contamination in a processing area. In some plants, *Listeria* spp other than *Lm* were more frequently isolated. The detection of these species that shared with *Lm* the same environment and niches should result in a more strengthened GHPs implementation [12,7]. Moreover, has to be underlined the relevance of *Lm* contaminations in these plants, since the majority of *Lm* isolates were setted into lineage I (4b) and II (1/2a), which are involved in human Listeriosis. As well, the virulence genes investigated were detected in all strains.

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III-P133: Food Safety: Salubrity Characteristics of “Fiore Sardo” Cheese

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Summary

Fiore Sardo represents one of the three Sardinian DOP cheese; it is processed in craft way till today with ancient traditional production technologies. Aim of this study is to demonstrate the microbiological Fiore Sardo salubrity using the results of a scientific research on support and promotion of traditional product. The dairy sheep farm where the study was carried out consisted of about 800 dairy Sarda ewes and their milk was daily used to make Fiore Sardo. The HACCP system was applied by a self-control manual elaborate by us. During two years 4 processing, on February, March, April and May were followed. Samples from raw milk, curd milk, 48h curd milk, 1 month cheese, 3 month cheese and 6 month cheese seasoning were carried out and analysed for chemical-physical (pH and a_w) and microbiological (*Salmonella* spp, *Listeria monocytogenes*, coagulase positive staphylococci, *Escherichia coli*,) parameters; then, the microbial populations dynamic, controlling survival times, was observed. In conclusion, about pathogen micro-organisms considered, Fiore Sardo cheese made with traditional technologies and hygiene parameters of HACCP system has salubrity characteristics.

1. Introduction

Fiore Sardo is the only sheep's cheese produced from raw milk in Italy.

It is important to underline that an important consequence of not heating the milk is the survival of a microbic flora in the curd. It is particularly varied and comes from the milk but also from the areas in which the sheep graze.

The product is typically handcrafted (traditional cheese prepared by shepherds in sheep-pens) although attempts to produce it industrially are now taking place.

Its production, which is more or less confined to the Barbagia of Ollolai (above all in Gavoi and Fonni), entails a lengthy ripening period (no less than 7 months). It is still considered a niche product (annual production is about 7000 quintals) but is in ever-increasing demand because of its exceptionally typical characteristics.

Milk and milk products are a frequent source of consumer's toxic infections due to food, representing, from a micro-biological point of view, an optimal culture medium for the growth of contaminating and pathogenic micro-organisms.

Among the numerous illnesses transmitted to man through milk and its by-products, the micro-organisms most often responsible for pathologies from foods derived from sheep's milk are: *Salmonelle* spp, *Escherichia coli*, *Stapylococcus aureus* and its *Enterotoxins*, *Listeria monocytogenes*.

2. Material and methods

The research was conducted over two years in a dairy situated next to a sheep-breeding farm with a flock of 800 sheep in Sardinia, (Sassari). For two consecutive years (2005-2006) samples of primary materials, semi-finished goods and final products were taken. These were monitored four times a year in different periods: February, March, April and May.

During the course of each monitoring, 6 samples were taken (Raw milk, curd, curd 48 h, cheese 30 days, cheese 90 days, cheese 180 days), and the exams done (*Salmonella* spp, *Listeria monocytogenes*, coagulase positive staphylococci, *Escherichia coli*).

The taking of samples for analysis was carried out as follows:

- FIL-IDF 50C:1995 (taking samples of milk and milk products).
- APHA,1992 (Taking environmental samples)

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The isolation of *Listeria monocytogenes* was performed by means of a method derived from Norma FIL-IDF 143A:1995 "Lait et produits laitiers: recherché de *Listeria monocytogenes*".

The search for *Salmonella spp* was performed by means of a method derived from the application of the following norms:

- ISO 6785: 1985 "Lait et produits laitiers – Recherche des *Salmonella*", (preparation of samples from milk and its derivatives)

- AFNOR NF V 08-052:1997 AFNOR NF V 08-052 : 1997. Microbiologie des aliments – Recherche des *Salmonella*.

The search for *Escherichia coli* β -glucuronidasi positiva was performed by means of a method derived from the application of the following norms:

- ISO 16649-2 "Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli* – Part. 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl- β -D-glucuronide".

The search for *Staphylococcus coagulase* + was performed by means of a method derived from the application of the following norms:

- UNI 10984 – 2: 2002 "Routine horizontal method for the enumeration of *Staphylococcus coagulase* positive. Plate count technique at 37°C without confirmation of colonies" .

- ISO 6887 - 1: 1997 "Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination".

- FIL-IDF 122B: 1992 "Milk and milk products – Preparations of samples and dilutions for microbiological examination"

- ISO 7218: 1996 "Microbiology of food and animal feeding stuffs – General rules for microbiological examinations.

3. Results and discussion

Matrixes Raw Milk, Curd, Curd at 48 hrs: the course of total Coliform and *Staphylococcus coagulasis*+ parameters, known as contaminators shows a decided improvement compared to the outcome of the two previous years. In these two years, these value parameters are also significant in the analysis of variability.

The same cannot be said for *Escherichia Coli*, which does not always show significant differences when analysed. This may partially be explained by the fact that the presence of this micro-organism (with faecal origin) shows values which are never worrying, not even in the first year (this testifies the firm's good hygiene level from the beginning). Therefore, improvement which was verified during the second year could not affect the final result.

It should be underlined that for the 3 parameters above, both in the first and second year, contamination values were reduced to zero. This was already noted on the control carried out in the first month of maturation, maintaining the same value up to the end of maturation. As for the *Salmonella spp*, all research of the first and second year continually gave negative results, on all the matrixes concerned.

During the first year *Listeria Monocytogenes* was isolated from raw milk, curd, curd at 48 hrs and cheese brine. Findings were verified in three processes in the first year. Unsuccessful₂ isolation in the second year would appear to indicate an improvement in Firm hygiene.

The a_w values of the product, at the end of ripening time, were about 0,820.

4. Conclusions

The data collected on the microbiological characteristics of Fiore Sardo cheese at the end of maturation proves that the product presents unexceptionable characteristics of healthiness with regards to the pathogens considered. This is linked to the technologies of production through salting, smoking and lengthy maturation, which allow us to 'cure' a product which initially had rather high contamination levels and important pathogens like *Listeria Monocytogenes*.

This is also confirmed by the course of the a_w values of the product, which even from the first month of maturation present values which are decidedly incompatible with the survival of pathogens like *Salmonella spp* and *Listeria Monocytogenes*. These present a limit value of survival of 0.940 and 0.950 respectively.

Table 1: *Listeria monocytogenes* in 25g of product in the 4 manufacturing processes in 2005

2005	Raw milk	Curd	Curd 48 h	Cheese 30 days	Cheese 90 days	Cheese 180 days
Februar	+	+	+	-	-	-
March	+	+	+	-	-	-
April	+	-	-	-	-	-
May	+	-	-	-	-	-

Table 2: *Listeria monocytogenes* in 25g of product in the 4 manufacturing processes in 2006

2006	Raw milk	Curd	Curd 48 h	Cheese 30 days	Cheese 90days	Cheese 180 days
February	-	-	-	-	-	-
March	-	-	-	-	-	-
April	-	-	-	-	-	-
May	-	-	-	-	-	-

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III-P134: Physicochemical and Sensorial Properties of Hand Made Cheeses from La Gomera (Canary Islands, Spain)

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Summary

In this study, the characterization of the traditional cheese of La Gomera has been made. Cheeses were cylindrical with about 1 kg of weight. There were not significant differences in chemical cheese composition or in pH. The rinds of cheeses are homogeneous with brownish-yellow colours due the smoking process. Cheeses with sheep milk were more elastic and had oral sensations with fresh butter flavour. In texture the geometrical characteristics are mainly floury. Predominant odour and flavour was caramel and burn wood together with milky and citric acid. Taste was also sour (citric taste more than milky sour), medium salted and lightly bitter.

1. Introduction

The origin of the cheese is similar to that in the rest of the Canary Isles. The primitive settlers had sheep and goats, so it is certain that some form of preserving the milk were used. The way of making cheese is similar to that used by the Spanish conquerors, although it has been adapted to an island situation. The farms had semi-extensive management; the size of the herd was between 150 and 300 animals. The animals graze on properties which either belong to the farmer or have been rented, in many cases the pasture is composed of spontaneous grasses and also edible species of autochthonous bush. Most farms are mixed arable and livestock and the diet is complemented with arable sub-products. In arid zones the animals frequently eat piteras (*Agaves spp*), tuneras (*Opuntias spp*), vinagreras (*Rumex lunaria*) etc.

Cheeses are made with raw goat milk or a mix of goat and sheep milk, still some farmer's use natural kid's rennet. All cheeses are smoked with either the woody stalks from heather (*Erica arborea*) and jara (*Cystus spp*).

There is a low level of mechanisation compared with other Canary islands. Gomeran cheese is made using natural rennet and a manual press. Most cheeses are consumed fresh although there is a good market for semihard cheeses.

2. Material and methods

A wide enquire of nearly all La Gomera goat and sheep farmers were completed (Fresno et al, 2005). From these producers, 20 were chosen (90% of the cheese-makers with sanitary registration). Two types of cheeses (goat milk; goat + sheep milk) were analysed at two ripening periods, fresh (3-5 days) and semihard (20-30 days) for physicochemical (Instalab 600), textural (Texture Expert Exceed Analyzer XT2i), colour (Minolta CR-400) and sensorial characteristics. Statistical methodologies were made with SPSS 11.0.

3. Results and discussion

Cheeses were cylindrical with about 1 kg of weight. There were not significant differences in chemical cheese composition or in pH (data not shown). The rinds of cheeses were homogeneous with brownish-yellow colours due the smoking process. Fresh cheeses showed higher lightness and colour tone but lower yellow intensity (Table 1). Semi-hard cheeses were more fracturable and cohesive and presented higher values for hardness and gumminess parameters while cheeses with sheep milk were more elastic. Analysing the sensorial profile (Table 2) goat cheeses were more firm, adhesive and astringent while cheeses with sheep milk were sweeter and had oral sensations with fresh butter flavour. Semi-hard cheeses showed higher roughness and pungent values but were less sweet and drier. In texture the geometrical characteristics are mainly floury. Predominant odour and flavour was caramel and burn wood together with milky and citric acid. Taste was also sour (citric taste more than milky sour), medium salted and lightly bitter.

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Table 1: Effects of ripening and type of milk on textural and colour characteristics

	type of milk(m)		ripening(r)		RSD	Level of significance		
	goat	goat + sheep	3-5d	20-30d		Effect(m)	Effect(r)	m x r
	LSM					Effect(m)	Effect(r)	m x r
L	68.93	63.35	72.53	59.74	2.71	0.259	0.019	0.518
C*	30.13	33.69	31.19	32.63	1.21	0.239	0.624	0.820
H*	79.83	83.54	85.55	77.82	1.62	0.274	0.034	0.957
a	5.50	3.95	2.55	6.89	0.85	0.365	0.022	0.892
b	29.38	33.33	30.96	31.75	1.19	0.190	0.787	0.888
Fracturability	87.75	68.56	52.89	103.41	9.18	0.207	0.004	0.517
Hardness	201.69	166.92	115.21	253.41	24.39	0.371	0.003	0.495
Cohesiveness	0.14	0.13	0.12	0.15	0.01	0.486	0.005	0.462
Adhesiveness	1.92	2.48	2.56	1.84	2.10	0.282	0.168	0.928
Elasticity	53.08	63.21	59.63	56.66	56.28	0.010	0.385	0.038
Gumminess	1503.84	1522.25	851.79	2174.29	215.04	0.959	0.003	0.899

LSM: Least square mean.

RSD: Residual standard deviation.

Table 2: Effects of ripening and type of milk on sensorial characteristics

	type of milk(m)		ripening(r)		RSD	Level of significance		
	goat	goat + sheep	3-5d	20-30d		Effect(m)	Effect(r)	m x r
	LSM					Effect(m)	Effect(r)	m x r
Roughness	2.32	2.08	1.97	2.43	0.10	0.197	0.023	0.311
Moisture	2.35	2.77	2.99	2.14	0.21	0.222	0.024	0.086
Elasticity	2.04	1.67	2.27	1.45	0.26	0.494	0.148	0.382
Firmness	4.34	2.38	3.04	3.68	0.35	0.014	0.365	0.666
Friability	5.31	4.65	4.96	5.00	0.14	0.142	0.937	0.242
Adhesivity	4.11	3.15	3.52	3.74	0.19	0.002	0.386	0.002
Solubility	4.72	4.97	4.74	4.95	0.17	0.565	0.637	0.547
Granulosity	1.95	1.73	1.75	1.94	0.10	0.339	0.403	0.403
Sweetness	0.09	0.52	0.61	0.00	0.11	0.041	0.007	0.041
Bitterness	0.96	0.65	0.67	0.94	0.20	0.550	0.584	0.658
Acidity	2.69	1.80	2.61	1.89	0.22	0.079	0.144	0.727
Pungent	1.82	1.51	1.03	2.30	0.25	0.572	0.034	0.568
Astringent	0.81	0.00	0.42	0.39	0.13	0.007	0.881	0.881
Taste persistence	4.93	4.83	4.14	5.63	0.25	0.827	0.006	0.548
Odour intensity	4.74	4.47	4.31	4.90	0.18	0.537	0.190	0.413
Flavour intensity	5.17	4.72	4.47	5.43	0.21	0.331	0.051	0.212

LSM: Least square mean.

RSD: Residual standard deviation

4. Conclusions

As it is common for hand - made cheeses, there were differences between them; but with the results of this research it will be possible to establish a protocol to get a quality label protection, as an AOC, for these traditional, singular and high quality products.

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III-P135: Evaluation of Oxidative Changes of Goat Milk Powders by Sensory and Analytical Tests

B. Hart¹, J. Jetten²

Summary

The aim of this work was to study the oxidative changes in goat milk powder during storage. Fresh produced full cream goat milk powder and skimmed goat milk powder were stored at -20°C (control) and 30°C up to 307 days. At several moments during storage the milk powder itself and recombined milk made from the milkpowders were organoleptically evaluated. The powders were analysed on peroxide value, free fatty acids, volatile components and the use of oxygen. From the analyses performed, it can be concluded that levels of aldehydes and ketons in combination with the rate of oxygen decrease can be useful to predict the shelflife of goat milk powders.

1. Introduction

Goat milk and goat milk powders are becoming important ingredients in today's food industry. Due to the specific fatty acid composition of goat milk fat, it is often thought that goat milk products are very prone to the formation of off-flavours. In this study we evaluated the aging of goat milk powder by sensory and analytical tests.

2. Material and methods

Freshly produced full cream goat milk powder (FCGMP-fresh), skimmed goat milk powder (SGMP-fresh) and 10 month old full cream goat milk powder (FCGMP-old) were stored at -20°C and 30°C up to 307 days.

At day 0, 6, 12, 147 and 307 the powders were analysed on:

- Sensory changes: presence of rancid and lipolysis notes in powders and recombined milk (10% (w/w) solution) were sensorially evaluated on rancidity (card board-like) and lipolysis (acid, cheese-like) notes by 4 experienced pannellists.
- Peroxide value (only for full cream milk powder) by method of Wheeler. Fat was isolated by solvent extraction and determined according to NEN-ISO 3960 (1998).
- Free fatty acids and other volatile components were isolated by a combined steam distillation-extraction according to Likens and Nickerson. The extracts were methylated and analysed with GC-MS using an apolair DB5-MS column (30m x 0.25mm, df 1.0µm).
- Oxygen consumption: milk powders were packed in glass containers with and without headspace. At day 0, 6, 12, 47, 146, 243 and 286 the oxygen concentration was measured by an optical technique developed by TNO (OxySense™).

3. Results and discussion

Rancid taste notes were reported for some solutions after 147 days at 30°C. Rancid odor notes for powder as well as solutions were reported later. Lipolysis-notes were hardly reported for any of the samples.

The peroxide values are shown in table 1. In samples marked with * an off-flavour in the solution was reported. It is clear that the relation between peroxide values and reported flavours is not consistent. This is a well known fact.

The most common volatile components in all samples were C6-, C8-, C10-, C12- and C14-acids. In SGMP C16-acid and C18:1-acid were main components too.

In all goat milk powders various aldehydes and ketones were formed during storage. These

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components are resulting from lipid oxidation. The formation started earlier in FCGMP ($t=147$) at 30°C than in SGMP ($t=307$) at -20°C . Other examples of components that increased are: butanoic acid methylester, 2-octanon, octanal, 2-nonenal, 2-decanal. The concentration of hexanal, a well known lipid oxidation marker, increased during storage at 30°C for both powders.

Decrease of oxygen concentration was as expected detected at first in samples without headspace at 30°C . At -20°C (reference condition) the usage of oxygen is very low but still noticeable.

In figure 1 the oxygen concentration for the samples stored at 30°C without headspace is shown.

The samples could be divided in two groups: stable and unstable samples. Once decrease of oxygen concentration was noticed, the rate of usage of oxygen seemed to be about $0,02\%$ O_2/day for the unstable samples ($R^2=0.99$ and 0.97). For stable samples the rate of usage of oxygen was at least 3 times lower (0.007% O_2/day , $R^2= 0.81$ and 0.77).

From the data it was calculated that for containers without headspace, usage of oxygen for fresh samples could be measured after 83 days and for old samples already after 12 days.

Table 1: Peroxide values (mmol/kg fat) in fresh and old FCGMP during storage at -20°C and 30°C for various days

Storage time:	0 days	6 days	28 days	147 days	307 days
FCGMP 30°C fresh	< 1.0	< 1.0	4.9	4.3* / 4.8*	3.7*
FCGMP -20°C fresh	< 1.0	< 1.0	<1.0	0.5 / 1.1	4.8
FCGMP 30°C old	<1.0	2.0	<1.0	3.6*	7.4*
FCGMP -20°C old	not analysed	<1.0	<1.0*	1.3	1.2

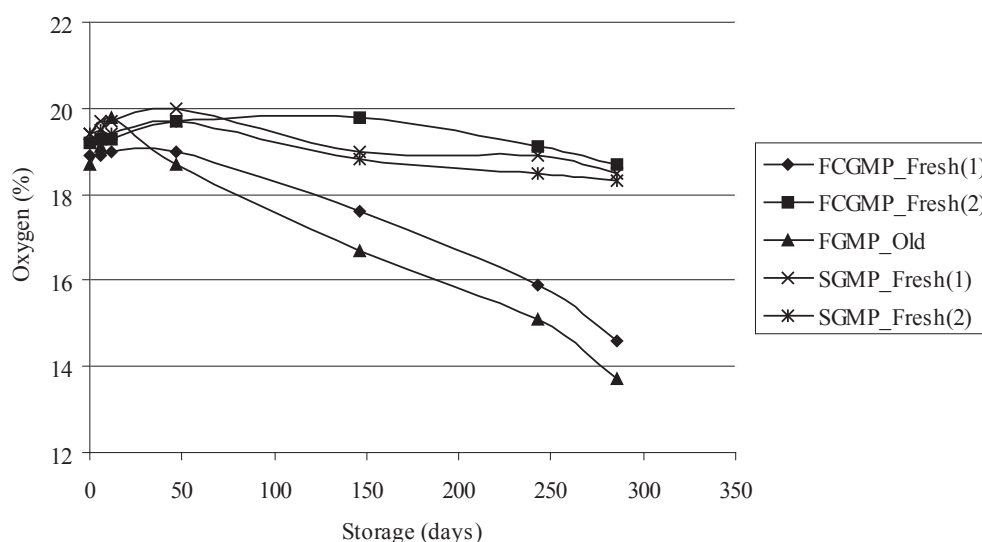


Figure 1. Oxygen% in samples stored at 30°C in containers without headspace.

4. Conclusion

Lipid oxidation is the most important cause of deterioration of goat milk powders. Peroxide values, although being a standard quality parameter, do not give adequate information on sensory quality of goat milk powders. Levels of aldehydes and ketones in combination with the rate of oxygen decrease, can be useful to predict the shelf life of goat milk powders. More research needs to be done on the observed variation the influence of variation between product batches

III-P136: Effect of Whole Citrus Inclusion in The Diet of Lactating Ewes on Cheese Characteristics and Changes During Ripening

D.P. Jaramillo¹, T. García¹, B. Guamis¹, M. Rodríguez², A.J. Trujillo¹

Summary

The aim of this study was to evaluate the effect of whole citrus inclusion in the rations of milking ewes on cheese characteristics. Ewes were distributed in 4 groups that received diets containing citrus at 0, 10, 20 and 30% of dry matter. Cheese composition, colour (CIELab) and microbiology analysis were performed during ripening. Chemical composition changed during cheese ripening as expected: total solids, fat and nitrogen increased, and pH decreased. The inclusion of 30% of citrus in the diet lowered fat and dry matter content of cheeses ($P < 0.05$). Soluble nitrogen and total free amino acids increased with ripening but differences were not significant between cheeses. Colour *b* value increased with the amount of citrus and ripening time, whereas a value was higher in control cheese ($P < 0.05$). Microbiology analysis showed that total counts decreased after 30 days of ripening and was not influenced by diet. The inclusion of citrus did not affect negatively the overall characteristics of cheeses.

1. Introduction

Crops waste reduction has become an important issue in order to reduce environmental problems and management costs. Citrus by products has been tested and offered as ruminant feeds mainly as fresh citrus pulp, citrus silage or dried citrus pulp (Bampidis and Robinson, 2006). Whole fresh citrus has been less studied; however it can be included in the diet of lactating ewes. For this purpose our aim was to evaluate the inclusion of whole fresh citrus in the diet of lactating ewes on the cheese characteristics. Changes in cheese composition, microbiology and colour were evaluated through ripening.

2. Materials and methods

A flock of 28 ewes (Guirra breed) were distributed in 4 homogeneous groups and fed with isoenergetic and isoprotein diets containing 0 (Control), 10 (Diet 1), 20 (Diet 2) and 30% (Diet 3) of dry matter of whole citrus replacing cereal grain and beet pulp. Bulk milk was collected three times from each group and was pasteurized before cheese productions (63°C, 30 min). Manchego-type cheeses (control, CH1, CH2 and CH3) were elaborated according to the procedure described by Trujillo et al. (1999) and cheese samples were collected at day 0, 15, 30 and 60 of ripening. Cheese chemical composition (fat, F; total nitrogen, TN; dry matter, DM; pH; soluble nitrogen, SN; and free amino acids, AA) was evaluated according to standard methods, whereas colour analysis (CIELab scale) was performed using a MiniScan Colorflex (Hunter Associates Laboratory Inc., Virginia, USA). Cheese microbiology was performed for total counts, lactobacilli, lactococci, *Enterobacteriaceae*, enterococci and *Micrococcaceae*. Data was subjected to statistical analysis using the SAS program including in the model the fixed factors: diet, days of ripening and batch production and the evaluated dependent variables.

3. Results and discussion

Table 1 shows the physico-chemical characteristics of cheeses at days 1 and 60 of ripening. Significant differences were found at the end of ripening in fat and dry matter content of CH3, and lower pH value was observed for CH2. Lower fat and dry matter contents of CH3 was expected since milk from the group of animals fed with 30% of citrus had less fat content. Cheeses pH

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and water content decreased during ripening, whereas SN and AA increased due to the residual chymosin and bacterial enzymatic activity. At the end of the ripening period there were not statistical differences in SN and AA between cheeses. Evaluation of cheese colour showed that with the increase of citrus in the diet the *b* value increased and the *a* value decreased (Table 2). The total colour difference observed in the experimental cheeses vs. control could be related to the high content of carotenoids in the citrus fruit, which could be transferred to the milk through the diet. Colour changes during ripening were significant for all the cheeses. In general, cheese microbiology after 30 days of ripening was similar in all the evaluated samples. Total counts, lactococci and *Micrococcaceae* decreased during this period, while *Enterobacteriaceae* decreased up to not detectable levels. Lactobacilli and enterococci increased during the first 15 days and then remained constant.

Table 1: Chesse physico-chemical composition (% DM) during ripening

Cheese	DM (%)		TN (%)		F (%)		pH	
	1	60	1	60	1	60	1	60
Control	49,85±1,08 ^{a,b}	70,57±0,84 ^a	6.72±1.15	5.79±0.06	54.34±2.97 ^a	55.95±2.71 ^a	5,42±0,14	4,90±0,04 ^a
CH1 (Diet 1)	50,86±1,52 ^a	69,61±1,44 ^a	6.74±0.96	5.76±0.34	52.87±2.51 ^a	54.58±1,96 ^a	5,40±0,05	4,87±0,06 ^a
CH2 (Diet 2)	49,25±1,15 ^{a,b}	70,79±1,89 ^a	6.95±1.09	5.74±0,47	50.90±5.52 ^{a,b}	54.61±2,28 ^a	5,38±0,13	4,81±0,05 ^b
CH3 (Diet 3)	47,86±1,71 ^b	67,83±3,33 ^b	7.32±1.31	5.99±0,64	48.01±5.07 ^b	50.71±3.73 ^b	5,48±0,04	4,90±0,03 ^a

^{a,b} means within columns without a common superscript are significantly different (P<0.05)

4. Conclusion

Our results showed that the inclusion of whole citrus (up to 30% DM) in the diet of lactating ewes did not affect negatively the characteristics of cheeses. The fat and dry matter content of CH3 was lower compared to the control group but similar to values reported for other ewe milk cheeses (~50%DM).

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III-P137: NIR Spectroscopy Application to Pressed Cheese

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Summary

Near infrared spectroscopy (NIR) is the measurement of the wavelength and intensity of the absorption of near-infrared light by a sample. Near-infrared light spans the 4000 – 10.000 cm⁻¹ range and it is energetic enough to excite overtones and combinations of molecular vibrations to higher energy levels. However, there have only been a few cases where results for the non-destructive assessment of major constituents have been presented. The purpose of the present study was to evaluate the use of NIR spectroscopy for the non-destructive quantification of major constituents of Piacentinu Ennese cheese. The data collected were evaluated by chemometric software using about the 70% of the samples to calibrate and the other 30 % to confirm the method. R-Coefficient was for fat 0.95, protein 0.97, salt 0.99 and moisture 0.99 respectively. The calibration range [%] of measure used to predict the results of Piacentinu Ennese was for fat 22.25-41.00, protein 23.33-34.36, salt 0.1-7.30, moisture 24.50-51.00.

1. Introduction

Near-infrared spectroscopy has been widely studied for the rapid determination of major cheese constituents such as protein, fat, moisture and total solids (Frank and Birth, 1982; Wehling and Pierce, 1988; Pierce and Wehling, 1994). The objective of this research was to investigate the use of NIR spectroscopy for measurement of fat, protein, and moisture content in Piacentinu cheese samples. Piacentinu Ennese is a hard, raw cheese produced with traditional techniques and traditional tools (Campo and Licitra, 2006). The technology and tools are the same as those utilized for Pecorino Siciliano with the only exception of the added saffron to the milk before coagulation. The aging period is about 1 month for the fresh type called "primosale" (first salt), 2-4 months for the semi-aged and over 4 months for the aged one.

2. Material and methods

Calibrating a near infrared reflectance spectroscopy instrument involved a set of samples to develop a mathematical relationship between spectra generated by the instrument and values obtained from a laboratory reference method. The samples were processed with NIRFlex N-500 (spectral range 4.000-10.000 cm⁻¹ and resolution of 12cm⁻¹) in the reflectance mode. All spectra have been elaborated with NIRCal 5.1 a Chemometric Software by Buchi. The samples at 0 days, 2 and 4 months of ripening were chemically analyzed, according to the AOAC methods, and by NIR spectroscopy using a calibration curve previously obtained with 500 Pecorino PDO cheese samples. The samples were analyzed in glass Petri dishes (100 mm size) at 20°C. Each sample was scanned three times for a total of almost 1000 spectra.

3. Results and discussion

Four reference values were determinate in the experimental samples using official analytical methods: total protein, salt, moisture and fat. The same samples were validated by a specific validation set. These results were used to develop the four single calibration quantitative curves for each parameter (protein, salt, moisture and fat). The calibration methods are reported in figures 1-2-3-4 and in table 1.

4. Conclusion

The calibration assays developed for "protein," "salt" "moisture" and "fat" showed a significant relationship between Official Analytical Chemistry calibration and the measurement obtained by FT-NIR spectroscopy. The sample assay in diffuse reflectance obtained a R value between 0.92

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and 0.99. The most interesting result however was reached in verifying that estimate errors are similar when comparing data obtained during internal validation (i.e. during model optimization) versus data obtained with the set of totally independent external validation.

