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of the International Dairy Federation

IDF International Symposium on Sheep, Goat and other non-Cow Milk

16-18 May 2011, Athens, Greece

GREECE

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IDF International Symposium on Sheep, Goat and other non-Cow Milk

Foreword

These are the proceedings of the sixth[1] international symposium on properties, production, technology, nutrition, markets and policies with regard to milk of ewes, goats and other non-cattle milking species. These proceedings are published thanks to the generous support of the IDF National Committee of Greece.

IDF thanks the fellow organizers of the event, the IDF National Committee of Greece and the Hellenic Ministry of Rural Development and Food. About 250 delegates from 31 countries from all parts of the word attended the Symposium, in which 30 lectures and 180 posters were presented. The new element of this event was the rather high number of scientific contributions on non-cow, non-ewe, non-goat milk. A selection of presentations is being published in the International Dairy Journal[2] and 46 of them are published in these proceedings.

Objectives of the Symposium:

The overall objective of the Symposium was to provide a renewed vision of knowledge on husbandry and milk production, technology, chemistry, physics, microbiology and nutrition and to highlight the significance of markets and appropriate policies as they have evolved since 2007. The event was addressed to scientists and other professionals involved in the sheep's, goats' and other non-cows' dairy sectors including milk producers, dairy processing industry, trade associations, academia, research institutes, and governments.

Specific objectives:

To create an opportunity to exchange ideas and strengthen existing links between countries involved with sheep's, goats' and other non-cows' dairy production, between international organizations and institutes concerned with production, processing and nutritional qualities of sheep's, goats' and other non-cows' milk products.

Enhance contact and communication between scientists and technologists, producers and manufacturers, nutritionists and analysts.

Stimulate improvement in the quality of the raw materials and the products and promote the expansion of existing and development of new markets.

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[1] IDF International Symposium on Sheep, Goat and other non-Cow Milk, 16-18 May 2011, Athens, Greece.
[2] Special Issue: IDF International Symposium on Sheep, Goat and other non-Cow Milk, International Dairy Journal, Vol. 24, Issue 2 (2012), 50-152, Guest Editors: Effie Tsakalidou & Efstathios Alichanidis.

Improve knowledge of the requirements of international markets concerning quality, safety, regulations and economics to assist the sectors to achieve these outcomes.

Lead to an improved image for the traditional products and to the development of new products and new market.

IDF thanks the Scientific Programme Committee: Golfo Moatsou (GR) – Chair, Mohamed Abd-El_Salam, Efstathios Alichanidis (GR), Christos Kehagias (GR), Silvia Orlandini (IT), Dimitrios Papageorgiou (GR), Antonio Pirisi (IT), George Psathas (CY), Merxte de Renobales (ES), Andrea Rosati (IT), Joerg Seifert (IDF), Yvette Soustre (FR) and Effie Tsakalidou (GR), as well as the members of the Organizing Committee, from the Greek National Committee side: Effie Tsakalidou (Chair), Emmanuel Anifantakis, Ioannis Kandarakis†, Christos Kehagias, Christina Ligda, Chrysa Matara, Ekaterini Moschopoulou, Ioanna Pappa, Anna Polychroniadou, Eudoxios Psomas, Afroditi-Nectaria Vamvakaki, Ioannis Vastardis, George Zervas and Ioanna Ztaliou, Andreas Marangos from Cyprus Milk Industry Organization and from IDF side: Joerg Seifert.

IDF also thanks the many speakers and presenters of posters and, last but certainly not least, the participants in the symposium for their contributions to its success.

Christian Robert
Director General of IDF,

Brussels, April 2012

Session 1: Social Economic & Environmental Aspects

Posters

1.1. (S1.3) Buffalo-Milk Production Potential and its Comparative Milk Qualities

A. Iqbal[1], B.B. Khan[1], A. Kausar[2], M.J. Aftab[2]

Summary

Pakistan, India and China are the leading buffalo countries in the world. Indo-Pakistan possess promising dairy buffalo breeds. Nili-Ravi from Pakistan is the best breed,also called Black Gold. Its average milk yield is reported 1800-2500 L in 305 days lactation. Water buffalo contribute 5 % towards world milk production. Globally, Pakistan and India produces 90 % of buffalo milk while the remaining is contributed by Italy and China. The present paper will review the milk production potential of buffaloes in different ecological regions of the world along with comparison of its milk with other dairy animals in terms of its nutritional characteristics, therapeutic value and general usefulness.

Introduction

The world has two main types of buffaloes 1) Riverine and 2) Swamp, whereas the third type known as Mediterranean is from these two major types. The Riverine is found in South Asia and South West Asia whereas Swamp is present in East Asia and South East Asia. The Mediterranean buffalo are found in Italy, the Balkan states, Turkey and in some parts of Russia.

There are 46 countries including Asia, Africa, Latin America and Australia inhabiting 172.2 million Buffaloes. Asia has 166.4 million buffaloes i.e. 96.36% of the world's population. In Asia the three major buffalo populated countries are the India, Pakistan and China with the buffalo population of 96, 27 and 22 million respectively. The other countries with lesser buffalo population include Indonesia, Malaysia, Thailand, Srilanka, Nepal, Bangladesh Iran, Bhutan, Italy, Brazil.

The best buffaloes of the world are found in the Subcontinent and the leading buffalo country is the India which produces 96 million tons of milk annually. Pakistan produces 27 million tons annually. Pakistan has 2 best sub-tropical breeds of buffaloes; Nili-Ravi and Kundi, having the potential of giving over 5000 liters of milk per lactation, making Pakistan the 2nd highest buffalo milk producing country in the world. Buffaloes milk is ranked second after cows milk in the world as the buffalo milk that is produced is more than 12% of the world's milk production. 80% of the total buffalo milk is produced by India and Pakistan.

Composition of Buffalo Milk

Buffalo milk is thicker than cow milk because of higher percentages of total solids i.e., 16% than cow's milk, with 12-14%. The butterfat content of buffalo is 6-8% but may increase in well fed animals and in the milk of Swamp buffaloes which are not used for milking normally. On the other hand the fat content of cow is 3-5%: that is why the energy level of buffalo milk is higher than cow milk. Buffalo milk contains a higher level of casein, albumin & globulins than cows milk. Buffalo milk is richer in minerals such as calcium, magnesium & inorganic phosphates.

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Usefulness of Buffalo Milk

Better calcium and phosphorus ratio and less sodium and potassium in buffalo milk than in cows milk makes it a better nutrition supplement for infants. Buffalo milk is preferred over cows milk because of its higher butter fat content i.e. more than 7% and higher solids-not-fat content i.e. 9 to 10.5% than cow's milk. Its average fat globule size is high (2.04 µm) as compared to cows milk i.e. 1.86 µm. Similarly its calcium level is high and cholesterol level is low (0.65mg/g) as compared to cows milk (3.14 mg/g). Buffalo milk has 11.42% more protein than cows milk. Buffalo milk also has high level of natural antioxidant named tocopherol peroxidase. Cow milk allergic patients can benefit from consuming buffalo milk.

Presence of immunoglobulin, lactoferrin, lactoperoxidase & lysozymes in buffalo milk make it a good dietary and health food. Buffalo milk has pro-biotic characteristics. Traditional fermented buffalo milk (Dahi) has beneficial impact on human health which is due to the property of lactic acid bacteria (LAB). Buffalo milk has better emulsifying power than that of cows milk because buffalo milk has higher percentage, that is 50%, of butyric acid having triglycerides as compared to cows milk which has only 37%. Due to this factor more butter & ghee is obtained from buffalo milk. Buffalo milk is also less prone to hydrolytic rancidity than cow's milk.

Significance of buffalo milk

Characteristics of buffalo milk make it highly valuable for processing. To produce 1 kg of cheese only 5 kg of buffalo milk is required compared with 8 kg of cows milk required to produce the same quantity of cheese & 10 kg of buffalo milk is required as compared to 14 kg of cows milk for production of 1 kg of butter. Buffalo milk is preferred by dairies because it is best for making mozzarella cheese. Cheddar cheese from buffalo milk is also superior to cow milk cheese because of its better nutritional value and acceptability.

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1.2. (S1.7) The Situation of Sheep and Goat's Milk in Iran

M. Soltani[1], D. Say[2], N. Güzeler[1]

Summary

Iran's total raw milk production is 7 905 406 tons and accounts for the 1.12% of the total world production. 84.43% of total raw milk produced in Iran is cow's milk, 7.10% is sheep's milk, 5.26% is goat's milk and 3.20 % buffalo's milk. About 60% of total raw milk produced in Iran is industrially processed. A small part of milk which is used in dairy factories for dairy products manufacture is sheep's milk. Sheep's and goat's milk are usually used to produce various traditional cheeses such as Lighvan cheese, Khikki cheese, White cheese and traditional fermented dairy products such as concentrated yoghurt, Kashk, liquid Kashk and yoghurt drink. The aim of this review is to assess the situation of sheep and goat's milk in Iran and to introduce Iran's dairy products which are made from them.

1. Sheep and goat's milk production in Iran

According to the some estimates more than 750 million goats and 1 billion sheep are scattered around the world [1]. Dairy sheep and goats have an important role in dairy industry of Mediterranean and Middle East region countries [2]. Iran's total raw milk production is 7 905 406 tons and accounts for the 1.12% of the total raw milk that is produced in the world [3]. Like the other parts of the world, much more cow's milk is produced than milk of other lactating animals in Iran, such that a large amount of raw milk produced is cow's milk (84.43%). This is followed by sheep's milk (7.10%), goat's milk (5.26%) and buffaloes' milk (3.20%).

Iran ranks 7th in the world and 4th in Asia for sheep milk production with 534000 tonnes and is 0.26% of world sheep milk production approximately (Table 1). The ratio of sheep milk production to sheep population in Iran is lower than elsewhere in the world. It seems that this subject is related to some parameters such as feeding, race and weather conditions [4].

Table 1: World and Iran sheep population and sheep milk production in 2005-2008 [4]

	2005	2006	2007	2008
World Sheep Population	1091375497^{m}	1104192341^{m}	1105610121^{m}	1086307458^{m}
Iran Sheep Population	52219000*	52219000**	53800000*	53800000*
World Sheep Milk Production (tonnes)	194101483^{m}	207399826^{m}	210343406^{m}	205304768^{m}
Iran Sheep Milk Production (tonnes)	534000*	543935**	534000*	534000*

* FAO estimated data; ** Official data; ^{m} May include official, semi-official or estimated data

Iran goat milk production is 410 000 tonnes and is constituted of 0.22 % of world goat milk production approximately (Table 2). According to amount of goat milk produced, Iran qualified 8th in the world and 4th in Asia. Because of some parameters such as race, feeding and environmental conditions the ratio of goat milk production to goat population in Iran is lower than elsewhere in the world [4].

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Table 2: World and Iran goat population and goat milk production in 2005-2008 [4]

	2005	2006	2007	2008
World Goat Population	826905503[m]	827789739[m]	836893906m	864400877[m]
Iran Goat Population	25807000*	25833000**	25531000*	25300000*
World Goat Milk Production (tons)	169617781[m]	170635173[m]	174557394[m]	179085902m
Iran Goat Milk Production (tons)	396000*	410056**	410000*	410000*

* FAO estimated data; ** Official data; [m] May include official, semi-official or estimated data

2. Use of Sheep and Goat's Milk in Iran Dairy Industry

A large part of milk which is used in Iran dairy factories in order to produce industrial dairy products is cow's milk and relastively little of it is sheep milk. There is no record about the industrial use of goat's milk in Iran dairy industry. Sheep and goat milk generally is used for traditional dairy production such as lighvan cheeses, concentrated yoghurt, doogh (yoghurt drink), kashk and traditional liquid kashk.

Lighvan cheese, one of the most popular cheeses in Iran, is produced in small local dairies in Lighvan, a region of east Azerbaijan Province in north-west of Iran [5]. It is a semi-hard and starter-free traditional Iranian cheese and is produced from a mixture of raw sheep and goat's milk [6]. Khikki cheese is traditionally made from whole sheep's milk or mixed sheep and goat's milk using natural rennet in the Assalem region in the north of Iran. Cheese produced is ripened and stored in brine in sheep-skin or goat-skin bags [7].

Concentrated yoghurt is made from cow's milk at industrial level and from sheep and goat's milk or mixture of them at traditional level. The traditional production method consists of straining yoghurt using a cloth bag. The long time taken by production, nutrient value reduction and contamination are the disadvantages of the traditional production method [8]. Doogh (yoghurt drink) is a native beverage in Iran and it consists an important part of families' daily beverage consumption. Similar products exist as ayran in Turkey, tahn in Armenia and lassi in Southern Asia. It is usually produced by mixing set or stirred yoghurt and water at the same rate and addition some aqueous extracts of local herbs [9,10], some spices such as thyme, cucumber and garlic essence or a mixture of them.

Kashk, a concentrated yogurt-type product, is produced traditionally with dehydration of yogurt by sun-drying especially in west and north-west of Iran by villagers and nomads, from sheep and goat's milk [11]. It can be kept for long time at ambient temperature without loss of nutritional value or spoiling [12]. The origin of kashk is in Middle East and it has different names such as kashk in Iran [13], kurut in Turkey [12], kishk in Lebanon, jub-jub in Syria and kushuk in Iraq [14].

Liquid kashk is a dairy product that is produced by milling, dilution, addition of salt and pasteurization of kashk in industrial dairy units. Kashk is made traditionally from sheep and goat's milk used to traditional liquid kashk production [11]. It is produced according to the standard defined by Iran Standard and Industrial Research Institute.

3. Conclusion

A wide variety of traditional dairy products such as lighvan cheese, khikki cheese, concentrated yoghurt, doogh and kashk are produced from raw sheep and goat's milk under non-standardized conditions in the different rural areas of Iran. Due to the absence of a standard production method, depending on processing method and storing conditions, the compositional characteristics vary. Also, the microbiological quality of these traditional dairy products is low generally. However, traditional liquid kashk does not have these problems because of the industrial

production method. Thus, it is possible to transfer a traditional dairy product to industrial production and ensure consumer health.

Acknowledgment

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1.3. (S1.11) Industrial Designs of Feta Cheese Packaging Using Advance CAD Systems

V. Alexopoulos[1], Dr Ing. P. Kyratsis[1,2], N. Efkolidis[1], S. Peristeri[1]

Summary

Feta is a cheese traditionally made in Greece. Since 2002, Feta has been a protected designation of origin product. According to the relevant EU legislation, only those cheeses produced in a traditional way in some areas of Greece and made from sheep's milk or from a mixture of sheep's and goats' milk (up to 30%) of the same area, may be named "Feta". The term "Feta" in Greek means "slice", which is based on the way that Feta cheese is cut into slices in order to be served as a starter. Although, it is one of the most famous products of Greece and in a number of cases synonym of the Greek food quality, its packaging is very simple geometrically and its promotion is based on label design and graphics.

1. Introduction

The present research aims to propose a number of different innovative packaging designs for such a product, using the principles of industrial design engineering and advanced CAD systems [1, 2]. The proposed packaging designs stress its name origin and can be used for both the everyday consumption and for touristic promotion of the Feta cheese. These proposals are expected to offer a further added value in the already world renowned Feta cheese product, increase its sales and deliver higher income to the Greek Feta cheese producers. At the same time, the product can be clearly distinguished from the other white cheeses available in the market, that in a number of cases compete with the original Feta cheese.

2. Results and Discussion

Feta and olive oil with oregano

The proposed packaging combines the Feta cheese with olive oil and oregano or thyme (a combination that is a tradition in Greece). Oregano and thyme, herbs with a strong flavor, also have antibacterial activity when combined with Feta cheese. Olive oil is an ingredient that the consumers prefer for its taste, flavor and the expected health benefits.

It is a packaging that offers the opportunity to the non native consumer to taste these ingredients at the same time, as the Greek tradition requires. Thus it increases the possibility for the consumer to be satisfied by the taste of the product and prefer it again in the future. As a result, the milk and Feta cheese producers will be able to promote further their final product and acquire a higher income, which will increase their capacity for further investments.

In this packaging, the main body of a rather ordinary Feta cheese container is extended, to make an olive shaped sheath, which contains the extra virgin olive oil together with oregano or thyme and is air tight sealed with aluminum foil (figure 1). The fact that the ingredients are offered in separate containers, emphasizes the increased quality of the both products, while at the same time the consumer has the opportunity to serve the cheese by himself.

The promotion of such a product is expected to be very successful not only in all Mediterranean countries, but in all over the world too, since it promotes a very healthy way of life, which is recognised globally.

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Figure 1. Packaging of Feta with olive oil and oregano

Feta slices

The proposed packaging is divided into four slices, thus its shape refers to the name of the cheese (figure 2). Every slice of the package has a seal made of aluminum foil that is air tight, avoiding the main factor of deterioration. The slice-shaped packaging can easily be detached, so that the consumer can take only one portion of the product and leave the rest of the cheese untouched for later consumption.

Figure 2. Packaging "Slices"

The concept of the sliced Feta comes to satisfy consumer needs that the current packaging designs do not fulfill. It is a packaging that makes possible the consumption of the Feta gradually, while it prolongs the time that the product can be stored in a refrigerator.

In addition, it could be possible in the proposed packaging to reduce the content of salt, since salt is an ingredient primarily used as a preservative in Feta rather than a taste enhancer. The possibility of reducing the salt content without lowering the shelf-life of the product is an area of research that needs further investigation. Attempts to reduce NaCl in Feta cheese have already been made by the industry and the academic community. The proposed packaging attempts to make a further step towards this goal from the industrial design point of view. It is a packaging concept that targets a group of consumers who are health conscious, since it is well known that excessive consumption of salt and saturated fat has been linked to health problems in the general population (high blood pressure and elevated cholesterol levels).

Feta and extra virgin olive oil

This box contains two products: Feta and extra virgin olive oil in a bottle (figure 3). On the top surface, two openings allow the consumer to see the contents. On the label, a table recalls a homemade product. The two Greek products, the colors and a rather common table cloth pattern, make this package a perfect candidate for a souvenir or a present from Greece. In the inside, a paper frame supports the two products in order to be easily transferred, without the

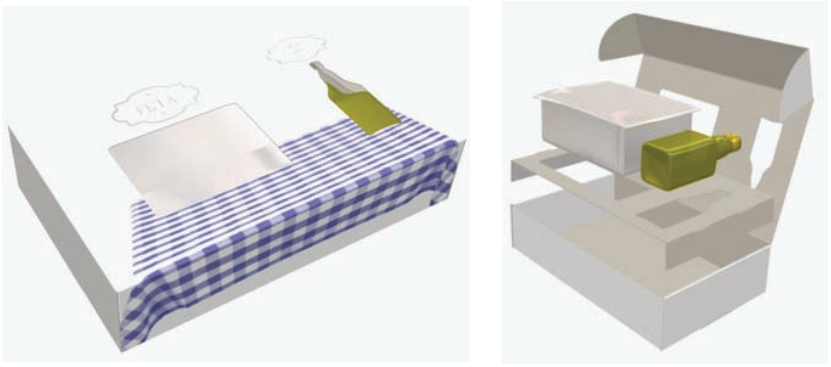

Figure 3. Packaging box with Feta and olive oil

risk to be damaged e.g. in an airplane or a train.

Feta block

This package design is intended to be offered as a present. It is a packaging that resembles a block of Feta cheese and contains two pieces of Feta in vacuum packs (figure 4). On its surface there is an embossed pattern of round glossy cheese holes in order to attract attention. On the front surface, a hot foil stamp provides information about the content, for the purpose of promoting the design concept. On the back surface, there embossed a design similar to those that can be found on Feta blocks, when sold in bulk, that traditionally have the stamp of the producer.

Figure 4. Packaging box with Feta and olive oil

3. Conclusion

The use of advanced CAD systems eliminates the need for real prototypes and makes possible to create realistic digital images of the designed packaging, ensures their manufacturability and drastically reduces the time to market. The use of the principles of industrial design engineering reinforces the dynamics of targeting different customer groups and expands the appeal of products like Feta cheese in countries all over the world.

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Session 2: Raw Milk

Posters

2.1. (S2.10) Comparative Study of Terpenes Concentration in Blood Plasma and Milk of Sheep and Goats Fed Indoors

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Summary

The aim of the present study was to investigate the use of terpenes as feed tracers for the identification of animal products. In a 20 day experiment, 8 ewes and 8 goats were divided in two equal groups, representing control (C) and treatment (T). In T groups 1g/head of each of the following terpenes, α-pinene, limonene and β-caryophyllene, was administered daily for 18 days. Milk production was recorded daily and blood plasma and milk samples were also collected. Blood plasma samples were extracted with organic solvents and Solid Phase Micro-extraction Method used for milk samples before terpenes were identified on a GC-MS. Dosed terpenes were found in blood plasma and milk samples of T group for both sheep and goats. β-caryophyllene was not detected in goats' milk. It was concluded that terpenes can be integrated in certification schemes as biomarkers in animal products, but always used together with other indicators.

1. Introduction

Food tracers can be used to certify quality aspects and origin of animal products and to control public health risks arising from product consumption [1]. Monoterpenes α-pinene and limonene and sesquiterpene β-caryophyllene were chosen as a representative of the substances that originate from pasture and can be used as biomarkers for the identification of animal products [2].

2. Materials and Methods

In a 20 day experiment, 8 adult lactating ewes and 8 adult lactating goats were divided into two equal groups, representing control (C) and treatment (T) group. Animals of the T group were orally administered with 1g of each of the following terpenes, α-pinene, limonene and β-caryophyllene. A 10 ml mixture of the three terpenes in vegetable oil (soybean oil) was prepared and dosed orally each morning to provide 1g of each of the before mentioned terpenes, to each of the T goats, for a period of 18 days, while the C goats respectively were receiving 10 ml of plain vegetable oil. Milk production was recorded daily for both species. Blood and milk samples were also collected at 1, 2, 3, 5, 7, 15, 18, 19 and 20th day of the experiment. Blood plasma samples were extracted with organic solvents and the Solid Phase Micro-extraction Method, using PDMS/CAR fiber, was used for milk samples, before terpenes were identified on a GC-MS.

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3. Results and Discussion

The results indicated that terpenes did not have any effect on milk production for both goats (Table 1) and sheep (Table 2).Dosed terpenes were found in blood plasma of T group for both sheep and goats, whereas no differences between species were observed. All three terpenes were identified in ewes' milk, while β-caryophyllene was not detected in goats' milk. Moreover, milk α-pinene and limonene contents varied greatly ($P<0.001$) between species and days indicating differences in transfer mechanisms for both ewes and goats.

Table 1: Evolution of goat's milk production (kg/head/day) for the Control (C) and Treatment (T) groups of animals

	Day										
Treatment	1	2	3	5	7	15	18	19	20	SE	P
C	1.18	1.33	1.59	1.30	1.35	1.28	1.13	1.24	1.20	0.015	ns
T	1.06	1.10	1.14	1.30	1.15	1.11	1.01	1.01	1.03	0.015	ns

Table 2: Evolution of sheep milk production (kg/head/day) for the Control (C) and Treatment (T) groups of animals

	Day										
Treatment	1	2	3	5	7	15	18	19	20	SE	P
C	0.65	0.60	0.48	0.78	0.70	0.70	0.51	0.93	0.75	0.018	ns
T	0.79	0.65	0.71	0.70	0.78	0.78	0.50	0.90	0.88	0.018	ns

The different concentrations determined within species, with monoterpenes α-pinene and limonene to be transferred to a higher degree than β-caryophyllene, to both blood and milk, urge us to carefully consider the use of these substances as biomarkers. Moreover the variability of terpene concentration observed in milk samples between the two species can probably be attributed to the physiological differences between sheep and goats [3].

Table 3: Evolution of average terpenes concentration in blood plasma of goat and sheep for the Treatment (T) group of animals

		Day										
Substance	Species	1	2	3	5	7	15	18	19	20	SE	P
α-pinene	Goat	0.000	0.010	0.002	0.007	0.040	0.001	0.007	0.007	0.000	0.0052	ns
	Sheep	0.000	0.001	0.004	0.005	0.000	0.002	0.006	0.004	0.002		
Limonene	Goat	0.000	0.001	0.002	0.026	0.041	0.015	0.001	0.001	0.000	0.0053	ns
	Sheep	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.002		
β-caryophyllene	Goat	0.000	0.000	0.001	0.000	0.003	0.000	0.000	0.000	0.000	0.0001	ns
	Sheep	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000		

Table 4: Evolution of average terpenes concentration in milk of goat and sheep for the Treatment (T) group of animals

		Day										
Substance	Species	1	2	3	5	7	15	18	19	20	SE	P
α-pinene	Goat	0.000	0.000	0.000	0.058	0.061	0.159	0.486	0.286	0.201	0.9218	***
	Sheep	0.000	0.766	2.314	1.395	2.351	3,737	1.887	1.919	0.852		
Limonene	Goat	0.000	0.000	0.000	0.038	0.000	0.089	0.023	0.170	0.114	1.3071	***
	Sheep	0.000	1.009	1.006	8.855	0.776	2.997	2.289	1.642	0.484		
β-caryophyllene	Goat	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.1776	***
	Sheep	0.000	0.101	0.435	6.762	5.028	4.876	3.720	1.689	0.439		

3. Conclusion

It was concluded that terpenes can be integrated in certification schemes as biomarkers in animal products, providing that they are considered along with other indicators.

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2.2. (S2.12) Growth of Psychrotrophic Bacteria in Raw Goat Milk and Correlations with Lipolytic and Proteolytic Activities

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Summary

This study investigated psychrotrophic bacteria growth in raw goat milk stored at 4 °C for 1, 3 and 5 days and its relation to the lipolytic and proteolytic activities in milk, using casein/true protein ratio (C/TP) and free fatty acids (FFA) content. Linear increase of lipolytic and proteolytic psychrotrophs growth during storage was observed. The C/TP ratio did not change significantly ($P<0.05$) during storage, indicating low proteolytic activity in milk. However, a linear increase ($P<0.05$) in FFA content was observed, showing high level of lipolytic activity during storage ($y = 0.3465x + 0.1223$). Strong correlations ($P<0.01$) were found between the growth of psychrotrophic microorganisms and the increasing of lipolytic activity in raw milk during the storage. It is concluded that the storage of raw goat milk at 4 °C favors the growth of proteolytic and lipolytic psychrotrophic bacteria which is related to undesirable biochemical changes in milk.