Table 1: Results relating the calibration

Parameter	Samples set	N. spectra	Range %	R	Sec/Sep %
Protein	C-set	321	23.05 – 33.75	0,96	106
	V-Set	114	23.78 – 32.67	0,97	106
Salt	C-set	312	0.05 – 7.3	0.99	99.60
	V-Set	141	0.07 – 7.13	0.99	99.60
Moisture	C-set	592	24.47-50.95	0.99	87.47
	V-Set	294	24.54-48.06	0.99	87.47
Fat	C-set	327	24.75 – 40.81	0,94	92.83
	V-Set	144	23.87 – 40.97	0,92	92.83

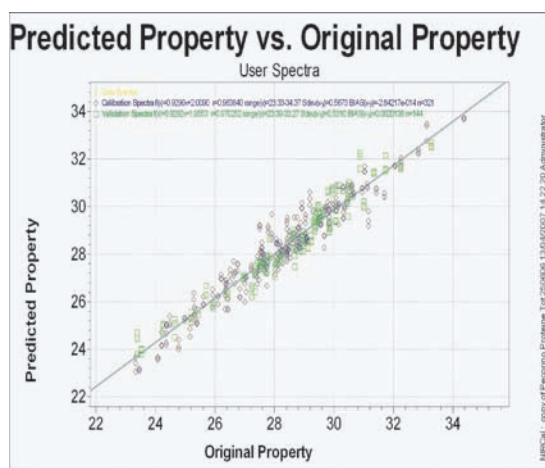


Figure 1. calibration curve of protein.

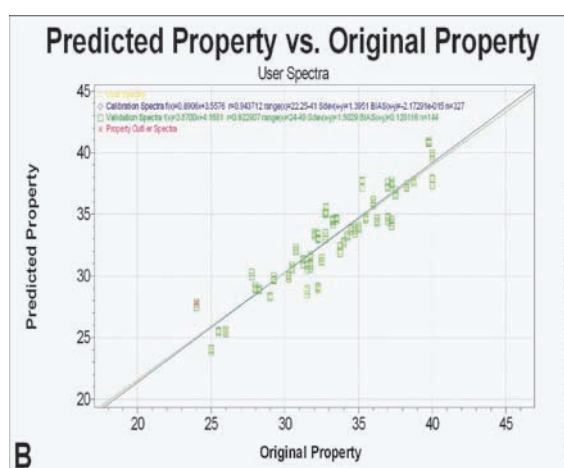


Figure 2. Calibration curve of salt.

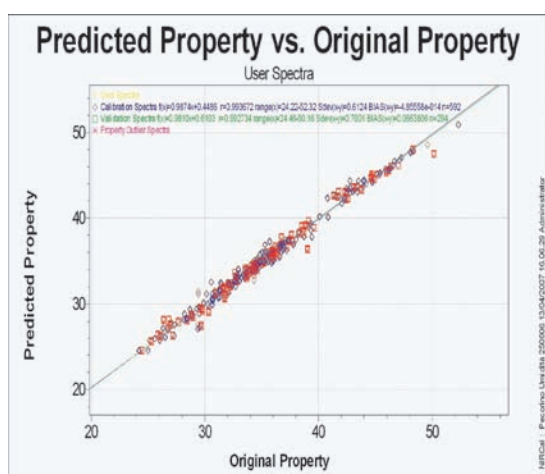


Figure 3. Calibration curve of moisture.



Figure 4. Calibration curve of fat.

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III-P138: Detection and Quantification of Bovine, Ovine and Caprine Milk Percentages in Dairy and Soybean Products Using Electrophoresis and HPLC of Caseins and Whey Proteins

H.K. Mayer¹, I. Schüller¹, M. Moritz¹, B. Raba¹

Summary

Isoelectric focusing (IEF) of γ -caseins according to the EU reference method was well suited to detect cows' milk even in matured cheese, but could not discriminate ewes' from goats' milk. IEF and cation-exchange high performance liquid chromatography (HPLC) of para- κ -caseins was appropriate to differentiate bovine, ovine and caprine milk in mixed cheese. Urea-polyacrylamide gel electrophoresis (PAGE), IEF and reversed-phase (RP) HPLC of whey proteins was useful for the species identification of whey cheeses. The detection of cows' milk percentages in dairy-like soybean products was successfully performed by urea-PAGE, SDS-PAGE and IEF. Thus, electrophoretic and HPLC methods were appropriate for species authentication in milk and soybean products.

1. Introduction

Dairy products made from ewes' and goats' milk are of considerable economic importance, particularly as a result of widespread acceptance of traditional cheeses. However, the substitution of ewes' and goats' milk for cows' milk is a fraudulent practice in the dairy industry. The seasonal changes and the much lower milk yield of ewes and goats, together with the much lower price of bovine milk are the main reasons for this adulteration. As a consequence, an adequate methodology is required to control authenticity of milk and milk products. Moreover, soybean dairy-like products (e.g., soybean milk, soybean infant formulas, yogurt- and cheese-like products), which are an alternative for people suffering from an allergy against animal whey proteins, have to be checked to prevent potential adulterations resulting from the addition of milk proteins to these products and their adverse effects on allergic people (Mayer, 2005).

The objective of the present study was the detection and quantification of bovine, ovine and caprine milk percentages in milk and soybean products using electrophoretic and HPLC techniques.

2. Material and methods

Standard mixtures of milk from different species as well as model cheeses of different ages were analyzed using different electrophoretic and chromatographic methods to study their applicability for the qualitative detection of cows' milk as well as the quantitative determination of bovine, ovine and caprine milk percentages in cheese made from mixed milks. In addition, soybean milk was spiked with different percentages of bovine milk to enable quantitative analysis of milk protein in soybean products. Electrophoretic techniques were performed as described by Mayer (2005). RP-HPLC analysis of soluble whey proteins was carried out according to the IDF-Standard 178 (IDF, 1996) with slight modifications.

3. Results and discussion

Urea-PAGE and anion-exchange HPLC of caseins was restricted to the adulteration control of milk only. The official EU reference method (Commission Regulation, 2001), which is based on the IEF of γ -casein fractions, was a reliable tool to detect cows' milk even in matured cheeses made from milk of other species, but could not discriminate ewes' from goats' milk (Mayer, 2005). IEF and HPLC of para- κ -caseins was appropriate to differentiate bovine, ovine and caprine milk in mixed cheeses (Mayer, 2005). The detection of cows' milk in soybean milk products was

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carried out using different electrophoretic techniques. Separation of proteins according to their molecular weight using SDS-PAGE was suitable to a limited extent only (Fig. 1), due to the risk of false-positive results.

However, both caseins and whey proteins could be used as indicator for the presence of cows' milk in soybean milk products using urea-PAGE (Fig. 2). IEF according to the EU reference method was also applicable, although false-positive results are possible (Fig. 3).

RP-HPLC of whey proteins enabled the differentiation of cows', ewes' and goats' milk as the variant A of bovine β -lactoglobulin had the highest retention time and was used as an indicator for the detection and quantification of cows' milk percentage in milk of other species.

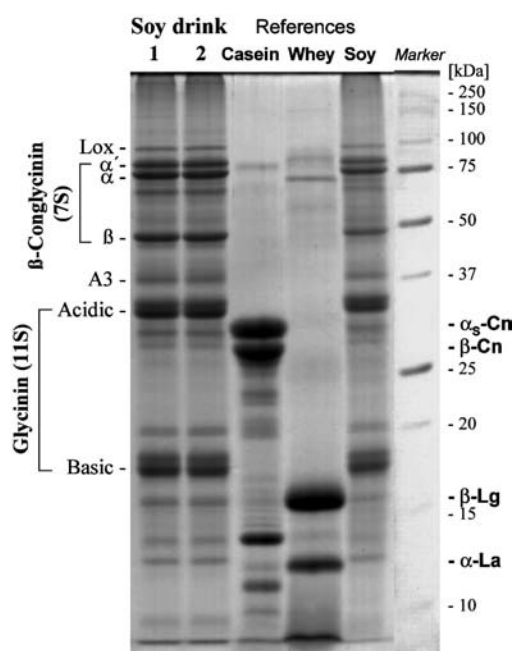


Figure 1. SDS-PAGE of proteins from soybean and bovine milk (caseins and whey proteins).

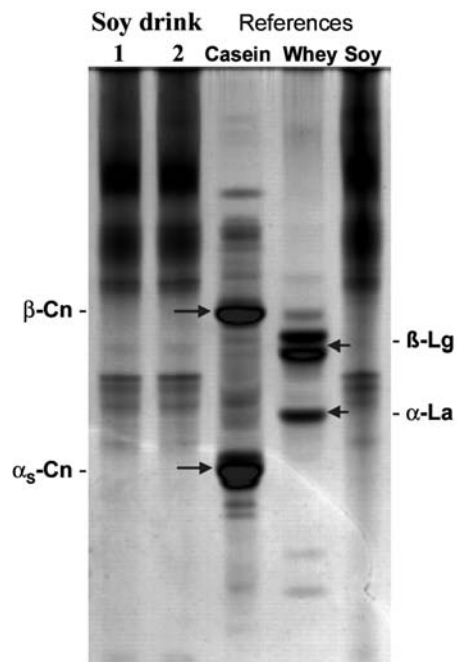


Figure 2. Urea-PAGE of proteins from soybean and bovine milk (caseins and whey proteins).

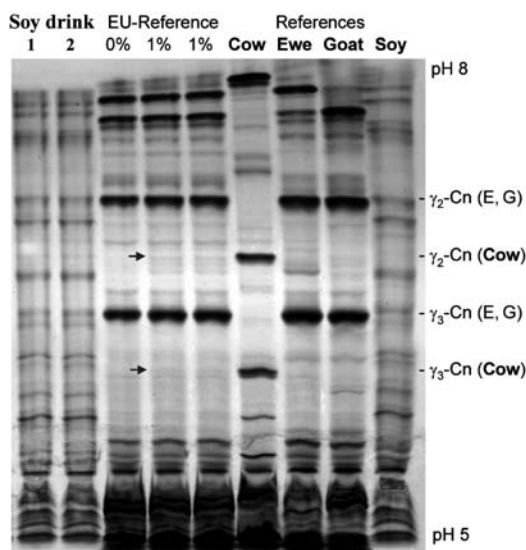


Figure 3. IEF of soybean milk and γ -caseins of milk according to the EU reference method

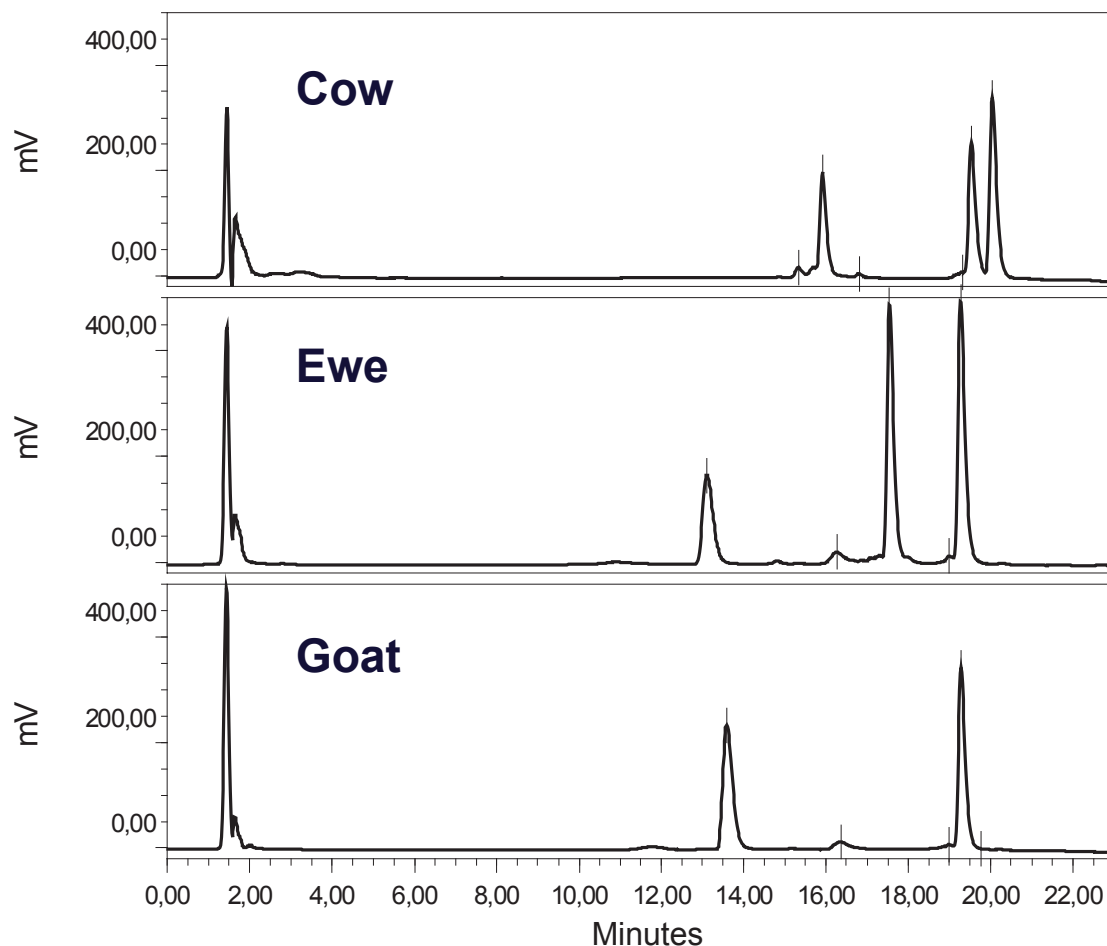


Figure 4. RP-HPLC chromatograms of bovine, ovine and caprine whey proteins.

4. Conclusion

The analytical procedures used were appropriate for the detection and quantification of bovine, ovine and caprine milk percentages in dairy products. Moreover, RP-HPLC and electrophoresis enabled the detection of adulterations of dairy-like soybean products by addition of animal caseins and whey proteins.

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III-P139: RP-HPLC Analysis of Caprine and Bovine Glycomacropeptide to Detect Rennet Whey Solids in Dairy Products

H.K. Mayer¹, B. Raba¹, M. Moritz¹

Summary

A fast, sensitive and reliable reversed-phase high-performance liquid chromatography (RP-HPLC) method was established that enabled not only the separation of α -lactalbumin and β -lactoglobulin in bovine and caprine whey samples, but also simultaneous determination of glycosylated and non-glycosylated caseinomacropeptide (CMP) in milk powder. RP-HPLC analysis of CMP was well suited for adulteration control of dairy products to detect fraudulent admixtures of sweet whey to milk powders and UHT milk. On the other hand, analysis of CMP proved to be an appropriate tool to monitor the percentage of caprine sweet whey powder in goats' milk powder, where it was thought to be a potential ingredient for functional foods.

1. Introduction

Glycomacropeptide (GMP) present in cheese whey (or sweet whey) is the C-terminal hydrophilic part, f(106→169), of κ -casein, from which it is released by the action of the endopeptidase chymosin [EC 3.4.23.4] during renneting of milk. GMP lacks aromatic amino acids (phenylalanine, tyrosine, and tryptophan), and is the sole glycopeptide in milk. Glycomacropeptide has been isolated from bovine sweet whey and its chemical composition has been analyzed. The macropeptides have a rather heterogeneous composition with respect to genetic variation and posttranslational modifications. In addition to the non-glycosylated macropeptides, which contribute to about 50-60% of total CMP, the glycoside fraction (also referred to as glycomacropeptide, GMP) is composed of varying amounts of sugars including N-acetylneuraminic acid (sialic acid), galactose, and N-acetylgalactosamine. At least five different O-glycosidic saccharides are supposed to be randomly linked to four threonine residues (131, 133, 135 and 135) or possibly one serine residue (141), varying phosphorylation adds to the high degree of heterogeneity. From the 11 known genetic variants of κ -casein, only the two major variants A and B are commonly present in GMP or sweet whey produced from bulk milk, but each variant inherits the complex series of the post-translational products (Elgar et al., 2000; Thomä et al., 2006). Although there is relatively limited information available concerning chemical and quantitative analysis of caprine GMP, amino acid sequence in goat GMP differs from that of bovine GMP (Silva-Hernandez et al., 2002).

In adulteration control of dairy products, GMP has been used to detect fraudulent admixtures of sweet whey to milk powders and UHT milk (Commission Regulation, 1999). In recent years, GMP has been the subject of growing interest due to its beneficial biological and physiological properties including growth-promoting effects on bifidobacteria, suppression of gastric secretion, binding of cholera and *Escherichia coli* enterotoxin, the inhibition of viral or bacterial adhesion to intestinal epithelial cells, and modulation of immune system (Brody, 2000). Thus, GMP has a number of biological activities, and is thought to be a potential ingredient for functional foods and pharmaceuticals.

The objective of the present study was to establish a fast and reliable RP-HPLC technique to determine the glycosylated and non-glycosylated caseinomacropeptide in bovine and caprine milk powder.

2. Material and methods

Samples of authentic sweet and acid whey of cows' and goats' milk were prepared as references. Standard mixtures of either cows' or goats' milk powder containing increasing concentrations

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of sweet whey powder were prepared on a laboratory scale. RP-HPLC analysis of glycosylated and non-glycosylated GMP was carried out according to the method described by Thomä et al. (2006) with slight modifications.

3. Results and discussion

A sensitive and reliable RP-HPLC method was developed to enable not only the determination of α -lactalbumin and β -lactoglobulin in bovine and caprine whey samples, but also simultaneous measurement of glycosylated and non-glycosylated CMP in milk powder. Using an optimized time table gradient, the main fractions of bovine rennet whey were separated within 30 minutes (Fig. 1). The overall elution order was: glycosylated CMP (GMP) < non-glycosylated CMP variant A < non-glycosylated CMP variant B. In contrast to the results of Commission Regulation (1999), the resolution was remarkably enhanced with respect to the separation of the non-glycosylated and glycosylated CMP. Figure 2 shows the relationship between total CMP peak area and rennet whey powder percentage in cows' milk powder. A typical RP-HPLC chromatogram of caprine rennet whey is shown in Figure 3. Although caprine CMP peak area was smaller in comparison to bovine sweet whey, a proper calibration curve was established when plotting the total CMP area against the whey powder percentage in goats' milk powder (Fig. 4).

RP-HPLC analysis of CMP is appropriate for adulteration control of dairy products to detect fraudulent admixtures of sweet whey to milk powders and UHT milk. Furthermore, CMP percentage may be checked in milk powders due to its beneficial biological properties.

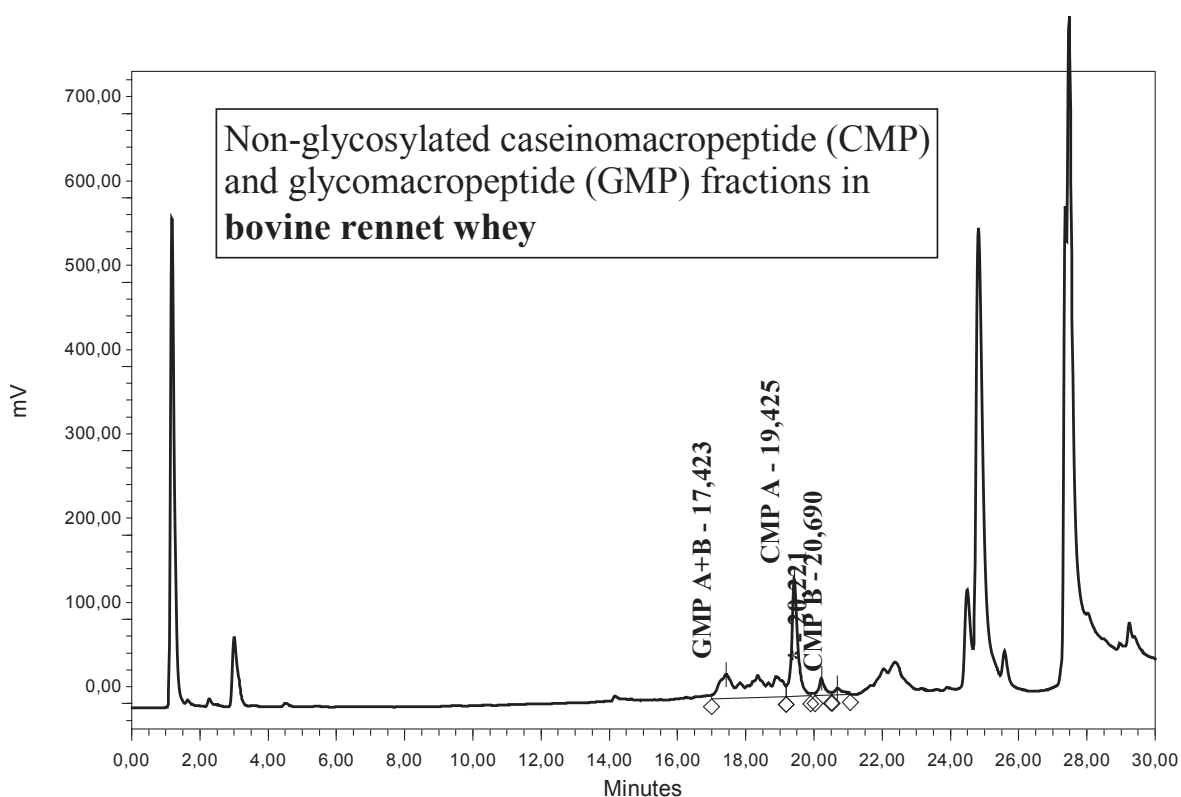


Figure 1. RP-HPLC chromatogram of bovine rennet whey.

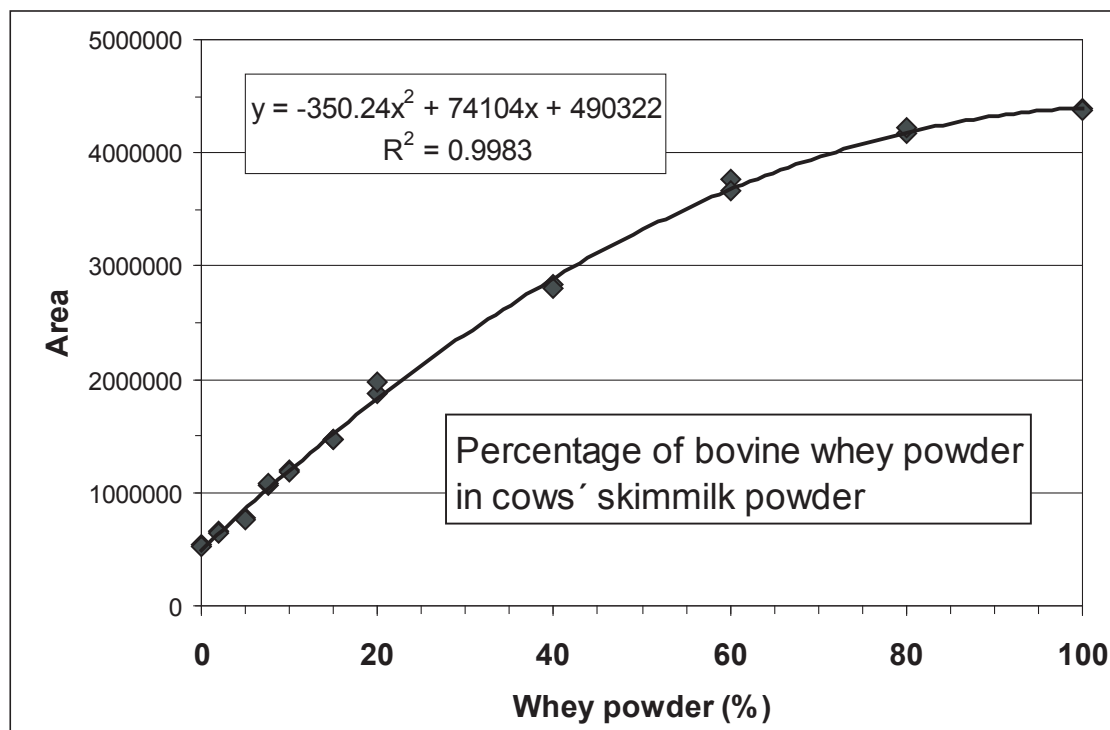


Figure 2. Relationship between CMP peak area and rennet whey powder percentage in cows' milk powder.

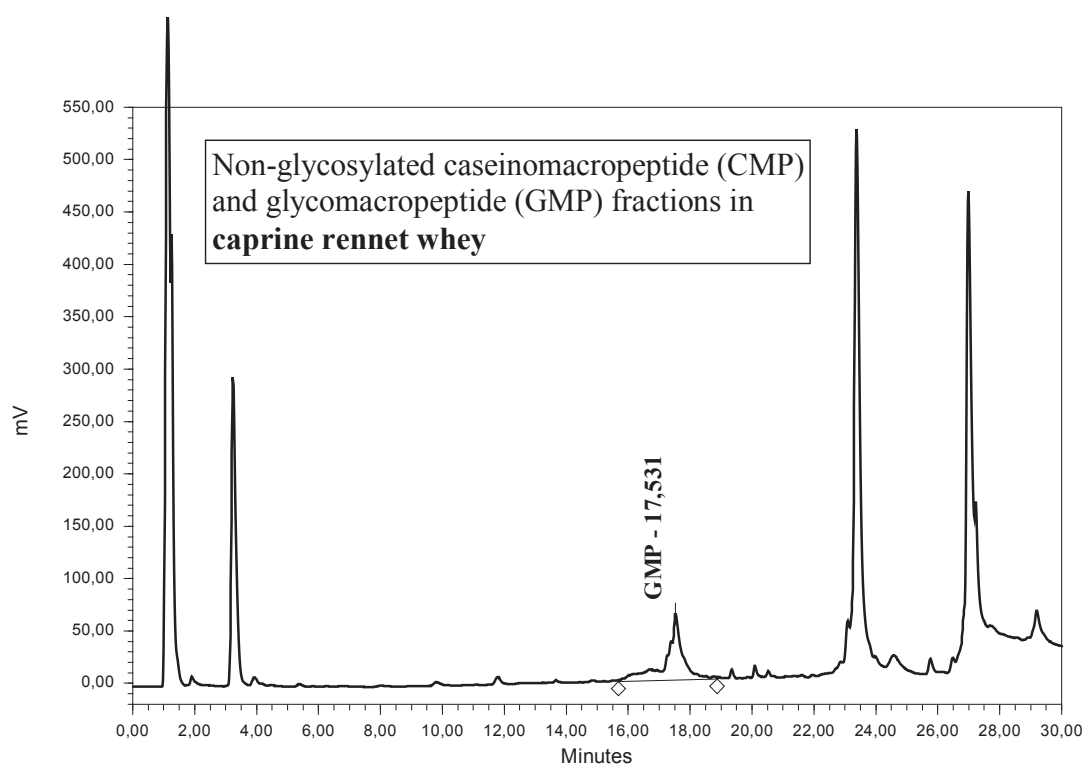


Figure 3. RP-HPLC chromatogram of caprine rennet whey.

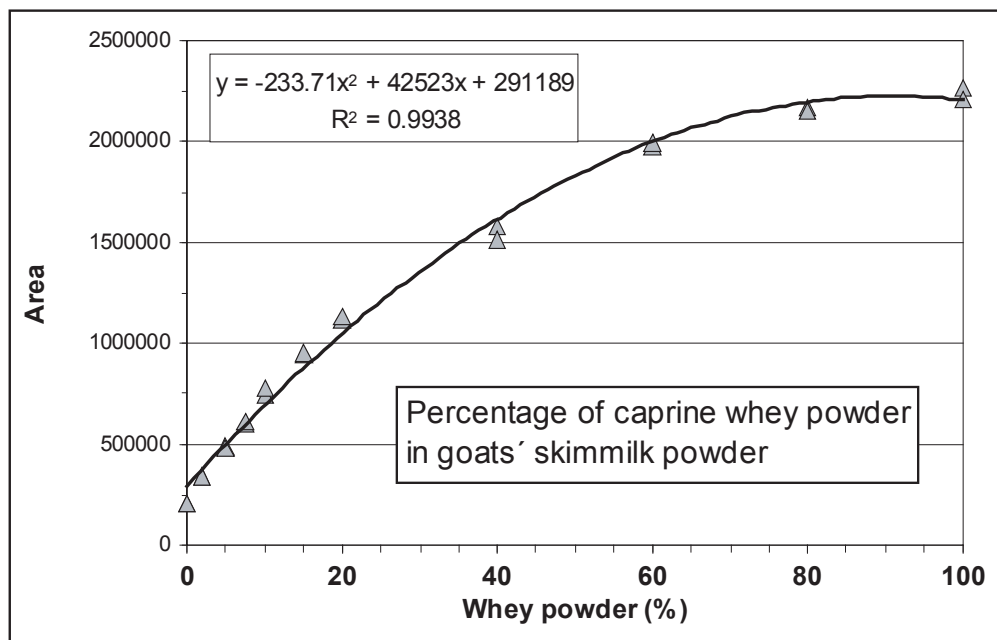


Figure 4. Relationship between CMP peak area and rennet whey powder percentage in goats' milk powder.

4. Conclusion

RP-HPLC analysis of CMP proved to be an appropriate tool to monitor the addition of a certain concentration of caprine sweet whey powder to goats' milk powder, where it was thought to be a potential ingredient for functional foods.

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III-P140: Fatty Acid Composition of Milk and Cheese from Sheep Fed Rough or Cultivated Pasture

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Summary

Aim of this paper was to investigate the fatty acid (FA) composition of milk and two month old cheese obtained from the same flock of Sarda ewe fed two different types of pasture: mixture of *Lolium perenne* and *Trifolium squarrosum* (P1) and rough pasture mainly consisting of botanical families different to legumes and grass (P2). Milk and cheese from ewe fed P1 pasture resulted in a high content of medium chain FA, saturated FA (SFA), CLA, and vaccenic acid (VA), and in a lower content of long chain FA and PUFA. The effectiveness of the FA composition to discriminate between dietary regimens was confirmed by the factorial analysis performed on 13 individual FA. Two main factor were extracted that explained more than 90% of the total variance. The first factor was positively associated to SFA, VA and CLA, while the second factor with palmitic, oleic, linoleic, and alfa-linolenic acid.

1. Introduction

A number of studies have highlighted the role of pasture based diet in affecting the composition of milk and cheese from dairy ruminant species, with emphasis on the content of conjugated linoleic acid (CLA) and polyunsaturated fatty acids (PUFA) (Pulina et al., 2006). Nevertheless, the contribute of different kind of pasture has been less studied. In dairy sheep, differences in milk fatty acid composition has been previously reported when ewes grazed different kind of cultivated pasture (Addis et al., 2005). Aim of this paper was to investigate the fatty acid (FA) composition of milk and six month old cheese obtained from the same flock of Sarda ewe fed two different types of pasture.

2. Material and methods

A Sarda ewe flock grazed on 2 different types of pasture: a cultivated pasture consisting of a mixture of *Lolium perenne* and *Trifolium squarrosum* (P1) and a rough pasture (P2) consisting of (on dry matter basis) 15% Gramineae family; 8% Pea family; 77% other families, including 61% Crucifers, 5% Polygonaceae; 5% Ranunculaceae. Each grazing cycle lasted 3 weeks. The milk obtained during the last week of each cycle was used to produce a 6 months old cheese (Caciotta). Three bulk milk samples were collected for analysis the day before the cheese making. Three Caciotta cheeses from the same batch of production, were collected on the day after cheese making and after 1, 2, and 6 months of ripening. Milk and Cheese fat was extracted and analysed for FA composition according to Secchiari et al. (2003). Data of cheese FA composition were analysed according to a linear model including pasture (with two levels: LT or R), time of ripening (with 4 levels: 0, 1, 2, and 6 months), and the interaction as fixed factor, whereas milk FA composition was analysed with a linear model including only pasture as fixed effect (SAS, 1999). In order to verify the effectiveness of the FA composition to discriminate milk and cheese obtained from ewes fed different pastures, factorial analysis (SAS, 1999) was performed on 13 selected FA (C4; C10; C14; C15; C16; C18; *anteiso* C15; *iso* C16; *trans*-11 C18:1; *cis*-9 C18:1; *cis*-9, *cis*-12 C18:2; *cis*-9, *cis*-12, *cis*15 C18:3; *cis*-9, *trans*-11 C18:2).

3. Results and discussion

Since, in any case, the FA composition of cheese reflected the composition of raw milk, suggesting any influence of the cheese making procedure and ripening on this parameter, table 1 reported only least square means of the FA composition of cheese from ewes grazed P1 or P2 pasture.

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Cheese from ewe fed P1 pasture resulted in a high content of medium chain FA, odd- and branched-chain FA (OBCFA), rumenic acid (RA, *cis*-9, *trans*-11 CLA), and vaccenic acid (VA, *trans*-11 C18:1), and in a lower content of long chain FA, monounsaturated FA, and PUFA. Since pasture was the only dietary source for the ewes and the FA composition of P1 and P2 pasture was similar (data not shown), the difference in cheese FA composition may be due to changes of rumen environment and bacterial population. According to the literature (Vlaemink et al., 2006), changes in the rumen microbial population are reflected in milk OBCFA. In particular, rumen conditions that promote synthesis of bacterial BCFA are detrimental to bacteria that are involved in the last phase of rumen biohydrogenation of dietary PUFA, leading to an increase in milk of *trans* monoenes and a decrease of C18:0. Therefore, the lower levels of PUFA and the higher levels of OBCFA and VA in cheese from ewes grazed pasture P1 (table 1) may be due to rumen conditions that favoured the bacterial populations hydrogenating dietary PUFA to *trans* monoenes, at the expense to bacteria hydrogenating *trans* monoenes to C18:0. The lower level of C18:0 in cheese from ewes grazing P1 pasture seemed to confirm this hypothesis (7.23 vs. 9.98 g/100 g lipids, $P < 0.01$, for cheese from ewes grazed P1 and P2 pasture, respectively). As a consequence of the higher level of VA in cheese from ewes grazed P1 pasture, RA was higher as well. In fact, these FA were strongly and linearly related ($P < 0.01$). The effectiveness of the FA composition to discriminate between dietary regimens for milk and cheeses obtained from ewes fed different pastures, was confirmed by the factorial analysis performed on 13 individual FA. Two main factor were extracted that explained more than 90% of the total variance (figure 1). The first factor was positively associated to SFA, VA and RA, while the second factor with palmitic, oleic, linoleic, and alfa-linolenic acid.

Table 1: Fatty acid composition of cheese from ewes grazed P1 (mixture of *Lolium perenne* and *Trifolium squarrosum*) or P2 (rough) pasture

	Pasture		SEM	P
	P1	P2		
SCFA	10.14	11.04	0.04	**
MCFA	51.36	46.66	0.33	**
LCFA	31.21	35.43	0.25	**
OBCFA	4.99	3.81	0.02	**
SFA	68.19	67.34	0.37	
MUFA	20.32	21.49	0.16	**
PUFA	4.18	4.29	0.04	*
VA	2.73	2.47	0.03	**
RA	1.41	1.07	0.01	**

$P < 0.05$; ** $P < 0.01$.

SCFA: short chain fatty acids; MCFA: medium chain fatty acids; LCFA: long chain fatty acids; OBCFA: odd and branched chain fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; VA: vaccenic acid; RA: rumenic acid.

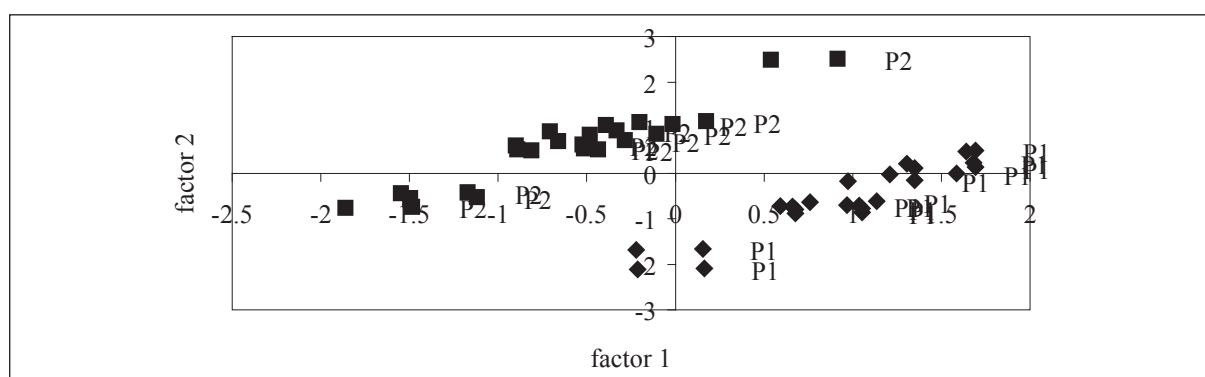


Figure 1. factorial analysis of selected fatty acid composition of cheeses from ewes grazed P1 (mixture of *Lolium perenne* and *Trifolium squarrosum*) or P2 (rough) pasture.

4. Conclusion

In conclusion, the botanical composition of pasture significantly affected the milk and cheese FA composition, probably as a consequence of differences in the energy and protein content of pasture P1 and P2 that led to changes in rumen environment and bacterial population. Moreover, cheese FA composition could be a useful tool to discriminate the dietary regimen of ewes.

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III-P141: Phosphopeptides in Kefalograviera Cheese: Detection and Crude Fractionation

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Summary

The aim of this work was to investigate whether Kefalograviera, a Greek PDO ovine cheese, could be regarded as a potential source of casein phosphopeptides (CPPs) given their possible biological functions on calcium bioavailability and anticariogenic activity.

It was found that the molecular mass of the peptides in concern ranged between 4.0 and 12.5 kDa. Further investigation is needed in order to isolate and characterize these CPPs.

1. Introduction

CPPs are casein-derived peptides that have phosphorus bound, via monoester linkages to seryl residues. Given their highly negatively charged structures arising from phosphorylation, CPPs have the ability to bind a range of macroelements and trace elements [1]. Specific CPPs can form soluble organophosphate salts and lead to enhanced calcium absorption by limiting the precipitation of calcium in the distal ileum. However, more cell culture and human studies are necessary to demonstrate the potential of CPPs to enhance dietary mineral bioavailability and to modulate bone formation. [2]. Another interesting property associated with CPPs is their potential to enhance mucosal immunity. Moreover, it has been shown that Ca-binding phosphopeptides have anticariogenic effects via inhibition of caries lesion through recalcification of the dental enamel [5].

CPPs have been isolated and characterized during ripening of various hard and semi-hard cheeses. The presence of CPPs in the microenvironment of the cheese might improve its nutritional characteristics based on all the aforementioned reasons. Therefore, it was interesting to investigate whether Kefalogra-viera, classified as a hard cheese, could be regarded as a potential source of CPPs.

2. Materials and methods

CPPs were precipitated from the pH 4.6-soluble fraction (NCN) of 3 mo old cheese by addition of 10% (w/v) CaCl₂ to a 100 mM final concentration and ethanol to a 50% (v/v) final concentration. The suspension was centrifuged (12000×g) and the supernatant discarded. The precipitate was freeze-dried and stored at -20°C. The NCN extract was kept also at -20°C. Before RP-HPLC analysis, the lyophilized crude CPP preparation (10 mg) was dissolved in 1 ml of sequencing grade 0.1% (vol/vol) trifluoroacetic acid solution, sonicated for 5 min, and filtered through a 0.45-mm cellulose acetate filter. Reversed-phase HPLC was performed according to [3], except for the gradient. Separations were conducted with eluent A for 5 min and a linear gradient from 0 to 100% of eluent B for 50 min. The column was finally eluted with 100% eluent B for 10 min. For analysis 50 µl of the diluted CPP preparation and 30 µl of NCN were used. A discontinuous polyacrylamide gel electrophoresis (PAGE) followed by staining with "Stains all" reagent was initially used for the detection of CPPs in the enriched preparation. Molecular mass of CPPs was estimated by SDS-PAGE followed by the phosphoprotein staining procedure of Myers et al. [4]. Lyophilized CPP crude preparation (25 mg) was also dissolved in 1 ml of distilled water and chromatographed on a 56×1.6 cm- Sephadex G-50 column using 50 mM NaCl as the eluent at a flow rate of 6 ml/h. Three-ml fractions were collected, and their absorbance was measured at 280 and 214 nm. The column was calibrated using protein standards of molecular mass of 6.25 to 67.0 kDa.

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3. Results and discussion

The RP-HPLC peptide profile of NCN and the crude CPP preparation showed that most of the CPPs were eluted at relatively low acetonitrile concentration. As estimated by SDS-PAGE, the molecular mass of the peptides detected was 4.04, 8.80, 9.20, 10.50, and 12.40 kDa. Fractionation of the enriched preparation using gel filtration chromatography confirmed the above values and revealed that CPPs with a lower molecular mass were also present. Further investigation is needed in order to isolate and characterize these CPPs.

4. Conclusions

Preliminary results indicated that Kefalo-graviera cheese could be regarded as a potential source of CPPs. Further research is needed

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III-P142: Proteolysis and Ace-inhibitory Activity in Kefalograviera Cheese

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Summary

The objective of this study was to monitor proteolysis in Kefalograviera cheese, a Greek ovine PDO hard cheese and to follow the ACE-inhibition during ripening. ACE-inhibition increased with developing proteolysis and decreased when proteolysis exceeded a certain level. These preliminary results indicate that ACE-inhibitory peptides liberated during ripening can be further degraded to inactive fragments. Further investigation is needed to confirm this observation and identify and characterize these antihypertensive peptides.

1. Introduction

The angiotensin I-converting enzyme (ACE, peptidyl dipeptide hydrolase, EC 3.4.15.1) has been associated with the renin-angiotensin system, which regulates peripheral blood pressure. Inhibition of this enzyme can exert an antihypertensive effect [4]. Previous research studies indicate that ACE-inhibitory peptides are liberated during ripening of different cheese varieties [5]. It was therefore interesting to study the relationship between proteolysis and ACE-inhibitory activity in this ovine PDO hard cheese.

2. Materials and methods

Three batches of Kefalograviera cheese were manufactured and cheese samples were analysed for pH, moisture, fat, protein and salt at 2, 4, 6 and 8 months of ripening. Four methods were used to monitor proteolysis during aging. Water-soluble N (WSN) and N soluble in 12% trichloroacetic acid (TCA-SN) were determined in aliquots of the same water-soluble cheese extract (WSE) prepared by cheese:water ratio 1:4 [3]. The concentration of total free amino acids (FAA) in the WSE of the cheeses was determined by the Cd-ninhydrin method of Folkertsma and Fox [2]. All analyses were carried out in duplicate. Cheese samples and their WSE were analysed by urea-PAGE [3].

The ACE-inhibitory activity was measured by the spectrophotometric assay of Cushman and Cheung [1] as modified by Nakamura et al. [6]. For determination 50 μ l of the WSE, diluted 1:10 with water, were used.

3. Results and discussion

The typical physico-chemical characteristics of Kefalograviera cheese are given in Table 1.

Urea-PAGE electrophoretograms of Kefalo-graviera cheese samples of various ages (not shown) demonstrated that in all samples, α s1-casein was hydrolysed to a greater extent than β -casein.

The levels of WSN (%TN), TCA-SN (%TN) and total FAA in Kefalograviera cheeses during aging are given in Table 2. As can be seen, all nitrogen fractions increased during ripening. Furthermore, the ACE-inhibition index (%) is also presented. When the changes in WSN (%TN), TCA-SN (%TN) and ACE-inhibition % of Kefalograviera cheese during ripening were plotted on the same diagram (Figure 1), it was apparent that inhibition increased with cheese maturation but started to decrease after 6 months of ripening. This finding is in agreement with the results of Meisel et al. [5], who also detected higher ACE-inhibitory activities in middle-aged Gouda cheese than in short-termed or long-termed ripened cheese. It is likely that with more extensive proteolysis potent bioactive peptides could be broken further down to less active smaller peptides and free amino acids. Further studies are needed in order to precisely evaluate the relationship between proteolysis and ACE inhibition in the environment of this particular cheese.

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Table 1: Moisture, fat, moisture in non-fat-substance (MNFS), protein, salt in moisture (S/M) and pH values of Kefalograviera cheese ripened for 4 months. Values are means of three batches.

Kefalograviera Cheese	Values
Moisture (%)	37.8
Fat (%)	30.62
MNFS (%)	54.59
Protein (%)	26.08
S/M	7.67
pH	5.49

Table 2: Water-soluble nitrogen (WSN), nitrogen soluble in 12% TCA (TCA-SN), total free amino acids measured by reaction with Cd-ninhydrin at 507 nm (A_{507nm}) and ACE-inhibition index %, during ripening of Kefalograviera cheese. Values are means of three batches

Age of cheese (months)	WSN (%TN)	TCA-SN (%TN)	TCA-SN (%WSN)	Total free amino acids (A_{507nm})	ACE-inhibition index %
2	13.35	8.89	66.59	1.84	22.93
4	14.79	10.76	72.78	2.11	30.79
6	17.89	13.49	75.43	2.18	41.39
8	20.17	15.29	75.80	2.24	38.51

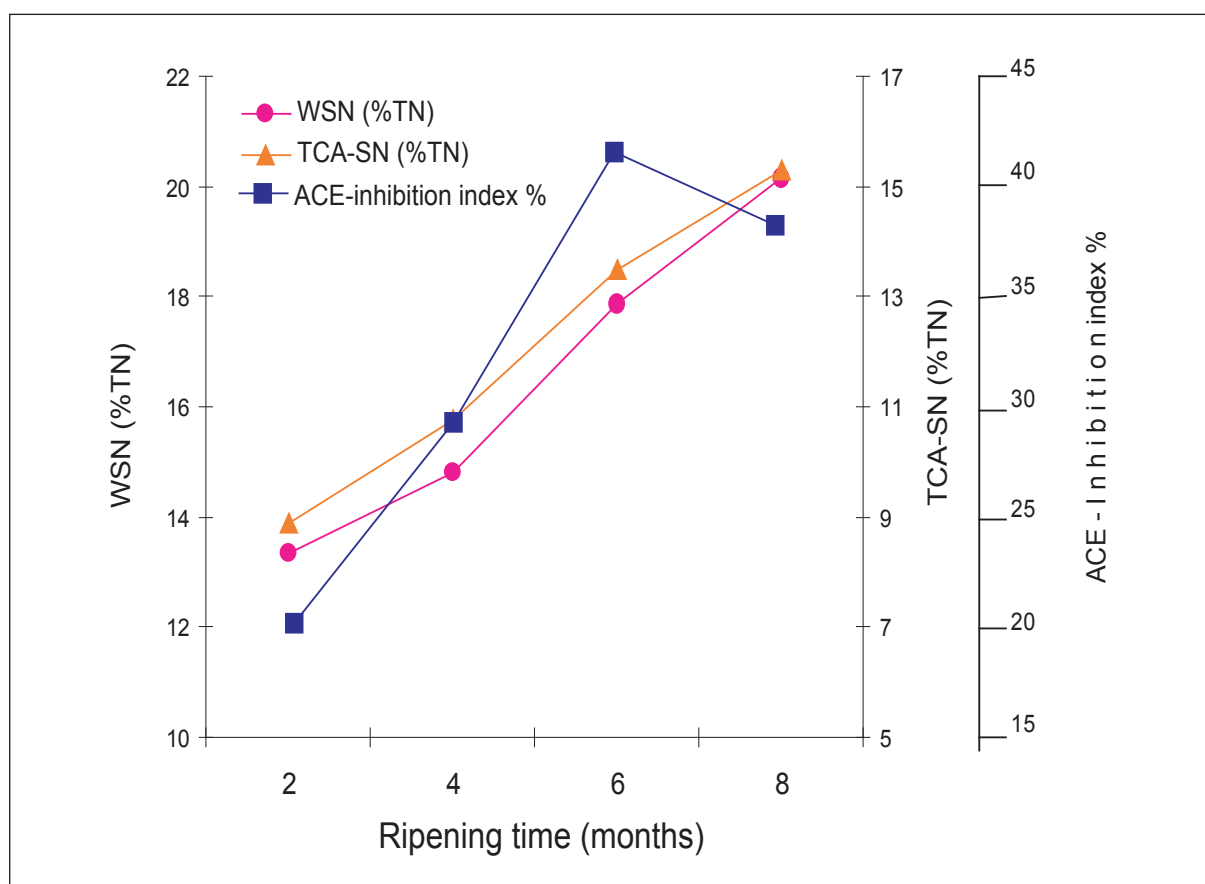


Figure 1. Change of ACE – inhibition index % in Kefalograviera cheese during ripening.

4. Conclusions

The ACE-inhibitory activity increased as proteolysis advanced, however, the bioactivity decreased when proteolysis during ripening exceeded a certain level. More studies are still required to confirm these results and to identify and characterize the peptides responsible for ACE-inhibition.

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III-P143: Genotypic and Phenotypic Diversity of *Staphylococcus Aureus* Isolated from Dairy Products

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Summary

The purpose of this study was to characterize and to analyze the genotypic and phenotypic differences among 122 *S. aureus* isolated from bovine, goat, sheep and buffalo dairy products.

Cow isolates showed a prevalence of β -hemolysis, double hemolysis in goat strains and homogeneous distribution among α , β and double-hemolysis in *S. aureus* isolated from sheep dairy products.

Among the cow isolates, 73% were SE producers; SEA, SED toxins and *sej* gene were more frequently found. 55% of goat *S. aureus* could produce enterotoxins and SEC toxin and *sel* were predominant. A similar toxin pattern was noticed in sheep isolates. Among the new *se* genes, *seg*, *sej* and *sel* were predominant. In all isolates where production of classical SE was identified by SET RPLA, the presence of SE was confirmed by the multiplex PCR technique.

1. Introduction

Staphylococcus aureus is considered the world's third most important cause of food-borne illnesses.

The presence of *S. aureus* in milk and dairy products could represent a serious problem for public health because some strains have the ability to produce a variety of enterotoxins (SEs). Five major antigenic types of SEs have been recognized (SEA-SEE) but in recent years new types enterotoxins genes (*se*) were described (*seg-seu*) (Cremonesi et al 2005).

The present study used a multiplex-PCR to analyze the diversity of *S. aureus* isolated from dairy products and to show the distribution of genes encoding *sea*, *sec*, *sed*, *seg*, *seh*, *sei*, *sej* and *sel* in *S. aureus* isolated from bovine, goat, sheep and buffalo raw milk and milk products. The results of this multiplex PCR (mPCR) method were compared with the corresponding SE shown by reverse passive latex agglutination (SET-RPLA) assay.

2. Material and methods

Identification of *S. aureus* strains

A total of 122 *S. aureus* strains (81 from cow, 22 from goat, 17 from sheep and 2 from buffalo) were isolated from different dairy products (milk, curd, 1–2 month old cheeses, butter and whey).

The strains were isolated using Baird Parker RPF agar and *S. aureus* identification was done by Gram staining, catalase activity determination, heat stable nuclease (TNase) test using Toluidine blu agar.

A miniaturized biochemical system (Biolog) was used to confirm the staphylococcal species.

Haemolysis on blood agar

Haemolytic activity were tested using blood agar.

Detection of *sea*, *sec*, *sed*, *seg*, *seh*, *sei*, *sej* and *sel*

DNA was extracted according to Cremonesi et al. (2006) and genes for classical and recently-discovered SE were searched by multiplex PCR, according to Cremonesi et al (2005).

Production and detection of SEA, SEB, SEC and SED

The ability for enterotoxin production was evaluated used SET-RPLA according to the manufacturer's instructions.

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3. Results and discussion

According to phenotypic properties, all the 122 strains studied were identified as *Staphylococcus aureus* (Data not shown).

***S. aureus* hemolysis on blood agar**

The majority of strains isolated from cow dairy products showed a prevalence of β -hemolysis (62%), while most of the *S. aureus* strains derived from goat (64%) showed a double hemolysis on blood agar.

An homogeneous distribution among α - and β -hemolysis was observed for strains isolated from sheep dairy products. The two strains isolated from buffalo dairy products were β -hemolytic on blood agar.

Detection of *S. aureus* enterotoxins

Comparing the distribution of SE and the presence of *se* genes, a wide diversity was observed among the strains from cow, goat and sheep milk products

Of the strains isolated from cow dairy products, 73% were enterotoxin producers (Figure 1). Among these isolates, we have detected a high prevalence of combination SEA, SED enterotoxins and *sej* gene. Among isolates from goat, 12 over 22 (55%) could produce enterotoxins and SEC toxin and *sel* gene were predominant. A similar toxin pattern was noticed in *S. aureus* isolated from sheep dairy products. The two strains isolated from buffalo did not produce staphylococcal enterotoxins.

Six *S. aureus* from cow and two from sheep were positive for one or more of the recently described *se* genes (*seg* – *sel*) without producing classical SEs or having the corresponding genes.

Among the new *se* genes, *seg*, *sej* and *sel* were predominant in the 122 isolates. *seg* and *sei* in combination were found in 4% of the isolates.

In all isolates, the presence of SE (identified by SET RPLA) was confirmed by the multiplex PCR technique.

In contrast, in 13 isolates (9 from cow, 2 from goat and 2 from sheep) toxin production was not verified of SET RPLA, despite the corresponding gene set was detected by PCR. This result can be explained by a lower sensitivity of the SET RPLA, or by the lack of expression of *Se* genes or activity of the SE toxins (Loncarevic et al 2004).

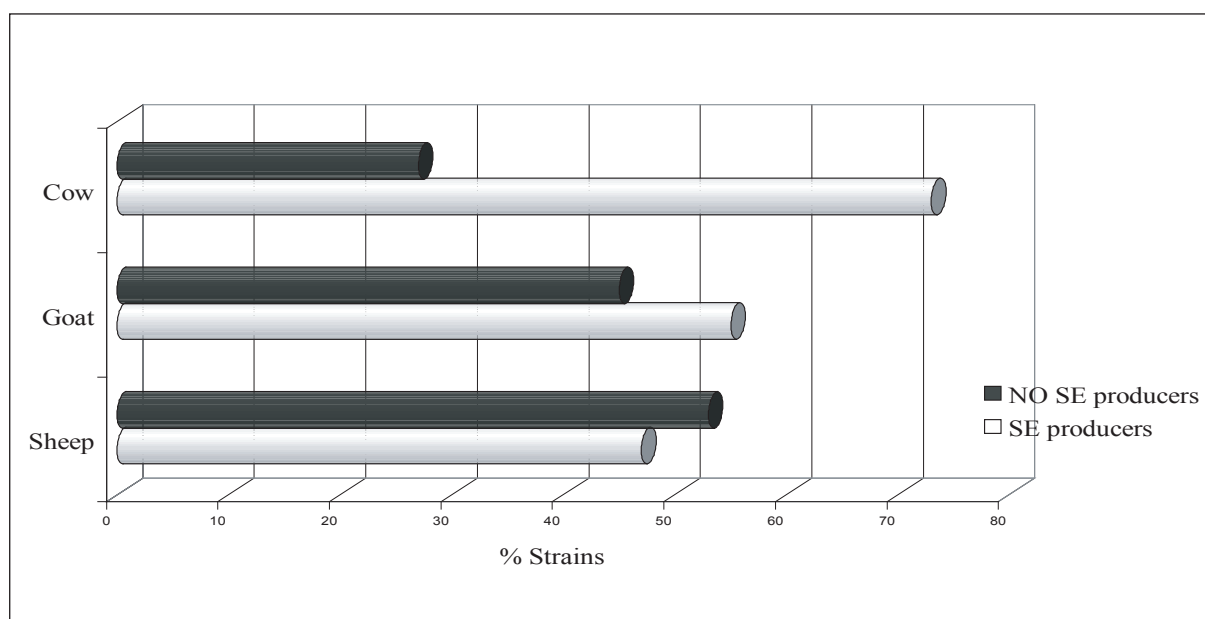


Figure 1. Detection of enterotoxin in *S. aureus* isolated from raw milk and dairy products.

4. Conclusion

The strains of *S. aureus* isolated from cow, goat and sheep raw milk and dairy products have an heterogeneous enterotoxigenic potential.

The immunoassay methods used to detect SE in bacterial strains can detect only classical SE (from SEA to SEE), thus an underestimation of the potential SE producing isolates must be expected if only these methods are used.

However the availability of DNA sequence information on the described *se*, and developed PCR methods, now give the opportunity to evaluate which strains harbor the *seg-seI* toxin genes undetectable by immunoassay.

The question that still remains concerns the evaluation of the effective hazard which these new toxins present and whether the strains harboring *se* produce these toxins in sufficient amount to cause food intoxication.

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III-P144: Seasonal Variation of Vaccenic Acid, Conjugated Linoleic Acid and n-3 Fatty Acids of Goat's Milk Fat and Their Transfer to Cheese and Ricotta

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Summary

The seasonal variation of conjugated linoleic acid (CLA), vaccenic acid (VA) and omega-3 fatty acid (n-3 FA) contents in goat milk and the extent of their transfer from milk to cheese and ricotta fat were investigated. Samples of milk and of the derived fresh cheese and ricotta were collected in two milk processing plants located in Middle-Eastern Sardinia (Italy) every two weeks from early spring (March-April) to early summer (July). Concentrations of CLA and VA did not differ among milk, cheese and ricotta (c9,t11 CLA content 0.64, 0.70, 0.62 mg/100 mg FA; VA content 1.14, 1.16, 1.20, respectively for milk, cheese and ricotta) whereas the concentration of n-3 FA in ricotta tended to be lower than milk (0.75 vs 0.91 mg/100 mg FA; $P < 0.10$). The FA composition of milk and dairy products was significantly affected by period of sampling.

1. Introduction

The relationship between fatty acids concentration of unprocessed milk and the derived dairy products has been previously investigated in sheep (Nudda et al., 2005).

The main aim of the present study was to evaluate the extent of transfer of CLA, VA and n-3 FA from goat milk to cheese and ricotta. The seasonal variation of CLA and VA in goat milk and dairy products was also investigated.

2. Material and methods

Samples of milk and of the derived fresh cheese and ricotta were collected from two milk processing plants located in Middle-Eastern Sardinia (Italy) every two weeks from early spring (March-April) to early summer (July). The concentrations of individual fatty acid in each sample of milk, cheese and ricotta were determined by gas chromatography (Nudda et al., 2006). Data were analyzed with a linear model that included the effect of processing plant, period and source (milk, cheese and ricotta).

3. Results and discussion

Concentrations of CLA and VA did not differ among milk, cheese and ricotta. The concentration of n-3 FA in ricotta tended to be lower than milk whereas was intermediate in cheese. The FA composition of milk, cheese and ricotta was significantly affected by period of sampling: the mean concentration of VA and n-3 FA decreased from early spring to early summer (Figure 1). On the other hand, the means concentration of c9,t11 CLA decreased from March to April, then remaining stable until early summer (Figure 1). The different pattern of individual fatty acids could be attributed to the farming system of goats found in this geographic area, where natural pasture and Mediterranean maquis shrubs are the main feeding source. The reduction of n-3 FA in milk, for example, may reflect the reduced availability of grass, which is the main source of C18:3 n-3 fatty acid, in late spring-summer. No differences were observed between the two cheese plants for c9,t11 CLA and VA concentration in milk, cheese and ricotta, whereas the concentration of n-3 FA differed significantly between the two cheese plant (0.72 vs 0.98 mg/100 mg FA).