1. Introduction

Due to the low quantity of goat milk produced in small farms, it must be kept under cold storage for long periods before processing in the dairy industry. The bacterial growth in goat milk during this storage period depends primarily on refrigeration. Psychrotrophic bacteria may grow readily at temperatures below 7 °C although their optimum temperature is higher [1]. These micro-organisms have been studied owing to their production of proteolytic and lipolytic enzymes. Proteases hydrolyse milk proteins, forming smaller compounds that are responsible for bitter flavor in milk. The lipolytic enzymes are responsible for fat hydrolysis, increasing the FFA in milk, resulting in rancid, soapy and fruity flavor to milk [2]. This study evaluated the growth of psychrotrophic bacteria in raw goat milk during the cold storage for different periods and correlated their growth with the proteolysis and lipolysis of raw milk.

2. Material and methods

About 100 l of raw bulk goat milk (immediately after milking) were collected in the premises of the College of Animal Science and Food Engineering of University of São Paulo – Brazil, within three different weeks, during July and August 2009. The milk was refrigerated at 4.0 ± 0.1 °C for 1, 3 and 5 days.

Mesophilic bacteria were counted in Plate Count Agar (Merck) after incubation at 37 °C/48h. Proteolytic psychrotrophic bacteria were counted in Calcium Caseinate Agar (Merck) and lipolytic psychrotrophic bacteria were counted in Tributyrin Agar (Merck), after 10 days of incubation at 7 °C.

The goat milk proteolysis was quantitatively estimated through the ratio casein (C)/true protein (TP), expressed as nitrogen-protein equivalent, using *Kjeldahl* method, as described by AOAC [3]. The FFA quantification was realized according to Deeth et al. [4], where 4 ml of milk were added to a mixture of isopropanol: petroleum ether: H_2SO_4 and then titrated with methanolic solution of KOH. Results were expressed in $meq.kg^{-1}$ of milk.

The Complete Randomized Design with factorial arrangement (3 storage times and 3 repetitions of experiment) experiment was submitted to statistical analysis using SAS® software [5], using the *proc glm* procedure and subsequent regression study by orthogonal contrasts. The Pearson

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correlation coefficients (r) between the values of the log of counts of mesophiles, proteolytic and lipolytic psychrotrophs and the rates of lipolysis and proteolysis were performed using the *proc corr* procedure.

3. Results and discussion

The Brazilian regulations for raw goat milk [6] require maximum level of 5 x 10^5 cfu.ml^{-1} of mesophilic bacteria. The quality of milk used in this study complied with the legislation between the first and the third day of storage (4.3 x 10^5 and 4.6 x 10^5 cfu.ml^{-1}, respectively), but not in the fifth day, when mesophilic bacteria population reached 3.6 x 10^6 cfu.ml^{-1}. The populations of lipolytic psychrotrophic bacteria ranged from 5.2 x 10^4 to 2.1 x 10^6 cfu.m^{l-1} and those of proteolytic bacteria from 2.1 x 10^5 to 6.0 x 10^6 cfu.ml^{-1}. As happened with the mesophilic counts, the growth of psychrotrophs was higher after the third day of storage. Between 1 and 3 days of storage, the populations of lipolytic and proteolytic psychrotrophs were similar. The increase was linear ($P<0.05$), of mesophiles ($y = 0.4845x + 4.7626$; $R^2 = 0.96$), lipolytic ($y = 0.8045x + 3.9855$; $R^2 = 0.97$) and proteolytic ($y = 0.7278x + 4.5022$; $R^2 = 0.95$) psychrotrophs in raw goat milk during storage. This effect was expected, since it is known that psychrotrophs can grow at low temperatures. Furthermore, psychrotrophs have optimal growth in mesophilic temperature range, which explains the observed linear increase of mesophilic population in raw goat milk during storage.

The proteolysis and lipolysis of raw goat milk during the storage on the fifth day was 0.85 for C/TP and 1.088 meq.L^{-1} for milk for FFA content. Between first and third days, the FFA contents were similar (0.530 vs 0.693 meq.l^{-1} of milk). The C/TP ratios were also similar (0.88 and 0.89, respectively). The higher values of lipolytic activity on fifth day of storage coincide with the higher growth of the lipolytic microorganisms in raw milk, showing increasing linear effect ($P<0.05$) during the storage time ($y = 0.3465x + 0.1223$). However, no effect ($P>0.05$) of the storage time was observed on the proteolytic activity in milk.

Highly significant ($P<0.01$) correlations were found between the growth of microorganisms and the proteolytic and lipolytic activities in raw milk (Table 1). The correlations between the proteolytic activity and the counts of mesophiles, proteolytic and lipolytic psychrotrophs were -0.70, -0.70 and -0.49, respectively, indicating that the growth of these bacteria decreases the C/TP ratio and consequently, increases the proteolytic activity during the storage. The correlations between the FFA contents and the same microbial group were 0.87, 0.81 and 0.74, respectively, showing a high positive correlation between the growth of these microorganisms and the lipolytic activity in milk during storage.

Table 1: Correlation coefficients (r) between mesophiles, proteolytic and lipolytic psychrotrophs growth and the lipolysis (FFA content) and proteolysis (ratio casein/true protein) in raw goat milk stored for up 5 days at 4 °C

	C/TP		FFA	
	r	P-value	r	P-value
Mesophilic counts	- 0.70	0.001	0.87	<0.001
Proteolytic psychrotrophs	- 0.70	0.001	0.81	<0.001
Lipolytic psychrotrophs	- 0.49	0.039	0.74	0.003

4. Conclusion

The storage of raw goat milk at 4 °C favors the growth of proteolytic and lipolytic psychrotrophic bacteria and this group of bacteria is significantly correlated with the increase of proteolysis (by decreasing the ratio casein/true protein) and lipolysis (by increasing free fatty acids content) in

milk. To keep quality of milk in relation to proteolysis and lipolysis, it is recommended that raw goat milk should not be kept at 4 °C for more than 3 days.

Aknowledgement

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2.3. (S2.19) Assessing The Charm II Bacterial Receptors System to Detect Antibiotics in Sheep's Milk

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Summary

The aim of this study is to assess the Charm II bacterial receptor system with sheep´s milk using a specificity and sensitivity study and different types of milk (lyophilised cow´s milk, lyophilised sheep´s milk and raw sheep´s milk) to establish the control point. To carry out the study into the specificity of the methods antimicrobial free milk samples were analysed. To calculate sensitivity 10 milk samples were analysed for the different antibiotics under study aminoglycosides, betalactams, macrolides, sulfamides and tetracyclines at the Maximum Residues Limits (MRL). Specificity was higher when using lyophilised sheep's milk for the control point. The sensitivity of the Charm II system method was 100% for ampicillin, cloxacillin, penicillin G, cephalexin, ceftiofur, cefoperazone, gentamicin, neomicym, tetracycline, oxitetracycline, sulfadiazine, sulfadimethoxine, erythromycin, espiramycin and tylosin at the MRL concentration when using lyophilised sheep's milk for the control point. Therefore the Charm II system is appropriate to analyse antimicrobial residues in sheep's milk.

1. Introduction

Antibiotic residues in milk can cause toxicological problems that affect public health [1] and could also pose a problem for the manufacturing of fermented products such as cheese and yoghurt [2].

Screening methods have been developed for the detection of antibiotics in milk, among which we find the bacterial receptors binding system known as Charm II. This test can detect a wide range of residues in meat tissues, milk, animal urine, farmed fish, farmed shrimp, water, eggs, feed and honey [3]. For the detection of any antimicrobial drug, Charm II uses a radioactively-labelled antimicrobial drug ([^{14}C] or [^{3}H]) which competes for specific binding sites on a cell or a ribosome (binding reagent) with the contaminating drug. When the binding reagent is added to the milk with antimicrobial drugs, the contaminating antimicrobial drugs bind to receptors in the cell. This prevents the [^{14}C] or [^{3}H] antimicrobial drug from binding to these sites. Thus, the more radioactively-labelled and antibiotic bound, the less contaminating the antibiotic is in the sample. Furthermore, the counts per minute (cpm) are inversely proportional to the amount of antibiotic present in the sample [4].

It is necessary to point out that a large number of detection methods for residues of antimicrobials has been developed and assessed in analysis of cow's milk and that assessment studies in sheep's milk are very scarce [5]. Therefore, this study aims to assess the use of the Charm II system with sheep´s milk by means of calculating the specificity and sensitivity for the different antimicrobials groups.

2. Materials and Methods

The milk samples employed in this study were obtained from the sheep in the University Polytechnic of Valencia's experimental flock throughout the lactation period. Animals were healthy and did not receive any kind of treatment during the experiment.

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In order to calculate the specificity of the tests, 250 antimicrobial-free milk samples were analysed (50 samples per test). In the sensitivity study, 10 different milk samples were analysed for 2 aminoglycosides, 6 betalactams, 3 macrolides, 2 sulfamides and 2 tetracyclines at an equivalent concentration to the MRL.

To carry out the study, Charm II equipment (Charm LSC 7600, Charm Science Inc. Lawrence, Massachusetts, USA) was employed and the tests included: aminoglycosides (GTBL-100K), betalactams (TBL8MRL-100K), macrolides (ETBL-100K), sulfamides (SULFAMRL-100K) and tetracyclines (TMRL-100K) following the manufacturer's recommended instructions. It was necessary to establish a control point to calculate and interpret the results. In order to determine the control point in this study, different types of milk were used: lyophilised cow´s milk (Charm Sciences Inc. Lawrence, Massachusetts, USA), lyophilized sheep's milk (Zeu Inmunotec. Zaragoza, Spain) and raw sheep´s milk. The results were interpreted as follows: if a sheep´s milk sample (cpm) > control point (cpm) the sample was assumed negative; if the sheep´s milk sample (cpm) ≤ control point (cpm) the sample was taken as positive.

3. Results and Discusssion

Table 1 shows the control point (CP) values calculated from lyophilised cow´s milk (CP_1), lyophilised sheep´s milk (CP_2) and raw sheep´s milk (CP_3); values were lower in all cases for CP_2. This table also presents the mean values and standard deviations obtained in 50 sheep's milk samples, as well as specificity. As observed below, when lyophilised sheep´s milk and raw sheep´s milk were employed, specificity was higher (91-100%) than for lyophilised cow´s milk (34-100%).

Table 1: Charm II parameters (cpm) and specificity (%) for sheep´s milk samples

Group	CP (cpm)			Sheep´s milk samples mean (cpm) ± SD	Specificity (%)		
	CP1	CP2	CP3		CP1	CP2	CP3
Aminoglycosides	588.0	255	524	992.2±209.2	98	100	100
Betalactams	793.4	501	473	559.2±197.3	34	94	91
Macrolides	857.7	426	715	1121.6±306.9	80	100	92
Sulfamides	808.5	425	508	986.6±289.1	76	100	94
Tetracyclines	1093.0	844	972	1361.7±193.5	92	98	96

cpm: counts per minute; CP_1: control point of lyophilized cow´s milk; CP_2: control point for lyophilized sheep´s milk; CP_3: control point for raw sheep´s milk; SD: standard deviation; Specificity: negative samples/total samples x 100

Figure 1 illustrates the counts per minute (cpm) obtained in 10 sheep´s milk samples with betalactams at the MRL. The lines correspond to the different control points. In all cases, the cpm of the milk samples were lower than those calculated for all the control points; thus, sensitivity was 100% for all betalactam antibiotics used.

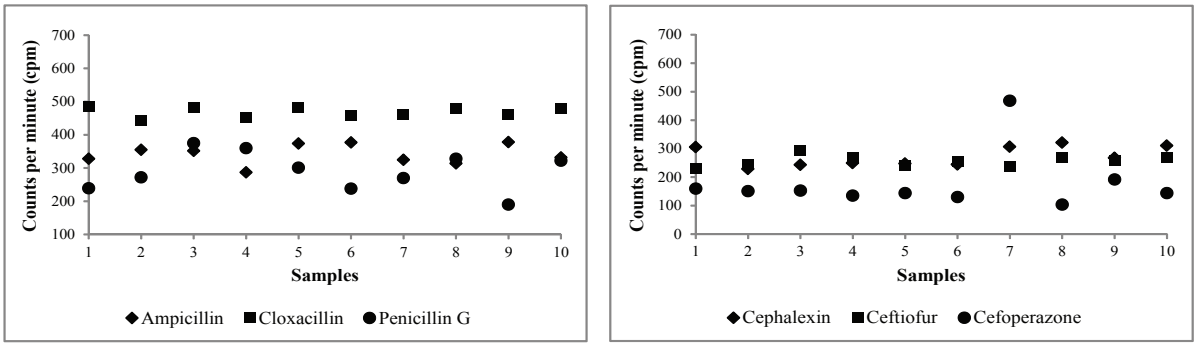

Figure 1. Charm II counts of sheep´s milk samples with betalactam antimicrobial at MRL
(• • • •: CP_1 lyophilised cow´s milk; ▬ ▬ : CP_2 lyophilised sheep´s milk; ▬▬ : CP_3 raw sheep´s milk)

Figure 2 depicts the results corresponding to non-betalactam antibiotics. The milk with aminoglycosides and sulfamides also presented cpm below the three calculated control points; therefore, sensibility was 100%. However, the cpm values of the milk samples came close to those obtained with the control point corresponding to lyophilised cow's milk (CP_1). Conversely with tetracyclines and macrolides, the control point corresponding to lyophilised sheep's milk (CP_2) obtained greater sensitivity.

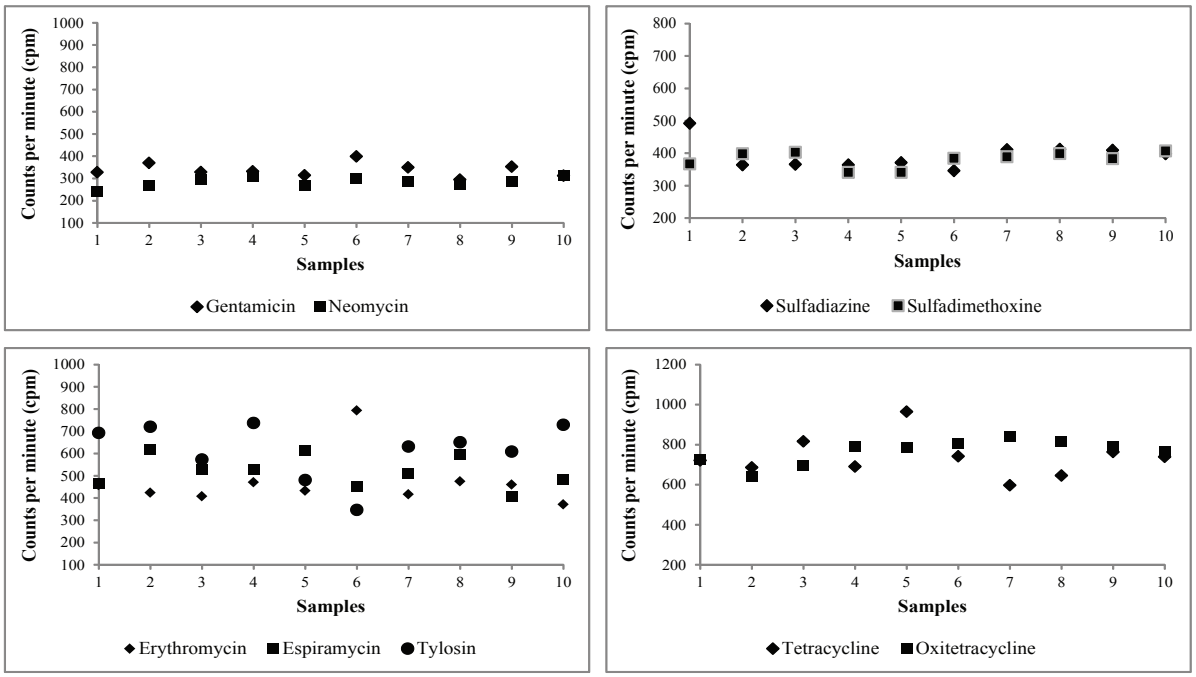

Figure 2. II counts of the sheep´s milk samples with non-betalactam antimicrobials at MRL
(• • • •: CP_1 lyophilised cow´s milk; ▬ ▬: CP_2 lyophilised sheep´s milk ; ▬▬ : CP_3 raw sheep´s milk)

4. Conclusion

Specificity was optimal (between 94-100%) for the different tests in the Charm II system when the control point was calculated using lyophilised sheep's milk. Moreover, the sensitivity of the Charm II system was always 100% for all the assayed antimicrobials when applying the control

point calculated with lyophilised sheep's milk and raw sheep´s milk. In conclusion, the Charm II system is appropriate to analyse antimicrobial residues in sheep's milk when the control point is calculated with lyophilised sheep´s milk or raw sheep´s milk.

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2.4. (S2.20) Effect of Freezing Sheep's Milk on Microbiological Methods to Detect Antibiotics

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Summary

The milk samples collected during quality controls are sometimes frozen in laboratories to confirm results at a later date. Given the possible degradation of some molecules during storage at low temperatures, a study was proposed to assess the influence of freezing on the sensitivity to betalactam antibiotics in some microbiological screening methods used to detect residues in milk. Antimicrobial-free sheep's milk samples fortified with 6 betalactam antibiotics at the Maximum Residue Limits equivalent concentration were used. Each milk sample was divided into 2 aliquots: without a preservative and with acidiol; frozen at -20ºC, -40ºC and -80ºC and analysed after 0, 3, 7, 15 and 30 days using the CMT Copan, Delvotest MCS and Eclipse 100 microbiological screening tests. In general, freezing milk samples affects the response of microbiological screening methods. Thus, it would be advisable to avoid freezing sheep's milk samples to detect betalactam antibiotics with these tests.

1. Introduction

The presence of antibiotic residues in milk can be related to serious problems for public health and have negative consequences for the dairy industry [1, 2]. It is therefore necessary to establish control measures to ensure that milk does not contain drug residues. Usually tests for antibiotics detection are done on refrigerated milk, and even freezing raw milk samples is a common practice in laboratories to repeat analyses and to confirm results.

Some authors have studied the influence of freezing on the stability of antimicrobials and have generally indicated loss of antimicrobial activity with freezing time [3, 4]. There are very few studies available on the effect of freezing milk samples on antibiotic detection by microbiological methods. Therefore, the aim of this study was to assess the influence of freezing sheep's milk samples on the sensitivity to betalactam antibiotics in some microbiological screening methods used to detect residues in milk.

2. Materials and methods

This study employed 16 antibiotic-free sheep's milk samples, according to international standard ISO 13969/IDF 183 [5]. Each milk sample was divided into 2 aliquots: without a preservative and with acidiol. Milk samples were fortified with 6 betalactam antibiotics (penicillin, ampicillin, cloxacillin, cephalexin, cefoperazone and ceftiofur) at the Maximum Residue Limits (MRLs) equivalent concentration. When antibiotics were not detected at this concentration, they were analysed at 2MRL. Afterwards, milk samples were frozen at -20ºC, -40ºC and -80ºC and were analysed after 0, 3, 7, 15 and 30 days using the CMT Copan, Delvotest MCS and Eclipse 100 microbiological screening tests. To assess the effect of freezing temperature, freezing time and acidiol on the sensitivity (positive results*100/total samples) of the microbiological methods, a statistical analysis was performed using the SAS statistical package [6] and the following logistic regression model:

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$$L_{ijk} = \text{logit}\ [\ P_{ijk}\ ] = \beta_0 + \beta_1\ F_i + \beta_2\ T_j + \beta_3\ A_k + \varepsilon_{ijkl}$$

where: L_{ijk}= logistic model; $[P_{ijk}]$= positive or negative probability; β_0 = intercept; β_1, β_2 and β_3= estimated parameters, F_i = freezing temperature as dummy variables: -20ºC: z_1=0 and z_2=0; -40ºC: z_1=1 and z_2=0; -80ºC: z_1=0 and z_2=1; T_j = freezing time (n=5); A_k= presence of acidiol as dummy variables: without acidiol, z=0; with acidiol, z=1 and ε_{ijkl} = residual error.

3. Results and Discussion

The sensitivity of the microbiological methods for betalactam antibiotics calculated from the non-frozen sheep's milk samples (freezing time=0) was 100% for all cases, while the percentage of the positive results obtained lowered when using frozen milk samples. Table 1 offers the percentages of the decrease in the positive results when samples were frozen regardless of freezing temperature and time. Freezing-defrosting of sheep's milk samples interferes with the results of the microbiological tests for antibiotic detection, mostly for penicillin G, ampicillin and cefoperazone and for cloxacillin, cephalexin and ceftiofur but to a lesser extent.

Table 1: Percentage of the decrease in the positive results in microbiological tests when freezing sheep's milk samples

Antibiotics	Microbiological Tests		
	CMT Copan	Delvotest MCS	Eclipse 100
Penicillin G	69	48	73
Ampicillin	52	48	69
Cloxacillin	11	7	21
Cephalexin	24	17	23
Cefoperazone	54	20	54
Ceftiofur	6	2	5

The statistical analysis of the factors of variation related to the response of the tests to detect betalactam antibiotics reveals that the different temperatures studied had no significant effect ($p>0.05$). Freezing time significantly ($p<0.001$) affected the response of the CMT Copan test for cloxacillin, cephalexin and cefoperazone, and that of Delvotest MCS for ampicillin, cloxacillin and cefoperazone (Figure 1).

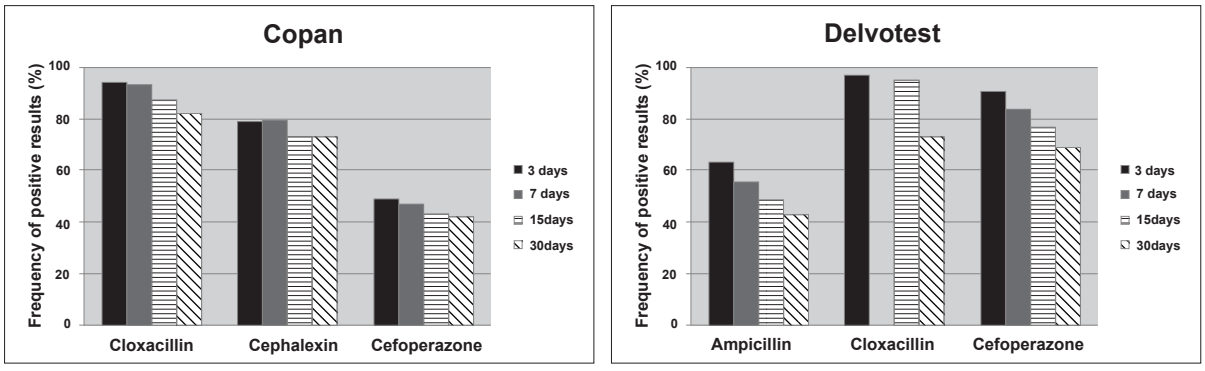

Figure 1. Effect of acidiol on the microbiological screening tests' sensitivity

Moreover, the presence of acidiol had no significant effect on the response of CMT Copan and Delvotest MCS, except for the detection of cloxacillin ($p<0.001$) which was affected by the preservative. In contrast, the use of acidiol affected the response of Eclipse 100 ($p <0.001$) for the detection of penicillin, ampicillin, cephalexin and cefoperazone, with a higher percentage of positive results compared with the milk samples frozen without a preservative (Figure 2).

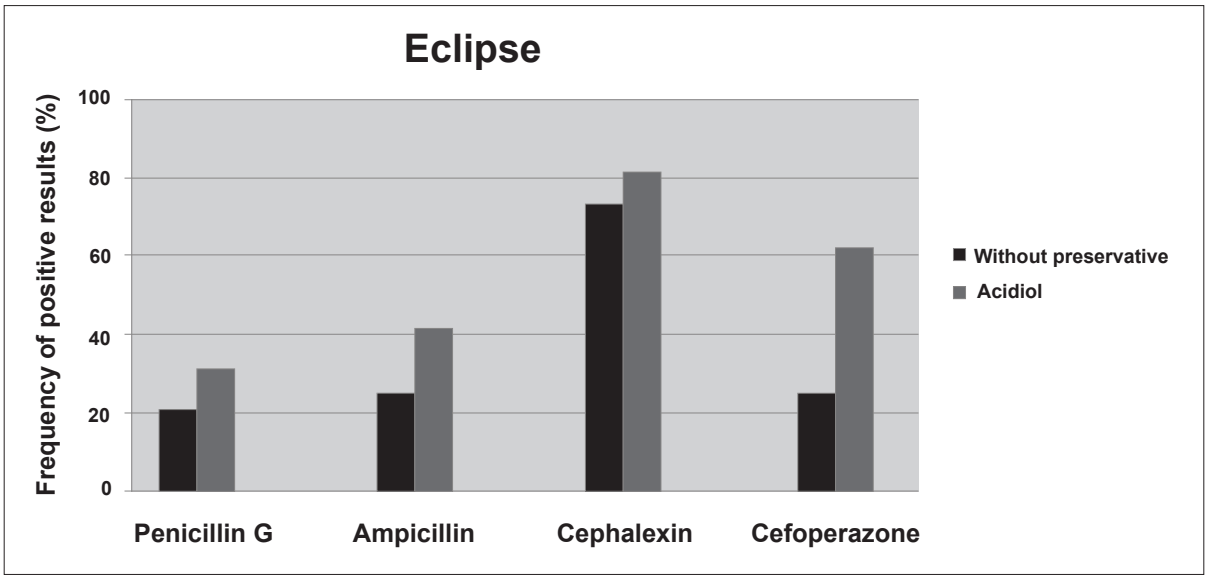

Figure 2. Effect of acidiol on the results of Eclipse 100

4. Conclusion

Freezing-defrosting of sheep's milk samples interferes with the results of the microbiological tests for antibiotic detection, mostly for penicillin G, ampicillin and cefoperazone, and for cloxacillin, cephalexin and ceftiofur but to a lesser extent. The freezing temperature of milk does not influence the response of the microbiological tests for the detection of betalactam antibiotics. In some cases, a prolonged freezing period may affect the response of CMT Copan and Delvotest MCS. When applying the Eclipse 100 test to sheep's milk, it is advisable to add acidiol as a preservative before freezing. In any case, it is suggested to avoid freezing sheep's milk before the detection of antibiotics with microbiological tests because it could affect the reproducibility of the results.

Acknowledgements

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2.5. (S2.21) Effect of Detergent Residues in Goat's Milk on The Response of Screening Methods for Antibiotic Detection

M.C. Beltrán[1], T. Romero[1], O.G. Nagel[2], R.L. Althaus[2], M.P. Molina[1]

Summary

This study aimed to asses the effect of the presence of residues deriving from detergents used to clean and disinfect milking machines and milk storage tanks on the response of two screening methods to detect antibiotics in goat's milk. Antibiotic-free milk samples spiked with different commercial detergents (5 acids, 5 alkalis and 5 domestic dishwashing liquids) and 2 disinfectants at different concentrations were used. Spiked milk samples were analysed by a microbiological method (Eclipse 100) and by a protein receptors binding test (Twinsensor B/T). Acid detergents and disinfectant products did not affect the response of the methods employed as the obtained results were negative at the tested concentrations. Alkaline detergents and domestic dishwashing liquids gave positive results when concentrations were 4-6 ml/l depending on the product and method employed. These relatively high concentrations are very difficult to encounter if good practices for cleaning milking machines and milk storage tanks on farms are implemented.

1. Introduction

Nowadays, residues of antibiotics in raw milk are controlled on livestock farms, in the dairy industry and/or in quality control laboratories which use microbiological methods and protein receptor binding tests for a specific group of antibiotics (betalactams and/or tetracyclins). Screening methods for antibiotic detection can be affected by different factors such as the milk's physico-chemical characteristics, natural inhibitors, preservatives, detergents and/or disinfectants and other substances that are capable of producing non compliant results [1, 2]. Nevertheless, very few studies about the effect of these factors have been carried out. Thus, this study was about the effect of the presence of detergent or disinfectant residues on the response of antibiotics detection methods with goat's milk.

2. Materials and Methods

Antibiotic-free goat's milk samples spiked with a selection of specific commercial detergents for cleaning and disinfection of the milking machine as well as some domestic dishwashing liquids that are sometimes employed for manual cleaning of milk storage tanks and milking utensils were used. Eight different testing concentrations were prepared for each of the detergents and disinfectants considered. These were: 0, 2, 4, 6, 8, 10, 12 and 14 ml/l for alkaline detergent, disinfectants and domestic dishwashing liquids and 0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.50 and 2.0 ml/l for acid detergents.

Spiked milk samples were analysed in duplicate by a microbiological method, Eclipse100 (Zeu-Inmunotec. Zaragoza, Spain), and by a protein receptor binding test, Twinsensor B/T (Unisensor S.A. Angleur, Belgium), following each manufacturer's indications. The Eclipse 100 results were visually interpreted by three trained technicians, while the "ReadSensor" equipment (Model RS00650) was used with the Twinsensor B/T results. All the results were classified as negative (compliant) and positive (non compliant).

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Table 1: Characteristics of detergents and disinfectants used

Detergent	Brand Name	Active Material (%)	Recommended dose (%)
Alkali	Basix	sodium hydroxide/sodium hypochlorite (5-15/5-15%)	0.5-1
	Circoaction AF	sodium hydroxide/sodium hypochlorite (<20/<10%)	0.5-1
	Clor FW	sodium hydroxide/sodium hypochlorite (5/5%)	0.5-1
	Grupaclor	sodium hydroxide/sodium hypochlorite (5/10%)	0.5-1
	Manobactyl	sodium hydroxide/sodium hypochlorite (5-10/5-10%)	0.5-1
Acid	Cid	phosphoric acid/sulfuric acid (15-30/5-15%)	0.5-1
	105 Nifos	phosphoric acid/sulfuric acid (25/5%)	0.5-1
	Grupacid	phosphoric acid/sulfuric acid (25/5%)	0.5-2
	Manocid	ortho-phosphoric acid/nitric acid (25/10%)	0.5-1
	Circoaction SF	ortho-phosphoric acid/sulfuric acid (20-30/5-10%)	0.5-1
Disinfectants	Bleach	sodium hypochlorite (1-5%)	Unknown
	Quacide P40-EC	binguanida hydrochloride (7%)	
Domestic dishwashing liquids	Carrefour	anionic tensioactive (5-15%)	Unknown
	Coral	sodium laureth sulfate/benzenesulfonic acid (5-10/5-10%)	
	Fairy	sodium laureth sulfate/ethanol (20-30/1-5%)	
	Mistol	ethoxylated fatty alcohol (5-10%)	
	Tenaz	sodium hydroxide (<1%)	

3. Results and Discussion

Incorporation of acid detergents or disinfectants products into the milk did not affect the response of the methods employed at any test concentration (specificity 100%). However, Table 2 shows that alkaline detergents influenced the response of the methods at 4 ml/l for Eclipse100 and at 10 ml/l for Twinsensor B/T, only for detection of betalactam antibiotics.

Table 2: Effect of alkaline detergent concentration on the specificity of Twinsensor B/T and Eclipse 100

ALKALINE DETERGENTS										
Detergent	Concentration (ml/l)		0	0.5	1	2	4	6	8	10
Basix	Twinsensor	B[1]	100	100	100	100	100	100	100	100
		T[2]	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	100	0	0	0	0
Circoaction AF	Twinsensor	B[1]	100	100	100	100	100	100	100	100
		T[2]	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	100	0	0	0	0
Clor FW	Twinsensor	B[1]	100	100	100	100	100	100	100	100
		T[2]	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	100	0	0	0	0
Grupaclor	Twinsensor	B[1]	100	100	100	100	100	100	100	0
		T[2]	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	83.3	0	0	0	0
Manobactyl	Twinsensor	B[1]	100	100	100	100	100	100	100	0
		T[2]	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	100	91.7	0	0	0

1-Betalactams, 2- Tetracyclines

Table 3 shows the results for the milk samples with domestic dishwashing liquids, whose presence had no effect on the response of Eclipse 100. However, positive results were obtained with Twinsensor B/T with concentrations from 6 ml/l, but only for the detection of tetracyclins.

Results obtained in this study are similar to those reported by other authors in sheep's milk [3] and cow's milk [2, 4] who also indicated that there were interferences in the microbiological methods when the detergent concentration is near or equal to the recommended usage dose.

Table 3: Effect of domestic dishwashing liquids concentration on the specificity of Twinsensor B/T and Eclipse 100

ALKALINE DETERGENTS										
Detergent	Concentration (ml/l)		0	0.5	1	2	4	6	8	10
Basix	Twinsensor	B1	100	100	100	100	100	100	100	100
		T2	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	100	0	0	0	0
Circoaction AF	Twinsensor	B1	100	100	100	100	100	100	100	100
		T2	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	100	0	0	0	0
Clor FW	Twinsensor	B1	100	100	100	100	100	100	100	100
		T2	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	100	0	0	0	0
Grupaclor	Twinsensor	B1	100	100	100	100	100	100	100	0
		T2	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	83.3	0	0	0	0
Manobactyl	Twinsensor	B1	100	100	100	100	100	100	100	0
		T2	100	100	100	100	100	100	100	100
	Eclipse 100		100	100	100	100	91.7	0	0	0

1-Betalactams, 2- Tetracyclines

As for protein receptor binding methods, results indicate that only high concentrations of detergent interfere with the results. This coincides with other authors [2] who suggest that such methods are affected by the detergent at concentrations equal to the recommended usage dose.

4. Conclusion

Acid detergents and disinfecting products do not affect the Eclipse 100 and Twisensor B/T methods. However, alkaline detergents and domestic dishwashing liquids affected them when concentrations were high, at around 4-6 ml/l. All this suggests that, if good farming practices are implemented for cleaning and disinfecting milking machines and milk storage tanks, residual high detergent concentrations that can cause interferences in screening methods for antibiotics cannot be found.

Acknowlegments

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2.6. (S2.25) Variation in Cell Population and Lymphocyte Subpopulation in Milk from Sarda Dairy Sheep in Relation to The Total Somatic Cell Content

S. Dore[1], P. Bonelli[1], P. Nicolussi[1], R. Re[1], G.A. Pilo[1], E.A. Cannas[1]

Summary

In this work the correlation between somatic cell count (SCC) and the variability of the cell population and lymphocyte subpopulations in Sarda sheep milk at different SCC class levels is studied. A negative correlation between SCC and epithelial cells and a positive correlation between SCC and polymorphonuclear leucocytes (PMN) were observed; an inversion of CD4+/CD8+ ratio in samples with SCC> 2 000 000 cell/ml was found. Evident increase of PMN was observed at SCC level > 300 000 cell/ml suggesting the use of this value as cut-off in diagnosis of udder infection in dairy sheep.

1. Introduction

Somatic cells in milk from a healthy udder consist of epithelial cells, macrophages, lymphocytes and polymorphonuclear leucocytes (PMN). Somatic cell count (SCC) in milk represents the best indicator of udder health status in lactating animals and several studies demonstrated the importance of PMN in defence of the mammary gland. The aim of this study is to evaluate the correlation between SCC and the variability of the cell population and lymphocyte subpopulations in sheep milk at different levels of SCC class as shown in Table 1.

Table 1: Classes of somatic cell content in milk

SCC Class	Value (cell/ml*1000)
I	<300
II	301-500
III	501-1000
IV	1001-2000
V	>2000

2. Material and methods

Seventy-two half-udder milk samples from Sarda dairy ewes were collected and tested for SCC by fluoro-opto-electronic method using a Fossomatic FC instrument (Foss Electric, Hillerød, Denmark) according to UNI EN ISO 13366-2:2007 (IDF 148-2: 2006), differential cell count by microscope method (ISO 1366-1 IDF 148-1:2008) and lymphocytes subsets by flow cytometry (FACS-Calibur, BD) using 2 different four-colour staining protocols (CD25/CD8/CD45/CD8; WC1/CD8/CD45/CD4). In statistical analysis (ANOVA) every single half-udder was considered as an independent sample.

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3. Results and discussion

Results showed a negative correlation between SCC and epithelial cells (ghost cells and cellular debris included) and a positive correlation between SCC and PMN. A significant increase of PMN ($P<0.001$) and a decrease of epithelial cells ($P<0.001$) were found from the I to the V SCC class; no significant differences were observed in lymphocyte and macrophage percentage content among the different SCC classes (Figure 1). Lymphocyte subsets analysis evidenced a CD4+/CD8+ inversion in V class samples (Figure 2).

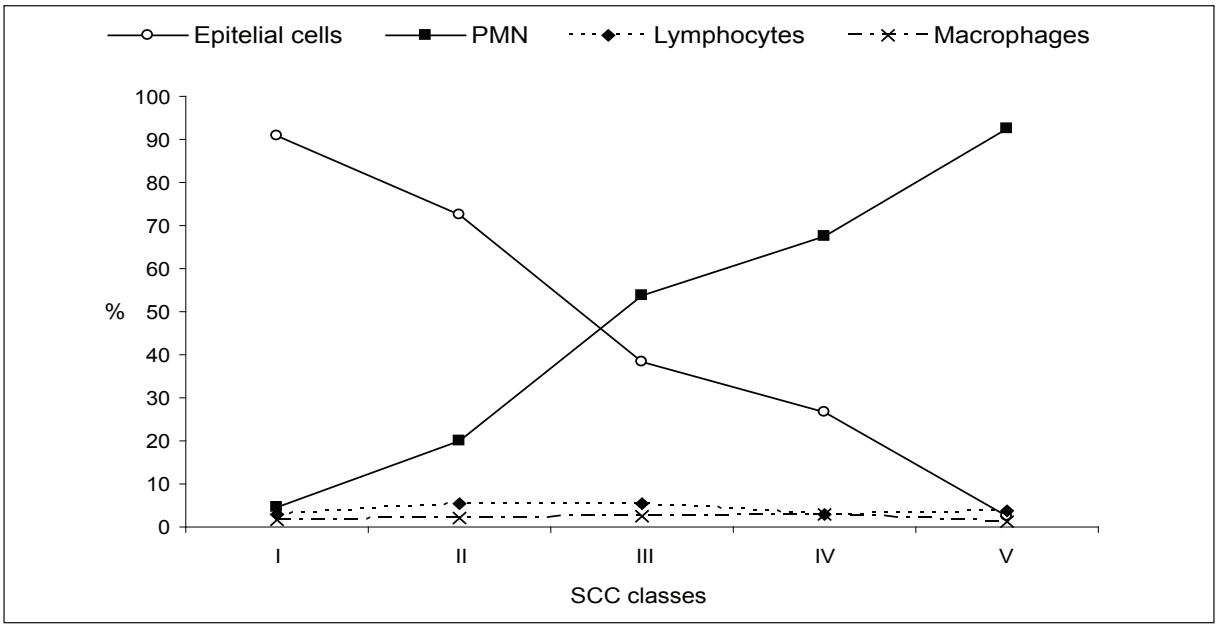

Figure 1. Percentage distribution of each somatic cell type at different levels of SCC classes

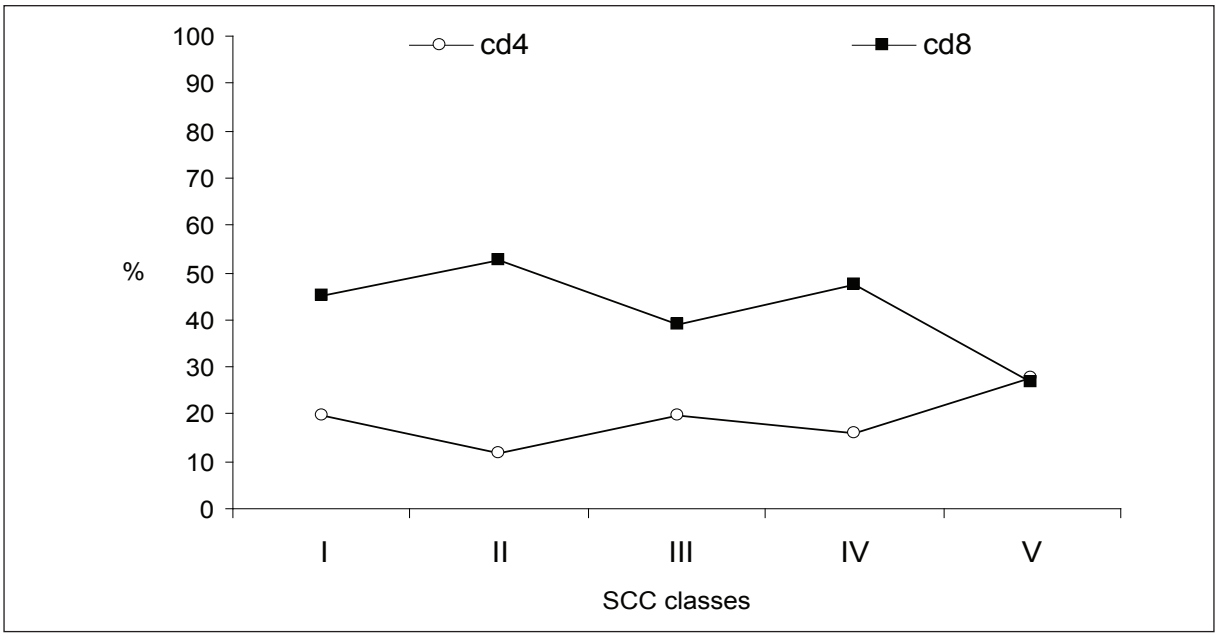

Figure 2. Percentage distribution of lymphocyte subpopulations at different levels of SCC classes

4. Conclusions

PMN become the predominant cell type (>50%) in the III SCC class, but their increase is evident (19,9 ±7,1 %) in the II SCC class that corresponds to the thresholds proposed by several authors to diagnose an inflammatory udder status; this cut-off value can be properly used in mastitis control programs based on milk somatic cell content in dairy sheep. Milk samples with higher SCC value are characterized by an increase of CD4+ T-lymphocyte percentage and IL-2 receptor co-expression. Presumably this larger presence of regulatory T-cells could be related to their ability to regulate PMN function in mammary gland inflammation.

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2.7. (S2.29) Milk Production Parameters and Cheese Efficiency of Ewes Risen in Two Different Feeding System

O. Maamouri[1,2,3], N. Atti[1], S. Smeti[1,4], M.H. Othmane[1,3]

Summary

For this experiment on milk production and cheese efficiency, thirty ewes were divided into three groups. Two groups were reared on a feed lot (FL), receiving silage (FL-S) or hay (FL-H) ad libitum. The third group was reared on pasture. The concentrate supply was 500 g/day/ewe for all groups. Milk production of pasture group (540 ml) was significantly higher than FL-H group (439 ml) while FL-S group had an intermediate level (510 ml). Fat percentage did not differ between groups, while protein percentage was significantly higher (P=0.05) for FL-S group. Milk fat and protein content (g/day) were higher for ewes in FL-S and pasture groups than for FL-H. However, milk urea (mg/dl) was higher for pasture (29) than both FL groups (23). Milk yield significantly decreased (P<.0001) while fat and protein percentage significantly increased (P<.0001) as lactation stage advanced. Cheese efficiency was significantly higher (P=0.01) for FL-S group than other groups.

1. Introduction

In the Mediterranean region, sheep's milk is used for the manufacture of cheese due to its richness in useful compounds [Bocquier and Caja, 1993, Othman et al., 2002a]. Control of its composition, including levels of fat and protein is important, since these parameters largely determine the cheese yield [Pellegrini et al., 1997]. However, milk yield and cheese efficiency vary as a result of animal genotype and environment characteristics. Among these factors, animal feeding plays an important role. The objective of this experiment was to study the milk production parameters and cheese efficiency of Sicilo-Sarde dairy ewes raised in feedlot (FL) in comparison to pasture feeding system.

2. Material and methods

The experiment was carried out in the dairy experimental farm (Lafareg) of the National Institute of Agricultural Research (INRAT). The region has a sub humid climate with 650 mm annual precipitation. Thirty dairy ewes in middle lactation were divided into three groups. Two groups were lodged on FL, receiving silage (FL-S) or hay (FL-H) ad libitum. The third group was reared on triticale pasture (P) with rotational grazing system at a stocking rate of 57 ewes/ha. The concentrate supply was 500 g/day/ewe for all groups. Individual milk yield was weekly recorded and samples were analysed for milk fat, protein and urea using Milkoscan 4000 (Foss Electric, integrated Milk Testing). The cheese yield was determined on individual basis for each sheep. The process of making cheese until coagulation was applied in test tubes containing 10 ml of milk [Othmane et al., 2002a].

Data of milk production and composition, and cheese efficiency were analysed by using the MIXED procedure of SAS® (SAS Institute Inc., 2000, Cary, NC, USA) for block designs with repeated measures. The statistical model included: diet treatment, time of sampling and their interaction as fixed effects. Data recorded during pre-experimental period (milk yield, milk

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composition and cheese efficiency) were used as covariates and included in the model. Differences between diets were performed using the procedure of least squares means [SAS®, 2000], at (P<0.05) unless otherwise declared.

3. Results and Discussion

Chemical composition of various feed resources was determined in the Animal & Forage Production laboratory at INRAT (Table 1). Chemical composition and nutritive values of experimental feeds were determined following the method described by Sauvant [1981]. The contents in triticale and oat hay of crude protein were low (9.7% and 7.5%, respectively). These results are due to the lack of nitrogen fertilization during the growing cycle of the plant. Similarly, oat silage presents a low crude protein (9%) probably due to inadequate conditions of storage of silage causing leaching of crude protein [Nefzaoui and Chermiti, 1989].

Table 1: Chemical composition of experimental feeds

	Triticale	Oat Hay	Oat Silage	Concentrate
DM %	23.7	84	26.2	88.1
Ash %	6.5	8	11	3.3
CP %	9.7	7.5	9.0	16.2
CF %	27.2	28.5	39	15.2
Energy (FU/kg DM)	0.6	0.6	0.5	1

DM: dry matter; CP: crude protein; CF: crude fiber; FU: fodder unit

Data concerning milk production, composition and cheese efficiency were reported in Table 2. Milk production of P group (540 ml) was significantly higher than FL-H one (439 ml) while FL-S group had an intermediate level (510 ml). Milk yield significantly decreased (P<0.0001) as lactating stage advanced. Fat percentage did not differ between groups despite the superiority for FL-S group which may be due to crude fiber content of oat silage, while protein percentage was significantly higher (P=0.05) for FL-S (5.4%) group than FL-H and P ones (5%). Fat and protein percentage significantly increased (P<.0001) as lactation stage advanced. Milk fat and protein content (g/day) were higher for ewes in FL-S and P groups than for ewes of FL-H one. However, milk urea nitrogen (mg/dl) was higher for P (29) than both FL groups (23), but the difference was not significant. The values of this parameter are close to the recommended levels for sheep milk [Cannas, 2002]. Cheese efficiency values followed the same trend of fat content throughout the recording period with a medium value of 34.8%. This could be due to the abundance of fat content in the milk of sheep and its importance in determining cheese yield [Othmane et al., 2002b]. Cheese efficiency was significantly higher (P<0.01) in FL-S group (40.5%) than in the FL-H and P groups by 33.6% and 30.4%, respectively. This result may due to silage nutrients properties like soluble nitrogen content. As lactating stage advanced, cheese efficiency significantly decreased (P<.0001). A low milk urea and high protein content (casein content) is often linked to improved cheese yield.

Table 2: Milk yield, composition and cheese yield according to experimental diets

	Diets[μ]			ESM	Pr > F		
	P	FL-H	FL-S		diet	time	diet*time
Milk yield (ml/d)	540 ± 36.7	438.5 ± 53	510 ± 55	8.17	0.3135	<.0001	0.0011
Fat (%)	7.33 ± 0.23	7.6 ± 0.35	8.23 ± 0.34	0.12	0.1354	0.0009	0.3375
Fat yield (g/e/d)	39.6[a] ± 2	27.3[b] ± 3.16	39.7[a] ± 3.04	0.93	0.0126	0.0005	<.0001
Protein (%)	4.88[b] ± 0.09	5.14[ab] ± 0.15	5.35[a] ± 0.13	0.04	0.033	<.0001	ns
Protein yield (g/e/d)	25.3[a] ± 1.27	19.4[b] ± 1.94	26.2[a] ± 1.90	0.52	0.041	0.0155	ns
Milk urea (mg/dl)	28.7 ± 2.47	23.1 ± 3.52	22.5 ± 3.52	0.96	0.2674	0.1031	0.2164
Cheese yield (%)	30.4[b] ± 1.48	33.6[b] ± 2.19	40.5[a] ± 2.18	0.63	0.0094	<.0001	0.0008

[μ] P: pasture regimen; FL-H: group in feedlot receiving hay; FL-S: group in feedlot receiving silage
[a,b] Means within rows with same superscript letters are not significantly different (P>0.05).
ESM: mean standard error.

4. Conclusion

In conclusion, for the sub-humid area of Mediterranean region, the cultivated pasture resulted in higher ewe's milk production comparing to feedlot system. Oat silage, resulting in high cheese efficiency, should be used successfully as forage source for dairy ewes.

Acknowledgement

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2.8. (S2.30) Levels of PCDDs, PCDFs and Dioxin-Like PCBs in Sheep Milk Collected in Sardinia, Italy

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Summary

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) are widespread contaminants with important implications for environmental and human health. Milk and dairy products consumption has been classified as one of the primary pathways of human exposure to these toxic chemicals. In this study we evaluated the levels of 17 laterally substituted PCDD/Fs and 12 DL-PCBs in 45 sheep milk samples collected from 15 farms in Sardinia (Italy). Mean concentrations of PCDDs and PCDFs were 2.31 and 3.11 pg.g^{-1} fat basis, respectively. Among DL-PCBs, only PCB 118 was detected in 51.1% of samples. Contamination of milk by PCDD/Fs and DL-PCBs (0.92 pg WHO-TEQ g^{-1} fat) being within the permissible limit set by the European Commission (6 pg WHO-TEQ g^{-1} fat) gives no indication of particular health risk. However, continuous surveillance in milk is needed to correctly evaluate both the environmental impact and the human health risk.

1. Introduction

Dioxins and Polychlorinated biphenyls (PCBs) are chlorinated heterocyclic organic compounds characterized by stability and toxicity to humans and animals. The Dioxins include 75 polychlorinated dibenzo-*p*-dioxins (PCDDs) and 135 polychlorinated dibenzofurans (PCDFs). They are volatile organic compounds released in the environment as consequence of organic material and urban refuse combustion, during industrial activities and woodland fires. PCBs are subdivided in two groups: the dioxin-like (DL-PCBs) and the non-dioxin-like (NDL-PCBs). PCBs have been widely used in numerous industrial and commercial applications, as fluid for thermal and hydraulic loops, adhesive components, paints and flame retardant. Among dioxins, the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the main toxic compound classified by the International Agency for Research on Cancer (IARC) as "cancerogenous agent for the human" (group 1). Although DL-PCB show similar toxicity as dioxins, they are classified by IARC as group 2A "probable human cancerogens". Dioxins and DL-PCBs, highly persistent in the atmosphere, can be found in waters, sediments, air and soil. Due to their lipophilic character and low biodegradability, PCDD/Fs and DL-PCBs are accumulated in foods, especially those with high lipid content. After ingestion of contaminated vegetables, the toxic compounds are absorbed in the gastrointestinal tract of the animals and concentrated in the liver and in the body fat. Milk represent the main route of excretion of dioxins in human and animals [1]. The average level of contamination reported in milk ranged between 0.6 and 1.0 pg TEQ g^{-1} of fat for the PCDDs and PCDFs, and from 0.6 to 1.3 pg TEQ g^{-1} of fat for DL-PCBs [2]. The aim of the present research was to assess the level of PCDD/Fs and DL-PCBs contamination in raw bulk tank milk of sheep reared in 15 different husbandry areas in Sardinia.