4. Conclusion

The results of this survey showed that CLA and VA concentrations in fresh cheese and ricotta fat were primarily dependent on the fatty acid content of the unprocessed raw milk. As lactation

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progressed, The C18:3 n-3 concentration in milk fat and, consequently, in dairy products fat decreased, probably due to variation in pasture availability and fatty acid composition of grass lipids.

Table 1: Fatty acid profile of goat milk and dairy products

	source			P level		
	milk	cheese	ricotta	source	season	plant
C4:0-C12:0	16.8	17.1	16.4	ns	**	†
C14:0-C17:0	47.9	49.0	48.1	ns	ns	ns
C18:0	11.5	10.8	10.9	ns	ns	ns
C18:1 c9	25.5	25.3	25.0	ns	**	ns
VA; 18:1 t11	1.14	1.16	1.20	ns	**	ns
LA; 18:2n-6	2.52	2.47	2.36	ns	**	**
ALA; 18:3n-3	0.80	0.73	0.67	†	**	**
CLA, c9,t11	0.64	0.70	0.62	ns	*	ns
n-3 FA ¹	0.91	0.83	0.75	†	**	**
n3/n6 ²	0.31	0.29	0.27	†	**	**

**P < 0.01; *P < 0.05; †P < 0.10; ns = not significant

¹ [C18:3 n-3 + C20:5 n-3 + C22:6 n-3].

² [C18:3 n-3 + C20:5 n-3 + C22:6 n-3]/[C18:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:4 n-6].

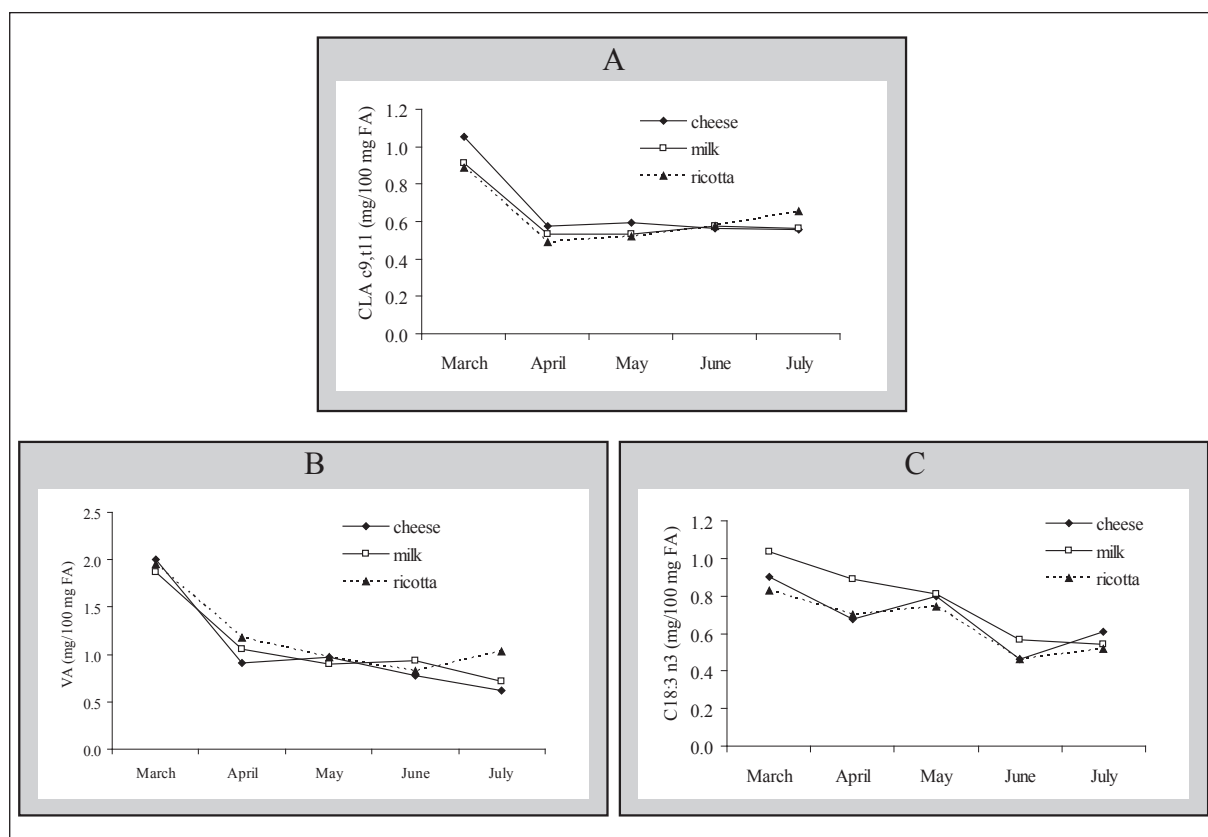


Figure 1. Seasonal evolution of c9, t11- CLA, t11- C18:1, C18:3 n-3 (mg/100 mg FAME) and CLA/VA in goat milk, cheese and ricotta sampled every two weeks from March to June in two milk processing plants located Middle-Eastern Sardinia (Italy).

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III-P145: Sources of Contamination by *Staphylococcus Aureus* of Goat Milk and Cheese Determined by MLVA

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Summary

Multiple Loci Variable number tandem repeat Analysis (MLVA) and staphylokinase gene amplification were used to track the origins of *S. aureus* contamination in caprine dairy environments. The diversity of MLVA profiles (57 profiles among 454 strains) allowed identification of several sources of cheese contamination, most often bulk milk through *S. aureus*-shedding goat milk and occasionally milking machine or human. The staphylokinase gene was always present in human *S. aureus* strains. Contamination by a given MLVA type can persist one or two years.

1. Introduction

S. aureus is a public health concern as some strains have the ability to produce enterotoxins and cause food-poisoning. Controlling contamination of milk and cheese by *S. aureus* remains a challenge for milk producers and the dairy industry, especially for raw milk derived products. A simple and efficient typing method would be helpful in understanding *S. aureus* sources and spread and is a prerequisite for the development of control programs.

Our objective was to propose a MLVA system combined with the investigation of the staphylokinase gene (*sak*) to discriminate *S. aureus* strains and to track putative origins of contamination.

2. Material and methods

Samples were collected from 12 caprine farms producing raw milk cheeses. 454 coagulase-positive staphylococci isolates were collected from bulk milks, cheeses, goats (milks of *S. aureus*-shedding females and udder skin), milking machine biofilms, human noses and hands over three periods through the year and over two years for two farms. Amplification of *sak* gene as potential marker of strains from human origin and variable number of Tandem Repeats (TR) found in *clfA* and *clfB* (Clumping factors A and B), *coa* (Coagulase), *fnb* (Fibronectin binding proteins) and *SAV 1078* genes was carried out by PCR assay (Gilbert et al, 2006). Then, alleles for each locus were defined at 90% of similarity between the profiles analysed by the unweighted pair group method using arithmetic average and Jaccard coefficient. Strains were finally characterized by a MLVA profile defined by the alleles for the 5 TRs and the presence or absence of *sak* gene.

3. Results and discussion

Diversity of strains

Our results confirmed the existence of a genetic heterogeneity among *S. aureus* strains for the different loci studied although one or two variants were always prevalent in each environment. Five alleles have been differentiated for TRs of *SAV1078*, *coa*, *clfA* et *clfB* loci. The TR *fnb* was more discriminant with 10 alleles retrieved. A total of 57 different profiles was identified. 41% of strains contained *sak*: all the strains from human hands and noses, 61.2% from udder's skin and *S. aureus*-shedding goat milk samples. The percentage of *sak*-positive strains from bulk milk was 57% whereas it was only 11% in cheese (table1).

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Table 1: Number of positive strains for staphylokinase gene (*sak*) according to their origin

Origin of strains:	Nb of strains positive for <i>sak</i> gene/ Nb total of strains	%
Shedding goat milks	18 / 33	54,5
Goat udder's skins	31 / 47	66,0
Farmers'noses	24 / 24	100
Farmers' hands	5 / 5	100
Biofilms (milking machine)	1 / 12	8,3
Bulk milks	88 / 154	57,1
Cheeses	20 / 179	11,2
Total	187 / 454	41,2

Origin of bulk milks and cheeses contamination

Our results suggest that milk and cheese contamination by *S. aureus* has multiple origins. Cheese contamination was generally associated with that of bulk milk. Most often, strains from bulk milk originated from *S. aureus*-shedding goats. In one farm, milk could have been contaminated with *S. aureus* via the milking machine, highlighting the importance of milking hygiene. Contamination of bulk milk by human strains was scarce (one case for noses strains).

Persistence of *S. aureus* contamination

Some VNTR profiles were identified over different sampling periods within the same year in milks and cheeses, but also during two consecutive years.

Table 2: Sources of *S. aureus* strains with a MLVA profile identical to that identified in bulk milks

Bulk milks		Number of identical profiles				
		Goats		Biofilm	Farmers	
Number of strains	Number of profiles	Shedding goat milks	Udders' skin		Noses	Hands
154	33	10	4	1	2	0

MLVA : Multi Locus VNTR Analysis.

Table 3: Sources of *S. aureus* strains with a MLVA profile identical to that identified in cheeses

Cheeses		Number of identical profiles					
		Bulk milks	Goats		Biofilm	Farmers	
Number of strains	Number of profiles		Shedding goat milks	Udders' skin		Noses	Hands
179	30	17	7	4	1	1	0

MLVA : Multi Locus VNTR Analysis.

4. Conclusion

It can be concluded from this study that i) milk and cheese contaminations by *S. aureus* have multiple origins and not only shedding *S. aureus* goats ii) generally a main source exists in each herd iii) MLVA is a powerful tool for discrimination of isolates in dairy products and identification of putative contamination sources.

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III-P146: Effects of Distinct Diets Fed to Lactating Ewes on the Volatile Profile of Raw Milk Cheese

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Summary

Most sheep milk is used for cheese production — so establishment of relationships between feed and cheese characteristics is useful in rationalization of off-flavors, as well as in identification of potential tracers of specific geographical origins and production systems of the final product that actually reaches the consumer. Towards this purpose, a group of sheep were consecutively fed 3 different types of diet (viz. pasture, hay and silage) — with repetition of the first diet (pasture) at the end of the sequence. Control of the major groups of microorganisms found natively in milk, and in cheese throughout ripening was sought; the microflora did not differ significantly among batches. The volatile fraction of cheese fat was extracted via purge and trap, and analysed by GC-MS. Of the studied groups, only ketones, alcohols, esters, organic acids, benzenic and phenolic compounds showed significant differences in cheeses originated from the types of feed used in this work. Terpene fraction did not prove statistically different between cheeses and no sesquiterpenes were extracted from these samples. Phenolic compounds showed highest concentration in cheeses associated with the silage group, and differed significantly between the 2 pasture treatments. Sensory analysis revealed higher overall appreciation of cheese produced when animals were fed the first pasture treatment.

1. Introduction

Volatile fraction of cheeses is, among other origins, linked to animal feed [2], chiefly when cheese is produced from raw material with little processing; thus, volatile profiling allows for the portrayal of chemical and sensory properties, associating animal production factors to final product characteristics, proving an essential tool in product traceability. Molecules related to feed may derive from ruminant metabolism, for instance fatty acids [1], or be directly originated from plants, such as terpenes [5] and phenolic compounds [3]. However, impact on the volatile fraction of dairy products is not exclusive to animal parameters and may be attributable both to technological parameters and microbial synthesis occurring during cheese ripening [1,4].

2. Material and methods

Experimental Conditions

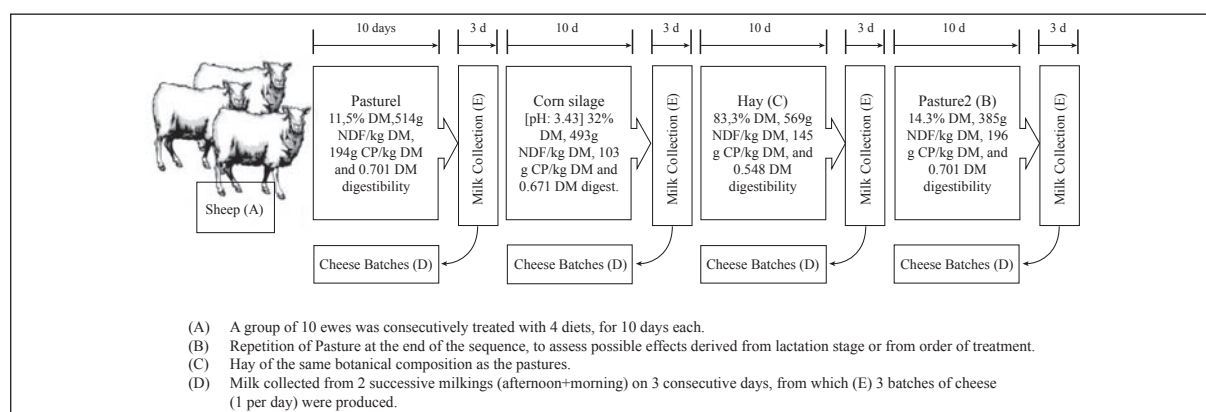


Figure 1. Flow chart of experimental design.

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Cheesemaking – Experimental cheeses were produced from raw milk, using commercially available chymosin as coagulant and ripened for 6 weeks.

Microbiological control – Bulk milk and 0, 15, 30 and 45 d-old cheeses were sampled and analysed for major groups of microorganisms, namely: total mesophilic count, total lactic acid bacteria, lactobacilli, streptococci, enterococci, *Pseudomonas* spp., *Leuconostoc*, enterobacteriaceae and coagulase-positive staphylococci.

Volatile Analysis – Quadruplicates (2 cheese replicates x 2 analytical replicates) of 45 d cheese fat (obtained by centrifugation of blended cheese) had their volatile fraction extracted by dynamic headspace in a HP 7695 purge and trap apparatus, and analysed by GC-MS with a HP6890 chromatograph equipped with an MS detector. Peak identification was made by comparison of retention times and ion spectra from previously analysed and characterized samples and spectra comparison from the Wiley 275 library.

Sensory Analysis – Duplicates of 45 d-old cheese were evaluated by a sensory panel of 12 trained assessors, using a hedonic discrete scale (0-5) for various properties of flavor and aroma.

Data Analysis – Data from volatile profiles was tested using ANOVA (post-hoc Bonferroni) tests, and pairwise multiple comparisons. Microbiological data was compared using a Kruskal-Wallis test. Analysis was performed using the SPSS software v. 15.0 for Microsoft Windows (2006).

3. Results and discussion

Microbiology

Microflora did not differ significantly in milk for any of the controlled microorganisms; few significant differences occurred in some cases, throughout ripening, that might have led to changes in the volatile profile due to microbial metabolism; even those differences are not certain to produce a direct effect observable in the volatile profile; for instance, lactic acid bacteria had significantly different ($P < 0.01$) viable counts in 0d-old cheeses (Figure 2), which could have led to differences in diacetyl formation [4] – although, as observed in Figure 3, differences in total diketone concentration were not found to be significant ($P < 0.05$).

Volatile Analysis

Groups of major compounds found in the volatile fraction of fat extracted from 45 d-old cheese is depicted in Figure 3. A total of 135 compounds were found, of which 117 were identified. The terpene fraction was highest for cheeses associated with the initial pasture treatment, and lowest for the hay treatment, although these differences were not proven to be significant ($P > 0.05$). Terpene fraction as a whole was very low in the volatile profile of these cheeses and no sesquiterpenes were observed, which might indicate low botanical diversity in the pasture and hay used in this study (alfalfa, white clover and fescue), since dicotyledonous species have been shown to be more terpene rich than other plant species [5]. Phenolic compounds were significantly higher in cheeses associated with the silage group, although they also differed significantly between the 2 pasture treatments; since no significant differences were observed in the microbiology of the cheeses from both pasture treatments (that could be responsible for variations in phenol concentration, for example), these were most likely due to physiological changes in amino acid catabolism by the ruminants, with the progress of lactation. Alcohols were present at low levels in these samples; ketones (highest values in Hay – Figure 3) and aldehydes which are normally reduced to alcohols (lowest values in Hay – Figure 3) during ripening were present at higher levels, which leads us to believe that the redox potential was particularly elevated in these cheeses, impairing alcohol formation. Benzenic (aromatic) compounds were significantly higher in the Pasture2 treatment compared with the Pasture1, however the association between these compounds and the stage of lactation remains to be ascertained.

Sensory Analysis

Sensory analysis revealed higher overall appreciation of cheese produced when animals were fed the Pasture1 treatment – even when compared with Pasture2.

Silage cheese had a characteristic bitterness in flavor (score: 4 in 5), possibly due to higher phenolic concentration [5] (Figure 3), among other factors associated with bitterness, such as the proteolysis.

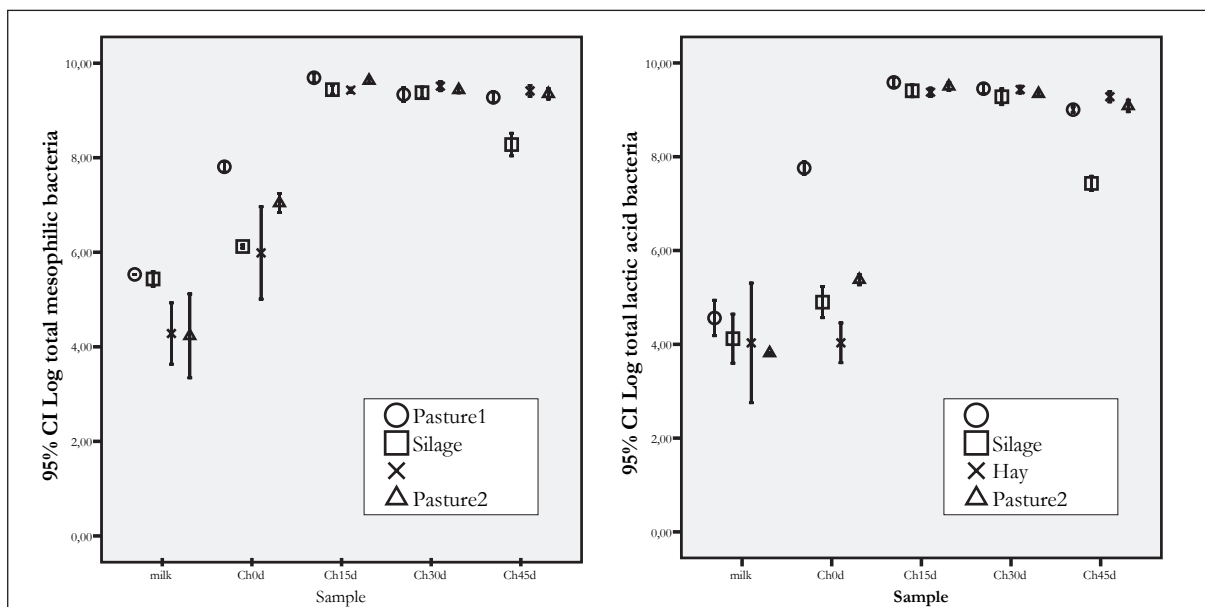


Figure 2. Plot of mean values and error bars of 95% Confidence Interval (CI) of mean for the total microbial count and lactic acid bacteria in milk and 0, 15, 30 and 45 d-old cheeses obtained from different types of feed.

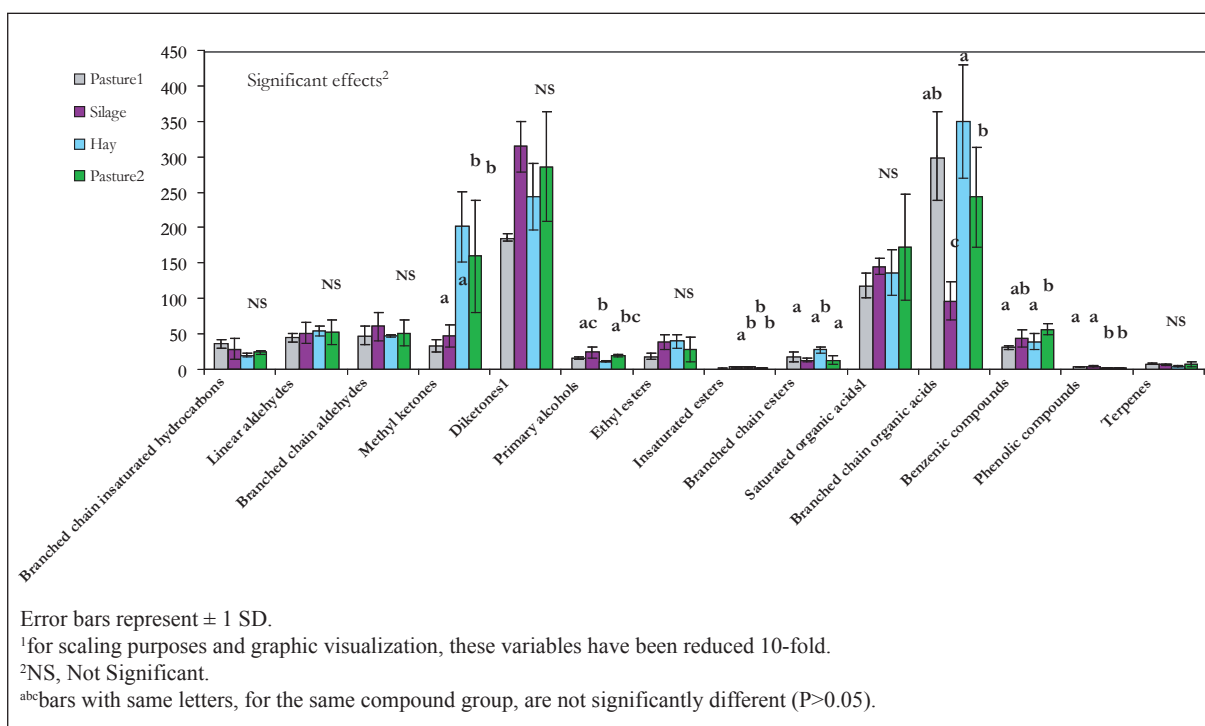


Figure 3. Plot of mean values for the volatile profile of main groups of compounds detected, scaled as relative abundance of the cyclohexanone (IS) peak.

4. Conclusion

Microbiological and technological profiles prevailing during cheese manufacture in production and ripening are largely responsible for the heterogeneity found within ewe cheese obtained according to traditional protocols; nevertheless, when cheese can be produced and ripened under controlled conditions, sensory changes and variations in the volatile fraction can be attributed to dietary differences and other factors of animal production, such as lactation stage and season. Feed is one of the influencing factors of volatiles in cheese, as observed in this study by differences in extracted ketones, alcohols, esters, organic acids, benzenic and phenolic compounds, that were not attributable either to technology (controlled experience) or microbiology. Botanical composition of feed that is specific to geographical regions associated with DOP cheeses are to be considered in further studies pursuing the objective of markers that can be traced back to animal production in traditional ewe products.

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III-P147: ACE-inhibitory and Antioxidant Activity in Ovine Casein Hydrolysates

J.A. Gómez-Ruiz¹, A. Philanto², I. Recio¹, M. Ramos¹

Summary

This work shows the potential role of the different ovine casein fractions and their hydrolysates to exert antioxidant and antihypertensive activity. Casein fractions, β - , κ - and α_s -CN, were hydrolysed by two different enzyme preparations (pepsin-trypsin-chymotrypsin [A] and pepsin-Corolase[®] PP [B]).

Antioxidant activity was evaluated by using the ABTS^{•+} decolorization assay while antihypertensive activity was assessed by measuring the ability to inhibit the Angiotensin Converting Enzyme (ACE). Although intact and hydrolysed α_s -CN fractions were those with the highest antioxidant activity (TEAC 1.63 mg/mL and 1.76 mg/mL, respectively), κ -CN fraction hydrolysed with the enzymatic preparation A increased its antioxidant activity almost threefold to reach a TEAC value of 1.5 mg/mL. Concerning ACE-inhibitory activity, fractions under 3000 Da obtained after casein hydrolysis with preparation A showed the highest activity. Hydrolysates from κ -CN and α_s -CN were the most active with striking IC₅₀ of 6.73 μ g/mL and 7.72 μ g/mL, respectively.

1. Introduction

One of the main precursors of bioactive peptides are milk proteins (1). Research has traditionally focused on bovine milk caseins, paying less attention to caseins from other animal species. Among the different bioactive peptides, antioxidant and antihypertensive peptides have been described. While there are several mechanisms involved in the antioxidant activity of bioactive peptides, antihypertensive-food-derived peptides generally act by inhibiting the Angiotensin-Converting Enzyme (ACE), which is associated with the renin-angiotensin system regulating peripheral blood pressure. The aim of this work was to cover, at least partially, this gap evaluating the presence of bioactivity in sheep milk casein hydrolyzed with different gastrointestinal enzymes. In order to achieve this, the whole casein and its fractions were hydrolysed with two different enzymatic preparations, pepsin-trypsin-chymotrypsin [A] and pepsin-Corolase[®] PP [B] and their antioxidant and ACE-inhibitory activities assessed.

2. Material and methods

- FPLC (Fast Protein Liquid Chromatography) with a cationic exchange column was used to isolate ovine caseins (2)

- ACE-inhibitory activity was measured by the method of Cushman and Cheung (3) and the antioxidant activity was measured by the ABTS^{•+} method (4). Casein fractions, β -, κ - and α_s -CN, were hydrolysed by two different enzyme preparations (pepsin-trypsin-chymotrypsin [**Preparation A**] and pepsin-Corolase[®] PP [**Preparation B**]). After hydrolysis, fractions were ultrafiltrated to obtain the water soluble extracts under 3000 Da (WSE < 3000 Da).

3. Results and discussion

Isolation of ovine casein fractions by FPLC

Casein fractions were collected and analysed by CE and HPLC (results not showed) to confirm their identity. Figure 1 shows the FPLC chromatogram with the three fractions identified as β -CN, κ -CN and α_s -CN (α_{s1} -CN + α_{s2} -CN).

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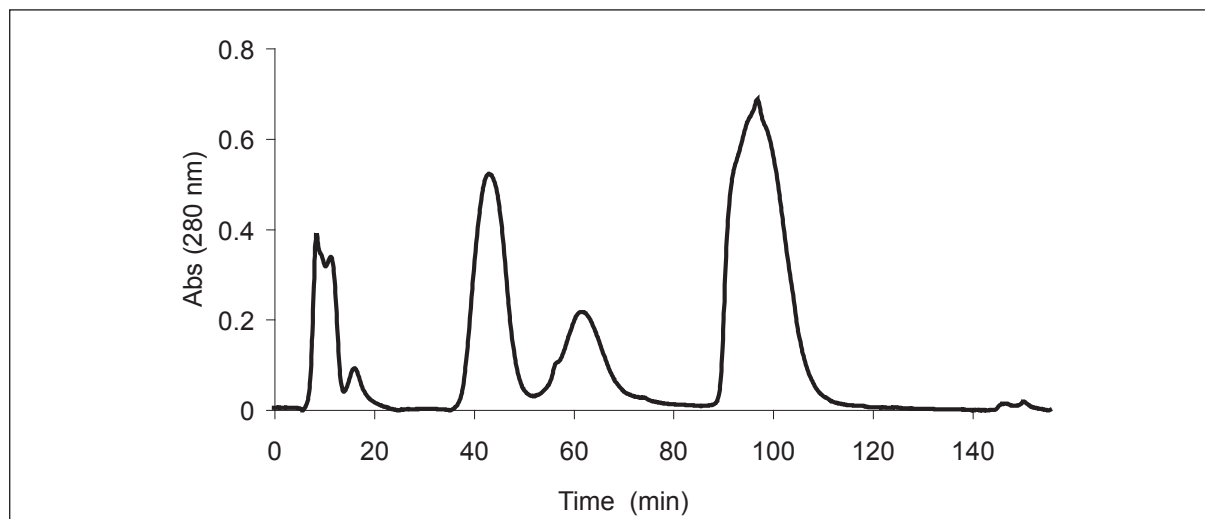


Figure 1.

ACE-inhibitory activity

Figure 2 shows the ACE-inhibitory activity of the fractions under 3000 Da after the hydrolysis either with Preparation A (pepsin, trypsin and chymotrypsin) or with Preparation B (pepsin and Corolase® PP). Fractions hydrolysed with Preparation A were the most active, showing the lowest IC_{50} . The most important results were obtained for the hydrolysates of κ -CN and α_s -CN, with κ -CN hydrolysate showing an IC_{50} of 6.73 μ g/mL and the later an IC_{50} of 7.72 μ g/mL.