2. Materials and methods

The level of contamination with PCDDs, PCDFs and DL-PCBs was determined in 45 raw sheep bulk tank milk samples collected from 15 extensively managed flocks in Sardinia (Italy). From

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each flock single bulk tank milk samples were collected monthly for three consecutive months (from March to May). 12 of the 15 flocks were located in an high risk contamination area near a major industrial pole. 6 farms (group A) were located within 7 Km and 6 farms (group B) between 7 and 13 km from the industrial sites. 3 farms served as "control" (group C) and were >140 Km from the main industrial site. The elevation above sea level (a.s.l.) of groups A and B was between 10 and 49 m, while for group C was between 185 and 400 m a.s.l. Raw milk samples were collected into glass flasks, transported refrigerated from the site of collection to the laboratory where they were stored at -20 °C until analysis. Milk was extracted, partitioned, cleaned up, fractionated and analyzed by high resolution gas chromatography-mass spectrometry (GC/MS Polaris Q, Thermo) according to US EPA Method 1613 modified to include DL-PCBs [3]. The extraction of lipids for the determination of the DL-PCBs has been carried out by Erney method [4]. The congeners investigated in the sheep raw milk samples were: PCDDs (2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDD; OCDD), PCDFs (2,3,7,8-TCDF; 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,4,6,7,8-HpCDF; 1,2,3,4,7,8,9-HpCDF; OCDF), non-*ortho* DL-PCBs (PCB 77; PCB 126; PCB 169) and mono-*ortho* DL-PCBs (PCB 105; PCB 114; PCB 118; PCB 123; PCB 156; PCB 157; PCB 167; PCB 189). The PCDD/Fs and Dl-PCBs contaminations are expressed as concentrations of toxic equivalents (TEQs), using the toxic equivalency factors (TEFs). Comparison between mean concentrations was performed by the Least Significance Difference (LSD) test (statistical significance level $P<0.05$). The effect of the experimental variables was estimated using the following generalized linear model: $Y_{ijkm}=m+G_i+C_j+A_k+e_{ijkm}$, where the concentration of the polluting agent Y_{ijkm}, is the dependent variable, m is the mean, G_i is the farms group, C_j is the effect of the sampling date, A_k the altitude and *eijkm* the residual error. Statistical analysis was performed using Statgraphics Centurion XVI (StatPoint Technologies, Warrenton, VA, USA).

3. Results and discussion

Milk samples collected near the industrial sites (groups A and B) showed widespread contamination by PCDDs and PCDFs. PCDDs mean content was 2.31 pg.g^{-1} fat basis and ranged between 0.11 and 7.13 pg.g^{-1} fat basis, while PCDFs mean concentrations was 3.11 pg.g^{-1} fat basis and ranged between 0.20 and 7.47 pg.g^{-1} fat basis. Among DL-PCBs, PCB 118 was detected in 48.9% of milk samples with mean concentration of 0.73 ng.g^{-1} fat basis, while other congeners were never found at detectable levels. PCDDs and PCDFs concentrations were lower in the first sampling as compared to the second and third ($P<0.05$). The location of the farms (groups A, B and C), the date of sampling and the altitude had a significant effect on PCDDs and PCDFs concentrations ($P<0.05$), while no effects were observed for congener PCB 118. Among PCDDs, OCDD was the 2,3,7,8-substituted congener detected at the highest levels in most of the samples examined (0.78 pg.g^{-1} fat basis), followed by 2,3,7,8-TCDD (0.41 pg.g^{-1} fat basis), 1,2,3,7,8,9-HxCDD (0.41 pg.g^{-1} fat basis) and 1,2,3,4,6,7,8-HpCDD (0.39 pg.g^{-1} fat basis), while 1,2,3,7,8-PeCDD exhibited the lowest concentration (0.01 pg.g^{-1} fat basis). Among furans, the lower chlorinated penta PCDFs were in general more represented than the higher chlorinated PCDFs (hepta and octa PCDFs). In particular, the prevalent congeners were 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF which collectively accounted for 40.9% of the PCDFs, followed by OCDF (19.9%), 1,2,3,4,7,8,9-HpCDF (13.5%) and 1,2,3,4,6,7,8-HpCDF (10.3%) and to a lesser extent by HxCDFs (1.2-9.0%), while 1,2,3,6,7,8-HxCDF was below the detection limit in all samples analyzed. Comparison between dioxins and furans showed that 2,3,7,8-TCDD, the most toxic congener, was present in 77.8% of milk samples, while 2,3,7,8-TCDF was detected in only four out of 45 samples. However, in all cases concentrations of 2,3,7,8-TCDD (0.41 pg.g^{-1} fat basis) were higher than 2,3,7,8-TCDF (0.03 pg.g^{-1} fat basis), as well as HxCDDs (0.15-0.41 pg.g^{-1} fat basis) and OCDD (0.78 pg.g^{-1} fat basis) were prevalent compared to HxCDFs (0.04-0.28 pg.g^{-1} fat basis) and OCDF (0.62 pg.g^{-1} fat basis). In contrast, the levels of PeCDFs (0.52-0.75 pg.g^{-1} fat basis) and HpCDFs (0.32-0.42 pg.g^{-1} fat basis) were higher than the corresponding dioxin homologue groups (PeCDD: 0.01 pg.g^{-1} fat basis; HpCDD: 0.39 pg.g^{-1} fat basis). Contaminations of the samples of groups A and B were not influenced by the distance from the major industrial pole,

while concentrations increased during the lactation period. These data confirm the influence of the season on the mechanisms of "dry and wet deposition" of the pollutants on soil and vegetables. The average concentrations of PCDDs and PCDFs in the samples of the groups A and B are considerably higher than those found in two of the three control farms while, in one of these, the concentrations was comparable with those observed in groups A and B. This difference was probably accountable to a woodland fire which occurred in the surrounding area the year previous to sampling. However, the content of PCDD/Fs and DL-PCBs (0.92 pg WHO-TEQ g^{-1} fat) was within the limit set by the European Commission (3 pg WHO-TEQ g^{-1} fat for PCDDs and PCDFs and 6 pg WHO-TEQ g^{-1} fat for total TEQs). Of the total TEQs the contribution of PCDDs was the prevalent (0.50 pg WHO-TEQ g^{-1} fat), followed by PCDFs (0.35 pg WHO-TEQ g^{-1} fat), while the contribution of Dl-PCB (0.07 pg WHO-TEQ g^{-1} fat) was negligible.

4. Conclusions

The content of PCDD/Fs and DL-PCBs was within the EC limits. The present research shows that sheep milk collected in Sardinia does not represent a specific health threat. However, continuous surveillance on PCDD/Fs and DL-PCBs levels in milk is needed in order to monitor environmental pollution and potential risk to human health. The presence of a widespread contamination, although at low levels, nearby the risk area and in raw milk collected from the "control" group, highlights the need to develop the monitoring system. A revaluation of the actual Sites of National Interest (SIN) for the environment polluting agents in food of animal origin is also advisable.

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2.9. (S2.36) Evaluation of a microbial indicator test for antibiotic detection in ewe, goat and cow milk

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Summary

The presence of residual antibiotics in milk could cause potentially serious problems in human health and have technological implications in the manufacturing of milk products. The aim of this study was to evaluate the ability of Delvotest® T in detecting seven antibiotics (penicillin G, tetracycline, gentamicin, sulfadiazine, oxytetracycline, ceftiofur, and ampicillin) in ewe, goat and cow milk. Twenty-seven samples of raw, whole, refrigerated bulk-tank milk were analyzed. Higher detection ability was observed for cow and goat milks, compared with ewe's milk samples. Four out seven antibiotics were detected at or below the EU-MRL for all milk samples, while oxytetracycline and ampicillin over. Tetracycline was detected below the EU-MRL only in cow milk. Correlations ($P<0.05$ or $P<0.01$) between results obtained and some milk parameters were observed for tetracycline and oxytetracycline. The milk composition affects the bioavailability of oxytetracycline and tetracycline, and might slightly influence the detection of these drugs.

1. Introduction

Antibiotics are widely used for therapeutic and prophylactic purposes in human and veterinary medicine. In regard to dairy animals, to avoid risks related to drug residues in milk, maximum residue limits (MRL) have been established by law in many countries for each antimicrobial agent. In the European Union, the MRLs (EU-MRL) in foodstuffs of animal origin are established by the Codex Alimentarius Commission [1], the Regulation (EC) n. 470/09 [2], repealing the Council Regulation n. 2377/90 [3], and the Commission Regulation (EU) n. 37/10 [4]. Different methods of analysis for the detection of residues of inhibitors, mostly in cow milk, have been developed and evaluated [5], whereas few studies have been carried out so far for ewe and goat milks [6].

The aim of this study was to evaluate the ability of a prototype of Delvotest® T (DSM Food Specialties - Delft, the Netherlands) in detecting the presence of seven antibiotic residues in ewe, goat and cow milk, according to what is stipulated in FIL-IDF STANDARD 183:2003 "Milk and milks products – Guidelines for the standardized evaluation of microbial inhibitor tests" [7].

2. Materials and methods

Milk samples and analyses. Twenty-seven samples of raw, whole, refrigerated bulk-tank milk (13 ewe, 7 goat, and 7 cow milk samples) were analyzed. According to the guidelines [7] all the animals were in good health condition, free from antibiotics for at least 8 weeks before milk collection. Milk samples were analyzed with MilkoScan 4000 (FOSS Electric A/S, Hillerød, Denmark) to determine the chemical composition (Fat, Protein, Lactose). Somatic cells count (SCC) was measured with Fossomatic 5000 (FOSS Electric A/S). The pH values were measured with a pH meter (pH302 Hanna Instruments, UK). Bacteria levels were verified by Standard Plate Count (SPC) [8].

Antibiotics evaluation trials. The chosen antibiotics, the tested concentrations, and the respective EU-MRL are reported in Table 1. The experimental plan was designed in 2 phases: 1) 3 concentrations (1, 2 and 3) of each antibiotic were tested on milk samples of each lactiferous

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species; 2) additional concentrations (4, 5 and 6) were tested, when the detection limit was too high (Amp for goat and cow milk) or not determined in phase 1 (Amp, Tetra and Oxy-tetra for ewe milk).

Table 1: List of the antibiotics used for the evaluation test, the concentrations assessed, and the respective EU-MRL

Antibiotics	EU MRL (ppb)	concentrations (ppb)					
		1	2	3	4	5	6
Penicillin G potassium salt (Pen G)	4	2	4	6	-	-	-
Tetraclycine hydrochloride (Tetra)	100	50	100	150	200	-	-
Gentamicin sulfate salt hydrate (Genta)	100	50	100	150	-	-	-
Sulfadiazine sodium salt (Sulf)	100	50	100	150	-	-	-
Oxitetracycline hydrochloride (Oxy-tetra)	100	50	100	150	200	250	300
Ceftiofur (Ceft)	100	20	50	100	-	-	-
Ampicillin trihydrate (Amp)	4	2	4	6	8	-	-

Milk samples spiked with the same antibiotic concentration were inoculated into 4 separated Delvotest® T ampoules and incubated at 64°C. Incubation time was about 3h and 20min for ewe milk, 3h and 10min for goat milk, and 3h for cow milk. Four negative control ampoules were incubated in parallel with each spiked milk sample. The assays were analyzed both visually, comparing with standard samples, and using the Delvo® Scan system. Delvotest® T detection limit (DL) of each antibiotic was calculated for each lactiferous species and expressed as the two antibiotics concentrations (ppb) between which the intersection of the dose-response curve and the 95% positive results line lies.

Statistical Analysis. The possible correlation between the percentage of spiked milk samples detected and the variations of the milk parameters (pH, fat, protein, lactose, SCC, and SPC) was calculated, for each antibiotic concentration, as Pearson correlation coefficient. Statistical analysis was performed using Minitab statistical package, release 15 (Minitab Inc., State College PA, USA).

3. Results

Milk analyses. Milk analyses results are reported in Table 2. Ewe and goat milk composition correspond to the characteristics of milk produced at the end of lactation, especially with regard to the level of somatic cells (SCC).

Table 2: Chemical characteristics, Standard Plate Counts (SPC) and Somatic Cell Counts of the ewe, goat and cow milk samples

	pH[a]	Fat[a]	Proteins[a]	Lactose[a]	SCC[a] (x 1000)	SPC[a] (Log UFC/ml)
Ewe milk	6.74 ± 0.08	6.49 ± 0.80	5.29 ± 0.36	4.51 ± 0.35	1474 ± 303	4.34 ± 0.81
Goat milk	6.72 ± 0.03	3.62 ± 0.46	3.04 ± 0.07	4.21 ± 0.10	2052 ± 474	6.25 ± 0.20
Cow milk	6.71 ± 0.09	3.26 ± 0.17	3.30 ± 0.11	4.53 ± 0.06	555 ± 352	5.06 ± 0.67

[a]average ± sd

Antibiotics evaluation trials. Higher detection ability was observed for cow and goat milks, compared with ewe's milk samples (Table 3).

Penicillin G, gentamicin, sulfadiazine and ceftiofur were detected at or below the EU-MRL for all milk samples. The range of DL found for oxytretacycline (cow milk: 100-150 µg/l; goat milk: 100-150 µg/l; ewe milk: 250-300 µg/l) and ampicillin (cow milk: 4-6 µg/l; goat milk: 2-4 µg/l; ewe milk: 6-8 µg/l) was higher than EU-MRL, especially for ewe milk samples. Tetracycline was detected in cow milk at 50-100 µg/l, below the EU-MRL, while in ewe and goat milk the range of detection was 150-200 µg/l and 100-150 µg/l, respectively. All ampoules Delvotest® T containing milk (ewe, goat and cow) not supplemented with antibiotics were negative.

Table 3: Antibiotics tested, MRL and detection limit determined in ewe, goat and cow milk

Antibiotics	EU MRL (ppb)	Phase 1			Phase 2		
		Range of detection limit (ppb)			Range of detection limit (ppb)		
		Ewe milk	Goat milk	Cow milk	Ewe milk	Goat milk	Cow milk
Penicillin G	4	2	1-2	1-2	-	-	-
Tetraclycine[a]	100	> 150[a]	100-150	50-100	150-200	-	-
Gentamicin	100	50-100	50-100	50-100	-	-	-
Sulfadiazine	100	50-100	50-100	< 50	-	-	-
Oxitetracycline[a]	100	> 150[a]	100-150	100-150	250-300	-	-
Ceftiofur	100	20	< 20	< 20	-	-	-
Ampicillin[a]	4	> 6[a]	4-6	4-6	6-8	2-4	4-6

a: Using IDF STANDARD 183: 2003 it was not possible to determine the detection limit in ewe milk.

-: not tested.

Statistical Analysis. No correlation between the percentage of spiked milk samples detected and the variations of goat milk parameters was found. A correlation ($P<0.05$) between percentage of positive ewe milk samples detected and SPC was found for tetracycline concentrations 100 and 150 ppb. For oxytetracycline (150 ppb) statistical analysis showed: a negative correlation between the percentage of positive ewe milk samples detected and pH, fat, protein, and a positive correlation ($P<0.01$) with lactose content. For cow milk samples, a correlation ($P<0.05$) between the percentage of positive detected, at 50 ppb of tetracycline, and fat content turned out.

4. Conclusion

Delvotest® T microbial test proved to be an effective method to detect in milk residues of most of the drugs commonly used in veterinary medicine. In some cases, different results were obtained for the same drug depending on the lactiferous species. For tetracycline and oxytetracycline a few statistical correlations between the percentage of positive ewe and cow milk samples detected and the variations of some milk parameters were found. Indeed, the different milk composition has an influence on the bioavailability of oxytetracycline and tetracycline, and might slightly affect the detection of these drugs by microbial tests such as Delvotest® T.

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2.10. (S2.49) Sheep Milk: Yield, Composition and Potential Cheese Yield

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Summary

Ewes' milk is mainly used for cheese making. Data regarding cheese yield predictability relative to seasonal changes in sheep milk composition are scarce. Thus, the effect of lambing period and lactation day on ewes' milk yield, composition and potential cheese yield was investigated in a commercial dairy flock, where the lambing season is spread throughout the year.

Lambing group had no significant effect on average milk yield/ewe (1132 ml/day), but significantly affected fat (5.97% to 7.05%), protein (4.98% to 5.47%), lactose (4.75% to 5.06%), total solids (16.3% to 17.8%), and potential cheese yield (16.1 to 18.9 g MS/100 ml milk). Lactation day affected all parameters significantly: milk yield and lactose content decreased, while fat, protein and total solids contents, and potential cheese yield increased throughout lactation.

Results show that day of lactation had a significant impact on all the traits analyzed, and lambing season affected milk composition and potential cheese yield.

1. Introduction

Ewes' milk is mainly used for cheese making, which is an activity of great economic and social importance in rural Portuguese areas. For the same cheese type and cheese-making process, the cheese yield depends greatly on milk composition (fat, protein, total solids and casein contents, casein genetic variants), and hygienic quality (pH, somatic cell counts).

In Portugal, ewes' milk is paid by quantity regardless of its quality. Ultimately, it results in the substitution of low milk production autochthonous breeds by foreign breeds with higher milk productivity. However the adoption of milk payment based on milk components, should be considered for cheese making. The implementation of such a system could affect shepherds' flock managing decisions, like the lambing season. Moreover, the main lambing season occurs in September/October, but producers are willing to allow lambing all the year in order to overcome seasonality of cheese production. So, it is necessary to know the impact of date of lambing on cheese yield as milk composition changes throughout the lactation period, particularly in grazing ewes, since herbage allowance and quality change throughout the year. Thus, the objective of this work is to evaluate the effect of lambing period and lactation day on ewes' milk yield, composition and potential cheese yield in a commercial dairy flock, where the lambing season is spread throughout the year.

2. Materials and methods

Milk yield, milk composition (fat, protein, lactose and total solids contents) of 136 lactating ewes and potential cheese yield were evaluated from November 2009 till June 2010, considering six groups of lambing: November (Nov), December (Dec), January and February (Jan + Feb), March and April (Mar + Apr), May (May) and June (Jun), according to 1st machine milking date (on average 30 to 45 days after lambing).

Ewes grazed all year round, from Nov to Feb during the daylight and after mid March throughout the 24 hours/day. Sheep feeding was supplemented with hay from Nov to Dec and with concentrate daily at each milking (350 + 350g/day). The average crude protein (% DM)

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and the estimated metabolizable energy (MJ/kg DM) (MAFF, 1975) for pasture, hay and concentrate were respectively: 22% and 9.3 MJ; 8.3% and 8.2 MJ and; 25% and 13 MJ.

Ewes were machine-milked twice daily in a milking parlor at 06:30 h and 17:00 h and the milk yield of each ewe was recorded. Individual milk samples proportionally composited according to morning and evening milk yield at 30 and 90 days of lactation were analyzed for fat, protein, lactose and total solids contents (Milk-o-Scan 4000), and the potential cheese yield of each ewe was determined.

The methodology to evaluate potential cheese yield was as followed: duplicates of 20 ml of each individual milk sample, previously warmed to 30°C and mixed by shaking, were transferred to a pre-weighed tube. One microlitre of an 80 mg / 100 ml diluted solution of rennet (96 ± 2% Chymosin) was added. Rapid stirring ensured uniform distribution of the rennet. Coagulation was carried out at 32°C in a water bath for an hour. The gel obtained (curd + whey) was centrifuged at 3 500 g for 15 min at room temperature. After expulsion of the whey, the centrifuge residues (curd obtained after expulsion of whey by centrifugation) are weighed, dry matter (DM) was determined, thus potential cheese yield is expressed in g of DM per 100 ml of milk.

Milk production, composition and potential cheese yield data were analyzed by fitting a compound symmetry model to the repeated measures at 30 and 90 days of lactation (time) in a mixed statistical procedure testing the six groups of lambing (Littell et al., 1998). Prediction of potential cheese yield was tested by finding adequate regression equations derived from individual ewe's milk quantity and composition and potential cheese yield data.

3. Results and discussion

Considering milk yield on days 30 and 90 of lactation, lambing group had no significant effect ($P>0.05$) on average milk yield/ewe (1132 ml/day), but significantly affected ($P<0.0001$) fat (from 5.97% in Mar + Apr to 7.05% in Jun), protein (from 4.98% in Jan + Feb to 5.47% in Nov), lactose (from 4.75% in Jun to 5.06% in Mar + Apr), total solids (from 16.3% in Jan + Feb to 17.8% in Nov), and potential cheese yield (from 16.1 g DM/100 ml milk in Mar + Apr to 18.9 g DM/100 ml milk in Dec). Lactation day, affected significantly all the traits ($P<0.001$): milk yield (1386 vs 877 ml/day) and lactose content (5.06% vs 4.80%) decreased from day 30 to 90 of lactation, fat (5.91% vs 7.01%), protein (5.07% vs 5.30%) and total solids contents (16.4% vs 17.4%), and potential cheese yield (15.9 vs 17.8 g DM/100 ml) increased throughout lactation.

Interactions between lambing season and lactation day were significant for all the traits ($P<0.0001$). Milk yield follows the pattern of herbage allowance throughout the year: it decreased from Nov to Jan + Feb (minimum of 1101 ml/day), reflecting the decreasing availability of herbage during those months. In spring, milk yield increased rapidly to a maximum of 1730 ml/day in May, where herbage allowance was higher. The milk yield of the ewes from lambing groups Jan + Feb and Mar + Apr were similar at days 30 and 90 of lactation, those ewes had a lower peak yield and a higher persistency. The low persistency and the low peak yield in Dec shows that this is the most adverse season for ewes' milk production (Figure 1). It was observed that the major differences in fat, lactose, and total solids contents between the day 30 and 90 of lactation was observed in ewes lambing in Autumn (Nov, Dec). Lack of significance between day 30 and 90 of lactation was also observed for fat and total solids content in May and Jun); for lactose content on Mar + Apr; for protein content from Mar + Apr onward; and for potential cheese yield in all seasons but Nov.

The values of potential cheese yield were significantly lower at day 30 of lactation than at day 90 in Nov, and it is rather difficult to explain the sudden increase of its value at day 30, from Nov to Dec. The values of potential cheese yield on Nov and Dec at day 90 of lactation were significantly higher than in late Winter and in Spring. Milk from Jan to Jun has potential cheese yields similar to those obtained in "Saloia" breed, and higher than those of "Assaf" breed using the same methodology (Martins et al., 2009).

The prediction of potential cheese yield, by stepwise selection of variables, resulted in the following equation: Potential Cheese Yield (g DM/100 ml milk) = -1.14683 + 0.00034652 Milk (ml) + 8.17472 Fat (%) + 7.38509 Protein (%) + 8.15955 Lactose (%) - 6.69179 total solids (%).

Thus,in this example, the calculated potential cheese yield is 15.88 g / 100 ml of milk.The adjustment obtained is similar to the high variability observed throughout the lambing months in milk fat and protein contents.

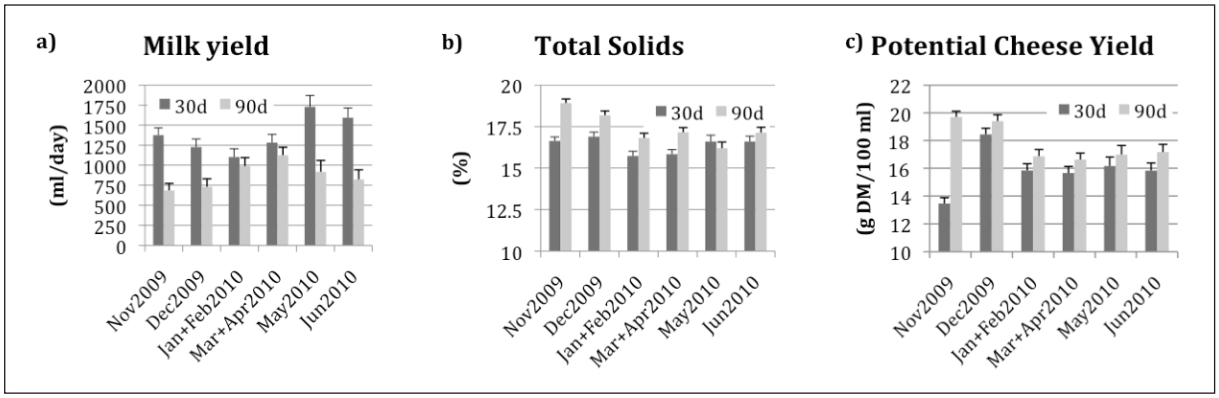

Figure 1. a) Milk yield (ml/day), **b)** total solids content (%), and **c)** individual potential cheese yield (g DM/100 ml of milk) according to lambing season and day of lactation

4. Conclusion

Results show that day of lactation had a significant impact on all the traits analyzed, and lambing season affected milk composition and potential cheese yield.

Acknowledgments

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2.11. (S2.50) Effects of Two Diets on Milk Production and Milk Composition of High Producing Dairy Goats Milked Once A Day

Y. Lefrileux[1], S. Raynaud[2], S. Morge[3], J. Barral[4], Y. Gauzere[5], E. Doutart[2], C. Laithier[2]

Summary

This study was about the effect of the goats diet on milk composition for farmhouse lactic cheese-making. On an experimental farm two groups of 60 goats were fed with two different diets for two months: one group with a high nitrogen level and the second one with a low nitrogen level. Individual zootechnical measures were taken every week and the milk composition of each group was measured once or twice a week. The group fed with the high nitrogen level diet gave more milk, but milk with a lower fat level. The level of urea in the milk and also dry matter content, fat, sodium chloride and citrate changed according to the diet. There was a small, yet significant, difference for total nitrogen, soluble nitrogen, non protein nitrogen and potassium. The urea content of the milk can be linked to the rumen degradable nitrogen balance ratio (PDIN-PDIE supplies/UFL supplies) of the diet.