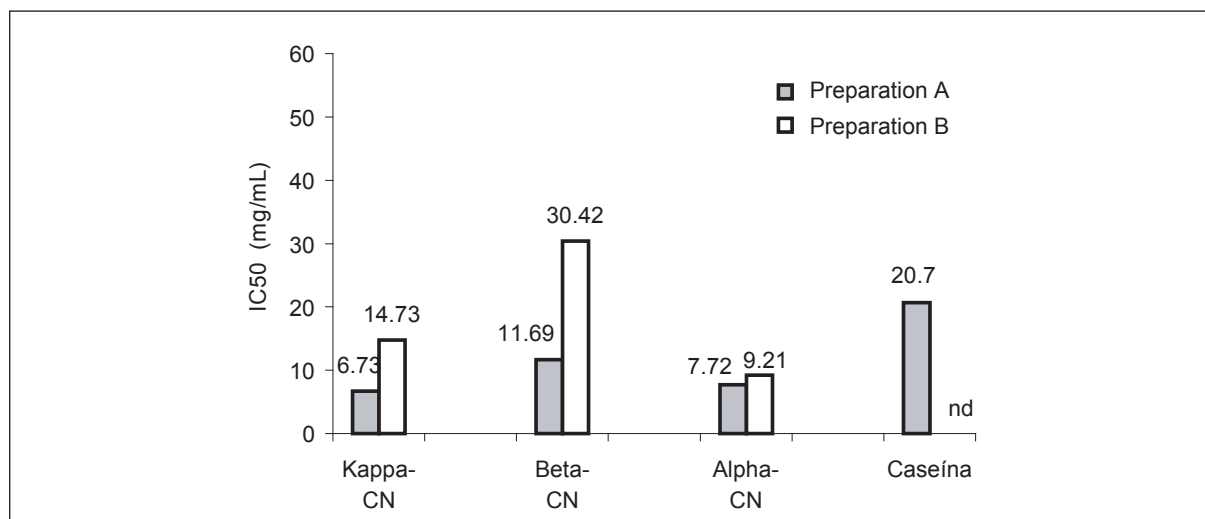


Figure 2.

Antioxidant activity

Figure 3 shows the antioxidant activity measured by the ABTS^{••} method after 15 min and expressed as Trolox Equivalent Antioxidant Capacity (TEAC). Hydrolysed α_s -CN fraction was the most active. However, while the hydrolysis of α_s - and β -CN fractions had almost no influence on their antioxidant activity, hydrolysis of κ -CN fraction resulted in 3-fold increase in this activity. Hydrolysis with pepsin-trypsin-chymotrypsin is only shown.

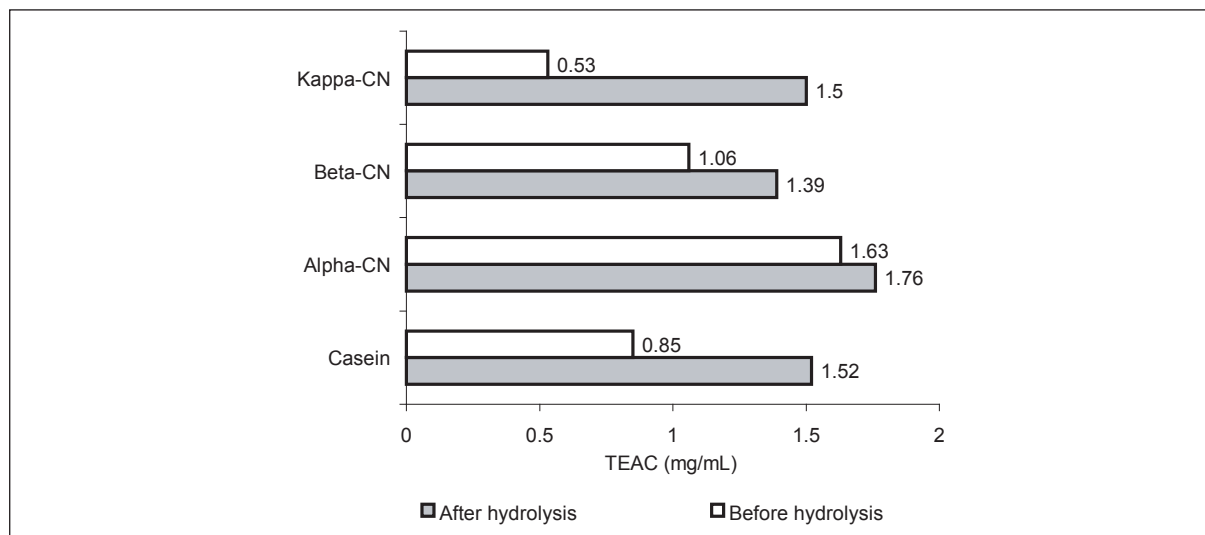


Figure 3.

4. Conclusion

1) Hydrolysis of ovine casein fractions with enzymatic Preparation A was more effective than Preparation B in terms of production of bioactive peptides. Although more peptides were found in the hydrolysates produced by pepsine and Corolasa PP (Preparation B), these peptides showed lower inhibitory activity. Ovine κ -CN and α_s -CN fractions under 3000 Da obtained with pepsin-trypsin-chymotrypsin (Preparation A) showed the highest ACE-inhibitory activity.

2) Intact ovine casein fractions showed antioxidant activity. In addition, hydrolysis of the different fractions with pepsin-trypsin-chymotrypsin increased this activity. This effect was more clearly observed in κ -CN fraction.

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III-P148: Cla and Vaccenic Acid Content in Traditional and Novel Polish Dairy Products from Ewe's Milk

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Summary

The objective of the present work was to determine the profile of fatty acids including *cis*-9, *trans*-11 CLA as well as vaccenic acid (C18:1 *trans*-11) in traditional and novel Polish milk products from ewe's milk. Raw ewe's milk, yoghurt, bioyoghurt, kefir, sour milk, traditional whey drink – żętyca and four kind of traditional cheeses – oscypek, bundz, bryndza and redykołka were analysed. The physicochemical analysis of the above products was performed using standards methods and the profile of fatty acids was estimated by gas chromatography. The level of CLA ranged 9.4 – 17.7 mg/g fat and vaccenic acid 15.1 – 41.6 mg/g fat.

1. Introduction

In recent years there has been an increasing interest in impact of some fatty acids, especially *cis*-9, *trans*-11 CLA, on human health. Some of the experimental data confirm its health benefits concerning cardiovascular diseases and cancer. According to the reports of some authors [7] there it is important to evaluate not only CLA isomers but also the vaccenic acid (C18:1 *trans*-11) level, which could be approximately at 20% converted into *cis*-9, *trans*-11 CLA in the human organism. The main source of above acids are milk products. In Poland ewe's milk was used for cheese making for centuries, and these products were consumed mostly by the local people. Recently in Poland the demand for small ruminant products like oscypek, bundz and bryndza is steadily increasing because of their special taste as well as of health-conscious consumers. Therefore, it is necessary to obtain the fatty acids composition, especially the CLA and vaccenic acid of ewe's milk products. Such information is needed to define the nutritional values of above products in Poland. The aim of this work was to evaluate the *cis*-9, *trans*-11 CLA and vaccenic acid concentration in regional products from ewe's milk.

2. Materials and methods

Ewe's milk was collected 3 times in May and June from Polish Mountain Sheep, and used for the production of fermented beverages. The treated milks were inoculated using (DVS) starter cultures at 2% yogurt culture (YC-180) probiotic culture (ABT-1), sour milk culture (CH-N-11) and (DVI) kefir culture (DA). The production of yoghurt, bioyoghurt, kefir and sour milk was as follows: pasteurization at 95 °C for 5 min; cooling to the temperature of inoculation: 45 °C (yoghurt), 38 °C (bioyoghurt), 22 °C (sour milk and kefir). The inoculated milks were incubated: yoghurt at 43 °C for 6 h, bioyoghurt at 38 °C for 10 h, sour milk and kefir at 22 °C for 16 h. After the incubation period the fermented milks were cooled and stored one day at 4 °C than evaluated.

Whey drink (żętyca) and four kind of cheeses – oscypek (smoked cheese), bundz (fresh cheese), bryndza (soft cheese) and redykołka (smoked cheese) were made traditionally by mountain people and evaluated as one day products.

In all products dry matter content [1], the protein content from the crude nitrogen according to the Kjeldahl method and the total fat using the Gerber methods [2] was evaluated. The pH was measured using a digital pH meter. The profile of fatty acids was determined by gas chromatography. Methyl esters were obtained following the methods described by Chaluard et al. Identification and quantification of fatty acids was determined using PYE-UNICAM gas chromatograph equipped with Rtx 2560 column (100m x 0.25 mm x 0.20 µm) Restek. The initial temperature was 70° C (1min), increased by 50C/1min to 100°C (held 3min) increased by 10°C/1min to 175°C (held for 40min) and increased by 5°C/1min to 220°C (held for 19min) for a total run time of 86.5 min.

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3. Results and discussion

The chemical composition and pH of evaluated products are presented in table 1. Values found for raw milk are in accordance with our earlier findings [6]. Dry matter content in all novel fermented beverages was similar and in cheeses ranged from 40.29% to 58.94%. Among cheeses the highest fat content was found for oscypek and the lowest for bryndza.

The profile of fatty acids for all products was similar to the profile of raw ewe's milk (table 2). Polyunsaturated fatty acids percent ranged from 3.54 to 6.71 and was comparable with those reported by the other authors [3,5].

The evaluated products differed in CLA content. Several factors have been identified that affect the concentration of CLA in milk products. The most important are: the kind of milk, stage of lactation, manufacture treatment. Differences in CLA concentration between novel (fermented beverages) and traditional (cheeses and whey drink) products could be explained by the seasonal factor. Ewe's milk destined for novel milk products was collected in May and June and for cheeses in September. In some reports [4] it was suggested that seasonal variation in CLA content may be due to changes in $\Delta 9$ -desaturase activity. In our study it was found that vaccenic acid in products also varied but the trend was adverse than that for CLA: traditional products were characterised by higher concentration of vaccenic acid than novel food.

Table 1: Properties of products from ewe's milk

Kind of product	Dry matter [%]	Protein [%]	Fat [%]	pH
Raw milk	17.25 ± 0.07	5.74 ± 0.03	6.48 ± 0.08	6.67 ± 0.04
Yoghurt	16.18 ± 0.04	5.19 ± 0.06	6.12 ± 0.12	4.62 ± 0.02
Bioyoghurt	16.94 ± 0.18	5.27 ± 0.01	6.22 ± 0.07	4.71 ± 0.04
Kefir	16.59 ± 0.02	5.50 ± 0.00	6.24 ± 0.15	4.51 ± 0.04
Sour milk	16.65 ± 0.07	5.34 ± 0.02	6.11 ± 0.03	4.52 ± 0.05
Whey drink (żętyca)	30.98 ± 0.93	3.38 ± 0.43	25.50 ± 1.02	5.02 ± 0.05
Oscypek	57.55 ± 4.23	28.03 ± 1.25	28.66 ± 1.20	5.55 ± 0.15
Bryndza	41.96 ± 0.36	18.42 ± 0.12	14.75 ± 0.31	5.00 ± 0.03
Redykołka	58.94 ± 0.81	24.15 ± 0.46	24.40 ± 2.93	5.67 ± 0.09
Bundz	40.29 ± 1.46	16.01 ± 0.90	24.37 ± 0.94	6.13 ± 0.14

Table 2: Profile of fatty acids (%), CLA and vaccenic acid content (mg/g fat) in products from ewe's milk

Kind of product	Fatty acids saturated	Fatty acids monounsaturated	Fatty acids polyunsaturated	CLA <i>cis-9, trans11</i>	Vaccenic acid
Raw milk	62.34	23.16	6.23	16.86 ± 0.21	15.86 ± 0.11
Yoghurt	61.57	23.54	6.61	17.55 ± 0.05	15.17 ± 0.09
Bioyoghurt	61.97	24.13	6.55	17.73 ± 0.06	15.73 ± 1.40
Kefir	62.52	22.78	6.71	17.54 ± 0.24	15.99 ± 0.23
Sour milk	62.12	22.97	6.63	17.33 ± 0.36	15.77 ± 0.54
Żętyca	60.09	28.13	3.75	10.03 ± 0.66	31.65 ± 1.11
Oscypek	59.66	28.71	4.19	15.25 ± 3.13	41.62 ± 6.59
Bryndza	59.78	27.40	3.81	9.42 ± 0.62	27.60 ± 2.33
Redykołka	57.14	30.36	3.93	10.97 ± 0.29	34.43 ± 0.71
Bundz	58.38	28.85	3.54	9.56 ± 3.12	30.61 ± 2.86

4. Conclusion

The results of the present research indicate that polish products from ewe's milk are a good source of CLA and of vaccenic acid. However it can not be omitted that these products differed in fat content and that the best source of the above important fatty acids were the cheeses.

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III-P149: Free Fatty Acids Profile of Traditional Polish Cheeses from Ewe's Milk

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Summary

The aim of this study was to estimate the profile of FFAs of four kinds of traditional Polish cheeses (bundz, bryndza, oscypek and redykołka) made from raw ewe's milk by the people living in the mountain region of Poland. The fat content of all products was analyzed according to the standard method and the profile of FFAs was determined using the method of de Jong and Badings. The statistical analysis of the experimental data showed significant differences in total FFAs and in most of the individual FFAs content among cheeses evaluated in the present research. The highest total FFAs amount was found for Bundz while the smallest for Redykołka. The main volatile fatty acids in all types of cheeses was acetic acid. Caproic and palmitic acid were predominant among medium and long chain FFAs respectively. The percentage of short chain fatty acids in total FFAs ranged 34.80 – 55.30, medium chains 17.50 – 32.80 and long chains 24.00 – 35.60.

1. Introduction

In the mountain region of Poland the ewe's milk is raw material destined especially for cheese production. Regional cheeses are: bundz (fresh cheese), oscypek (smoked cheese), bryndza (soft cheese), and redykołka (smoked cheese). The manufacture treatments are traditional and handed down from generation to generation. There are a few common characteristics of these cheeses: ewe's milk is obtained exclusively from breed Polish Mountain Sheep, processed milk doesn't undergo pasteurization, all of the cheeses are made manually, and traditional wooden tools are used throughout the whole production process.

Lipids present in cheeses can undergo hydrolytic degradation and as a result of these reactions free fatty acids (FFAs) are formed. Short chain fatty acids, which are in high concentration in milkfat, especially contribute to cheese flavour when liberated by lipolysis [5]. There are many factors that contribute to the formation of FFA: the kind of milk, lipases originating from milk and from starter culture, manufacture treatments [5].

The objective of the present work was to determine the extent of lipolysis and the profile of FFAs of the four traditional Polish cheeses from ewe's milk.

2. Materials and methods

One day cheeses made traditionally by mountain people were used as the material for analysis: oscypek (smoked cheese), bundz (fresh cheese), bryndza (soft cheese) and redykołka (smoked cheese). All products were evaluated to determine fat content according to the Gerber method [1]. Free fatty acids were extracted according to the de Jong and Badings procedure [2]. The profile of free fatty acids was performed using gas chromatograph PYE-UNICAM equipped with capillary column "Nukol" (length 30m). The carrier gas (helium) flow rate was 20 cm³/min, and the temperature was raised from: 70 °C to 200 °C at 5 °C/min, then held at 200 °C for 30 min. The experiment was repeated three times. Statistical analysis was carried out using the Statistica 6.0 computer's program.

3. Results and discussion

The total fat [%] content in the analysed cheeses was as follows: oscypek 28.40 ± 1.08; bundz 24.13 ± 0.9; bryndza 15.76 ± 0.67; redykołka 23.40 ± 2.11.

The highest concentration of the total free fatty acids was found in bundz and the smallest in redykołka. The main volatile free acid in all products was acetic acid but its amount differed

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largely in products and ranged from 23.9 to 114.8 mg/100g of cheese. Acetic acid is regarded to be produced rather by oxidative deamination of glycine, alanine, serine and by fermentation of lactose than by lipolysis [3]. A relatively high concentration of caproic acid was found and the differences among products were considerable and statistically significant. Palmitic acid was predominant in all cheeses among long chain fatty acids and the highest concentration was found in bryndza. The most important agents affecting lipolysis in cheeses which are produced from raw milk are: lipoprotein lipase, lipase originating from bacteria present in raw material, production methods.

The differences in profile of free fatty acids in evaluated products could be explained by the different manufacture treatment, since all of them were produced from the same ewe's milk. A higher concentration of total FFA was observed in bundz as well as in bryndza than in the two other products. Oscypek and redykołka are smoked cheeses. Smoking is the process which inhibits the growth and activity of bacteria, so it could be supposed that this step of production limits also their lipases activity.

Table 1: Free organic acid and fatty acids (mg/100g) in four kinds of cheeses made from raw ewe's milk

Acids	Oscypek	Bundz	Bryndza	Redykołka
C2	35.3 ± 1.3 AB	112.8 ± 23.2 AC	114.8 ± 20.6 BD	23.9 ± 2.2 CD
C4	3.4 ± 0.1 A	5.3 ± 0.5 ABC	2.6 ± 0.2 B	2.8 ± 0.2C
C6	2.8 ± 0.2 A	4.8 ± 0.7 ABC	2.2 ± 0.1 B	2.4 ± 0.1C
C8	22.7 ± 3.0 A	46.6 ± 7.5 ABC	17.6 ± 0.2 B	15.7 ± 0.6C
C10	21.1 ± 0.8	23.3 ± 3.6	23.7 ± 0.2	23.7 ± 0.5
C12	6.4 ± 0.3 A	23.7 ± 6.6 ABC	7.1 ± 0.3 B	5.6 ± 0.7 C
C14	16.4 ± 0.6 ab	16.2 ± 1.5 cd	20.0 ± 1.0 Aac	12.8 ± 0.6 Abd
C16	37.9 ± 2.8 Aa	44.3 ± 4.6 BC	63.7 ± 1.6 ABD	25.6 ± 3.2 CDa
C18	9.4 ± 0.4 Aa	13.2 ± 0.8 Ba	29.9 ± 1.8 ABCD	5.9 ± 0.9 CD
C18:1	8.1 ± 0.6 Aab	13.2 ± 0.8 ABC	10.3 ± 0.0 Ba	10.1 ± 0.5 Cb
Total	163.5 ± 5.0 AB	303.4 ± 24.8 AC	291.9 ± 17.1 BD	128.5 ± 2.4 CD

A,B,C,D – average in the line with the same letters differ statistically significantly $p \leq 0.01$.

a, b, c, d - average in the line with the same letters differ statistically significantly $p \leq 0.05$.

4. Conclusion

Data obtained in this study indicate the significant differences in profile of FFAs in traditional polish cheeses from raw ewe's milk. On the basis on our findings it could be stated that manufacture treatments were the most considerable factor that affected the concentration of free fatty acids in the evaluated cheeses. It seems that the smoking process could be a factor that limits the activity of microorganisms and thereby lipolysis.

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III-P150: Chemical and Microbiological Characterization of Slovenian Karst Ewe's Cheese

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Summary

Karst ewe's cheese is hard type cheese made from raw ewe's milk, derived from the limestone region of Slovenia. The volatile and semi-volatile organic compounds (VOC), fatty acids profile and prevailing microbial population of Karst ewe's cheese have been studied. A group of seven monoterpenes, determined by gas chromatography/mass spectrometry (GC/MS), was present at a characteristic concentration pattern which was different from patterns obtained for cheeses of other regions. Fatty acids profile of cheese samples which differed in cheese making season, ripening time and animal pasture (lowland, high land) were determined by GC. Cheeses contained on average high wt. % of short chain fatty acids (13,11 wt. %) and high level of conjugated linoleic acid (1,33 wt. %). They had favorable n-6/n-3 ratio (3,21 : 1). Microbiological analysis demonstrated that lactobacilli and enterococci are the most important microflora and *Enterococcus faecalis* and *Lactobacillus paracasei* a predominant species of traditional Karst ewe's cheese.

1. Introduction

Traditional foods are mainly marketed and readily recognized by consumers for their "regional identity" and/or "culinary" and sensory qualities. In addition to comply with the obligatory requirements, traditional cheeses have to satisfy the demands and sometimes contradictory expectations of consumers. For example, consumers demand products, which are completely safe with respect to microbiological hazards but are also minimally processed and of high nutritional and sensory value. Slovenia has a long tradition in cheese production and till now 4 cheeses have been certified by Ministry of Agriculture, Forestry and Food as PDO cheeses (Nanoški cheese, Tolminc, Mohant and Bovški cheese) while Karst ewe's cheese is in the procedure of certification. An important step in the procedure of nomination is adequate chemical and microbiological characterization of cheese, therefore volatile and semi-volatile organic compounds (VOC), fatty acids profile (FAP) and prevailing microbial population (PMP) of Karst ewe's cheese have been studied.

2. Material and methods

Nineteen cheeses from different seasons (animal pasture) were sampled according to ISO 707:1999 and stored till analysis at -20 °C.

VOC: Cryotrap-SPME pre-concentration technique followed by GC-MS analysis was used to obtain information on VOCs. 20 g of finely grated cheese samples were placed in a purging flask after conditioning to ambient temperature, then the volatiles were sampled by purging with helium (50 mL/min) and pre-concentrated in a specially designed liquid nitrogen cooled cryotrap filled with glass beads. After an hour pre-concentration step, the temperature was raised to ambient and VOCs were probed with SPME needle (100 µm PDMS fiber) in the headspace of the thawed cryotrap for 30 min. SPME needle was transferred to the injection port of GC (Varian Star 3600 CX equipped with RTX-5MS fused silica capillary column, 60 m x 0.25 mm i.d., 5 µm phase thickness) where thermally desorbed species were analyzed for targeted substances with Varian Saturn 2000 Ion Trap Mass Spectrometer.

FAP: Methyl esters for analysis of fatty acid composition were prepared in situ according to method described by Park and Goins (1994). Gas chromatography was performed using Agilent 6890 apparatus on a Varian CP 4720 capillary column (100 m x 0.25 mm) with a film thickness of 0.25 µm.

PMP: Samples of cheese (10 g) were homogenised in 90 mL of sterile 2% (w/v) tri-sodium citrate dihydrate solution in a Stomacher Lab-Blender. Decimal dilutions were plated on Rogosa

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agar for lactobacilli, M17 agar for lactococci, VRB agar for coliform bacteria and citrate azide tween carbonate (CATC) agar for enterococci. In total, 220 and 117 colonies were collected from Rogosa and CATC agar plates, respectively, purified and morphologically characterized. Biochemical fingerprinting was performed with PhenePlate™ LB and PhenePlatek FS (PhP-FS) systems, which grouped lactobacilli in 17 distinct and enterococci in 10 PhP types. From each PhP type, representative isolates were subjected to phenotypic and genotypic analyses (PCR reactions with genus- and species-specific primers; Dutka-Malen et al. 1995, Ward and Timmins, 1999, Walter et al. 2000, Deasy et al. 2000, Guarneri et al., 2001, Dubernet et al. 2002).

3. Results and discussion

It is well known that quality of animal feed amply translates into final characteristics of cheese. For this reason, several researchers have tried to identify a possibly tight relation between the VOCs in cheese and its geographic origin. Our attention was devoted to monoterpenes, since their presence in milk almost exclusively depends on pasture or feed. We found that in Karst ewe's cheese a group of seven monoterpenes, namely α -pinene, camphene, β -pinene, 3-carene, tricyclene, limonene and γ -terpinene were present at a characteristic concentration pattern which enable distinguishing this cheese from other Slovenian traditional cheeses.

The fatty acid pattern of cheese is more and more interesting feature for consumers. In addition it also reflects geographic peculiarity. The average content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in Karst ewe's cheese were 67,12, 32,88 and 6,04 wt. %, respectively. Cheeses contained on average relatively high amount of short-chain fatty acids (13,11 wt. %). Content of short-chain fatty acids was increasing during lactation. Moreover, cheeses had favorable n-6/n-3 ratio (3,21 : 1), but again the proportion was much better at the beginning of the lactation when the animal were on low-land pasture. High level of conjugated linoleic acid (1,33 wt. %) was also determined. CLA was increasing with lactation and correlated tightly with t-vaccenic acid from which CLA is synthesized in the rumen of ruminants.

Microbiological analysis demonstrated that lactobacilli and enterococci are the most important microflora of hard artisanal cheeses. Non-starter lactobacilli grew in all cheeses, made from milk of either lower or mountain pasture, and reached similar levels after 60 days of ripening. Although the indigenous population of non-starter lactobacilli in raw milk from mountain pasture was relatively low, ranging from 10^2 to 10^3 cfu/mL of milk, it increased markedly until the end of the ripening period. It seems that when the ripening process starts, lactobacilli quickly grow, reaching levels of 10^5 – 10^6 cfu/g of cheese within the first weeks of ripening, and up to 10^8 cfu/g until the end of the ripening period. All 117 non-starter Rogosa agar isolates were found to be Gram⁺ rods. Phenotypization revealed 50% of isolates as *Lactobacillus paracasei* members, 18% as *L. plantarum*, 18% as *L. brevis*, 4,5% as *L. rhamnosus*, 4,5% as *L. curvatus* and 5% as *Lactobacillus* ssp. PCR reactions with genus- and species-specific primers provided genus-specific DNA band with all PhP representatives, while species-specific PCR efficiently determined only *L. paracasei*, and some *L. brevis* and *L. plantarum* PhP types. The counts of enterococci in ripened Tolminc cheese varied between 1×10^5 and 3.7×10^8 cfu/g. In PCR reaction with *Enterococcus* genus specific primers, all of the 10 PhP-FS representative enterococci were confirmed as *Enterococcus* members. Furthermore, PCR identification at the species level identified PhP representatives as *E. faecalis* (100%) suggesting that *E. faecalis* is the predominant enterococcal species in Tolminc cheese. Similar observations were recently reported by many researchers. Andrighetto et al. (2001) stated that most of the strains isolated in Italian cheeses were identified as *E. faecalis*, likewise *E. faecalis* dominated in the milk and Irish Cheddar-type cheese as reported by Gelsomino et al. (2001).

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III-P151: Microbiological Characteristics of Ewe's Milk and Pecorino Romano PDO Cheese

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Summary

Pecorino Romano is a PDO cheese made from ewe's whole raw or thermised milk which is inoculated with a natural starter culture, curdled using lamb rennet paste.

Previous studies showed that thermophilic Lactic Acid Bacteria (LAB) represented the main microbial group in the cheese. Facultatively Heterofermentative Lactobacilli (FHL) were also found during cheese ripening.

The aim of this study was to evaluate the microbiological quality of ewe's raw and thermised milk destined to Pecorino Romano PDO manufacture, and the microbiological characteristics of cheese during ripening.

Four dairy plants were chosen and three controlled manufactures were made in each plant. The composition of lactic acid microflora and of non-lactic acid microflora present in milk and cheese samples was investigated by molecular techniques and conventional methods. The results showed that a low number of LAB and a high number of psychrophilic bacteria were present in ewe's raw milk destined to Pecorino Romano PDO manufacturing. Furthermore, thermic treatments drastically reduce LAB in the milk and therefore in the cheese, particularly mesophilic microflora.

1. Introduction

Pecorino Romano is a PDO cheese produced exclusively in Sardinia, Latium and in the province of Grosseto in Tuscany. It is made from ewe's whole raw or thermised (max 68 °C for 15 sec) milk which is inoculated with a natural starter culture (*scotta-innesto*) obtained by acidifying the residual whey from the manufacture of Ricotta. Previous studies showed that thermophilic lactobacilli (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lb. delbrueckii* ssp. *lactis*) and thermophilic cocci (*Streptococcus thermophilus*) represented the main microbial groups in the *scotta-innesto* and cheese made from thermised milk. In addition, FHL (*Lb. plantarum* and *Lb. casei*) were found during cheese ripening (Arrizza, 1972; Piredda et al. 1996).

The aim of this study was to evaluate the microbiological quality of ewe's raw and thermised milk destined to Pecorino Romano PDO manufacture, and the microbiological characteristics of cheese during ripening.

2. Material and methods

Four dairy plants (A, B, C, D) were chosen and three controlled manufactures were made from March to June in each plant. Samples of raw and thermised (68 °C for 15 sec) milk from each controlled manufacture were analysed. Cheese samples at 3 and 5 months of ripening were also analysed for each manufacture. Samples were prepared according to the FIL-IDF standard 122C, 1996. The methods used for microbiological analysis are reported in Table 1.

The presence of the main species of LAB in milk and cheese was investigated by genus or species specific PCR. One milliliter of decimal dilution of each sample was pre-enriched in M17 broth at 45 °C, MRS broth at 45 °C and FH broth at 37 °C by 48 hours incubation. DNA from 1 ml of each culture grown from each decimal dilution incubated was extracted by microwave oven treatment and amplified according to protocols reported in literature (Tab. 2). The highest PCR positive decimal dilution grown after incubation allows us to estimate the corresponding CFU/ml or g of each species respectively in milk and cheese.

3. Results and discussion

MPCA counts of raw milk employed for the three controlled manufactures ranged between 5×10^5 and 8×10^6 CFU/ml. Milk thermisation reduced MPCA counts 2-3 log. The number of coliform

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bacteria was very different in the 4 plants ranging from 2.1×10^4 CFU/ml (plant C) to 2×10^7 CFU/ml (plant A). However, they were completely removed by thermisation. Psychrophilic bacteria and *Pseudomonas* counts were very high (from 4.8×10^5 CFU/ml in plant C samples to 3.5×10^7 CFU/ml in A, B and D plant samples) even though thermisation managed to completely remove *Pseudomonas* and to reduce Psychrophilic bacteria counts about 3 log. The presence of sporogenous lactate-fermenting bacteria was not notable.

LAB were detected and enumerated by PCR as described above. Enterococci and *Streptococcus thermophilus* were the most abundant species detected in raw milk (up to 10^6 CFU/ml), and they did not decrease after thermisation, especially *S. thermophilus*. Mesophilic and thermophilic lactobacilli were detected at very low level (up to 10^2 CFU/ml) and they were almost whole removed by thermisation.

Cheese samples at 3 and 5 months of ripening were analysed for LAB, sporogenous lactate-fermenting bacteria. *S. thermophilus* was the most abundant species in all the cheese samples analysed (up to 10^6 CFU/g), during the whole period of ripening. This species, together with *Lb. delbrueckii*, is brought to the milk by adding *scotta-innesto*. The presence (always at lower level than *S. thermophilus*) of *Enterococcus*, *Lb. plantarum*, *Lb. casei* and *Lb. delbrueckii*, varied a lot among the four plants and during the ripening period of the cheeses (3 or 5 months). The species mentioned above were usually detected in the culture obtained from the third or fourth decimal dilution of the cheese samples. In some cases they were present at 5 months of ripening, even if they were not detected at 3 months. This is probably due to the low number of CFU/g (< 10) at this sample point. *Lb. plantarum* was the sole species that was not detected in any cheese sample from plant C and D, although it was present in raw and/or thermised milk.

Table 1: Media and incubation conditions used for bacterial group estimation

Bacterial Group	Methods
Total mesophilic microflora	FIL-IDF 100B, 1991
Total and fecal coliforms	VRBA agar with MUG at 37 °C in aerobiosis for 18-24 hours
Psychrophilic microflora	FIL-IDF 101, 2005
<i>Pseudomonas</i> genus	Pseudomonas agar base at 25 °C in aerobiosis for 48 hours
Sporogenous lactate-fermenting bacteria	Bottazzi et al., 1985

Table 2: Media, incubation conditions and primers used for LAB identification and estimation

Media and incubation temperature	Species	Primers	References
M17 broth 45 °C	<i>Enterococcus</i> gen.	ENT1-ENT2	Ke et al., 1999
M17 broth 45 °C	<i>S. thermophilus</i>	ST1-ST2	Lick et al., 1996
MRS broth 45 °C	<i>Lb. delbrueckii</i>	Ld1-Ld2	Lick et al., 2000
FH broth (Isolini et al., 1990) 37° C	<i>Lb. casei</i> group	SS1-CA1	Drake et al., 1996
FH broth (Isolini et al., 1990) 37° C	<i>Lb. plantarum</i> group	P16-Lpl	Berthier et al., 1998

4. Conclusion

A low number of LAB and a high number of psychrophilic bacteria were present in ewe's raw milk destined to Pecorino Romano PDO manufacturing. The high presence of psychrophilic bacteria is probably due to a long refrigeration time before cheese manufacture. This group could be responsible for many processes of degradation due to thermoresistant enzymes which may persist in the processed product, even if the microbial population that generated them has been destroyed by thermal treatment. It would be suitable to pay more attention to the duration of the refrigeration period. Furthermore, thermic treatments drastically reduce lactic acid microflora in the milk and therefore in the cheese, particularly the mesophilic microflora which is not brought by adding *scotta-innesto*.

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III-P152: Volatile Compound Profile of Milk and Cheese in Relation to Some Plants Grazed by Goats

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Summary

This study investigated the relationship between the volatile compound profile (VCP) of milk and cheese from grazing goats and that of six species particularly selected by goats. Altogether 101 compounds were detected in plants, versus 37 in milk and 40 in cheese. Ketones and sesquiterpenes were the most abundant categories in the plants, while ketones and monoterpenes in milk and cheese respectively. The highest difference between herbage and milk and cheese was observed for sesquiterpenes: only 8 compounds in milk and 5 in cheese were detected vs. 10 from plants in early flowering; in full flowering, only 11 compounds in milk and 7 in cheese vs. 20 in plants were detected. Many compounds found in milk and cheese were detected exclusively in some species (*Geranium molle*, *Asperula odorosa*, *Galium verum*).

1. Introduction

In the Mediterranean areas grazing goats deeply change their botanical composition according to the season. From winter to summer, grasses intake decreases while forbs' one increases (Fedele et al., 1993). According to botanical category and phenological stage, plant volatile compounds profile (VCP) changes (Mariaca et al., 1997). The relationship between these variation and any contingent changes occurring in milk and cheese has not yet sufficiently investigated. In a previous study Fedele et al. (2004) observed that milk and cheese VCP and content were influenced by diets enriched with some plants particularly preferred by goats.

In this study an evaluation was carried out to discriminate specific compounds capable to link milk and cheese to single species grazed by goats.

2. Material and methods

In the first year 15 Siriana goats grazed a native pasture for 8 h/d from late winter to summer. In winter, spring and summer, three cumulative milk samples were collected. Bulk milk was processed in Caciotta cheese and ripened for 20 days. Caciotta cheeses were sampled according to FIL-IDF procedures. In the second year, the six mostly preferred plants (Fedele et al., 1993) were sampled from a non-grazed area: *Dactylis glomerata*, *Lolium perenne*, *Asperula odorosa*, *Cichorium intybus*, *Galium verum*, *Geranium molle*, at the phenological stages of early and full flowering. The parts usually browsed by goats were collected. Alcohols, esters, ketones and terpenes profiles were determined in plants, milk and cheese by a modified headspace technique (Ciccioli et al., 2004), identifying volatile compounds on the basis of their mass spectra (GC-MS).

3. Results and discussion

In the six plants overall 101 different compounds were detected. Ketones and sesquiterpenes were the most abundant, with a total number of 25 and 23 compounds respectively, varying with the phenological stage. Conversely, only 37 compounds were detected in milk, among them ketones and monoterpenes (9 and 10 compounds respectively) dominated the VCP. Similarly, only 40 compounds were detected in cheese; ketones and monoterpenes (10 and 17 compounds respectively) were dominant. The highest difference was observed for sesquiterpenes, in fact only 8 compounds in milk and 5 in cheese were detected vs. 10 from plants in early flowering; in full flowering, only 11 compounds in milk and 7 in cheese vs. 20 in plants were detected (Table 1).

Every plant was characterized by a specific composition: grasses showed a dominance of alcohols and esters, while *Cichorium* and *Geranium* of ketones, and *Geranium*, *Asperula* and *Galium* of terpenes (Table 1). Overall, among the 101 compounds found in the six plants, only 14 molecules appeared able to differentiate the product in relationship to species selected at

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pasture by animals (Table 2). Thus these compounds could represent a specific attribute of the vegetal species.

Geranium m. was the richest plant in specific compounds transferred to milk and cheese, followed by *Cichorium* (Table 2). Varying the contribution of each species to the animal diet, the proportion among classes of compounds varied in milk and cheese.

Table 1: Total number of compounds for each class and for each phenological stage, detected in the six analysed plants, milk and cheese, indicating the two species that mostly contributed

Compound class	Plant		Plants mostly contributing	Milk		Cheese	
	Flowering			Flowering		Flowering	
	Early	Full		Early	Full	Early	Full
Alcohols	12	12	<i>Lolium p., Dactylis g.</i>	6	3	6	4
Esters	14	9	<i>Lolium p., Dactylis g.</i>	4	3	4	4
Ketones	12	14	<i>Cichorium i., Geranium m.</i>	9	9	9	10
Monoterpenes	10	13	<i>Geranium m., Asperula o.</i>	10	8	16	17
Sesquiterpenes	10	20	<i>Geranium m., Galium v.</i>	8	11	5	7

Table 2: Specific compounds detected in each plant and present as well in milk and cheese. In Italics the compounds in common among more plants are reported

Plant	Compound (class of VOC)
Dactylis	1-penten-3-ol(A); Heptanone (K); <i>Decanoic ac.(ME)</i> ; <i>Octanoic ac. (EE)</i> ; <i>Sabinene (MT)</i>
Lolium	<i>Decanoic ac. (ME)</i>
Asperula	Heptanol (A); α -Farnesene (ST) ; α -Terpinolene(MT)
Cichorium	1-octen-3-one (K); Cis-hexenyl isovalerate (E); Junipene (ST); Δ -Cadidene (ST); <i>α-Pinene (MT)</i> ; <i>Octanoic acid (EE)</i>
Galium	<i>1-octen-3-one (K)</i> ; <i>Hexanoic ac.(ME)</i> ; Phellandrene (ST); <i>dl-Limonene (MT)</i>
Geranium	Pentadecanone (K); 2-octanone (K); <i>Hexanoic ac. (ME)</i> ; β -Cubebene (ST); <i>α-Pinene (MT)</i> ; <i>dl-Limonene (MT)</i> ; Tricyclene (MT); β -Myrcene (MT); Δ 3-Carene (MT); <i>Sabinene (MT)</i> ; γ -Terpinene (MT)

Classes: (A) = alcohols; (K) = ketones; (E) = esters; (ME) = methyl ester; (EE) = ethyl ester; (MT) = monoterpenes; (ST) = sesquiterpenes.

4. Conclusion

The aromatic characteristics of milk and cheese from animals grazing on natural pasture changed in relationship to grazing season and to their selecting behaviour, and some species appeared more characterizing the VCP of products.

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III-P153: Furosine As a Quality Marker for Ovine Dairy Products

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Summary

The determination of objective quality markers for food has become more and more important. Among them, Furosine (ϵ -furoilmethyl-lisine, Figure 1) may be used in order to stress the correctness of production cycles, especially of thermal treatments; the Furosine content in dairy products is a marker of thermal damage and fraud. In view of the importance of the ovine dairy sector in Sardinia, experimental investigations were carried out in order to establish the Furosine concentration in ovine raw milk and in ovine dairy products: Fiore Sardo, Pecorino Sardo and Pecorino Romano (typical Sardinian ovine cheeses with Denomination of Protected Origin, DOP), ricotta cheese and cheese cream. The average Furosine content was 6.91 mg per 100 g of proteins in ovine raw bulk milk, while it ranged from 7.74 to 8.66 and to 22.94 mg per 100 g of proteins, respectively for Fiore Sardo DOP, Pecorino Sardo DOP and Pecorino Romano DOP; ricotta cheese and cheese cream showed higher values, 43.70 and 24.80 mg per 100 g of proteins, respectively. The results obtained show the possibility and the opportunity to use the Furosine content as an index marker of the genuinity of raw materials and the correctness of the production cycles, as well as of the compliance to the Production Principles established for the DOP cheeses considered.

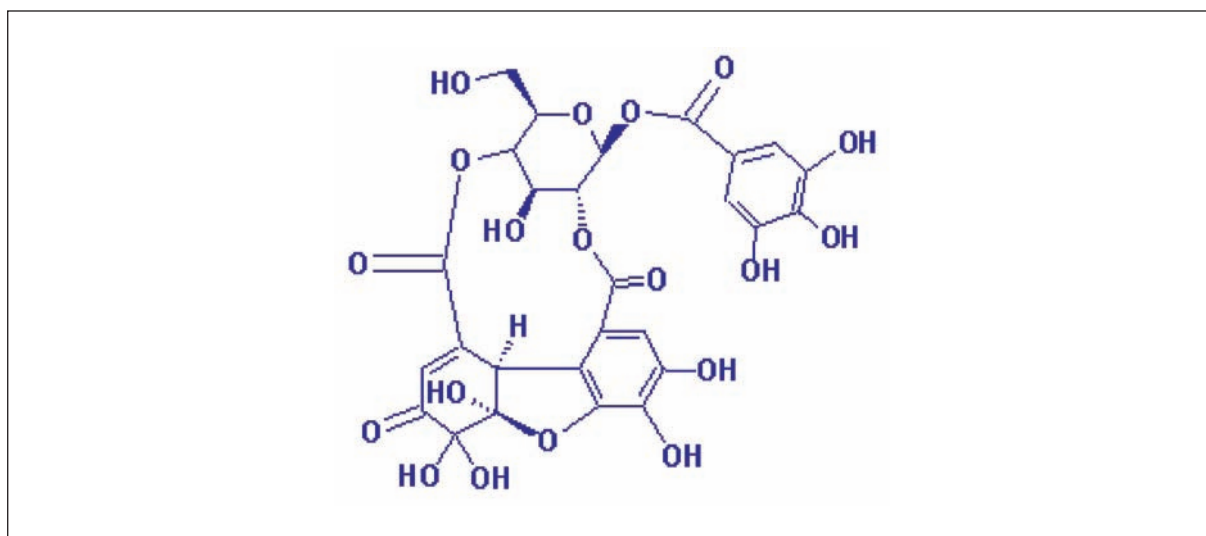


Figure 1. Chemical structure of Furosine.

1. Introduction

The leading position of Sardinia in the ovine breeding Italian sector is well known. As a matter of fact, according to the 5th General Census of Agriculture, of about 7 million heads living in Italy, almost 3 million of them (about 41%) are bred in Sardinia and annually yield more than 3,5 hl of milk (about 49% of the national production), most of which is employed in the dairy production. Among the numerous Sardinian dairy products, some of them, such as Pecorino Sardo cheese, Pecorino Romano cheese and Fiore Sardo cheese are well known and widely distributed, some, such ricotta cheese, have only a local distribution, and others, such as ovine cheese cream, have been recently introduced in the market.

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Among the quality markers, Furosine is usually employed as a thermal descriptor for assessing heat damage in different foods (milk and dairy products, pasta, eggs, rice, tomato derivatives, jams and fruit-based foods, meat products, honey). Furosine is produced by acid hydrolysis of the Amadori compound created by the Maillard reaction between reducing sugars and proteins. A number of investigations have been carried out about the Furosine content in cow milk and milk products, while only a few data in the literature refer to ovine milk and related products. Since the Furosine content in dairy products is a marker of thermal damage and fraud, and given the importance of the ovine dairy sector in Sardinia, experimental investigations were carried out in order to establish the Furosine concentration in ovine raw bulk milk and in the above mentioned ovine dairy products.

2. Materials and methods

Samples of ovine raw bulk milk (15), ricotta cheese (7), cheese cream (20), Pecorino Sardo DOP (12), Pecorino Romano DOP (5), and Fiore Sardo DOP (15), directly supplied by manufacturers, were analyzed. As to the cheeses studied, which were manufactured in compliance with their respective Production Principles, the Furosine content was monitored during the different phases of production and ripening.

Isocratic reversed phase liquid chromatography (HPLC) was applied to determine the Furosine content in the analyzed samples, by modifying the method proposed by Resmini et al. for the determination of Furosine in milk and dairy products. The analytical samples were prepared according to the official method of the Italian legislation; they were filtered through 0.45 µm PTFE-PP filters (Whatman Inc., Clifton, NJ, USA) before chromatographic analysis.

A Series 1100 liquid chromatograph (Hewlett-Packard, Waldbronn, Germany) was used, fitted with a variable wavelength UV-visible detector, and an autosampler with a 20 µl loop, connected to a Model 3396 reporting integrator (Hewlett-Packard, Avondale, PA, USA). The chromatographic separation of Furosine was performed by using a Spherisorb C8 (4.6 x 250 mm, 5 µm; Waters Corporation, Milford, MA, USA) column, with an analogous guard column. The mobile phase was 0.3% Acetic Acid (Carlo Erba, Milano, Italy) in ultrapure water, at the flow of 1.3 ml/min; the column was thermostatted at 32 °C; the detector was operated at 280 nm. The stock solution of Furosine was prepared with synthetic Furosine (Neosystem Laboratoire, Strasbourg, France) in HPLC grade 0,1 N Chloridric Acid (Carlo Erba) and stored at + 4 °C; work solutions were obtained daily by diluting the stock solution with 3 N Chloridric Acid. The calibration graph (external standard mode) was constructed by plotting peak heights vs. concentrations. A good linearity was achieved in the range 0.1-15 ppm, with a correlation coefficient of 0.9998. Each sample was analyzed in triplicate.

3. Results and discussion

Raw bulk milk

Raw bulk milk samples were collected, approximately every 15 days, during the entire sheep lactation period, from December to July. The Furosine content ranged between 4.26 and 8.94 mg/100 g proteins, with an average value of 6.91.

Pecorino Sardo DOP cheese

Twelve production cycles of Pecorino Sardo DOP cheese were considered, 6 of Pecorino Sardo "dolce" (short-term maturing, 30 days) and 6 of Pecorino Sardo "maturo" (long-term maturing, 120 days). Minimum, maximum and average Furosine concentrations in commercial products were, respectively, 5.21, 7.75 and 6.81 mg/100 g proteins for the "dolce" type, and 6.11, 11.43 and 8.66 mg/100 g proteins for the "maturo" type.

Pecorino Romano DOP cheese

The samples analyzed derived from 5 production cycles. Minimum, maximum and average values for the Furosine content were, respectively, 11.53, 36.64 and 21.04 mg/100 g proteins at 150th day (table cheese), and 11.02, 46.04 and 22.94 mg/100 g proteins at 240th day (ripen cheese).

Fiore Sardo DOP cheese

Fifteen production cycles were considered, 5 of the short-term maturing type (90 days, table cheese), and 10 of the long-term maturing one (180 days, table or ripen cheese). Minimum, maximum and average Furosine concentrations were, respectively, 6.10, 11.93 and 7.74 mg/100 g proteins for samples at 90th day, and 6.44, 15.83 and 9.85 mg/100 g proteins for samples at 180th day.

Ricotta cheese

Ricotta cheese is manufactured by re-cooking of the whey resulting from the production of cheese. Based on the cheese whose whey ricotta cheese is obtained from, different types of ricotta cheese are obtained in different geographical areas. Seven commercial samples of fresh ovine ricotta cheese were analyzed. The Furosine content ranged from 24.52 to 60.49 mg/100 g proteins, showing an average value of 43.70 mg/100 g proteins.

Cheese Cream

Twenty samples of cheese cream were analyzed, 18 of which were produced by the same manufacturer by mixing different ingredients in variable amounts (ovine or goatish cheese, ovine ricotta cheese, rennet), as well as polyphosphates and water, at a temperature between 75 and 100 °C. The Furosine concentration ranged between 9.95 and 41.53 mg/100 g proteins, with an average value of 24.80 mg/100 g proteins.

Average values of Furosine concentrations and processing temperatures of the products analyzed are reported in Table 1.

Table 1: Average Furosine concentrations and processing temperatures

Average concentration/ Processing temperature	mg/100 g proteins	°C
Raw bulk milk	6.91	-
Pecorino Sardo (30 th d)	6.81	35 - 38
Pecorino Sardo (120 th d)	8.66	40 - 43
Pecorino Romano (150 th d)	21.04	45 - 48
Pecorino Romano (240 th d)	22.94	
Fiore Sardo (90 th d)	7.74	33 - 35
Fiore Sardo (180 th d)	9.85	
Ricotta cheese	43.70	80 - 90
Cheese cream	24.80	75-100

4. Conclusion

The results summarized in Table 1 show the strict correlation of the Furosine content with the strength of thermal treatment used and the ripening time, though it was variable at a lower or greater extent within and among the different products studied. At present, in EU countries there is no legal limit for Furosine content in ovine milk and cheeses; normative references were established only for cow milk (8.6 mg/100 g proteins) and for Mozzarella cheese (12 mg/100 g proteins). As for the products analyzed, these limits were exceeded, respectively, in 13% of the samples of raw milk and in 80% of the samples of Pecorino Romano cheese.

The results obtained, though on a limited number of samples, indicate the possibility and the opportunity to use the Furosine content as an index marker of the genuinity of raw materials and the correctness of the production cycles, as well as of the compliance to Production Principles of the cheeses with Denomination of Protected Origin; in other words, as a quality marker for ovine dairy products.

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III-P154: Molecular Beacon Technology for Rapid and Specific Detection and Quantification of *Staphylococcus Aureus* from Dairy Products

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Summary

The aim of this study is to develop a new diagnostic molecular tool to quickly identify *Staphylococcus aureus* in raw milk, curd and raw milk cheese.

We performed a new diagnostic method based on a new approach for DNA purification from milk products and designed a high specific fluorescent probe, molecular beacon, designed on *S. aureus femA* gene. Quantification results in Real Time PCR were compared with those obtained by the traditional cultural method.

1. Introduction

Different methods for the detection and viable count of *Staphylococcus aureus* are available but they usually are time-consuming, as the results are not obtained within 72 h. The aim of this work was to develop a new molecular tool based on Real-Time PCR for a rapid quantification of *S. aureus* in raw milk, curd and raw milk cheese.

2. Material and methods

A total of 61 sheep milk, 30 curd and 55 sheep cheese samples collected from the Tuscany region (Italy) were examined by specific cultural procedures (ISO6888-1-1999 and 6888-2-1999). The DNA extraction in all the samples was carried out with 3 methods: BIO-RAD® kit, QIAamp DNA Mini Kit (Qiagen) and with the methodical of CTAB. For cheese samples TeSeE™ Medium Beads (BIO_RAD) were used before DNA purification. After DNA extraction all samples were analysed by Real Time PCR using specific primers and Molecular Beacons (Tab. 1, Fig. 1) for direct detection of *S. aureus*. Molecular Beacons are single-stranded oligonucleotide detector probes that form a stem-and-loop structure. The loop contains a probe sequence which is complementary to the target amplicon and the stem is formed by the annealing of complementary arms sequence that are located on either side of the probe sequence. Fluorophores (reporter and quencher) are covalently linked to the ends of the stem. Molecular beacons do not fluoresce when are free in solution. However, when they hybridize to a *S. aureus* DNA target strand they undergo a conformational change that enables them to fluoresce brightly. Fluorescence is proportional to the number of amplicons synthesized during an assay. Molecular beacons are added to the assay mixture before carrying out the amplification, and fluorescence is measured by real time PCR. Moreover, positive reactions can be also detected by sight.

3. Results and discussion

We performed a new diagnostic method based on a new approach for DNA purification from milk products and on the design of a high specific fluorescent probe, molecular beacon, designed on *Staphylococcus aureus femA* gene. This sequence is deposited in GeneBank (<http://www.bioinfo.rpi.edu/applications/mfold>) with accession number NC_003923. Quantification results in Real Time PCR compared with those obtained by the traditional cultural method are shown in table 2.

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Table 1: Primer sequence designed on *femA* gene, sequence deposited in GeneBank (<http://www.bioinfo.rpi.edu/applications/mfold>) with accession number NC_003923

Gene	Primer	Primer sequence 5'-3'	Length and Position of Primer	Length of Fragment Amplified
<i>femA</i>	OG287	CATGAATTATTGAATGCAATG	21+/990	76 bp
	OG288	CAAAATAAACACCCAGAAGCA	20-/1046	

Molecular Beacon	Primer sequence 5'-3'	Length and Position of Primer
OG286	GAGCAAAACGGACGGCCC	18+/1016

Table 2: Quantification results in Real Time PCR compared with those obtained by the traditional cultural method in raw milk, curd and raw milk cheese (CFU/ml)

Number of samples	Raw milk		Curd		Raw milk cheese	
	ISO 6888-1-1999	Qiagen	ISO 6888-2-1999	BioRad	ISO 6888-2-1999	Qiagen
1	8,09x10 ³	Negative	1,65x10 ³	1,40x10 ⁴	2,70x10 ⁴	1,30x10 ⁴
2	80	Negative	1,57x10 ³	1,40x10 ³	40	Negative
3	60	Negative	1,35x10 ²	2,10x10 ⁴	6,80x10 ⁴	1,90x10 ⁵
4	1,63x10 ²	1,5x10 ⁴	1,60x10 ²	9,30x10 ²	4,00x10 ³	Negative
5	9,09x10 ²	1,4x10 ⁵	1,58x10 ⁴	1,80x10 ⁴	8,50x10 ³	1,20x10 ⁵
6	1,00x10 ²	Negative	3,00x10 ²	1,20x10 ⁴	2,70x10 ⁴	5,40x10 ⁴
7	1,73x10 ²	Negative			9,00x10 ³	Negative
8	2,45x10 ²	Negative			9,60x10 ³	7,80x10 ⁵
9	1,20x10 ²	Negative			4,80x10 ²	Negative
10					1,37x10 ⁴	2,00x10 ⁵
11					5,30x10 ⁵	9,50x10 ⁴
12					4,10x10 ⁴	1,60x10 ⁵
13					40	5,70x10 ³
14					1,25x10 ³	Negative
15					1,00x10 ⁴	1,12x10 ⁵
16					1,30x10 ⁴	1,50x10 ⁶
17					5,00x10 ³	5,60x10 ³
18					9,00x10 ²	Negative
19					5,50x10 ³	Negative
20					2,20x10 ⁴	8,00x10 ³
21					1,70x10 ³	Negative
22					5,00x10 ²	Negative
23					3,00x10 ²	1,00x10 ⁵
24					1,70x10 ³	2,60x10 ⁵
25					1,70x10 ²	1,00x10 ⁵
26					1,50x10 ²	5,20x10 ⁴

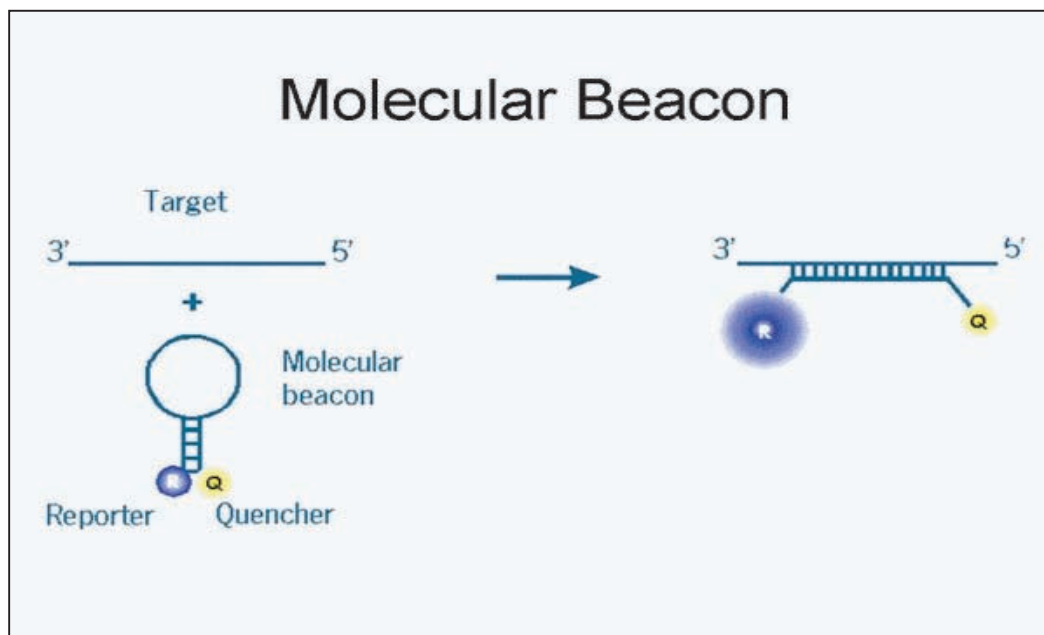


Figure 1. Molecular Beacon structure.

4. Conclusion

The detection with Molecular Beacons compared with traditional cultural methods is rapid and versatile for *S. aureus* detection in raw milk derivatives (curd and cheese) and for quantifying bacterial contamination. The quantitative real time PCR detected, after only 5 hours, UFC/ml *S. aureus*, showing a linear dynamic range of quantification (10³-10⁸ molecules of DNA/reaction) and a good correlation rate ($r=0.90$). Most diagnostic veterinary laboratories do not have real time PCR machines therefore the addition to the conventional PCR assays of the Molecular beacons, developing bright fluorescence in positive samples, would eliminate the post-PCR detection steps as gel electrophoresis.

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1. <http://www.bioinfo.rpi.edu/applications/mfold>

III-P155: Characterisation of Friuli Venezia Giulia Semistagionato Caprino Cheese

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Summary

The objective of this research was the chemical, physical and sensorial characterisation of *semistagionato caprino*, a traditional goat cheese of Friuli Venezia Giulia Region.

4 cheese-makings in three different farms (one per month from June to September) were monitored, recording process conditions and sampling milk and *lattoinnesto* (starter culture, farm-prepared).

After ripening (5 months, on average), 12 cheeses were collected. Cheeses were analysed for raw composition, nitrogen (N) fractions, fatty acid methyl esters (FAME), free fatty acids (FFA), texture profile (TPA) and sensory profile (QDA).

This study, although well qualifying certain characteristics, showed the complexity of defining a common substratum of quality parameters in traditional products due to the variability that is intrinsic in traditional productions and is to be regarded as an added value for artisanal products.

1. Introduction

The objective of this research was the chemical, physical and sensorial characterisation of *semistagionato caprino*, a traditional goat cheese of Friuli Venezia Giulia Region. This cheese is obtained from raw goat milk adding *lattoinnesto* (starter culture, farm-prepared) and goat kid paste rennet. It is a semi-cooked cheese with slightly pressed paste. Ripening lasts from 3 to 8 months.