1. Introduction

This study was part of a program dealing with the sustainability of the use of natural whey starter for farmhouse lactic cheeses made from raw goats milk. Its goal was to study the technological consequences of feed-related milk nitrogen composition variations, since urea excess in the milk has been suspected to create curd drawbacks for lactic cheeses or a slowdown in acidification during the making of semi-hard cheeses [8, 9].

The poster and the present article were only focused on the effects of the diet on milk composition. The technological consequences of these changes in milk composition are described elsewhere [6].

2. Materials and Methods

The experiment took place at the Pradel Experimental Farm (France). Two groups of 60 goats were fed with two different diets for two months: one group with a high nitrogen level (20 to 22% of total nitrogen content) and the other with a low nitrogen level (14 to 15% of total nitrogen content). The rumen degradable nitrogen balance ratios (PDIN-PDIE supplies/UFL supplies) were very different for the two diets: -4 for group 1 and 26 for the group 2.

Individual zootechnical measures were taken every week and milk composition of each group was measured once or twice a week. An analysis of variance for longitudinal data was carried out to study the diet effect on these zootechnical measures. It was impossible to analyse each individual milk sample in detail. In consequence, once a week for 7 weeks, we created 3 small groups of 8 randomly chosen goats and their milk was analysed. The diet effect was tested by analysis of variance.

SAS software was used for these analysis (SAS Institute Inc, Cary, NC, version 9.1).

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3. Results

The group fed with high nitrogen level gave more milk, with lower fat level (Table 1). No difference was detected between the two groups regarding the protein rate.

Table 1: Mean values and standard deviations (in brackets) of milk production, fat and protein rate for each group / diet (risk 5%)

Criteria	Group 1 (n=60)	Group 2 (n=60)	Group effect
Milk production (kg/goat/d)	3,51 *(0,93)*	3,77 *(0,99)*	***
Fat rate (g/kg)	33,08 *(3,83)*	30,02 *(3,96)*	***
Protein rate (g/kg)	32,31 *(2,05)*	32,23 *(2,04)*	ns

* p< 0,05 ** p< 0,01 *** p<0,001 ns : not statistically significant

The milk production increase and the fat rate decrease were in accordance with the results of a previous study [7]. The lower fat rate of the milk of the second group can be explained by a dilution effect or by a higher fat level in the feeds given to the group 1 [10, 11].

Other authors have already pointed out that a variation of the nitrogen content of the diet has no effect on the protein level of the milk as soon as the energy needs of the animals are covered by the diet [10].

Milk composition of the two groups was different for urea but also dry matter content, fat, sodium chloride and citrates (Table 2). There was a small, yet significant, difference for total nitrogen, soluble nitrogen, non protein nitrogen and potassium. There did not seem to be any difference between the groups regarding protein, caseins, lactose, calcium and phosphorus. Furthermore, the milk urea content could be linked to the rumen degradable nitrogen balance ratio (PDIN-PDIE supplies/UFL supplies) of the diet.

Table 2: Mean values and standard deviations (in brackets) of milk composition criteria for each group / diet (risk 5%)

Component	Group 1 (n = 7)	Group 2 (n=7)	Group effect
Dry matter (g/kg)	119,19 (1,43)	115,74 (2,54)	**
Fat content (g/l)	33,79 (1,7)	30,45 (2,55)	**
True Protein (g/l)	32,5 (0,80)	32,5 (0,45)	ns
Total Nitrogen (g/kg)	5,47 (0,14)	5,59 (0,09)	*
Soluble Nitrogen (g/kg)	1,27 (0,09)	1,42 (0,06)	***
Non protein nitrogen (g/kg)	0,33 (0,06)	0,44 (0,03)	***
Caseins (g/kg)	26,76 (0,74)	26,61 (0,65)	ns
Urea (mg/l)	**289,52 (152,88)**	**609,10 (73,05)**	***
Lactose (g/l)	44,0 (1,32)	43,3 (0,87)	ns
Calcium (g/kg)	1,14 (0,03)	1,12 (0,04)	ns
Phosphorus (g/kg)	0,87 (0,04)	0,88 (0,03)	ns
Sodium chloride (g/kg)	2,92 (0,20)	3,06 (0,11)	*
Potassium (g/kg)	0,76 (0,03)	0,80 (0,02)	**
Citrates (g/kg)	1,23 (0,05)	1,04 (0,10)	***

* p< 0,05 ** p< 0,01 *** p<0,001 ns : not statistically significant

Urea is known to be strongly influenced by the goat's or cow's diet [7, 10, 13]. Agabriel et al. have shown that the citrate content of the cow milk can be influenced by the grazing period or the type of farm and diet [1, 2].

The differences concerning the potassium, though significant, are very small. A higher sodium chloride content for the group 2 (+5%) could have been explained by mammary infections, but the average somatic cell counts are similar for the two groups.

Several criteria were not affected by the diet: lactose plays a role in milk production in the udder and remains stable in the milk [3], calcium remains stable because the animal can mobilize the body's calcium reserves [4]. If the energy and nitrogen needs of the goats are covered by the diet, the protein and casein contents of the milk are not affected by the nitrogen content of the diet [5].

4. Conclusion

This study has shown that:

- Nitrogen excess in the goat's diet not only has an impact on milk quantity and milk nitrogen components, but also on the minerals.
- The rumen degradable nitrogen balance ratio (PDIN-PDIE supplies/UFL supplies) of the diet influences the urea content of the milk.

Another part of this study (data not shown) focussed on the technological consequences of these differences in milk composition for farmhouse lactic cheeses made from raw goat's milk with natural whey starter.

Acknowledgments

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2.12. (S2.52) The Effect of Somatic Cell Count on The Plasminogen, Plasmin and Plasminogen Activator System in Ewe Milk

P.D. Angelopoulos[1,2], E.M. Zoidou[1]

Summary

The purpose of this study was to evaluate the effect of somatic cell count (SCC) on the plasminolytic system in ewe milk. The distribution of plasminogen activator in different fractions of milk was also examined. The results showed that the high-SCC milk had greater plasmin, plasmin + plasminogen and plasminogen activator activities when compared with the low-SCC milk, while plasminogen level and plasminogen:plasmin ratio were less. Greater plasminogen activator concentrations were found in high-SCC milk compared to low-SCC milk, mainly localized within the casein fraction, and in association with somatic cells. Two plasminogen activator types, tissue- and urokinase-type, were associated with casein micelles, while urokinase-type was the only form in milk serum and somatic cells.

1. Introduction

Plasmin (PL) which occurs in milk together with its inactive proenzyme, plasminogen (PG), is an important indigenous heat-stable milk proteinase, with a relatively broad specificity on caseins. The conversion of PG to PL is regulated by a complex system of molecular interactions between plasminogen activators (PA) -tissue-(t-PA) and urokinase-type (u-PA)- and specific PA inhibitors [5]. This system enters milk from blood. Somatic cell count (SCC) is a widely used marker for both udder health and milk quality. The aim of this study was to evaluate the effect of SCC on PL, PG, and PA activities in ewe milk. The type of PA in different fractions of ewe milk (casein, serum and somatic cells) were also examined.

2. Materials and methods

A total of 202 milk samples were collected from a Greek dairy ewe breed (Karagouniko) throughout a lactation period. The SCC was determined by a Fossomatic cell counter (Foss Electric, Hillerod, Denmark). Milk samples were also analyzed for PL and PG activities [3, 5]. A total of 24 milk samples were treated to obtain casein, serum and somatic cell fractions according to White et al. [6]. A colorimetric assay was used to measure PA activity [1]. To identify the type of PA present in each of the milk fractions, PA activity was determined in the presence and absence of fibrin (20 μg/min) or amiloride (1 mM). For statistical analysis the software Statgraphics Plus for Windows v.5.2 (Manugistics Inc., Rockville, Maryland 20852, USA) was used.

3. Results and discussion

3.1. Effect of SCC on the PG and PL activities

The PG and PL activities in ewe milk samples are shown in Table 1, as average values for four SCC groups (A, B, C, D). An increase of SCC from $< 5 \times 10^5$ /ml to SCC $> 20 \times 10^5$ /ml resulted in an increase to PL and PL+PG activities, while PG activity and PG:PL ratio were respectively

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decreased. The increase of PL and the decrease of PG:PL ratio in high-SCC ewe milk provide evidence that there is an increased influx of blood proteins by passing the blood-milk barrier, whilst at the same time an accelerated conversion of PG to PL occurs.

Table 1: Plasmin (PL), plasminogen (PG), PL+PG activities (Units/ml)[1] and PG:PL ratio in ewe milk as affected by SCC (cells/ml)

SCC	PG	PL	PG+PL	PG/PL
A: SCC <5 x 10^5	29.10[a]	12.98[a]	42.08[d]	4.27[a]
B: 5 x 10^5 <SCC< 10 x 10^5	23.86[ab]	19.81[b]	43.67[d]	1.79[b]
C: 10 x 10^5 /ml <SCC< 20 x 10^5	20.17[ab]	23.81[b]	43.98[de]	0.85[c]
D: SCC >20 x 10^5	19.10[b]	35.89[c]	54.99[e]	0.65[c]

[1] Values are means of 202 individual milk samples

[a,b,c] Means within the same column with different superscript differ significantly (Duncan test, P < 0.001)

[d,e] Means within the same column with different superscript differ significantly (Duncan test, P < 0.01)

3.2. Effect of SCC on PA activity in different milk fractions - Type of PA

The quantity and distribution of PA activity in different milk fractions as affected by SCC level is shown in Table 2. The PA activity presented as the average value for three SCC groups (I, II, III) was increased with the increasing level of SCC. This elevated PA activity contributed to the accelerated conversion of PG to PL (shown in Table 1) and maybe resulted from macrophages and neutrophils of high SCC milk [4]. PA activity in casein fractions of high SCC milk (> 20 x 10^5 /ml) was 2.9 and 1.5-fold higher (P < 0 .05) than that in the casein fractions of low (< 5 x 10^5 /ml) or medium (10 x 10^5 <SCC< 20 x 10^5 /ml) SCC milk respectively. The corresponding difference in PA activity for the high SCC serum fractions was 7.16 and 1.07-fold higher and for the somatic cell extracts was 2.04 and 1.40-fold higher (P < 0.05). It is interesting to note that the majority of PA activity across the SCC groups was in casein fractions and then in somatic cell extracts. The lower PA values in the serum fractions could be attributed to the occurrence of inhibitors in milk serum. These findings corroborate the observations by Heegard et al. (1994) [2] for bovine milk.

The effect of fibrin and amiloride on PA activity in each fraction of ewe milk samples is shown in Figure 1. There was a 43.7% increase in the PA activity associated with the casein fraction in the presence of fibrin, indicating that t-PA was present. The ability of amiloride to inhibit PA activity by 43.6% indicated the presence of u-PA in addition to t-PA. The PA activity associated with the somatic cell extracts and serum fraction remained almost unaffected by the presence of fibrin, but was strongly inhibited by amiloride (89% and 87,5% respectively), indicating that only u-PA was present in serum fraction and somatic cell extracts.

Table 2: Distribution of plasminogen activator (PA) activity (absorbance/h)[1] in ewe milk fractions as affected by SCC (cells/ml)

	PA activity		
SCC	Casein	Serum	Somatic cells (per 106 cells/ml)
I: < 5 x 10^5	0.175[a]	0.012[a]	0.075[a]
II: 10 x 10^5 <SCC< 20 x 10^5	0.340[b]	0.080[b]	0.109[b]
III: > 20 x 105	0.510[c]	0.086[b]	0.153[c]

[1] Values are means of 24 individual milk samples

[a,b,c] Means within the same column with different superscript differ significantly (Duncan test, P < 0.05)

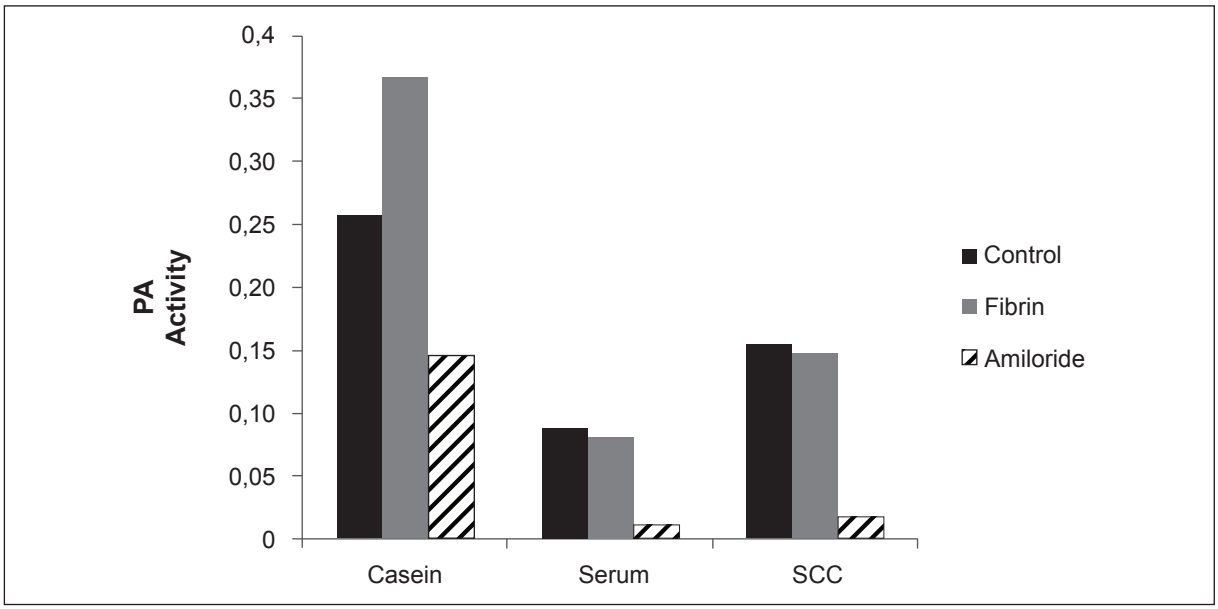

Figure 1. Effect of fibrin (20 µg/ml) and amiloride (1 mM) on plasminogen activator (PA) activity in the casein, serum and somatic cells fraction of ewe milk. PA activity expressed as absorbance/h. 24 individual milk samples were studied.

4. Conclusion

In conclusion PL-PG system in ewe milk was affected by SCC. Higher levels of PL activity and lower PG/PL ratio were associated with elevated SCC levels, suggesting that there is an increased influx of plasmin, whilst at the same time accelerated conversion of PG to PL occurs. This hypothesis was also supported by the simultaneously increased PA values. PA was associated mainly with casein, and somatic cells. The present study revealed the distribution of PA-types in ewe milk. U-PA was detected in casein, serum fractions and in association with somatic cells, while t-PA was confined only to the casein micelle fraction.

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Session 3: Processing and Products

Oral Presentation

3.1. (S3.oral) Relationship Between Technological Steps and Behaviour of French Goat Cheeses for Culinary Applications

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Summary

The use of goat milk cheese as an ingredient in ready made meals is increasing. However, the lack of data concerning its cooking properties could impede the development of such applications. Therefore, the culinary behaviour of goat milk cheeses currently sold on the French market (lactic and semi hard cheeses) was studied thanks to the development or adaptation of methods (sensory analysis, cooking tests coupled with photographs allowing the evaluation of flowability with image analysis and rheological measurement). The biochemical composition of cheeses was also assessed. Significant differences in colour and to a lesser extent of flowability were found between the cheeses studied but also differences concerning the appearance along with the occurrence of cracks for most trade products. The last step was dedicated to highlighting the main factors and mechanisms that are associated with the cooking ability of cheeses, using experimentally made cheeses.

1. Introduction

A major outlet for cheese exploitation is its use as an ingredient. According to Guinee [1], 35 to 45% of global cheese (all species and especially cow) was used as an ingredient in 2000. The distribution in Europe, given the dairy tradition of many countries, is as follows: 70, 20 and 10% of cheese consumed by retail sales, food services, and industry, respectively. In the U.S.A., these proportions are already distributed equally within the three sectors. The main uses are hot applications (80%) such as savoury tarts, lasagnes, pizzas and burgers but also industrial casseroles. Cheese can also be transformed into powders, or grated and then re-used in soups, sauces etc. The goat sector is directly connected to these applications. For some companies, this outlet is already a large volume market.. Many factors are involved in the variations of cheese functionalities [1, 2], such as cheese making parameters and cooking intensity of cheeses, but the greater part of the studies are about cow's milk cheeses, like Emmental, Mozzarella and especially low moisture partly skimmmed Mozzarella, Cheddar cheese and process cheeses. Several tests are commonly applied to measure the culinary properties of these types of cheeses [3]. No study concerned lactic cheeses, which correspond to 90% of French goat cheeses, have been performed to date. Therefore, this work aimed at first to set up and optimize tools and methods to evaluate culinary behaviour. The second step was the characterisation of the functionalities of commercial French cheeses and the third step was to establish the relationship between culinary behaviour and the processes by controlling key factors in experimentally made cheeses.

2. Material and methods

2.1. Sampling of goat milk cheeses

For the setting up and optimization of the tools in order to evaluate the culinary behaviour of

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cheeses, 3 classes of products have been targeted: fresh lactic cheeses (15 products) and ripened lactic cheeses (14 products), especially buchette type cheeses (mini log shape) and crottins, and finally semi-hard cheese (8 products) named Tome. In order to develop tests that are valid for a wide range of culinary behaviour, cheese composition used for this purpose had to be variable. For this purpose, commercial cheeses were used but experimental cheeses were also manufactured with different fat in dry matter (FDM, from 40 to 60%) and moisture in non fat substances (MNFS, from 60 to 79%).

For the evaluation of the culinary behaviour of the cheeses of the French market, the main studied cheese category was the ripened lactic cheeses because of their more frequent use in culinary sector: ripened lactic cheeses (17 products), fresh lactic cheeses (5 products) and semi hard cheeses (5 products). All the cheeses were studied at the same stage of ripening : 4 days after renneting for fresh cheeses and 26 days for the ripened cheeses.

Finally, in order to study the relationship between the culinary behaviour and process, lactic cheese makings were realised. The classical procedure was applied : starters CHN14 (Chr Hansen, Arpajon, France) 10g/100 l milk and bovine rennet at 520 mg chymosin/l (Carlin, Texel, Dangé St Romain, France) at 5 ml/100 l milk were added to pasteurised or raw milk and slow coagulation was conducted at 24°C for 24 h. Then fresh lactic curd was drained and salt was added. Cheeses were then packed (fresh lactic cheeses) or ripened for about 8 days at 12°C with surface ripening strains, and finally stored at 4°C (shelf life 45-50 days). Some parameters were modified in order to evaluate their impact:

- FDM (4 levels with constant MNFS).
- Ripening strains: cocktail A (PC PR1+ DHR form Cargill – dose ratio 4:1) and cocktail B (PCTN form Danisco +DHR from Cargill – dose ratio 4:1). Cocktail B has higher proteolytic and lipolytic activities than the cocktail A.
- Use of frozen lactic curd, 3 levels: 0% (only fresh curd), 50% frozen and 100% frozen curd. The fresh curd was frozen in a quick freezing room (from 4 to -20°C in 45 minutes) and then stored at -20° C. MNFS was 69% and FDM 47%.
- Freezing (and stored 30 days at -20°C) of slices of 7 day ripened lactic cheeses either with A or B cocktail. MNFS after drying was 70% and FDM 53%.

2.2. Biochemical analyses

The following analyses were realised: pH, dry matter [4], proteins by evaluating nitrogen fractions according to Kjeldhal method [5] , milk fat [6], cheese fat [7], lipolysis [8], calcium by complexometry, phosphorus [9], chlorides, ash (drying at 525°C), lactose and galactose with enzymatic kit (Boehringer test, Mannheim, Germany).

Fat organisation in six experimental cheeses of the final step was also observed by confocal laser scanning microscopy on 5 mm thickness slices of cheeses, using a lipid soluble Nile Red Fluorescent dye adapted from the protocol of Lopez et al [10].

2.3. Setting up of methods to characterise culinary behaviour

Adaptation or development of representative, repeatable and discriminative tests to assess culinary behaviour of goat cheeses was the first phase of this study. Six specific protocols were developed.

2.3.1. Cooking test

Heating protocols

The use of Petri dish (Schreiber test) was rejected soon after preliminary trials due to a too rapid cooking and the direct contact that is not representative of the real application. Other media types were tested: dry supports (toasts) and moist supports (pizzas). Different types of oven and treatment intensity were also tested. Regarding microwaves, an atypical behaviour

was observed for lactic cheeses, so this type of cooking was not used in the next steps. Finally, the most suitable protocol was: 5 mm thickness slice put on mini pizza (11 cm diameter) + 20 g of tomato sauce in glass Petri dish (without lid), heated in a fan oven during 8 min at 200°C (6 pizzas / batch, 5 replicates).

Flow ability

Before and after each cooking test, the cheeses were photographed in a standardized configuration and the cheese surface was evaluated by computer processing by image analysis made with the Image J Software (developed by National Institute for Health). The ratio (area after heating - area before heating) x100/ (area before heating) gave the flow ability value (%).

The ranges of variation observed % for fresh lactic cheeses, ripened lactic cheeses and semi hard cheeses (6-75%, 8-21% and 37-74 % respectively)were much larger than the standard deviations of repeatability and limit of repeatability and reproducibility. Repeatability was determined on 5 cheeses samples and 3 replicates . The variance of repeatability S^2r was 0.01 and the repeatability limit r (= 2.8 x Sr) which is the maximum acceptable difference between two measurements on the same sample, with a risk of error of 5%, was determined. On average, for an initial surface 20 cm^2, r = 1.2%. Reproducibility was evaluated by 3 operators on a sample, with 3 replicates. The average limit of reproducibility was 2.3% of the initial surface. The reproducibility of the complete tool "Heating step + flow ability measurement" was calculated with 4 replicates on 3 samples per type of product. Sr was 2.3, 4.8 and 4.6 % for fresh lactic cheeses, ripened lactic cheeses and semi hard cheeses respectively. Therefore, flow ability was considered as a discrimination tool for this study.

Sensory characteristics after cooking

Owing to a high heterogeneous colour of cheese samples after cooking and to their size, colour measurements with colorimeter tested in preliminary trials did not give repeatable results and this parameter was finally assessed only by sensory analysis. A list of sensory descriptors was generated by an internal panel (6 persons) for each type of cheese, including common descriptors: flow ability, colour, cracks, fat exudation, granular, dry and sticky. During the last phase (relationship between culinary behaviour and processes), 36 products were analyzed by 12 trained panellists from the laboratory of Maisons du Gout (Sensory Department of Actilait).

2.3.2. Exudation (laboratory test)

The method involving an aqueous extraction by centrifugation [11, 12] was applied to the uncooked cheeses. The standard deviation of repeatability for this method is less than 0.5g/100g cheese [12], equivalent to about 2 g fat / 100 g of cheese fat. Moreover, the ranges of variation observed for this parameter, increasing with high FDM values, were very large : 3-59, 11-70 and 50-75 g/100 g cheese fat for fresh lactic cheeses, ripened lactic cheeses and semi hard cheeses respectively. The tool is therefore suitable for assessing indirectly the fat mobility in the lactic cheese network, in terms of repeatability and discrimination ability.

2.3.3. Rheological methods

Two types of test have been optimised on uncooked cheeses: small amplitude oscillatory shear measurements and texture profile analysis (TPA).

Small amplitude oscillatory shear measurements

This test was conducted with thermal scanning on an AR1000 rheometer with a strain of 0.1% and a frequency of 1Hz. The modules were parallel planes. The cheese samples were 2 cm diameter (without rind for ripened cheeses) and 5 mm thickness. According to previous works [13, 14], the sample was protected from dehydration by use of silicone oil. The sample temperature was increased from 20 to 90°C in 20 minutes. The storage modulus (G'), the loss modulus (G'') and

the maximum ratio G''/ G' (tan δ max) were recorded. The temperature corresponding to tan δ max was generally used as an indicator of cooking performance and indirectly to flow ability. As the curves were very specific for lactic cheeses, 6 new descriptors selected according to their low coefficients of variation (from 0 to 7% for lactic cheeses) given in table 5 : G' and G" measured at 39°C, tan δ max, T°C for tan δ max, ΔG'39°C and ΔG"39°C

Texture profile analysis (TPA)

This type of measurement was adapted to the fresh goat lactic cheeses [15] and had to be validated on ripened lactic cheeses, especially concerning the sample shape. The apparatus used for this analysis was TAXTPlus texturometer (Stable Micro Systems Ltd. Godalming UK). A sample of cheese (20 mm diameter and 15 mm high) was compressed twice by a moving metal plate (7 cm diameter). The compression ratio was 25% of the initial height at 1 mm/s speed. The maximum forces recorded during successive compressions and areas under the curves were used to calculate parameters such as hardness (or firmness), adhesiveness, cohesiveness, springiness (or elasticity).

Comparison of standard deviations of fidelity with the ranges of variation (Table 1) showed that the method can discriminate for firmness, cohesiveness and elasticity. In contrast, the parameter measuring adhesiveness is not a discriminate factor because the limit of fidelity is 1 N.s for a ripened lactic cheese while the measured values varied from 0.6 to 2.6 N.s.

Table 1: Comparison of standard deviations of fidelity with the ranges of variation of TPA parameters

	Firmness	unit	Cohesiveness	unit	Elasticity	unit	Adhesiveness	unit
Fresh lactic cheeses (p= 6, n=3)								
Standard deviation Sr	0.3	N	2.2	%	1.9	%	0.2	N.s
Fidelity limit r	0.9	N	6.3	%	5.4	%	0.6	N.s
Variation range	6.6-13	N	40-57	%	59-85	%	0.4-1.3	N.s
Ripened lactic cheeses (p = 9, n=3)								
Standard deviation Sr	0.4	N	3.5	%	2.4	%	0.3	N.s
Fidelity limit r	1.2	N	9.9	%	6.7	%	1.0	N.s
Variation range	15-37	N	34-65	%	62-85	%	0.7-2.7	N.s
Semi hard cheeses (p= 11, n=3)								
Standard deviation Sr	0.5	N	0.9	%	0.8	%	0.3	N.s
Fidelity limit r	1.3	N	2.6	%	2.2	%	0.9	N.s
Variation range	16-30	N	67-75	%	59-92	%	1.0-1.8	N.s

p : number of products, n = replicates

3. Results - discussion

3.1. Culinary behaviour of cheeses currently sold on the French Market

The objective of this phase was to characterize a representative range of commercial cheeses to appreciate the diversity in terms of culinary behaviour with the tools previously developed. Their biochemical composition given in Table 2 was highly variable for each type of cheese. The levels of proteolysis and lipolysis remained within a normal range without generating sensory defects.

Table 2: Compositions of the studied French commercial goat milk cheeses

	Fresh lactic cheeses (4 days old)		Ripened Lactic cheeses (26 days old)		Semi hard cheeses (26 days old)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
pH	4.5 (0.1)	4.4-4.6	5.3 (0.3)	4.8-5.8	5.6 (0.2)	5.4-5.9
Dry matter (g/100g)	42 (4.2)	37-46	47 (3.4)	40-52	58 (3.5)	53-61
FDM (%)	50 (3.7)	46-55	51 (6.2)	48-57	51 (3.8)	47-56
MNFS (%)	74 (2.7)	77-76	70 (2.3)	66-75	60 (3.7)	55-64
Total Nitrogen/dry matter (%)	38 (3.3)	32-40	41 (14)	27-43	46 (8.3)	35-58
pH4.6 soluble N/TN (%)	9.7 (2.0)	6.8-12	27 (14)	10-55	23 (7.8)	14-30
Lipolysis (g Oleic Acid /100 g fat)	0.8 (0.6)	0.3-1.7	4.0 (1.9)	1.6-7.3	0.7 (0.2)	0.5-1.0
Calcium (mg/100g)	115 (37)	75-155	86(25)	36-115	708 (88)	608-828
Phosphorus (g/kg)	1.7 (0.1)	1.6-1.8	1.9 (0.2)	1.6-2.2	4.7 (0.4)	4.3-5.4

Concerning texture parameters (TPA) and especially flow ability and exudation (table 3), a high variability was observed. The firmness of cheeses, often inversely related to melting in the mouth, is generally correlated to the amount of intact non hydrolysed casein. For semi hard or hard cheeses, the fracture strength and hardness decrease during ripening due to proteolysis (breakdown of the para-casein matrix) and the concomitant increase in the hydration of casein. The preferential break-down of the αs1-casein by the residual chymosin is the most important factor as regards the reduction of firmness [1]. Subsequently, the firmness increases by decreasing of free water. For unpackaged cheeses and lactic cheeses, it seems that the loss of water accompanied by an increase in firmness occured early and could be the main explaining factor of our results. Cohesiveness and elasticity were correlated ($r = 0.7$, $p<0.05$) due to the viscoelastic nature of this category of cheese.

Table 3: Variation range of fat mobility , flow ability and TPA parameters of the commercial goat milk cheeses

	Fresh lactic cheeses (4 days old)		Ripened Lactic cheeses (26 days old)		Semi hard cheeses (26 days old)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Fat mobility (g/100g cheese fat)	30 (18)	12-50	50 (34)	27-118	64 (19)	46-96
Flowability (image analysis) (%)	17 (9.9)	3.3-29	25 (20)	3.4-84	55 (28)	21-96
Firmness (N)	6.1 (3.0)	3.7-9.5	22 (8.2)	11-36	30 (12)	21-48
Elasticity (%)	76 (6.2)	70-82	71 (11)	56-83	89 (2.1)	85-91

The viscoelastic characteristics of the commercial cheeses evidenced a high variability (Table 5) probably related to their composition and technological steps. An inverse correlation between tan δ at 39°C and flow ability of ripened lactic cheeses was observed but all these new interesting data need to be deeper analysed to be correlated to culinary behaviour.

Table 4: Identification of discriminating visco elastic parameters of goat milk cheeses

	Fresh lactic cheeses		Ripened lactic cheeses		Semihard cheeses	
	CV of the method[1]	Variation range for all the products	CV of the method[1]	Variation range for all the products	CV of the method[1]	Variation range for all the products
G'39°C (Pa)	5 %	5600-9700	7 %	9900-20700	16.5 %	5500-19600
G''39°C (Pa)	5 %	1800-3500	7 %	4900-7000	14 %	3300-7500
ΔG'39°C (%)[2]	3.5 %	56-70	4 %	51-81	4 %	63-85
ΔG''39°C (%)[3]	2.5 %	56-68	5.5 %	53-77	4 %	56-78
Tan δ max	0.5 %	0.35-0.40	2 %	0.33-0.59	15 %	0.91-1.47
T°C for Tan δ max (°C)	0 %	58-65	1.5 %	48-61	3 %	66-86

[1] 6 repetitions on each product for determination of CV; [2] ΔG'39°C (%) = (G'25°C-G'39°C)×100/G'25°C;
[3] ΔG''39°C (%) = (G''25°C-G''39°C)×100/G''25°C.

Sensory characteristics assessed after cooking were specific to each type of product but also very variable within each group (Figures 1 and 2), concerning especially the percentage of coloured surface, flow ability, granular texture, presence of cracks and exudation of fat.

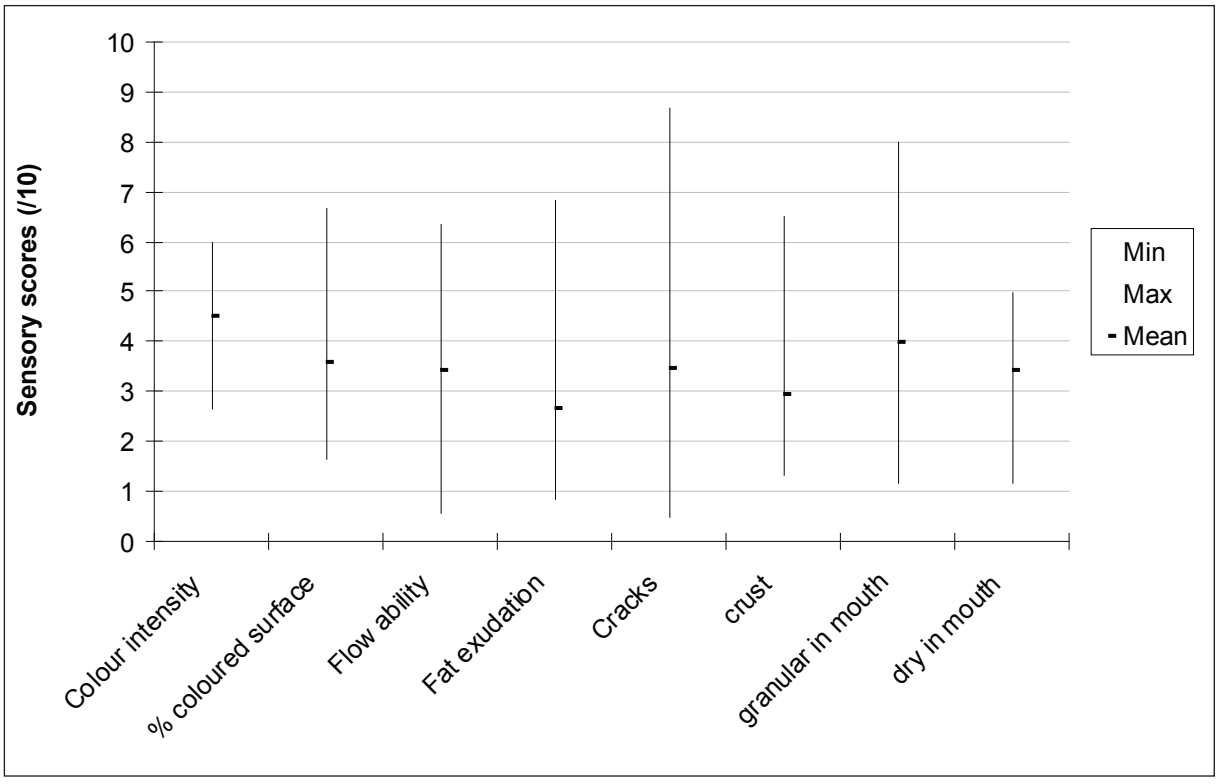

Figure 1. Sensory characteristics of ripened lactic cheeses, mean and variation range

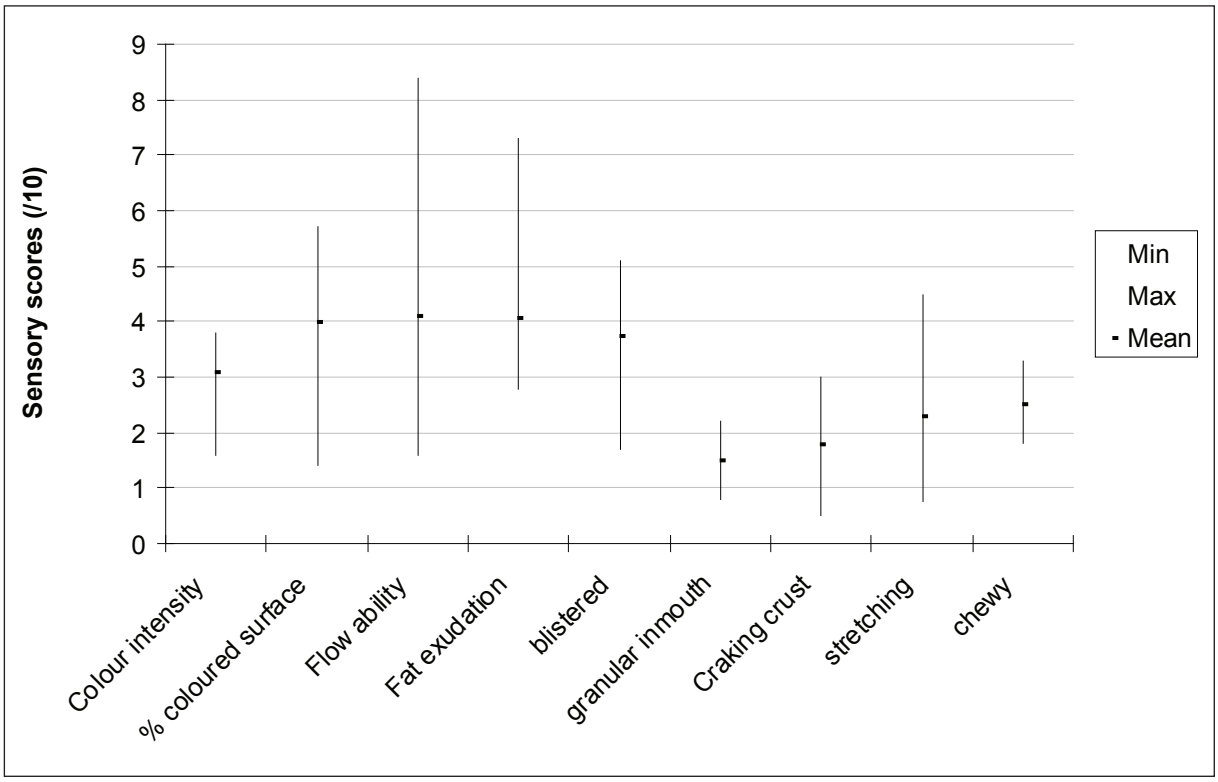

Figure 2. characteristics of semi hard cheeses, mean and variation range

This phase of screening allowed the identification and quantification of different behaviours during cooking that exist among many types of goat cheeses of the French market. This implies that improvement can be achieved if relationship between process, composition and culinary behaviour is understood.

3.2. Relationship between culinary behaviour and process

The behaviour of 79 ripened lactic cheeses with controlled characteristics was assessed by means of six sets of cheese makings.

3.2.1. Influence of proteolysis and lipolysis levels

The higher ripening degree (lipolysis and proteolysis levels) obtained with the cocktail B was associated with the larger coloured surface and a decrease in the granular texture (Figure 3).

Sensory analysis was the only reliable tool to assess the granular texture in the mouth, a defect frequently detected for cooked lactic cheeses. This descriptor was not linked to other sensory attributes of texture and also not linked to the parameters from the instrumental methods such as TPA [15, 16]. The granular texture could be related to the formation of aggregates of proteins under acidic conditions in the case of low enzyme activity of the cocktail (A).

The increase in coloured surface observed in cheeses with higher proteolysis levels corroborates previous works on goat milk Mozzarella [17]. Colour of cheese made with goat milk was less important than for cow's milk due probably to a lower proteolysis extend in the goat milk product. Maillard browning reaction is increased at high pH (above pH 6) and may be promoted by an extensive proteolysis [18].

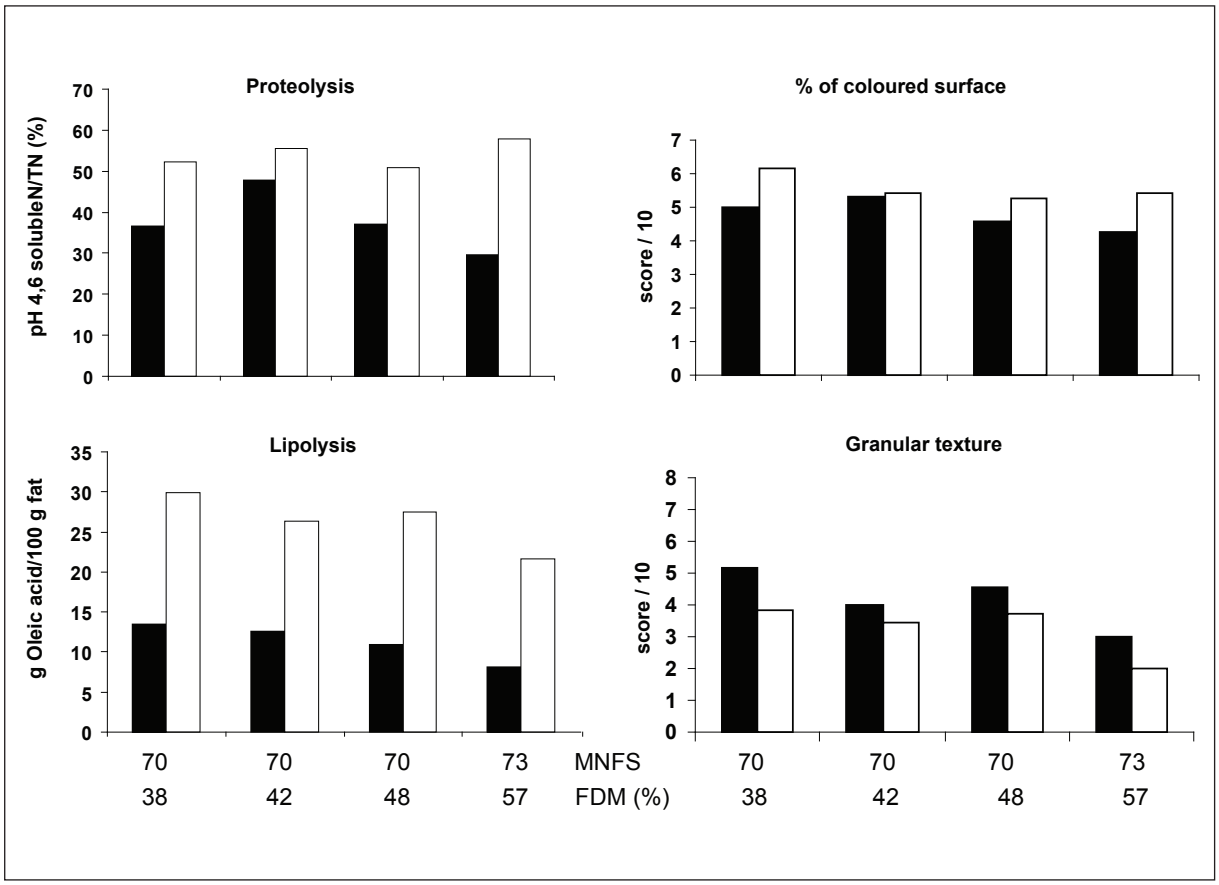

Figure 3. Effect of proteolysis level (higher for ripening cocktail A) of ripened lactic cheeses on the percentage of coloured surface and granular texture in mouth after cooking

3.2.2. Influence of the freezing

The incorporation of frozen curd was accompanied by changes in lipolysis level (a 0.5-6 g OA/100g fat decrease) and fat mobility (a 10-40 g/100 g fat increase), depending on the cheese composition, especially in the 100% frozen curd. These phenomena were sometimes associated with an increased fat exudation and a slight decrease in flow ability (sensory analysis results not shown). Only slight modifications were observed by confocal microscopy (figure 4) even using 100% of frozen lactic curd. The slight effect of freezing on goat milk lactic curd or fresh cheese on sensory value of cheese was previously studied and the freezing parameters were optimised (Gaborit, unpublished results). A minor impact of curd freezing was also observed by Van Hekken et al [19].

Freezing the slices of ripened cheeses seemed to induce greater changes in the supra molecular organisation of fat (Figure 4) and several changes in sensory quality such as the decrease in the coloured surface and flow ability and the increase in the granular texture and the presence of a crust and cracks (Figure 5). Other authors also found an impact of freezing method on Cheddar and Mozzarella microstructure [20].

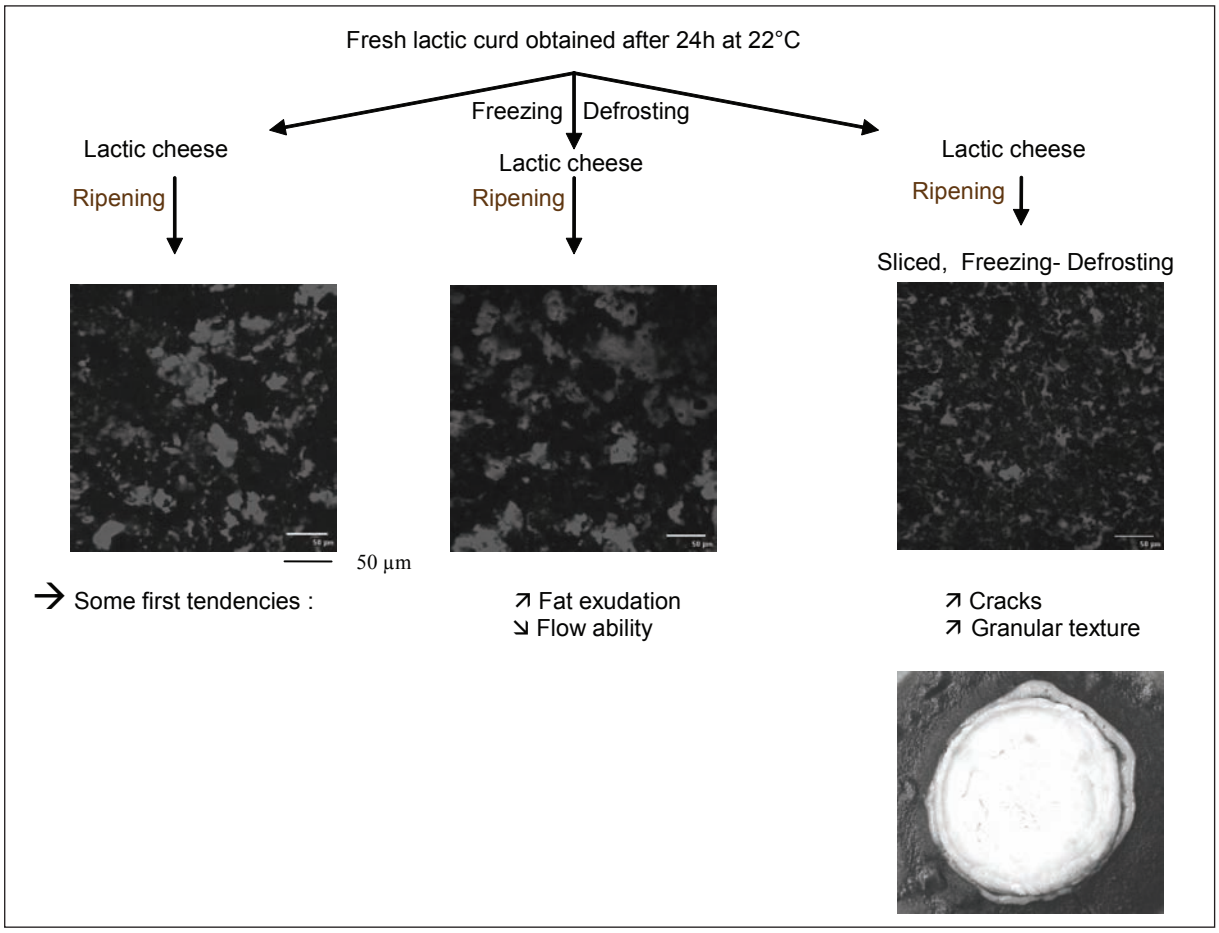

Figure 4. Impact of freezing at microscopic (confocal microscopy realised by MH Madec, INRA STLO, Rennes, fat in white or light grey and protein network in black) and macroscopic (ripened lactic cheese properties after cooking) scales: some tendencies

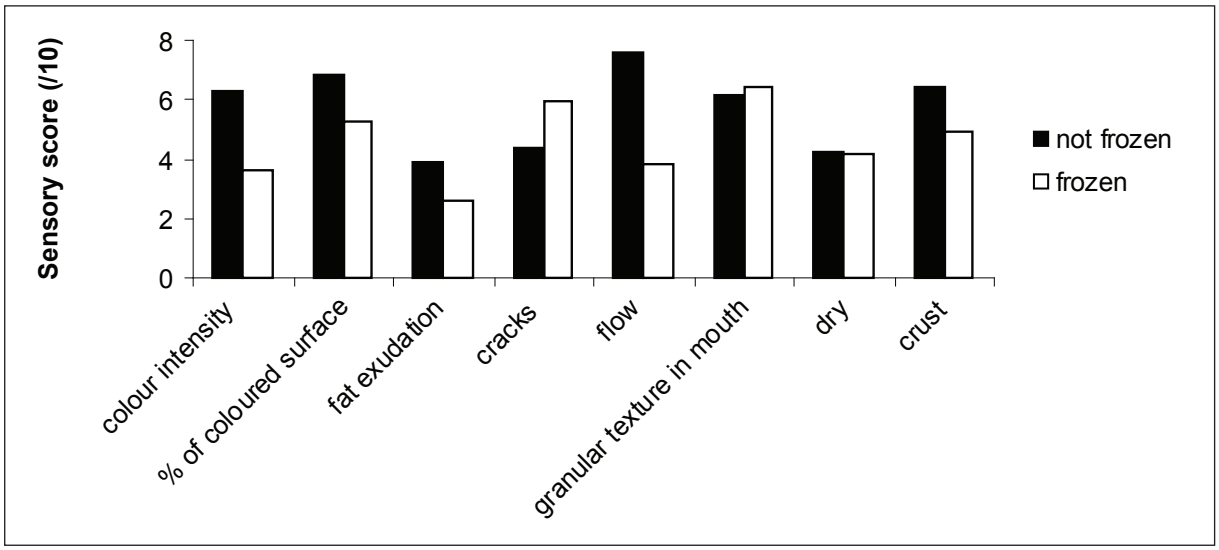

Figure 5. Impact of freezing on the sensory properties of ripened slices of lactic cheese after cooking on pizza

3.2.3. Compilation of the data obtained for the three steps

Finally, relationships between sensory and instrumental parameters were determined on the data obtained during the two last steps by Pearson correlation coefficients and principal component analysis (PCA). They confirmed the previously results for each step of the study and also showed an increase in flow ability when higher lipolysis and proteolysis levels were observed for lactic cheeses (Table 6). After melting of the fat, the integrity of the protein network and the interactions between proteins and water determine the extent of the protein flow. Anything that affects the amount and distribution of fat and strength of the protein network (as is the case during ripening) influences the flow ability [1, 21]. In Raclette type cheese, a positive impact of proteolysis level on its behaviour during heating and post cooking was evidenced [22], especially concerning 12% TCA soluble Nitrogen (NPN) fraction [23]. Concerning soft cheeses, a relationship between pH and flow ability of Cremoso Argentino was found [24], that is higher flow ability at pH 5.5 compared to pH 4.9 (with similar proteolysis levels).

The results obtained for ripened lactic cheeses also corroborate the inverse relation observed for Cheddar cheese between melting properties and content in Ca, P, lactose [25, 26].

Table 5: Correlations (Pearson) observed for the 84 ripened lactic cheeses characterised during the steps 2 and 3 (screening of the products of the French market and experimental cheese makings)

r (Pearson) N= 84	Flowability (sensory evaluation)	Granular texture
pH	0.8	
NCN/TN	0.7	-0.8
Fat/ Dry matter		-0.54
Ca	-0.88	0.67
Cl	0.64	
Lactose	-0.67	

4. Conclusion

The aim of this study was to characterize the most representative French goat cheese varieties, in which are highly variable in respect to biochemical composition, sensory characteristics, rheological properties and culinary behaviour. Many set-up or adapted methods were used for these specific lactic cheeses. This enabled the highlighting of different behaviours for culinary applications, in relation to some technological steps and the biochemical composition. These first data concerning a large panel of lactic cheeses need to be further analysed. Moreover, the observed tendencies need to be validated in a future research project in order to meet the producers and users' expectations (cheese makers, food industry, restaurants, consumers.) for texture, flow ability, colour and goat flavour of cooked cheeses.

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Posters

3.2. (S3.7) Jordan and Shor, Two Traditional Milk Products, Which are Produced in Azerbaijan Region of Iran

Y. Shafiei[1]

Summary

Jortan and Shor are two traditional milk products of Azerbaijan region of Iran which are mainly prepared from sheep milk. The main purposes of this study are to examine and to compare some properties of experimentally prepared products.

After preparation of these products, sensory and chemical properties of them were examined based on AOAC Official Methods. All of the stages repeated 5 times. For statistical analysis of data, Software-SPSS was used.

Considering the results, sensory properties of Jortan and Shor were different. Also there was a highly significant difference between most of chemical properties of two products ($p<0.01$), but there was no significant difference between acidity and pH ($p>0.05$).