2. Material and methods

The trials took place in three farms distributed from west to east in the Pre Alps area of Friuli Venezia Giulia region. All involved farmers, Alpine Goat breeders, are cheese self producers with small dairy plants.

4 cheese-makings per farm (one per month from June to September) were monitored for each farm, recording process conditions and sampling milk and *lattoinnesto* (starter culture, farm-prepared with milk thermally treated and incubated at 42°C until the desired acidity is reached: normally 8 to 12 hour are required).

Milk was analysed to determine fat, protein, lactose, total bacteria count, somatic cell count and spores of thermophilic gas-producing bacteria (STB). To assess starter cultures quality, main lactic bacteria groups were evaluated: *Lactococcus* spp., *S. thermophilus* and *Enterococcus-Pediococcus-Lactobacillus*.

After, on average, 5 months of ripening with humidity and temperature conditions measured by data loggers, 12 cheeses were collected. Cheeses were analysed for raw composition, nitrogen (N) fractions, fatty acid methyl esters (FAME), free fatty acids (FFA), texture profile (TPA), sensory profile (QDA).

3. Results and discussion

Milk analysis showed values of fat and protein content a little over the standard for Alpine Goat Breed (respectively 3.92 and 3.75 g/100g on average) with normal trend to grow toward the end of lactation.

Cheeses, on average showed 63.1% dry matter (DM) with 44.5% Protein (N*6.38)/DM, 43.0% Fat/DM and 7.6% ASH/DM. According to FAO [1], these cheeses were classified by MFFB (moisture on fat-free basis) mostly in "Firm/Semi-hard" category - the remaining in "Hard" category - and, by FAT LEVEL, in "Medium fat" category. N-fractions and proteolysis index ($N_{TCA\ soluble}/N_{tot}$ %), Table1, showed, for one farm, cheeses with degradation level double than the others, probably due to the different ripening conditions.

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Fatty acid methyl esters (FAME), grouped in saturated (SFA), mono (MUFA) and poly (PUFA) unsaturated, are reported in Table 2: SFA were, on average, 76.5%, MUFA 15.9% while PUFA less than 6%, this confirming the higher stability of SFA during ripening.

Most relevant free fatty acids methyl esters identified are reported in Table 3. Free fatty acids are of extreme importance among cheese flavour compounds. They exert their role directly or as precursors originating methyl ketones, alcohols, lactones and esters. During ripening, fatty acids with 4 or more carbon atoms can derive from milk fat lipolysis or from degradation of some AA. Lipolysis could be produced by the activity of endogenous milk lipases or from microbial ones, if not induced by moulds or some types of rennet. This is the case of *Semistagionato caprino*, in which production goat kid paste rennet is employed. Relevant differences were found for almost all determined FFA and, in particular, for C16:0.

TPA (data not reported), showed some physical differences between samples. S2 cheeses were quite different than the others showing less Hardness and more Cohesiveness and Springiness.

Sensory analysis (figure 1 - Spiderplot objects over attributes) recorded major differences for *hardness*, as expected S2 being significantly less hard than others, *fermented* (overripe fruit), *spiciness* and *saltiness*. Nevertheless the overall profile showed some common characters: medium high solubility, well defined odour and flavour, a little bitterness, a constant presence of aromatic characteristics of heterolactic fermentation a low presence of off-flavours as ammonia notes, products of lipid oxidation (rancid) or sulfuric compounds (egg odour).

4. Conclusions

This study showed the complexity of defining a common substratum of quality characteristics in traditional products. This difficulty originates from the variability that is intrinsic in traditional productions and is to be regarded as an added value for artisanal products. However the multi analytical approach via chemical and physical characterisation seems to be effective especially when there is the intermediation of sensory analysis to give an organoleptic significance to other analytical responses.

References

1. FAO-WHO; Codex General Standard For Cheese; Codex Stan A-6-1978, Rev.1-1999.

This project has received European Regional Development Funding through the Interreg IIIIB Community Initiative. Special thanks to the Associazione per la Valorizzazione dei Prodotti Ovini e Caprini del Friuli Venezia Giulia for the wide collaboration in trial organization and samples providing.

Table 1: Nitrogen Fractions

Sample	N _{sol} pH 4.6	Ripening coeff.	% N _{sol} TCA	Proteolysis index	% protein N	% non pro- tein N	N _{NH3}
S1	1.19	26.31	0.58	12.91	3.92	0.60	0.02
S2	1.00	24.18	0.27	6.59	3.88	0.24	0.01
S3	0.93	20.78	0.31	6.97	4.24	0.26	0.03
Average	1.04	23.76	0.39	8.82	4.01	0.37	0.02

Table 2: Fatty acids methyl esters

	S1	S2	S3
FAME mg/g FB	301.31	234.98	241.94
FAME g/100g FB	30.13	23.50	24.19
% SFA	76.56	74.01	79.02
% MUFA	16.11	18.20	12.63
% PUFA	6.50	5.23	6.08

Table 3: Most relevant free fatty acids methyl esters identified (mg ffa /g cheese)

ffa: mg / g cheese	S1	S2	S3
ffa: mg / g cheese	1.62	1.20	1.05
isoC4:0	0.90	1.88	2.56
C4:0	1.11	3.37	3.10
C5:0	0.06	0.16	0.00
C6:0	2.18	3.90	3.23
C8:0	3.05	2.43	3.76
C9:0	0.63	1.15	1.16
C10:0	10.71	13.04	15.91
C11:0	0.16	0.35	0.60
C12:0	2.37	3.87	3.56
C13:0	0.20	0.20	0.33
C14:0	7.57	3.01	3.01
C14:1w9	0.18	0.23	0.35
C15:0	1.27	1.34	1.54
C16:0	27.00	3.67	3.36
C16:1w9	0.72	1.01	0.52
C16:1w7	1.83	2.51	2.26
C17:0	0.25	0.40	0.34
C17:1	0.15	0.21	0.35
C18:0	10.74	17.35	16.23
C18:1w9 trans	1.64	2.38	4.07
C18:1w9 cis	17.71	20.34	16.83
C18:2w6 trans	0.41	0.93	0.67
C18:2w6 cis	4.45	7.19	8.35
C20:0	0.89	1.18	0.85
CLA 9,11	2.02	4.67	4.92
CLA 10,12	1.81	3.24	2.12

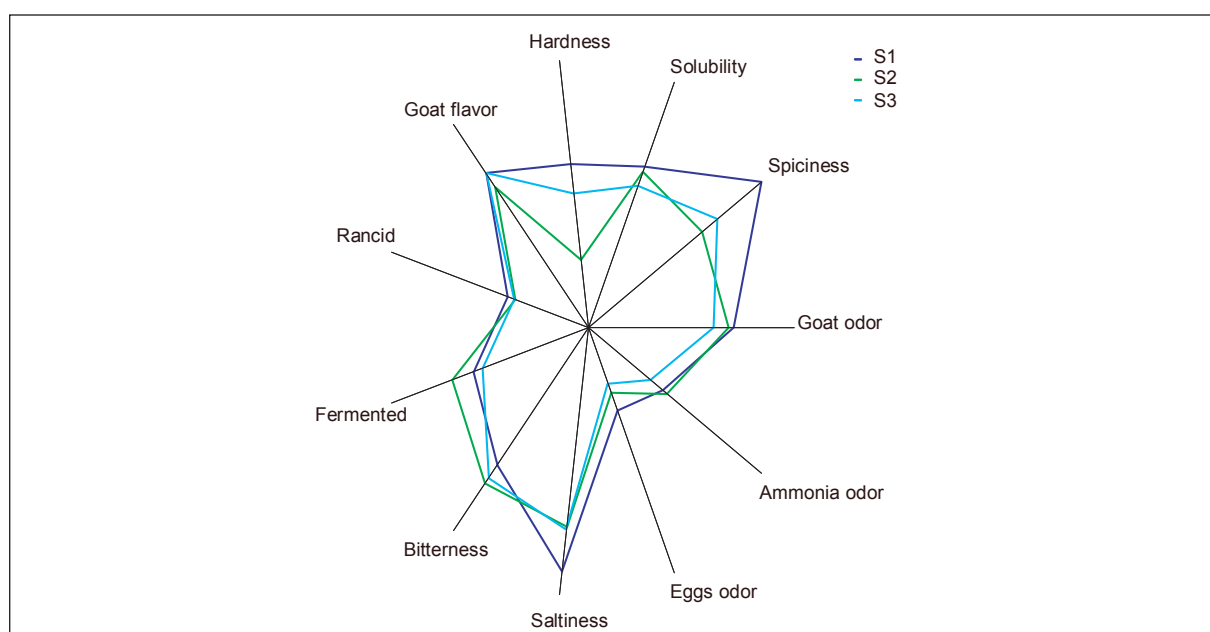


Figure 1. Spiderplot objects over attributes.

III-P156: Formaggella Del Luinese: A Traditional Goat Cheese

L. Vanoni¹, M. Brasca¹, R. Lodi¹, S. Morandi¹

Summary

“Formaggella del Luinese”, the only PDO Italian goat cheese, has been characterized. Several cheesemaking technologies and microbiological analyses performed on the milk, curd and ripened cheese were studied and compared. At the end of the ripening chemical and sensory analysis were performed.

1. Introduction

The “Formaggella del Luinese” is an artisanal goat cheese made only in the Valli of Luinese near Varese. The cheese has been produced for centuries, frequently made by marginal farmers who adopt traditional technology in their small-size milk production and processing activities.

When cheese is produced following traditional procedures from raw milk, the environmental microflora plays a fundamental role in fermentation and is one of the most important parameters affecting the cheese quality. In addition, the biodiversity of bacteria involved in cheese production can be considered a fundamental factor for the maintenance of the typical features of traditional cheese products. Recent investigations have shown that the indigenous microflora of raw milk influence the biochemical characteristics and flavour of cheeses (Demarigny, Y. et al 1997).

Since March 2006 this cheese has been granted transitory recognized protection at the national level while it awaits the approval of the European Community for Protected Denomination of Origin (PDO).

The cheese is cylindrical in shape and weighs, on average, 700-900 grams. The rind is natural, not hard, with the possible presence of mould while the cheese itself is soft and compact with a pleasantly sweet flavour; the colour is mainly white. The cheese undergoes a short term drying process at room temperature and is then left to mature for at least twenty days.

2. Material and methods

To better characterize this cheese, 9 different dairy farms in the province of Varese were selected and were followed throughout a production period of six months. For each production the samples of raw milk, curd and cheese (at 20 days of ripening) were collected.

Microbiological analysis (determined according to ISO/IDF): SBC, contaminating microorganisms, coliforms, *Escherichia coli*, coagulase positive staphylococci, lactic acid bacteria (LAB), propionibacteria, yeasts, moulds, *Salmonella* spp. and *Listeria monocytogenes*.

Chemical analysis (determined according to AOAC International): total protein, fat, free fatty acids and moisture.

Sensory analysis: descriptive sensory analysis was carried out in cheeses ripened for 20 days by a trained panel of 10 assessors, who were members of the ONAF and cheesemakers of Formaggella del Luinese.

A 0-10 scale was used, considering 11 criteria including taste (saltiness, acidity, bitterness and sweetness), aroma (overall aroma intensity), colour, smell intensity, and texture (elasticity, solubility, moisture and adhesiveness).

3. Results and discussion

The protocol for the production of Formaggella del Luinese is represented in Figure 1.

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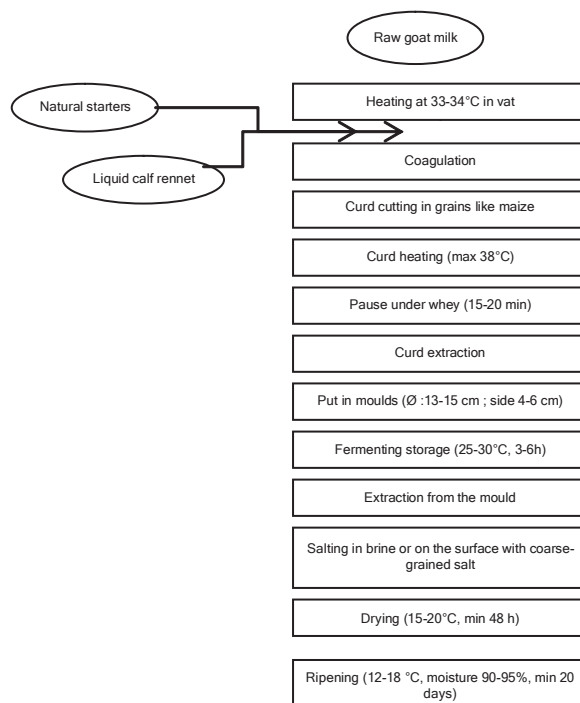


Figure 1. Formaggella del Luinese cheese process.

Microbiological analysis. The total mesophilic counts in milk (Figure 2) didn't exceed the allowed amount for goat milk (Reg CE 853/2004). Very good is the situation for total coliforms (2.20 ± 1.05 log cfu/mL), *E. coli* (1.05 ± 0.27 log cfu/mL) and coagulase positive staphylococci (2.50 ± 0.83 log cfu/mL); this showed the excellent hygienic conditions during the milking and the milk storage. All milks contained a high number of LAB (enterococci, lactobacilli, lactococci and obliged heterofermentative lactobacilli (OHL)).

In the curd LAB were increased 1-2 log unit, (there is a greater part of lactococci); there is also an increase of enterococci and OHL, it is very important because they have a flavouring role and help in forming characteristic flavours of raw milk cheese.

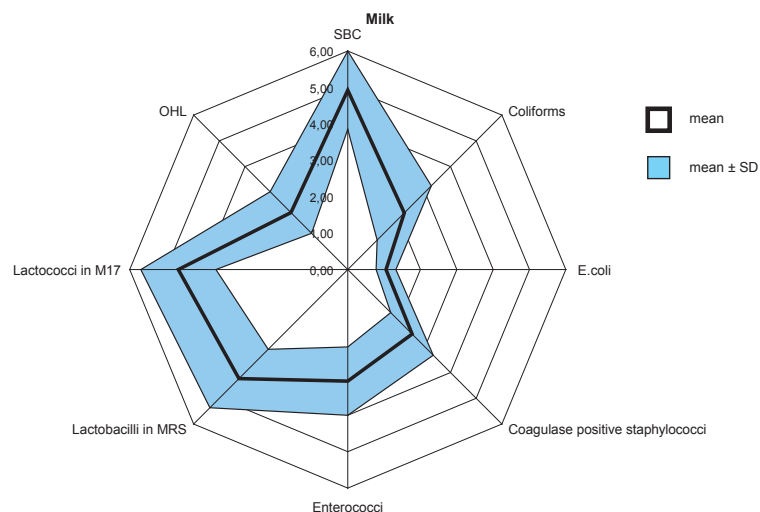


Figure 2. Counts of main microbial groups (log cfu/mL) in raw milk.

In the cheese (Figure 3) the acid lactic bacteria followed the same evolution than in curd, all samples showed high counts: LAB in MRS and M17 higher than 8.00 log cfu/g, enterococci and OHL showed an average count of 6.00 log cfu/g, the increase in LAB is important because the LAB activity produces a decline in pH that prevents the increase of undesirable bacteria.

Salmonella spp. and *Listeria monocytogenes* were not detected in all cheese.

Chemical analysis. Some differences were observed in the composition of the milks and the cheeses at the end of ripening, especially for the moisture content, probably because during the ripening they were record different parameters of temperature and moisture. The variation of the chemical composition of the milk depends on the effect that diverse factors such as type of breed, feeding, lactation stage, etc. (Auldust et al.,1998).

Sensory analysis. The Formaggella del Luinese cheese presented middle characteristics for the aroma and smell intensity, it was little salty and sweet, with a bitter taste, not negative. The texture was elastic and soluble (Figure 4).

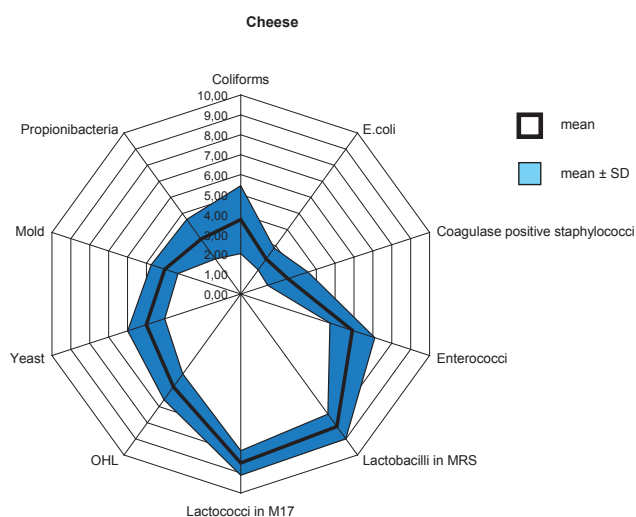


Figure 3. Counts of main microbial groups (log cfu/g) in cheese.

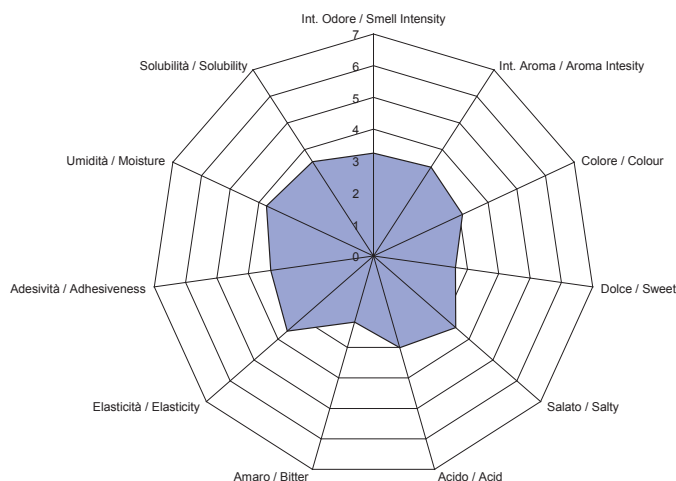


Figure 4. Sensory characteristics of Formaggella del Luinese cheese.

4. Conclusion

The results of this work suggest that the cheese made with traditional method doesn't present hygienic problems but presents an increase of the lactic acid bacteria in all shapes, this is a sign that it had a good fermentation process.

The positive results are important to encourage the denomination of PDO cheeses that assumes that a link exists between the area of origin, the traditional processing and the specific characteristics of the product.

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III-P157: Biogenic Amines in Spanish Cheeses Made With Sheep and Goat Milk

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Summary

The aim of this work was to characterize the BA content of Spanish cheeses made from sheep's raw milk (Manchego, Zamorano and Idiazabal; all of them with Protected Denomination of Origin) and one pasteurized goat's milk (Queso de Murcia Al Vino) elaborated with two different types of rennet: commercial bovine rennet and artisanal lamb rennet paste. Sheep's cheeses were made by commercial cheese makers and the goat cheese was made in the University pilot plant.

BA, derivatized with the AccQ-Flour™ reagent, were analyzed by HPLC in cheeses all along the ripening period. A total of eleven BA were detected. The total amount and the concentration of each BA increased along the ripening period and varied significantly ($p < 0,005$) between different type of cheeses. GABA exhibited the highest concentration in all cases, whereas histamine was present in very low amounts.

The type of rennet had no effect on the BA amount except on tyramine concentration that was higher when artisanal rennet paste was used.

1. Introduction

Biogenic amines (BA) are organic basic compounds which occur in fermented foods and beverages, such as cheese, wine and beer. BA are usually produced in foods by microorganisms that express amino acid decarboxylase activity (1). Toxicological problems, such as headache, hyper- or hypotension and several allergic disorders may result from the ingestion of food containing high levels of these compounds (1).

Cheese, especially from raw milk, has been often related to histamine intoxication episodes (2) Nevertheless, the amount of total and individual BA is very variable among different kinds of cheese and very little data are found about sheep's and goat's milk cheese.

Manchego, Zamorano and Idiazabal cheeses are Spanish raw sheep's milk cheeses, each one protected by its own PDO. On the other hand, Al vino cheese, from Murcia region, is elaborated with pasteurized goat milk also protected by its PDO.

The aim of this work was to characterize the BA content of these cheeses and to study the influence of using lamb rennet paste (nowadays used in the elaboration of some of the mentioned cheeses) on this content.

2. Material and methods

Sheep's cheeses were made by commercial cheese makers and the goat cheese was made in the pilot plant of the University of Murcia, in each case following the corresponding PDO protocol. On each elaboration day, two batches of cheese were made with the same milk. One batch was made with bovine commercial rennet and the other one with artisanal lamb rennet paste. Cheesemaking was repeated the following week. From each fabrication two cheeses were taken for BA analysis during ripening, up to 60 days for "Queso de Murcia al Vino" cheese, and up to 180 days for the rest.

BA were extracted from cheese in an acidic solution (3), derivatized with the AccQ-Flour™ reagent and analyzed by HPLC using diaminoheptane as internal standard for quantification (4).

3. Results and discussion

The total BA concentration increased with ripening time (figure 1) and varied significantly among

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cheeses (table 1). The concentration was lower than that found in the literature for cow's raw milk cheeses (5).

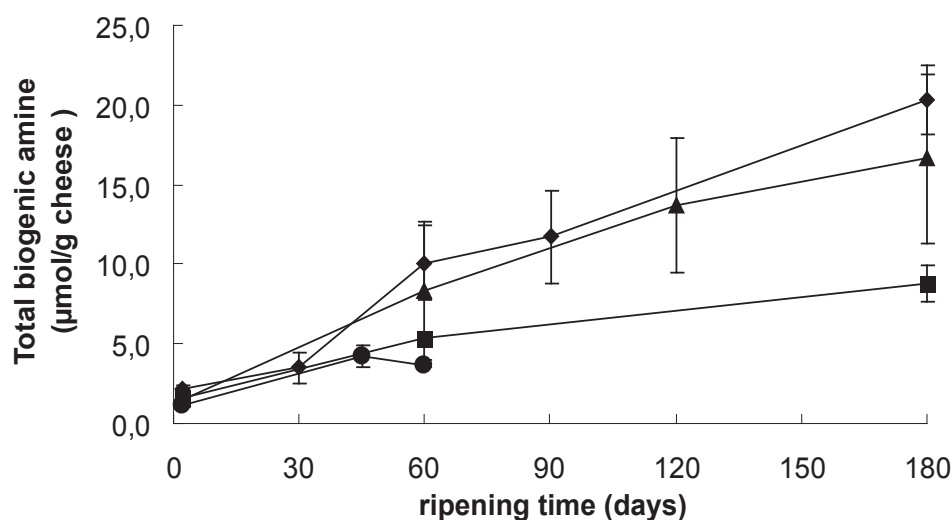


Figure 1. Total amine concentration during ripening of: ● "Al vino", ▲ Manchego, ■ Zamorano and ◆ Idiazabal cheese made with bovine rennet.

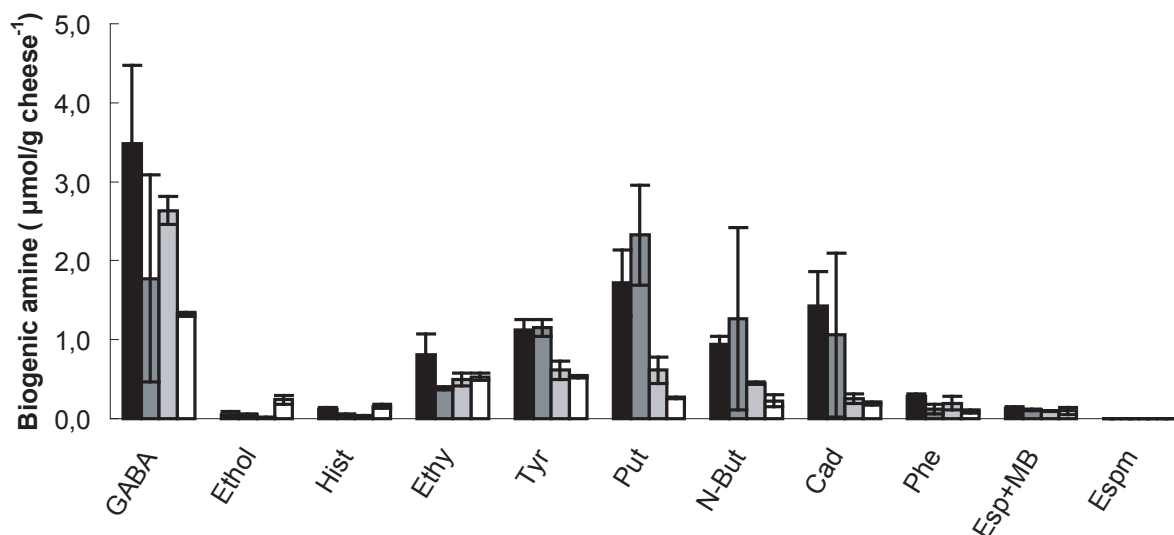


Figure 2. BA concentration in Idiazabal (black), Manchego (dark grey), Zamorano (light grey) and Al vino (white) cheese at 60 days ripening, made with bovine rennet GABA: γ -aminobutyric acid, Ethol: ethanolamine; Hist: histamine, Ethyl: ethylamine, Tyr: tyramine, Put: putrescine, N-But: n-butylamine, Cad: cadaverine, Phe: phenylethylamine, Esp+MB: espermine + methylbutylamine and Espm: espermidine

The concentration of individual BA in cheeses after 60 days of ripening is shown in figure 2. This ripening time was chosen for comparison because it is the longest ripening time for "Al vino" cheese. As the figure shows, GABA was the BA present in the highest amount in all cheeses, although concentration were significantly different among them (table 1). Other amines present

in high concentration are tyramine, putrescine and cadaverine. Similar results were found in other mature cheeses (6). In contrast, very low amounts of histamine were found in all cases.

The concentration of all individual BA varied significantly among cheeses, but the type of rennet affected only the concentration of tyramine (table 1). The concentration of this BA was higher in cheeses made with artisanal lamb rennet paste than in cheeses made with commercial bovine rennet.

After 60 days ripening, both the total concentration of BA and the concentration of the main individual amines were lower significantly ($p < 0,05$) in "Al vino" cheese than in other cheeses.

Table 1: Nested ANOVA analysis for effect of the factors "Type of cheese", "Type of rennet" and "Ripening time" in the total and individual BA amount. "Type of rennet" and "Ripening time" are nested with "Type of cheese".^a

Biogenic Amine	Type of Cheese	Type of Rennet	Ripening time
GABA	***	ns	***
ethanolamine	**	ns	***
histamine	ns	ns	***
ethylamine	***	ns	***
tyramine	***	*	***
putrescine	***	ns	***
n-butylamine	***	ns	***
cadaverine	***	ns	**
phenylethylamine	***	ns	***
ethylamine	***	ns	***
spermine+ methyl-butylam.	*	ns	***
spermidine	***	ns	***
total	***	ns	***

^a Significance levels: *** $p < 0.001$; ** $p < 0.01$; $p < 0.05$; ns: not significant.

4. Conclusion

Both the total amount of BA and the concentration of each BA increased along the ripening period and varied significantly ($p < 0,005$) among different type of cheeses. GABA exhibited the highest concentration in all cases, whereas histamine was present in very low amounts.

The type of rennet had no effect on the amount of individual BA except for tyramine, the concentration of which was significantly higher when artisanal rennet paste was used than when commercial rennet was used.

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III-P158: Vastedda Della Valle del Belice Cheese: Production and Microbiological Investigation

M.L. Scatassa¹, A.M. Di Noto¹, M. Todaro², S. Caracappa¹

Summary

The "Vastedda della valle del Belice" is a fresh "pasta filata" cheese, produced from whole raw milk of the Valle del Belice sheep. The various stages of making process for this traditional and historical cheese were examined by investigating technical and microbiological parameters at different stages of production.

The finished Vastedda cheeses exhibit good hygienic and sanitary conditions without pathogenic organisms such as *Listeria monocytogenes* and *Salmonella*. Lactic bacteria were present at an average concentrations of 10^7 to 10^8 cfu/g. The analysis of the cheese making processes showed a higher variability between each process.

1. Introduction

The "Vastedda della valle del Belice" is a fresh cheese obtained from whole raw milk without starter culture. It is made with the milk of a breed called Valle del Belice sheep which originated in the valley around the Belice river. This historical cheese is the only "pasta filata" cheese produced in Italy with sheep milk.

The raw milk is coagulated with traditional home-made lamb rennet paste at the temperature of 38°C. Milk coagulation occurs in approximately 30 min and the curd is cut into grains of about 0.5 cm with or without hot water added. The curd is poured into moulds kept at environmental temperature for about 6 to 48 h, depending on the temperature variations. During this time, because of the action of the microflora, the curd goes through a process of maturation. When about 5.2 pH is reached, the curd is stretched using water or whey at 80–90°C. The stretching is made manually and Vastedda cheese assumes the concave shape similar to the shape of a soup plate where it is put to dry for about 12 h. Afterwards, it is transferred in brine for about one or two hours. At completion, the cheeses will weigh from 500 to 700 grams.

The final characteristics of a typical product arise mainly from the specific raw materials employed, the environmental conditions, the area of production, the animal feeding and the traditional tools. The aim of this work was to investigate the productive process and the microbiological parameters at the various stages.