The findings suggest that these products have desired properties like that of cheese and yogurt and have a potential possibility to be produced in large industrial scale with high quality by utilizing modern dairy processing equipments and new technologies now available.

1. Introduction

In Azerbaijan region of Iran, many different milk products, which are traditionally prepared from ewe and goat milk, are still unknown elsewhere in spite of their valuable properties. Two of these products are Jortan (Cortan) and Shor (Şor), traditional fermented milk products obtained from yogurt after producing Ayran and separation of butter. These products are fat free or have a negligible fat content due to the extraction of fat from the yogurt as butter, so they are nutritionally healthful and they could be good alternatives for other high fat content products. Furthermore, in their manufacture no additional salt is used as preservative.

The main purposes of this study are: to introduce and to describe traditional production process of these products, to examine some sensory and chemical properties of experimentally prepared products with ewe milk, and to compare these properties in two products. Also, it suggests an industrial method and procedure for producing these products by utilizing modern dairy processing equipments and new technologies available today in order to produce them on a large industrial scale with high quality.

2. Materials and Methods

Fresh ewe milk was collected during lactation period in the East Azerbaijan Region of Iran, in April. Mixture of two starter cultures YC-X11 and YC-350 (Chr. Hansen, Denmark) were used by Iran Dairy Industries Co. (Pegah) of Tabriz as bulk starter culture, ready for inoculation. This mixture of yogurt starter culture consisting of Lactobacillus delbrueckii supsp. bulgaricus and Streptococcus salivarius subsp. thermophilus (1:1) was used for the yogurt [6] needed for Jortan and Shor production (Figure 1).

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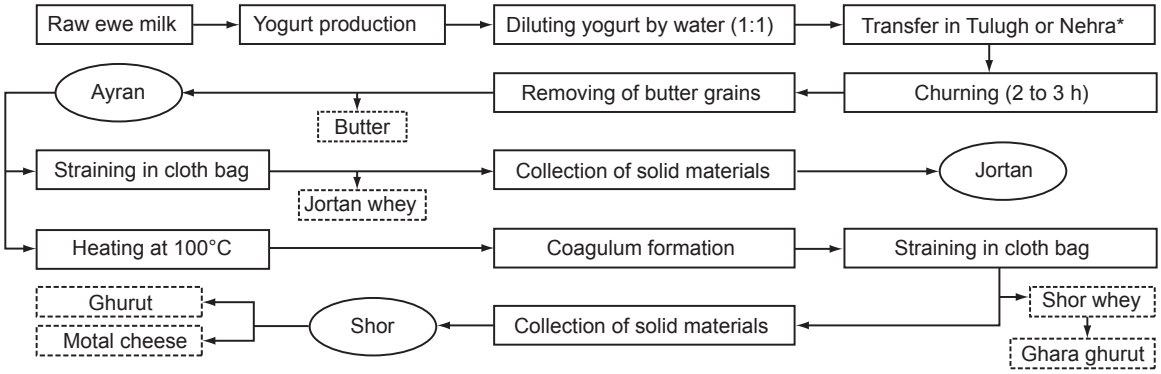

Figure 1. Schematic representation of traditional production procedure of Jortan and Shor.
* Tulugh and Nehra are traditional instruments which are used to shake the mixture of yogurt and water to separate butter and to produce Ayran

Jortan and Shor were analyzed for moisture, total solids, ash and titratable acidity according to AOAC official methods [1, 2]. To measure fat content of products Gerber Method was used [3]. All pH measurements were taken with a digital pH meter, model Metrohm 691, manufactured by Metrohm-co, Switzerland. Due to the lack of special standard tests for Jortan and Shor, we applied standard tests of yogurt for Jortan and those of white cheese for Shor owing to their organoleptic similarity.

Sensory evaluation of the samples was conducted by 30 untrained taste panelists. Each panelist received three samples of each test product and control product for comparison and was asked to evaluate using a sensory rating scale of 1 to 10.

Data were analyzed using Software-SPSS 11.5 [5]. The mean values and the standard error were calculated from the data obtained by the five replicate trials for chemical analysis. For comparison of mean values Independent t-test was used. The results of sensory evaluation were submitted to the analysis of variance (ANOVA).

3. Results and Discussion

Results of chemical analyses are shown in Table 1 and results of sensory evaluation of Jortan and Shor and other control products are shown in Table 2.

Table 1: Chemical properties of Jortan and Shor

Sam.	Moisture (%)	Total solids (%)	Fat (%)	Solids non-fat (%)	Ash (%)	Acid (%)	pH value
S1*	80.4 ± 0.4^{a}	19.6 ± 0.4^{a}	0.3 ± 0.009^{a}	19.3 ± 0.4^{a}	0.32 ± 0.05^{a}	0.26 ± 0.01^{a}	4.09 ±0.004^{a}
S2*	69.2 ± 1.8^{b}	30.8 ± 1.8^{b}	1.04 ±0.04^{b}	29 ± 1.78^{b}	0.56 ± 0.04^{b}	0.23 ±0.006^{a}	4.1 ± 0.009^{a}

$^{a, b}$ means in the column with no common superscript letters are significantly different (P<0.01).
* S1= Jortan, S2= Shor

Table 2: Mean score of Sensory evaluation of Jortan and Shor and comparison of them with sensory properties of control products

Evaluated Sample	Taste	Texture	Colour	Overall Acceptability
S1*	8.46 ± 0.08^{ac}	8.56 ± 0.06^{a}	8.5 ± 0.05^{ac}	8.5 ± 0.1^{abc}
S2*	8.23 ± 0.06^{cd}	8.2 ± 0.05^{b}	8.3 ± 0.05^{ab}	8.2 ± 0.06^{b}
C1*	8.6 ± 0.05^{a}	8.66 ± 0.03^{a}	8.63 ± 0.03^{c}	8.7 ± 0.05^{c}
C2*	8.33 ± 0.08^{ac}	8.26 ± 0.03^{bc}	8.36 ± 0.06^{a}	8.36 ± 0.03^{ab}
C3*	8.5 ± 0.05^{a}	8.56 ± 0.03^{a}	8.5 ± 0.05^{a}	8.6 ± 0.05^{c}
C4*	8.16 ± 0.03^{d}	8.23 ± 0.06^{bd}	8.16 ± 0.03^{bd}	8.2 ± 0.05^{ab}

$^{a, b, c, d}$ means in the column with no common superscript letters are significantly different (P<0.05).
* S1: Jortan, S2: Shor, C1: concentrated yogurt, C2: plain yogurt, C3: matured cheese, C4: fresh cheese

Most of chemical and sensory properties of Jortan and Shor are different, but both of these products have same characteristics as low acid and low fat content as compared with other milk products (Table 1 and 2). Also, they have low fat content but they are rich in milk fat globule membrane (MFGM) components that have valuable health effects.

4. Conclusion

Traditional methods have many disadvantages [4]. Hence, In Figure 2, we present a procedure for industrial manufacture of Ayran, Jortan and Shor.

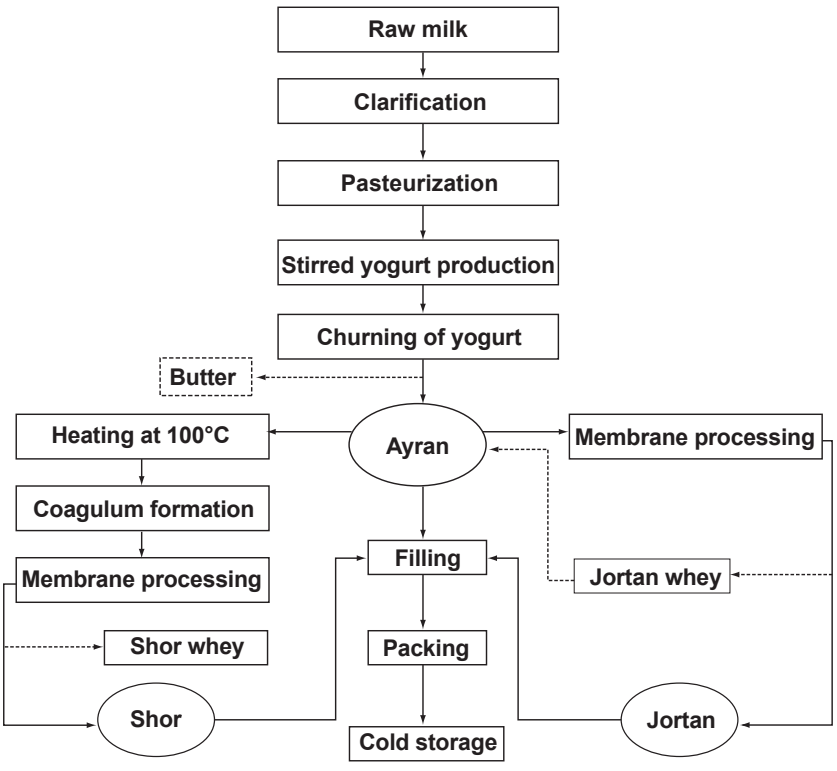

Figure 2. Schematic representation of the industrial production procedure for Jortan and Shor, as suggested by the author

This study indicated that Jortan and Shor could be appropriate candidates for new functional milk products, and could be produced industrially by utilizing available dairy processing equipments and modern technologies.

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3.3. (S3.9) Effect of Raw Goat Milk Storage Time at 4 °C on Goat Milk Powder Quality

C. R. Fonseca[1], K. Bordin[1], D.V. Neeff [1], C.A.F. Oliveira[1]

Summary

About 100 L of fresh goat milk was divided into 3 fractions and stored at 4 °C. On 1st, 3rd and 5th days, one sample was collected for microbiological (mesophilic and psychrotrophic bacteria) and biochemical analysis and the remainder of the fraction was spray dried. Immediately after manufacture and on 60th, 120th and 180th days, powders were evaluated by microbiological, proteolysis, lipolysis, fat oxidation and sensorial analysis. The raw milk storage time increased ($P<0.05$) peroxide value, caprilic, myristic and total FFA contents in milk powder, but there was no effect ($P>0.05$) on milk powder capric and rancid flavors. Milk powder time storage showed linear increasing effect ($P<0.05$) on peroxide value, caprilic, capric and FFA total amounts, capric odor and rancid flavor. It was concluded that the heat treatments used in milk powder processing were effective to destroy psychrotrophic bacteria, but not their thermoresistant enzymes.

1. Introduction

The drying process can be considered as an excellent alternative for extending the goat milk shelf-life without changing nutritional and sensorial characteristics. Also, the product volume decrease has technological and market advantages. Psychrotrophic bacteria may grow at temperatures below 7°C in raw goat milk and produce proteolytic and lipolytic enzymes that can make milk powder deteriorate faster, forming smaller compounds that are responsible for bitter, rancid, soapy and fruity flavor in milk powder [2]. This study evaluated the effects of raw milk cold storage on the quality of goat milk powder during its shelf life.

2. Material and methods

About 100 L of fresh goat milk was divided into 3 portions and stored at 4 °C. On 1st, 3rd and 5th days after storage, one of the fractions was submitted to pasteurization (65 °C for 30 min), vacuum concentration (40% of total solids) and spray drying. The powders were stored in nitrogen modified atmosphere packages at 25 °C. Immediately after the manufacture and on 60th, 120th and 180th days powders were analyzed as follows:

Proteolytic and lipolytic psychrotrophic and mesophilic bacteria were counted using Calcium Caseinate Agar, Tributyrin Agar and Plate Count Agar, respectively.

Proteolysis was quantitatively estimated through the relation casein (C)/true protein (TP) as described in AOAC [3]. The total FFA quantification was realized according to Deeth et al. [4] and the individual FFA was analyzed as described in De Jong & Badings [5]. Fat oxidation was measured by peroxide value analysis [3].

Seven trained panelists evaluated the capric odor, capric and rancid flavor and bitter taste of reconstituted goat milk (12%) samples using a non structured scale from 0 (no intensity) to 10 (strong intensity).

The Complete Randomized Design with factorial arrangement (3 storage times of raw milk, 4 storage times of milk powder and 3 repetitions) experiment was submitted to statistical analysis using SAS® software [6], using the proc glm procedure and subsequent regression study by orthogonal contrasts.

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3. Results and discussion

The equations obtained for the effects of storage times of raw goat milk and milk powder are shown in Table 1.

Table 1: Effects of raw goat milk storage time (A), milk powder storage time (B) and interaction between them (AXB) on the quality of milk powder samples

Parameters	A	B	A X B
Mesophilic counts	$y = 0.49x + 2.12$ ($R^2 = 0.99$)	*	*
Total FFA	$y = 0.28x + 0.23$ ($R^2 = 0.90$)	$y = 0.07z^2 - 0.29z + 1.01$ ($R^2 = 0.98$)	Significative
Caprilic acid	$y = 3.39x + 13.49$ ($R^2 = 0.78$)	$y = 4.10z + 11.03$ ($R^2 = 0.94$)	*
Capric acid	$y = -1.37x^2 + 6.14x + 8.52$ ($R^2 = 1.00$)	$y = 1.56z + 10.53$ ($R^2 = 0.96$)	*
Myristic acid	$y = 0.43x + 2.27$ ($R^2 = 0.98$)	*	*
Peroxide value	$y = 0.13x + 0.08$ ($R^2 = 0.97$)	$y = 0.15z - 0.03$ ($R^2 = 0.96$)	*
Capric odor	*	$y = 0.75z + 0.98$ ($R^2 = 0,95$)	*
Rancid flavor	*	$y = 0.27z + 0.12$ ($R^2 = 0.89$)	*
Bitter taste	$y = 0.25x - 0.05$ ($R^2 = 0.89$)	*	*

y = analysis parameters, x = raw milk storage time (days), z = milk powder storage time (days).
* Not-significant ($P > 0.05$).

The raw goat milk storage time increased linearly mesophilic populations, total FFA content, caprilic and myristic FFA, peroxide value and bitter taste. The goat milk powder storage time increased linearly caprilic, capric and myristic FFA, peroxide value, capric odor and rancid flavor. There was an interaction between the raw and powdered goat milk in relation to total FFA content, presenting higher level in powder sample stored at 25 °C for 180 days than that in raw milk stored for 5 days. Proteolytic and lipolytic psychrotrophic bacteria were not found in milk powder samples, indicating that these microorganisms are sensible to the heat treatments used during the milk powder manufacture. However, the effects of raw goat milk and milk powder storage times and also on the contents of some short-chain FFA (i.e. caprilic and capric acids) indicate that the lipases produced by the lipolyitic psychrotrophs during the storage of raw milk continued to act on milk fat during the milk powder shelf life, increasing the undesirable capric odor and the rancid flavor. Although no effect was observed any of the storage times of raw and powdered milk on the relation C/TP, there was a linear increasing effect of the raw milk storage time on the bitter taste of milk powder.

4. Conclusions

It was concluded that the heat treatments used in goat milk powder processing were effective to destroy psychrotrophic bacteria, but not their thermoresistant enzymes. Considering the results found, it is recommended that raw goat milk storage at 4 °C must not exceed 3 days to preserve the goat milk powder quality for up 180 days.

Aknowledgement

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3.4. (S3.13) Dairy Products Made from Sheep and Goat's Milk in Turkey

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Summary

Average total milk production in Turkey is around 13 million tons per year. Sheep's milk provides 5.85% of it , 1.53% is goat's milk and 0.26% is buffalo's milk. These kinds of milk are consumed locally and processed into dairy products such as cheese, yoghurt, ice cream and butter that are highly appreciated by the consumers. Their particular sensory characteristics are more preferred than those of the respective cow's milk products due to the composition of sheep and goat's milk. A variety of cheeses is produced: sheep's milk cheeses named Kashar, Tulum, Herby, and Orgu and goat's milk cheeses named Tulum and Carra. Also traditional yoghurt products are produced from sheep and goat's milk such as Tulum, Salted yoghurt and Kurut.The aim of this review is to introduce Turkey's dairy products, which are made from sheep and goat's milk and to describe their compositional characteristics.

1. Introduction

After China and Greece, Turkey is the third leading country in the sheep milk production with 734219 tonnes and this represents about 5.85% of the total milk production of the country. Also, Turkey ranks 13th in the world and 4th in Mediterranean countries after France, Greece and Spain based on goat milk production with 192210 tonnes. This amount represents 1.53% of the total milk production of the country [1]. Sheep and goat's milk is widely used for home consumption worldwide and to produce different cheeses and yoghurt, which makes them of particular economic value in countries. Delicious cheese and yoghurt are produced from sheep and goat's milk and these products are usually preferred to the ones produced from cow milk. Thus, raising sheep and goats is of great importance in Turkey. Similarly sheep and goat's milk are also used for manufacturing cheese in some countries such as France, Italy and Greece [2].

2. Cheeses from sheep and goat's milk

2.1. Kashar (Kaşar) cheese

Kashar cheese which is a semi-hard cheese is one of the the most important cheese varieties manufactured in Turkey. This cheese is known as maturated Kashar if produced traditionally. In cheese-making, curd is acidified and then it is scalded in hot water and kneaded. After ripening period it has a unique flavour, taste and aroma. Cheeses similar to this type are extensively manufactured in Balkan countries known as Kashkaval, Kasseri and Kachkawaj. The best quality traditional Kashar cheese is made from sheep milk[3].

2.2. Tulum cheese

Tulum, a hard type traditional cheese, is the most commonly produced cheese after white and kashar cheeses, respectively. The cheese is generally made from sheep's milk without starter culture. Its name, tulum, originates from the goat skin that is traditionally used as a casing material and ripened in it. Today, tulum cheese is commonly cased in plastic barrels or ceramic

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pots instead of tulum in some regions of Turkey [4]. Tulum is characterized as having a white or cream colour, high fat, high protein and high dry material content, butter-like aroma, obvious acidic taste, and a homogenous structure with no gas holes and not easily broken [5].

2.3. Herby (Otlu) cheese

Herby cheese a semi-hard, salty and herb added, is manufactured in small family businesses for their needs and commercial purposes from generally made from sheep's milk between May and June in the East and South east regions of Turkey. The herbs have noticeable effect on the taste and flavour of herby cheese, and this cheese is consumed almost every meal in all over the country with a good taste.

There are 25 kinds of herbs used for this aim. The main herbs used in this cheese are Allium sp. (local name 'sirmo'), Anthriscus sp. (local name 'mendo'), Ferula sp. (local name 'heliz'), Mentha sp. (local

name 'nane') and Thymus sp. (local name 'kekik') etc. Herby cheese is salty, with herb flavour, white to yellowish colour [6].

2.4. Carra cheese

Carra, which means pottery, is one of the popular semi-hard traditional cheese in Turkey. It is manufactured in South Anatolia region province in Hatay. Traditionally, Carra cheese is made from mostly non pasteurized goat's milk and heated acid curd (cokelek). The difference from the other cheeses is that the curd is sliced into 1 cm thick pieces, salt is put between the slices and then left for 2-3 days to harden. When cheese has reached the desired moisture content, it is coated with black cumin (Nigella sativa) and filled into a Carra, one layer rennet curd and one layer cokelek in alternating order until the carra is full. The pottery is turned up side down and stored in a cool place for a few days. Then, some salt and thyme (Thymus vulgaris) are sprinkled on the surface of the cheese before the potis closed with a piece of cloth. Finally, the mouth of the potis closed with mud or a mixture containing ash, salt, olive oil and water. When the mud or slurry dries, the pottery is buried in ground upside down and left for ripening at least for 3 months [7].

2.5. Orgu (Braided) cheese

Orgu cheese is one of the common traditional cheese types in Eastern region of Turkey from sheep milk, and becoming more popular and preferred almost all over Turkey. Orgu cheese production involves intensive manual handling during the manufacturing stage and requires skillful manipulation of the curd by the cheesemaker [8]. Acidified curd is scalded in hot water at 70-80°C for 5-6 minutes. The scalded curd is stretched like a string of 1 cm in diameter. Three strings are knitted together and then cut at approximately 10 cm in length. The Orgu cheese is usually consumed either fresh or ripened for 60 days in brine of 12-15% salt [9].

Table 1: Composition of cheeses from sheep and goat's milk

Cheese	pH	Acidity (LA%)	Dry Matter (%)	Fat (%)	Protein (%)	Salt (%)	References
Kashar	5.13	-	56.79	-	-	3.37	[10]
Tulum	-	1.02	50.04	25.05	20.42	2.85	[11]
Herby	5.52	0.48	45.80	17.83	21.37	5.19	[6]
Carra	5.63	0.85	53.43	24.86	18.86	8.83	[12]
Orgu	-	1.11	47.75	17.86	19.96	5.32	[9]

3. Yoghurt and yoghurt based products from sheep and goat's milk

3.1. Tulum yoghurt

"Tulum" yoghurt is made by spontaneous coagulation of milk in a specially prepared sheep's or goat's skin. 3-5 liters of milk are added to the skin every day and 100 g of salt every 2-3 days. The desired characteristic properties of Tulum yoghurt appear one week later. Tulum yoghurt has a high dry matter like concentrated yoghurt but higher protein and fat content as compared with concentrated yoghurt. It has granular structural and salty taste [13].

3.2. Salted yoghurt (ayran)

In Mediterranean and Southern regions of Turkey, the traditional way of prolonging the keeping quality of yoghurt is employed in production of "salted yoghurt". Goat milk is preferred for the production [14]. Flavour of salted yoghurt must be clean and mildy acidic. Body and texture are homogenously soft and smooth with no graininess. It should has good spreadability and no wheying off. Because of low moisture and high salt, salted yoghurt has good shelf life and lends itself for use in winter which is from 5-6 months to one year of age [15]. There are two procedures in the production of Salted yoghurt. The first procedure is the set-type yoghurt which is boiled and then salt is added. The second method is where yoghurt whey is removed by using cloth bag, boiled, and then salt (1-2%) is added in order to shorten cooking time. When the cooking stage is completed, salted yoghurt is transferred into another container and left for cooling generally stored under oils or fats [16]. It is sold in plastic trays in local markets and no federal standards for salted yoghurt composition exist.

3.3. Kurut

Kurut which is a traditional sun-dried dairy products is known as "dried yoghurt" and made in the villages of Eastern region of Turkey. Kurut is made in spring and consumed in winter while purchasing fresh milk and dairy products is quite impossible in the region. It is made from yoghurt or ayran in Anatolia. In production of kurut, salt (2-3%) is added into yoghurt and salty yoghurt is strained for 12 h and typical kurut dough is obtained. Then, dough is shaped into lumps of 40-60 g in weight and 3-5 cm in diameter. Kurut lumps are set aside in a shady place to be dried for 10-15 days. Kurut can be stored at ambient temperature for more than 6 months[17].

Table 2: Composition of yoghurt and yoghurt based products from sheep and goat's milk

Product	pH	Dry Matter (%)	Fat (%)	Protein (%)	Lactose (%)	Salt (%)	References
Tulum yoghurt	-	35.67	22.55	10.6	-	3.1	[13]
Salted yoghurt	4.09	23.27	6.94	8.05	5.85	2.76	[16]
Kurut	3.92	84.25	8.57	53.60	1.06	9.95	[17]

4. Conclusions

A large number of traditional dairy products from sheep and goat's milk are produced in Turkey and consumed generally in local regions and in the home market. Large scale sheep and goat farms could be a solution for solving this situation in the many regions, where only goats and sheep can still support a farm family. It also remains to be accepted that technical knowledge available can permito the manufacture of quality products from sheep and goat milk. Finally, the

success of the sheep and goat milk industry will be virtually dependant on the establishment of high producing dairy goat herds, production of high quality milk, improved and carefully control of product manufacturing, packaging, storage and distribution techniques.

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3.5. (S3.17) Study of The Coloring Properties of Powdered Saffron Spice in Powdered Skim Ewe Milk

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Summary

Color is one of the main food characteristics that influence consumer preferences, saffron spice (*Crocus sativus* L.) being highly appreciated for this property. Several milk products use saffron as an ingredient but its coloring properties in milk have never been reported. The aim of this work was to study the effect of saffron concentration (2 to 10 mg/ml), temperature (37, 50 and 70ºC) and extraction time (20, 40 and 60 min) in powdered skim ewe milk by tristimulus colorimetry. Saffron concentration influences color coordinates, but temperature and extraction time did not have any significant influence.

1. Introduction

Saffron spice (*Crocus sativus* L.) is an important Spanish product specially used in food, for its aromatic, flavour and colouring properties, having a Protected Designation of Origin named "Azafrán Mancha" [1]. This spice has been known since ancient times for its biomedical properties, such as antioxidant, anti-inflammatory, analgesic, and even by its antitumor and anticancer activity, as well as antidepressant, respiratory decongestant, antispasmodic, aphrodisiac, expectorant and sedative [2]. Many dishes are known around the world such as Spanish Paella, Italian Risotto, French Bouillabaisse and even some cheeses in Germany and Italy that include saffron, but the coloring properties of saffron in milk or the conditions for preparing these solutions have never been reported. The aim of this work was to study the effect of saffron concentration, and the extraction temperature and time on the coloring properties of saffron in powdered skim ewe milk solutions using tristimulus colorimetry.

2. Material and methods

A 16-litre batch of skim ewe powder milk (18% w/v; 0.01% milkfat; ZEU Inmunotec, Zaragoza, Spain) was used. Fifty-five grams of Spanish saffron spice (*Crocus sativus* L.) of the Protected Designation of Origin "Azafrán de la Mancha" were used from the 2007 harvest. Saffron was grounded according to ISO 3632 Technical specification [3]. The mixture was prepared in reconstituted ewe milk following the specification of the pending patent No.P200930912. The experiment consists on a multilevel factorial design based on the temperature of the saffron color extraction (37±4, 50±4 and 70±4 ºC); extraction time (20, 40 and 60 minutes) and saffron concentration (2, 4, 6, 8 and 10 mg/ml). All treatments were done by triplicate. Reflected color was measured using a Minolta CR-400 (Minolta Camera Co., Osaka, Japan) with a CR-a33f cone a D65 illuminant and an angle vision of 10º. CIE L*a*b*, CIEL*C*h coordinates were obtained. Statgraphics Plus 5.1 was used to carried out a General Linear Model (GLM) analysis.