2. Material and methods

A total of 42 cheese-making processes were studied and compared in 8 dairies in the Belice valley over a two-year period, considering their time, temperature and acidity. Samples were taken from the milk, curd, curd after acidification and the stretching as well as the final product for each cheese-making process. The samples collected were subjected to microbiological analyses using the following procedures: Coagulase-positive staphylococci ISO 6888-1:1999/A1:2003 and ISO 6888-2:1999/A1:2003; Enumeration of microorganisms at 30°C ISO 4833:2003; Coliforms ISO 4832:1991; *Escherichia coli* ISO 16649-2:2001; Enterococci on Rapid Enterococcus Agar (REA) incubated at 44°C for 48 h; Sulfite-reducing bacteria growing under anaerobic conditions ISO 15213:2003; *Salmonella* spp. ISO 6579:2002; *Listeria monocytogenes* ISO 11290-1:1996; Mesophile and Thermophile Lactococci on M17 medium, incubated respectively at 22°C for 48 h and 44°C for 72 h; Lactobacilli on acidified MRS medium and incubated under micro-aerobic conditions (5% CO₂) at 37°C for 72 h.

The concentrations of the microorganisms which were investigated in different samples by polynomial equation were fitted.

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3. Results and discussion

The results of the cheese making processes parameters were reported in Table 1 and evidence the high variability of the processes that could have more effects on Vastedda cheese characteristics.

Table 1: Cheese making processes parameters

Parameters	mean	SD
Milk pH	6.7	0.1
Rennet (g)	55	27
Clotting temperature (°C)	38.3	3.2
Clotting time (min)	33	16
Water (%)	8	8
Water temperature (°C)	76.7	13.1
Acidification time (h)	26	16
Curd pre-stretching pH	5.2	0.2
Stretching water temperature (°C)	77.2	10.6
Drying (h)	12	7
Brine time (h)	1.7	0.9
Vastedda cheese pH	5.3	0.2

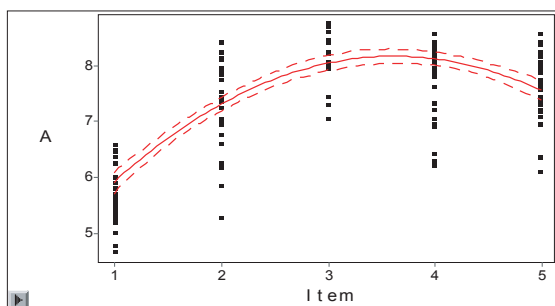


Figure 1. Total Bacterial Count evolution during cheese making processes ($R^2=0.61$).

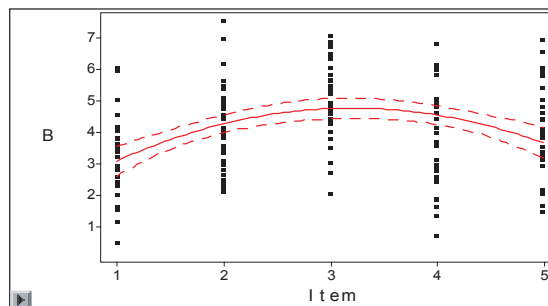


Figure 2. *E. coli* evolution during cheese making processes ($R^2=0.15$).

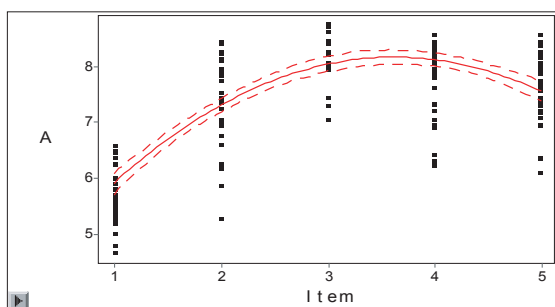


Figure 3. Enterococci evolution during cheese making processes ($R^2=0.21$)

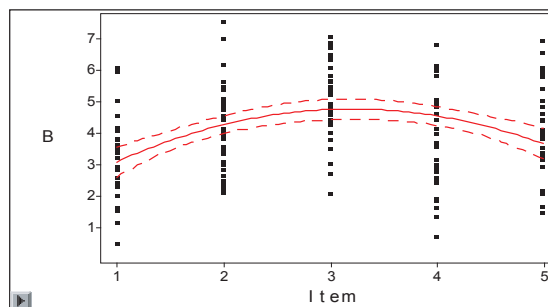


Figure 4. Lactobacilli evolution during cheese making processes ($R^2=0.46$)

Item: 1 milk, 2 curd; 3 curd pre-stretching; 4 cheese pre-brine; 5 Vastedda cheese

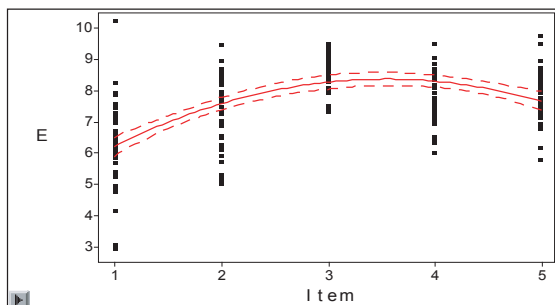


Figure 5. Mesophile Lactococci evolution during cheese making processes ($R^2=0.35$).

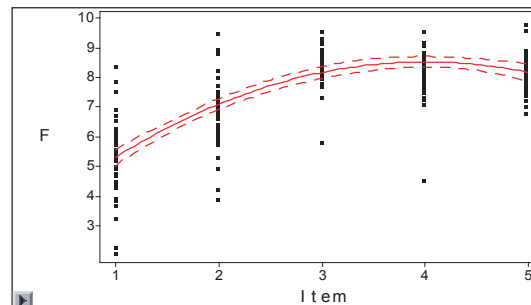


Figure 6. Termophile Lactococci evolution during cheese making processes ($R^2=0.60$).

Item: 1 milk, 2 curd; 3 curd pre-stretching; 4 cheese pre-brine; 5 Vastedda cheese

In the above figures the evolution of some microorganisms were reported. Coliforms and *E.coli* (Figure 2) were present in many samples, but after the stretching the numbers decreased significantly. In only two samples of cheese *E.coli* were present at concentrations between 10^5 to 10^6 cfu/g. Enterococci (Figure 3) are more resistant to high temperature; in the final product they are found in concentrations from 10^5 to 10^6 cfu/g. Total Bacterial Count and Lactic bacteria (Figure 1,4,5 and 6) were present at average concentrations of 10^7 to 10^8 cfu/g. No pathogenic organisms (including *sulfite reducing Clostridia*, *Listeria monocytogenes* and *Salmonella*) were observed in the sample. However, *S.aureus* was found in 15 milk samples but only in 7 cheese samples: In these latter ones *S.aureus* concentration was higher than 10^5 cfu/g in 4 of 7 but no enterotoxaemia activity was found.

4. Conclusion

The overall results presented in this work evidenced the good sanitary conditions of "Vastedda della Valle del Belice" cheese, since no pathogenic bacteria had been detected. Hygienic parameters are also quite acceptable since the presence of few *S. aureus* and *E. coli* are confined in some samples of raw milk and declined in the cheeses. These results strongly suggest that hygienic parameters are influenced by milk quality and cheese making procedures.

References

1. Council Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products.

Acknowledgements

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Posters Session IV. Market and Perspectives

IV-P159: Sheep and Goat Milk Production in Austria - An Economic Alternative?

J. Hambrusch¹, L. Kirner¹

Summary

As a result of the niche character economic calculations about sheep and goat milk production hardly exist in Austria. Based on selected economic indices the study provides insights into the profitability of sheep and goat milk production compared to alternative animal husbandry systems. The data were obtained from literature and supplemental oral interviews with sheep and goat farmers. Four different types of sheep and goat milk farms were specified within a farm model. The results reveal two important statements. First, the production of sheep and goat milk shows high productivity per ha grassland and second a quite low productivity per labour unit. Thus, the production of sheep and goat milk offers an economic alternative especially for small family farms.

1. Introduction

Sheep and goat farming plays only a minor role in terms of production value in the Austrian agriculture. In 2005 both industries contributed only 0.5% to the total agricultural production value of 5 420 Mio. € [1]. The bulk of sheep and goat farms is located in less-favoured grassland areas, traditionally dominated by dairy cattle farms. In recent years increasing competition and reforms of the Common Market Organisation put pressure on the dairy industry resulting in a decreasing number of farms. Because of similar demands on forage sheep and goat farming represents an alternative to cow milk production. Therefore, the study focuses on the economic performance of these businesses and provides comparisons between sheep and goat milk farms and alternative animal husbandry systems.

2. Material and methods

Additional to a literature review, for example [2] oral interviews with sheep and goat farmers provided specific information on production techniques, marketing, input costs and output prices (for example raw milk). Based on this information four different farm types were modelled for the economic analysis:

- MS-80: Sheep milk production, 80 ewes,
- MS-150: Sheep milk production, 150 ewes,
- MG-80: Goat milk production, 80 goats,
- MG-150: Goat milk production, 150 goats.

The economic evaluation comprises key figures like gross margins per animal, aggregated gross margins per ha and the ratio between total returns and total costs.

3. Results and discussion

On average, more than 70% of the total variable returns of milk sheep farms originate from milk sales, even more than 85% in case of milk goat farms (see Table 1). Concentrates represent the most important direct expense factor in both production activities. Figure 1 compares gross margins per ha grassland (including the costs for forage) for different farm types. According to these results sheep milk production exhibits higher gross margins per ha grassland than other production activities (for example milk cows, suckler cows, lamb fattening). In this context, only outstanding dairy cattle farmers can compete with sheep and goat milk producers. Otherwise,

¹ Federal Institute of Agricultural Economics, Marxergasse 2, 1030 Vienna, Austria.

the gross margins per labour unit of sheep and goat milk producers range from 4.8 €/h to 10.8 €/h and remain below those of cow milk producers.

Figure 2 refers to total returns and total costs per kg milk. The calculations incorporate beside variable costs also other cash costs, costs for depreciation and costs for labour, land and capital. Total costs vary significantly according to the farm types: from 1.56 € to 1.85 € on milk sheep farms and from 1.00 € to 1.26 € on milk goat farms. Considering different returns the variable costs are covered by milk returns in all model farms but only the two larger farms cover also their depreciation and cash costs. After the inclusion of returns from animal sales and direct payments also these costs are fully covered on each farm. However, no model farm can cover all opportunity costs for labour, land and capital.

Table 1: Gross margin per ewe and goat according to farm type

	Milk sheep farms		Milk goat farms	
Ewes/goats per farm	80	150	80	150
Variable Returns (€)	522	569	392	452
milk	380	427	330	390
lamb/kid	133	133	59	59
other returns	9	9	3	3
Variable costs (€)	198	208	169	178
concentrates	62	68	38	42
contributions	32	36	28	33
straw	15	15	15	15
others	89	89	88	88
Gross margin (€)	324	361	223	274

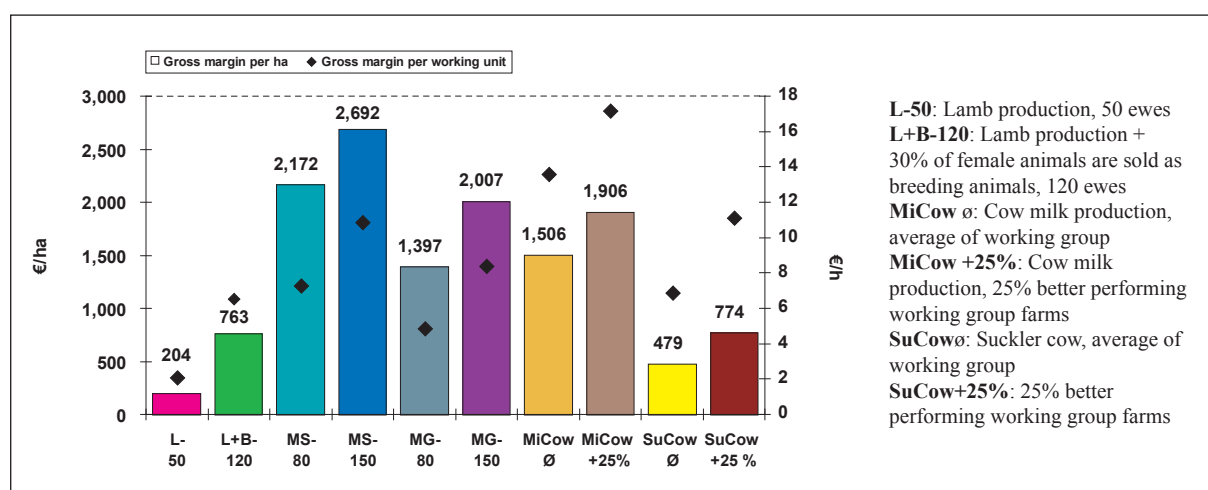


Figure 1. Gross margins per ha grassland and per working unit for different animal husbandry systems.

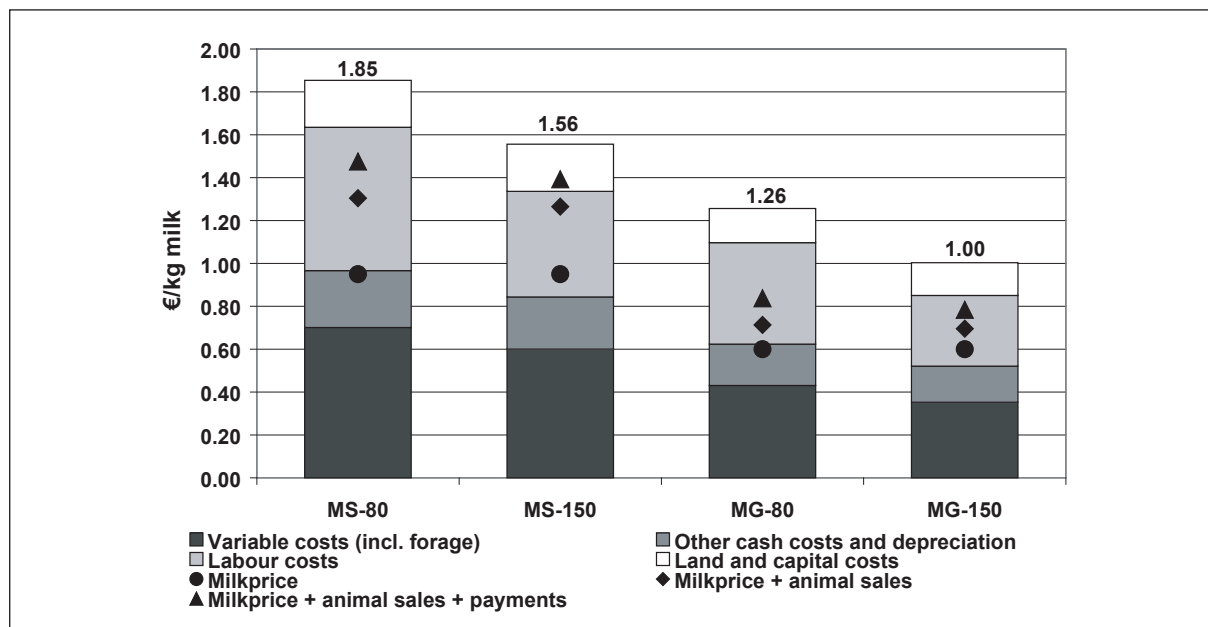


Figure 2. Total costs and total returns per kg milk of the model farms.

4. Conclusion

Like in other agricultural industries profitability depends mainly on the economic framework (for example price of milk), the management skills of the farmer and the production conditions. Therefore the study presented cannot fully clarify the question whether the production of sheep or goat milk is profitable or not. However, two tendencies can be derived unequivocally from the calculations. On the one hand, production of sheep or goat milk has a high productivity per ha grassland and may offer a good alternative for farms with less area. On the other hand, the production of sheep or goat milk is very labour intensive. Nevertheless, like in other agricultural industries (for cow milk production see [3]) not all costs can be covered by total returns. This means that the factor productivity of labour and capital is lower than in their use outside agriculture.

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IV-P161: Dairy Sheep Production in France, Diversity of the Breeding Systems in PDO Cheese

E. Morin¹, G. Lagriffoul²

Summary

In France, dairy sheep production is characterised by an association between one area, one (or several) local breed(s) and one PDO (Protected Designation of Origin) cheese: Roquefort cheese in the Roquefort area, Ossau-Iraty cheese in the western Pyrenees and Brocciu in Corsica island. The breeding systems reflect the characteristics of these 3 mountain areas while taking into account the PDO rules. Indeed, in France, more than 90 % of the dairy sheep farmers are involved into PDO production. Processing milk into PDO cheeses obliges the producers to respect some conditions of production which are specific to each PDO.

1. Three traditional areas

Every 10 years, the agricultural census is carried out by the statistical services of the ministry for agriculture. It makes possible to have a detailed picture of French agriculture.

The last census (year 2000) indicated that 5824 farms breed at least 25 dairy ewes. These farms are mainly located in the 3 traditional areas of sheep milk production: Roquefort area in the south of Massif Central (43% of the farms), French western Pyrenees (43%) and Corsica island (9%).

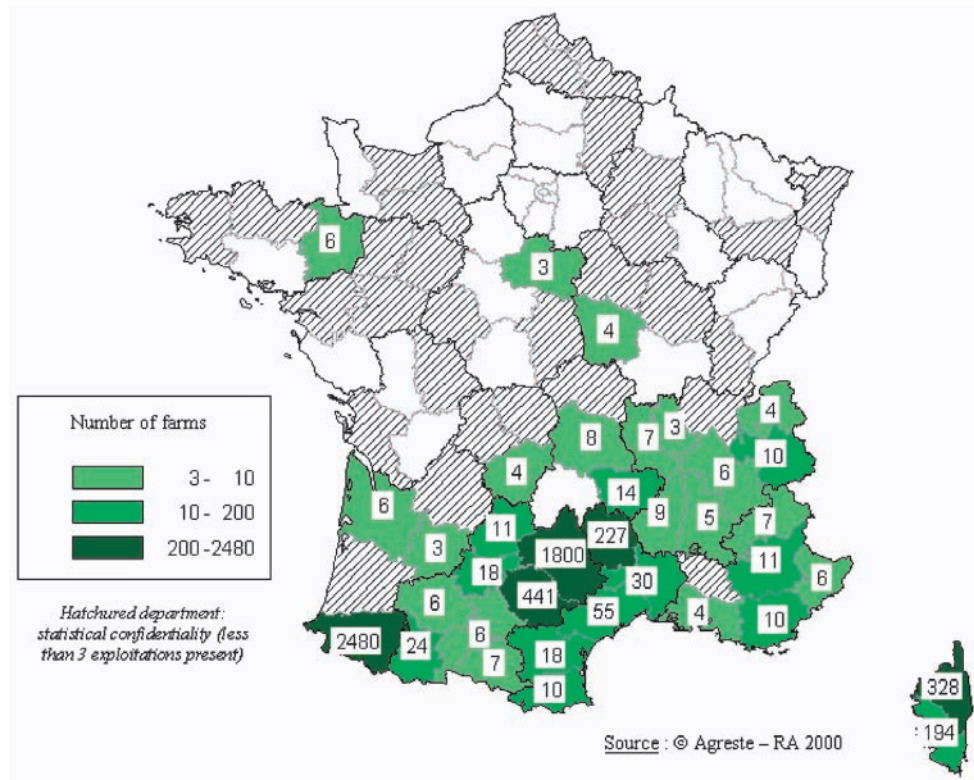


Figure 1. number of dairy sheep farms per department – 2000 (farms with at least 25 dairy ewes).

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² Comité National Brebis Laitières - Institut de l'Élevage, BP 42118, 31321 Castanet-Tolosan cedex, France.

2. Different breeding systems

Useful Agricultural Area (UAA) is very different from one area to another. In the French western Pyrenees, the farms are generally low-size: on average 24 ha UAA, mainly with natural grassland. In the Roquefort area the farms are bigger: on average 69 ha UAA, with a high proportion of temporary grassland.

In the three areas, sheep farmers often use pastoral lands in their feeding system: in Corsica island, 81% of the farmers have large areas of rangelands (on average 50 ha) and in the western Pyrenees, 74% of them use common mountain pastures during summer.

In the Roquefort area, the flocks have a relative large size: a little more than 300 dairy ewes on average. In the western Pyrenees and in Corsica island, the flocks are smaller, less than 200 ewes on average.

Table 1: Surfaces available in the 3 traditional areas

	Useful Agricultural Area (ha)	Main fodder area (ha)			Rangelands area		Farms with common mountain pasture (%)
		Annual forage	Natural grassland	Temporary Grassland	Farms %	Area (ha)	
Roquefort area	69.0	1.1	40.0	13.4	43	65	2
French western Pyrenees	24.4	1.6	6.0	15.6	74	12	74
Corsica island	29.9	1.0	6.7	18.7	81	50	38

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In the three areas, the ewes are local breeds, adapted to their environment and their production system: Lacaune ewes in the Roquefort area and Corsica ewes in the Corsica island. In the French western Pyrenees, there are three breeds: the Basco-Béarnaise ewes are in the Bearn area, Manech Black Face in the Basque mountains and Manech Red Face in the Basque hillsides.

Beside the flock of dairy ewes, the farmers often have another production. In the French western Pyrenees, 75% of the farmers have on average 15 meat cows (Blonde d'Aquitaine breed).

Table 2: Farm livestock

	Number of dairy ewes	Meat cows		Dairy cows		Meat sheep		Goats	
		Farm %	Number of cows	Farm %	Number of cows	Farm %	Number of ewes	Farm %	Number of goats
Roquefort area	302	28	23	3	10	12	132	2	8
French western Pyrenees	191	75	15	11	17	0	0	6	13
Corsica island	180	30	29	0	-	2	62	10	50

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3. A production for PDO cheeses

Most farmers are involved into PDO production: Roquefort cheese for Roquefort area, Ossau-Iraty cheese in the French western Pyrenees and Brocciu in Corsica.

The transformation of their milk into PDO cheese obliges the farmers to comply with PDO rules relating to the conditions of production. Firstly, in accordance with the PDO rules, only local breeds are allowed. As regards the food of the ewes, the farmers must privilege grazing and the feed must mainly come from the PDO area. Some rules are specific to one PDO cheese. For example, for Ossau-Iraty cheese, the animal productivity cannot be higher than 300 liters per ewe.

Table 3: Number of dairy sheep farms and total milk production (2004)

	Number of farms	Proportion of farms		Total milk production (millions of liters)
		α		
Roquefort area	2 315	α	100	180
French western Pyrenees	2 210	16%	83	53,6
Corsica island	426	27%	94	9,5

Interprofessions, syndicats AOC, INAO.

The most important feature for the dairy sheep production in France is the association between one PDO cheese, one production system and local breeds. In this background, a large majority of farms remain viable and the sector is dynamic.

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IV-P163: Study of Sheep Milk Production Systems in Serbia

M.P. Petrovic¹, D. Ružic-Muslic¹, M. Zujovic¹, V. Caro-Petrovic¹

Summary

Traditional system of milk and cheese production in Serbia exist during summer in the four region (Stara Planina, Sjenica, Svrljig and Homolje). Sheep production is conducted within extensive and intensive systems of private farms, with capacity of 20-500 sheep, where representatives of several local breed of sheep made the basis for the milk production.. Milkiness per animal is greater on intensive farms due to better nutrition conditions and they make much more attention to genetic improvement of milk production. Produced quantities of sheep milk are used for manufacture of quality cheeses. The most famous hard cheese is Pirot kachkaval from Stara planina, but too white cheeses from other three regions.

1. Introduction

Breeding dairy program in Serbia today are based on numerous factors which are included in analysis, all data relating to milk traits are considered to be important.

According to statistical indicators and recent researches (Petrovic et al., 2003., 2005) average milk production of more important breeds of sheep is small and ranges from 60-100 liters. In many Mediterranean and East European countries the attention is paid to discovering an optimum production system (Kukovics et al, 2001., Ugarte et al, 2001, Gabina, 2000., 2006). From the total number of sheep in Serbia, 80 percent are of the lokal Pramenka breed. Breeding dairy program in Serbia today are based on numerous factors which are included in analysis, all data relating to milk traits are considered to be important.

This paper draws on some major elements of the sheep systems in milk production in Serbia.

2. Material and methods

Researches were carried out in the four region (Stara Planina, Sjenica, Svrljig and Homolje). Based on the plan of research activities relating to the breed structure of sheep and parameters of milk traits. In order to evaluate the health conditions of the udder and quality of milk the somatic cell count was determined using the method of milk sediment smear on microscopic glass. Investigation included family farms which were engaged in breeding of sheep.

Milk recording was carried out using standard method once a month during the entire period of lactation. Laboratory analysis of milk was performed in dairy plant Dojkinci as well as in authorized institutions in Belgrade.

Processing of data was done using linear models which are applied in this fields of research.

3. Results and discussion

The results of influence of production systems on sheep milk quantity and quality are presented in table 1 and 2. These sheep populations are equally used for the production of milk, meat and wool, so the effects in milk production are poor. Milkiness during the lactation of 180 days ranges from 69.38 to 70.39 kg in extensive and 80.98 to 90.39 kg in intensive system of farming. We can see that there are no significant differences of milk yield between the various races, but there are significant differences between production systems.

Regarding the chemical composition of milk, it should be noted that the fat content is on average 6.94% in extensive and 6,46 in intensive system and, protein 5.33 % in extensive and 4,98 in intensive system and that there are no significant differences between the various races and systems.

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Table 1: Sheep milk production in extensive system of farming

Region	Breed of sheep	Milk yield (kg) LSM ± SE	Fat (%) LSM ± SE	Protein (%) LSM ± SE	SCC (000/ml)
Stara planina	Pirot pramenka	69.64 ± 1.91	7.11 ± 0.34	5.53 ± 0.19	280
Svrljig	Svrljig pramenka	70.01 ± 1.61	6.71 ± 0.26	5.31 ± 0.16	450
Sjenica	Sjenica pramenka	70.39 ± 2.58	6.93 ± 0.28	5.19 ± 0.20	400
Homolje	Svrljig pramenka	69.38 ± 1.97	7.01 ± 0.31	5.32 ± 0.18	330

Table 2: Sheep milk production in intensive system of farming

Region	Breed of sheep	Milk yield (kg) LSM ± SE	Fat (%) LSM ± SE	Protein (%) LSM ± SE	SCC (000/ml)
Stara planina	Pirot pramenka	82.63 ± 2.11	6.21 ± 0.24	5.08 ± 0.21	250
Svrljig	Svrljig pramenka	90.22 ± 2.89	6.52 ± 0.21	4.95 ± 0.20	280
Sjenica	Sjenica pramenka	90.39 ± 2.99	6.63 ± 0.18	4.88 ± 0.24	300
Homolje	Svrljig pramenka	80.98 ± 2.28	6.51 ± 0.30	5.01 ± 0.19	290

4. Conclusion

Sheep milk production in Serbia exhibit a great diversity of systems, from extensive to intensive management combined to a great diversity of genetic material.

The milk quantity and quality of sheep in both systems of production depend too from feeding condition. That is reason why good genetic material of sheep can not to give better milk production.

However, due to low milkiness of local sheep population it is necessary to devote more activities to genetic improvement of the populations that are being raised.

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Published in four separate parts

Part 4: Posters III-P124 to IV-P163

ABSTRACT

Scientific posters presented at the IDF 5th International Symposium on the Challenge to Sheep and Goats Milk Sectors, 18-20 April 2007, Alghero, Italy.

Presented in 4 sessions related to (1) raw milk, (2) processing and product, (3) characteristics of ewe's and goat's milk products and (4) market and perspectives.

Keywords: Acids; Artisanal; Breeding; Casein; Cheese; CLA; Ewe; Fat; Functional; Goat; Lactation; Lamb; Livestock; Market; Marketing; Milk; Non-bovine; Nutrition; Processing; Production; Protein; Quality; Raw; Rennet; Separation; Sheep; Technology; Udder; Vitamin; Yield

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? !.....	Half-space before and after question marks, and exclamation marks
±	Half-space before and after
microorganisms.....	Without a hyphen
Infra-red	With a hyphen
et al.....	Not underlined nor italic
e.g., i.e.,... ..	Spelled out in English - for example, that is
litre.....	Not liter unless the author is American
ml, mg,... ..	Space between number and ml, mg,...
skim milk	One word if adjective, two words if substantive
sulfuric, sulfite, sulfate.....	Not sulphuric, sulphite, sulphate (as agreed by IUPAC)
AOAC <u>International</u>	Not AOACI
programme	Not program unless a) author is American or b) computer program
milk and milk product	rather than "milk and dairy product" - Normally some latitude can be allowed in non scientific texts
-ize, -ization	Not -ise, -isation with a few exceptions
Decimal comma.....	in Standards (only) in both languages (as agreed by ISO)
No space between figure and % - i.e. 6%, etc.	
Milkfat.....	One word
USA, UK, GB.....	No stops
Figure.....	To be written out in full
1000-9000	No comma
10 000, etc.	No comma, but space
hours.....	ø h
second	ø s
litre.....	ø l
the Netherlands	

Where two or more authors are involved with a text, both names are given on one line, followed by their affiliations, as footnotes

for example A.A. Uthar¹ & B. Prof²

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IDF does not spell out international organizations

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