3. Results and Discussion

General Linear Model equations for the different color coordinates studied are shown in Table 1, where saffron concentration, temperature and time were included. The coordinates L*, a* and h had regression coefficients higher than 85%, but b* and C* explained no more than 5% of

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these variables. Saffron concentration was the only factor that was shown to be statistically significant for all color coordinates (p<0.007). On the other hand, temperature and extraction time did not have any influence on saffron color extraction (data not shown). As saffron concentration was increased, the extracts were less bright and yellow, but more red; besides, they became more vivid and tended to redder tones (Figure 1).

Table 1: General Linear Model equation for saffron extracts in skim ewe milk

Coordinate	Equation	R2	P
L*	75.16 - 1.66*S	85.49	0.000
a*	6.08 + 2.55*S	89.44	0.000
b*	76.73 - 0.28*S	2.64	0.007
h	85.53 -1.89*S	91.17	0.000
C*	75.98 + 0.41*S	5.41	0.000

S: saffron concentration

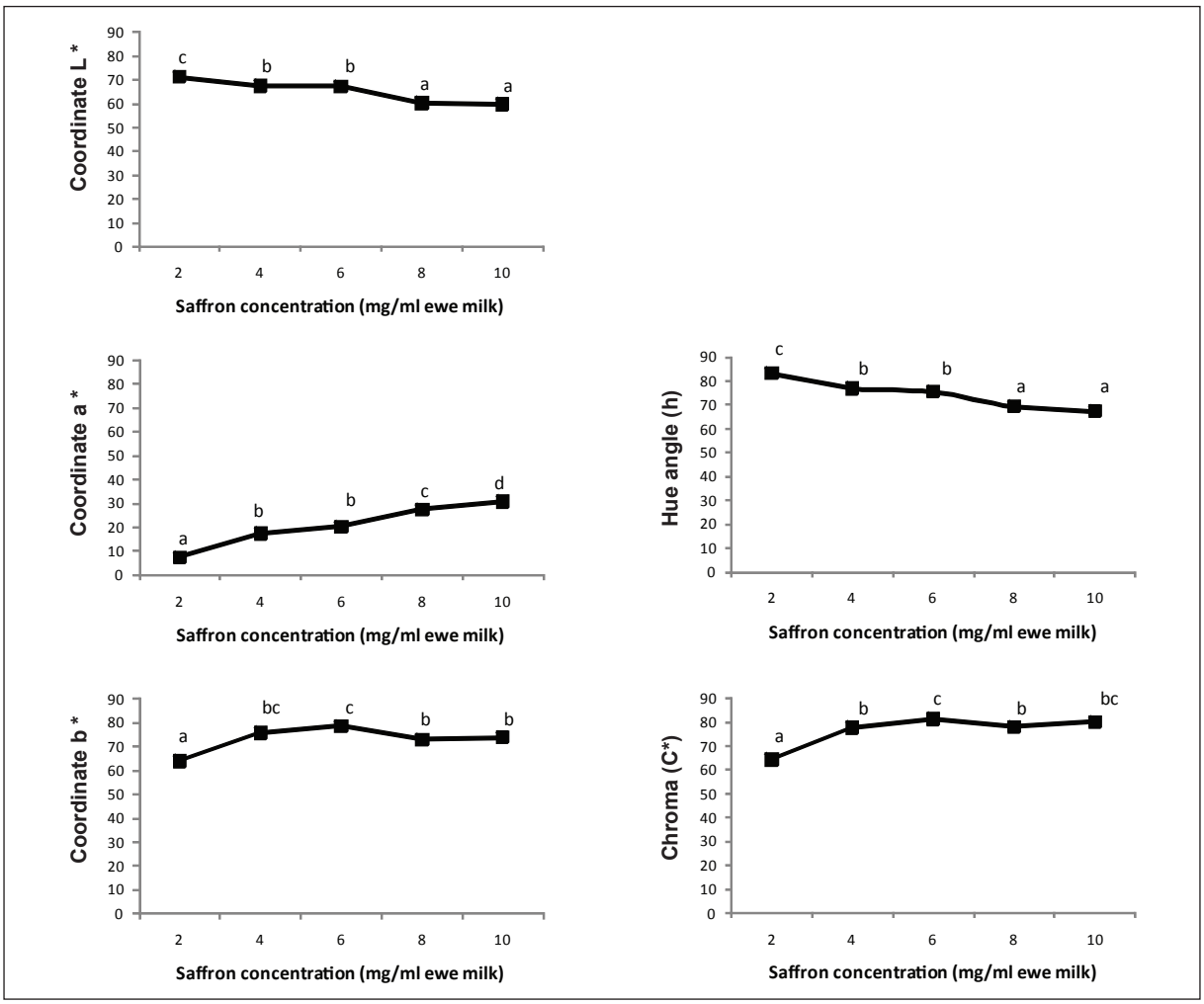

Figure 1. L*, a*, b*, Chroma (C*) and Hue angle (h) coordinate values for saffron color extraction in skim ewe milk at 37 °C during 20 minutes

Alonso et al. [4] obtained hue values for powder saffron water extracts at a concentration of 0.05 mg/ml from 50° to 53°, being lower than the values found in the saffron milk extracts, since prediction for hue at the same extraction conditions, using skim milk, will have values near 85° (Table 1), saffron extracts in ewe milk having a yellow hue, while saffron extracts in water tend to red hue. This color difference could be due by interactions between saffron coloring compounds and the different milk components, including proteins and lactose, showing the matrix effect of ewe milk in the saffron extracts, but further research is needed regarding these.

In saffron aqueous solutions, as well as in saffron ewe milk solutions, coordinate a* increased with saffron concentration, thus the color tended to red [4], demonstrated by the positive sign in the GLM equation (Table 1, Figure 1). In the other hand, in the saffron extracts coordinate b* decreased with saffron concentration, having an opposite behavior comparing with saffron aqueous extracts [4].

Even if temperature was not significant for any coordinate, it has been reported that this factor could cause isomerization, foaming and degradation of some milk and saffron compounds, such as crocetin esters, proteins, fat and lactose. In addition, heat treatment application to milk molecules has been proved to cause denaturalization, lactose degradation, and structural changes [5]. Some authors suggest the utilization of temperatures between 30 and 70°C for saffron aqueous extraction to avoid compound damage and hence losses of saffron coloring strength [6].

Extraction time was also not significant for any color coordinate studied, nevertheless some authors [5, 6] mentioned that long extraction periods are not recommended because they could cause loss of coloring strength, as well as saturation.

From the above, 37°C and 20 minutes were selected as suitable conditions for saffron extraction in skim ewe milk.

4. Conclusion

As saffron concentration was increased, a*and C* coordinates were increased, and L*, b* and h were decreased. Temperature and extraction time did not have any significant influence on saffron color coordinates, so that, the lower extraction conditions (37 ºC and 20 minutes) could be used to avoid milk and saffron damage.

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3.6. (S3.18) Effect Of Fat Content on The Coloring Properties of Saffron Spice in Ewe Milk

C. Licón[1], M. Carmona[2], A. Molina[1], M.I. Berruga[1]

Summary

Saffron spice (*Crocus sativus* L.) is widely used for its coloring properties in many dishes, but its coloring ability in a fatty matrix, such as milk, has not been yet studied. Due to fat concentration changes in sheep milk during lactation period, the aim of this work was to study by tristimulus colorimetry, the effect of fat content on saffron coloring properties. A 1% saffron solution in ewe milk was used, to consider the potential production of color in milk products. As milk fat content was increased, the saffron extracts were brighter, more yellow and less red, and the values of ΔE* coordinate were increased. Ewe milk was found an appropriate matrix for saffron color extraction to elaborate milk products; however, the period of lactation has to be considered when preparing these solutions.

1. Introduction

Saffron spice (*Crocus sativus* L.) is widely used for its coloring properties in many milk products such as Box cheese in Germany, Luneberg in Austria, Piacentinu Ennese in Sicily, spreadable cheeses in Sardinia, Bouchon allo Zafferano in Lombardia or Cacio allo Zafferano but its coloring ability on milk, has not been yet studied. Physico-chemical composition of milk is variable and mainly influenced by breed, feeding, milking system or lactation stage [1]. Particularly in ewe's milk, fat and protein concentrations vary significantly during lactation, from 4 to 10% and from 4.8% to 6%, respectively [2]. Standardization, which involves skimming or blending skim and whole milk, is a common practice used in dairy industry. But many other industries that process ewe milk do not practise this treatment resulting in variable milk composition during lactation. Consequently, this variation must be taken into account when dairy products are produced. The aim of this work was to study the effect of fat content on saffron coloring properties using a 1% saffron solution in ewe milk with different fat contents by tristimulus colorimetry, to research the possibility of future production of milk products.

2. Material and methods

Three 3 litre batches of ewe milk with different milk fat contents were used. Batch A, commercial semi skim UHT ewe milk, had a fat content of 1.6% (Gaza, Zamora, Spain); batch B, obtained from the Experimental farm "Las Tiesas", had a 6% of fat content (Albacete, Spain) and batch C, from the Experimental farm of Universidad de Castilla-La Mancha, had a fat content of 9% (Albacete, Spain). Thirty-three grams of Spanish saffron spice (*Crocus sativus* L.) of the Protected Designation of Origin "Azafrán de la Mancha" were used from the 2007 harvest. Saffron was ground according to the ISO 3632 Technical specification [3]. The mixture was prepared in milk following the specification of the pending patent No.P200930912. The temperature of the saffron color extraction was measured at 37 ± 4 ºC for 20 minutes at a saffron concentration of 1% (w/v). All treatments were done in triplicate. Reflected color was measured using a Minolta CR-400 (Minolta Camera Co., Osaka, Japan) with a CR-a33f cone, a D65 illuminant and an angle vision of 10º were used. CIE L*a*b* coordinates were obtained and ΔE*calculated according to the following formula: $\Delta E^* = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$, taking as a reference the corresponded

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milk without saffron color, comparing milks with the same fat content. Statgraphics Plus 5.1 was used for analysis of variance (ANOVA; $p<0.05$) to evaluate the effect of milk fat content. Tukey's test at a significance level of $p<0.05$ was used to determine differences between milkfat groups.

3. Results and discussion

The ability of ewe's milk for saffron color extraction was analyzed by tristimulus colorimetry because a technique able to determine the coloring properties of saffron in a milk matrix has not been yet developed.

Table 1 presents the color coordinates obtained for the ewe's milk used in this study. Values of L* and a* coordinates increased with milk fat, while values of b* decreased. The fat percentage between 1.6 and 6.0% is greater than the difference between 6.0 and 9.0% (4.4 vs 3.0%), but a* and b* values from 9.0% milk are significantly different from the rest.

Table 1: CIE L*a*b* coordinates in ewe milks according the fat content

Coordinates	Milkfat (%, w/v)			ANOVA
	1.6	6.0	9.0	
L*	85.03±0.85^{a}	87.28±0.70^{b}	88.34±0.95^{b}	0.002
a*	-3.58±0.13^{a}	-3.54±0.20^{a}	-2.83±0.35^{b}	0.004
b*	7.10±0.24^{b}	6.96±0.37^{b}	5.48±0.73^{a}	0.003

^{a,b,c}, different letters between rows mean significant differences ($p<0.05$)

Table 2: CIEL*a*b* and ΔE* coordinates on 1% (w/v) saffron milk extracts at 37 ºC and 20 minutes of extraction time

Coordinates	Milkfat (%, w/v)			ANOVA
	1.6	6.0	9.0	
L*	57.78 ± 0.76 a	60.46 ± 0.94 b	62.86 ± 1.31 c	0.000
a*	33.97 ± 0.83 c	32.39 ± 0.45 b	30.71 ± 1.04 a	0.000
b*	71.44 ± 1.38 a	75.47 ± 0.99 b	76.70 ± 2.58 b	0.000
ΔE*	78.57 ± 1.26 a	82.43 ± 0.84 b	82.02 ± 2.15 b	0.001

^{a,b,c}, different letters between rows mean significant differences ($p<0.05$)

The average values for the color coordinates and the ANOVA studied on the saffron milk extracts are shown in Table 2. All color coordinates presented positive values in the saffron milk extracts, approaching to light red and yellow colorations. The values of the coordinates L*, b* and ΔE* were increased as milkfat was increased ($p<0.001$), while a* decreased ($p<0.000$). This means that milk with more fat makes the extracts brighter, yellower and less red. The matrix effect of ewe milk was very important on the saffron milk extracts, especially in milk with 9.0% fat. This is particularly important because as lactation period progresses, milkfat level increases especially in ewe's milk, making saffron milk extracts brighter.

Frost et al., [4] indicated a perception of a brighter cow milk sample as milkfat was increased, as well as Popov-Raljic et al., [5], who suggested that milk fat provides a positive effect on L* coordinate, whole milk being brighter than semi skim milk. Frost et al., [4] also observed that as milk fat content increases; a yellower milk color is perceived. In ewe milk, b* coordinate decreases (Table 1), while according to other authors yellow perception increases. This could be caused by chemical differences between cow and ewe milk, since cow milk contains more carotenes than ewe milk, and these are responsible for yellow color in milk [5].

Carotenes have a lipophilic structure, so milk with higher fat content would have a greater influence in b* coordinate, and so that, contributing to make the saffron extracts yellower. Studies regarding saffron coloration focus mainly on aqueous solutions. Coloration of saffron milk extracts was different compared to saffron aqueous extracts, having yellow hues, while saffron extracts in water tend to have red hues at the same concentration. This fact is explained because the coloring compounds of saffron, crocetin esters, are water soluble substances that give a reddish color to the aqueous saffron solutions [6].

4. Conclusions

Ewe milk was was found to be an appropriate matrix for saffron color extraction. It can therefore be used to produce new products. However, the stage of lactation has to be considered when preparing these solutions.

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3.7. (S3.20) Teleme White-Brined Cheese from Sheep or Goat Milk

E.C. Pappa[1], I. Kandarakis[2], G.K. Zerfiridis[3], E.M. Anifantakis[2], J.A. Robertson[4],E.N.C. Mills[4]

Summary

Teleme white-brined cheese was manufactured from sheep and goat milk. The rate of pH decrease was faster in cheeses made from goat milk. The highest fat content and yield were in cheeses made from sheep milk; the highest protein content was in cheeses made from goat milk, whereas salt content was not affected by the kind of milk. The principal free amino acids were lysine, leucine, valine, glutamic acid and phenylalanine. Goat milk resulted in harder cheeses than cheeses made from sheep milk. The main groups of compounds found in the volatile fraction of Teleme cheese were aldehydes, ketones, alcohols, fatty acids, including large quantities of 2-butanone, ethanol, 2-butanol and acetic acid. Water-soluble extracts of Teleme cheese matured 120 days were analysed for constituent peptides and proteins using proteomics. Overall, the peptide profile of Teleme cheese is typical of other cheeses and the milk source species can be resolved through proteomics.

1. Introduction

Greece is one of the largest producers of sheep and goat milk within the European Union. Much of the milk is used to produce white-brined cheeses, like Teleme. Teleme can be produced using caprine, ovine or bovine milk, either alone or any mixture of them. Product quality may be affected by milk source so methods to ensure and maintain quality, in relation to composition and sensory attributes are important.

2. Materials and Methods

Teleme cheese was manufactured using (a) sheep milk of the ‘‘Boutsiko’’ breed (b) goat milk from the indigenous goat breed of Epirus region (Pappa, Kandarakis, Anifantakis, & Zerfiridis, 2006). Chemical and texture analyses used established methods. Proteomic analyses (Pappa, Robertson, Rigby, Mellon, Kandarakis, & Mills, 2008) were conducted to profile whey proteins present, as markers for source species of the ingredient milk. Statistical analysis was applied to the data.

3. Results and Discussion

The rate of pH decrease was higher in goat (G) cheese, thus transfer of the goat cheeses to the cold room occurred sooner (day 13) than for sheep (S) cheeses (day16). Teleme sheep cheese showed higher moisture, fat-in dry-matter content and organoleptic score (Table 1) and lower protein content than goat cheese. Goat cheeses were harder, with a higher force at the point of fracture and a higher compression at the point of fracture ($P<0.05$) than sheep cheeses.

Proteolysis was more pronounced in the sheep cheese than in goat cheese ($P<0.05$) as estimated on the basis of nitrogenous fractions, electrophoretic and chromatographic methods.

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The total free amino acid content (TFAA) in goat cheese was significantly higher than in sheep cheese ($P < 0.001$), with lysine, leucine, valine, glutamic acid, phenylalanine predominant at all stages of ripening, regardless of the milk source. Volatile compounds found in abundance in each Teleme cheese type and at all stages of ripening were 2-butanone, ethanol, 2-butanol and acetic acid. In general, levels of total volatile compounds (TVCs) were higher in sheep cheese than in goat cheese.

Table 1: Characteristics of Teleme cheese made from sheep (S) or goat (G) milk

	Milk	1st-day	Cold Room	60th-day	120th-day	180th-day
pH	S	4.97	4.45	4.23	4.17a	4.25
	G	4.92	4.45	4.32	4.33b	4.35
Moisture, %	S	60.62a	56.42a	55.53a	55.78a	56.25
	G	59.67b	56.46a	56.51b	56.55b	56.64
Salt in moisture, %	S	3.22a	4.63	4.75a	4.96	5.01
	G	2.81b	4.64	5.03b	5.02	5.14
Fat in dry matter, %	S	43.2a	55.1a	55.4a	53.9a	54.9a
	G	46.6b	49.6b	48.3b	47.1b	49.9b
Proteins, %	S	15.31a	15.47a	15.24a	15.17	15.05a
	G	15.89b	15.78b	16.31b	15.17	15.65b
TFAA[1)] (mg 100g-1 dry matter)	S	234.4	242.0a	339.2a	378.9a	496.1a
	G	264.9	329.0b	391.7b	454.8b	550.9b
TVCs[2)] content (AU)	S	24.77a	---	115.51a	---	346.84a
	G	14.45b	---	72.68b	---	227.67b
Organoleptic evaluation (max 100)	S	---	---	89.8a	88.4a	85.4a
	G	---	---	86.7b	85.6b	84.1b

1) total free amino acid content (TFAA), 2) total volatile compounds (TVCs)
Means (of three trials) in each column without the same letter differ significantly

Proteomic analyses of water soluble extracts (WSE) of Teleme cheeses prepared from sheep and goat cheese matured for 120 days were analysed for constituent peptides and proteins. The WSE from sheep cheese gave a broader spectrum of peptides than that from goat cheese, related to differences in protein and peptide hydrolysis rates during ripening. The more detailed analysis, from two-dimensional (2D) gel electrophoresis (Figure 1), with matrix-assisted laser desorption ionisation-mass spectrometry (MALDI-MS), identified proteins in WSE not hydrolysed during ripening (Table 2). These were mainly the whey proteins (α-lactalbumin, β-lactoglobulin, serum albumin). The use of high performance liquid chromatography in conjunction with mass spectrometry and Edman degradation increased resolution of 'aggregates' of proteins and peptides in WSE and identified peptides which could be either α-lactalbumin or β-lactoglobulin or could originate from α-casein or β-casein. Tandem mass spectrometry leads to the profiling of small peptides (with mass range from 3000 to 1500 Da) from the water soluble extract, originating from β-casein. Peptides and proteins identified in the WSEs from sheep and goat Teleme cheese were similar and consistent with the profile produced in other cheeses.

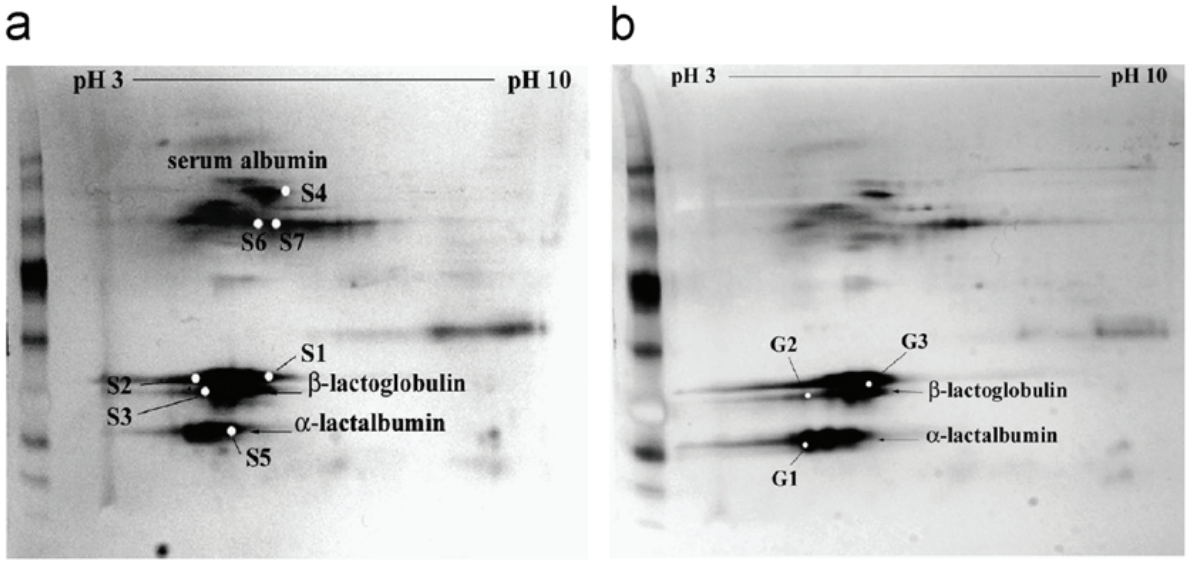

Figure 1. Two-dimensional electrophoresis of water-soluble extracts prepared from Teleme cheese manufactured from (a) sheep milk, (b) goat milk (S1-S7, G1-G3: spots selected for identification by MS)

Table 2: Identification of proteins from 2D electrophoresis gels following MALDI-MS

Spot	Protein identification (ID)	Observed molecular weight (Da)	MOWSE score[a]	ID*	Accession No[b]	Calculated molecular weight; pI
					Ovis aries	
S1	β-Lactoglobulin	20,362	99	I	P67976	18,151;5.26
S2	β-Lactoglobulin	20,362	114	I	P67976	18,151;5.26
S3	β-Lactoglobulin	20,362	149	I	P67976	18,151;5.26
S4	Serum albumin	71,244	70	U	P14639	66,328; 5.58
S5	α-Lactalbumin	14,200	84	I	P09462	14,255; 4.70
S6	Immunoglobulin gamma chain	~50k	61	U	gi 388235	52,332;5.65
S7	Immunoglobulin gamma chain	~50k	61	U	gi 388235	52,332;5.65
					Capra hircus	
G1	α-Lactalbumin	14,641	42	U	P00712	14,194; 4.92
G2	β-Lactoglobulin	20,362	112	I	P02756	18,191; 5.29
G3	β-Lactoglobulin	20,362	158	I	P02756	19,976; 5.50

* I = identified; U = uncertain. [a]MOWSE = Probability score [> 56 is significant ID].

[b] Accession No = SwissProt Acc. No., (http://expasy.org/sprot/) except S6 and S7 = Matrix Science (http://www.matrixscience.com).

4. Conclusion

High quality Teleme cheese can be produced from sheep or goat milk. Profiling of sheep and goat cheeses showed that although each differs in rate of maturation, flavour, texture and proteolysis, markers are similar in the final product. Since sheep milk is used mainly for the production of Feta cheese in Greece, then goat milk with account taken of composition differences can be used as a major ingredient for the production of Teleme cheese without compromising product quality.

Acknowledgments

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3.8. (S3.22) Changes in Lipolysis, Proteolysis and Volatile Profile of Xinotyri Cheese made from Raw Goat's Milk

Th.G. Bontinis[1], H. Mallatou[1*], E.C. Pappa[1], Th. Massouras[2], E. Alichanidis[3]

Summary

The changes in biochemical properties of Xinotyri goat's milk cheese (an artisanal variety produced in the mountains of Naxos; a Greek island of the Aegean Sea) during ripening, were investigated by determining the nitrogen fractions, caseins and their degradation products, the peptides profile, free fatty acids and volatiles profile. Values obtained for the nitrogen fractions and for caseins and their degradation products show that this cheese undergoes very little protein degradation. Both β- and α_s-caseins were slightly degraded. The free fatty acids increased considerably during ripening, resulting in a predominance of long-chain acids, followed by medium-chain acids. A total of 114 volatile compounds were detected. The most abundant volatiles isolated in Xinotyri cheese were alcohols followed by acids, ketones and esters. Ethanol, acetic acid and hexanoic acid ethyl ester were at the highest level during ripening.

1. Introduction

Xinotyri cheese is made from raw goats' milk by artisanal procedures, with no starter cultures, in Naxos island in the Aegean Sea (Cyclades complex), Greece. It is a hard cheese with an acidic and slightly salted taste, a smell of yeast, and a crumbly texture that becomes laminated and buttery in the mouth. The changes in compositional and microbiological parameters during the Xinotyri cheese ripening have been studied before (Bontinis, Mallatou, Alichanidis, Kakouri, & Samelis, 2008). The aim of this work was to study the evolution of proteolysis, lipolysis and volatile compounds during ripening.

2. Materials and Methods

Xinotyri cheese was manufactured according to the traditional method as described previously by Bontinis et al. (2008). Extraction of lipids, isolation of FFA and determination of FFA concentration was performed by gas chromatography according to De Jong and Badings (1990). The volatile profiles were studied by solid-phase microextraction (SPME) connected to GC–MS.

Proteolysis was monitored by Kjeldahl determination of the water-soluble nitrogen (WSN), the 12% TCA- soluble N (TCA-SN), the 5% PTA-soluble N (PTA-SN), urea-polyacrylamide gel electrophoresis (PAGE) of cheese proteins, and reverse phase high-performance liquid chromatography (RP-HPLC) analysis of the water – soluble extracts (WSE) of cheeses (Mallatou, Pappa and Boumba, 2004, Folkertsma and Fox, 1992).

3. Results and Discussion

The amounts of all individual FFA studied increased during ripening and storage. However, not all FFA increased by the same amounts and, as a result, the FFA composition of the cheese changed considerably over the 180 ripening d (Figure 1).

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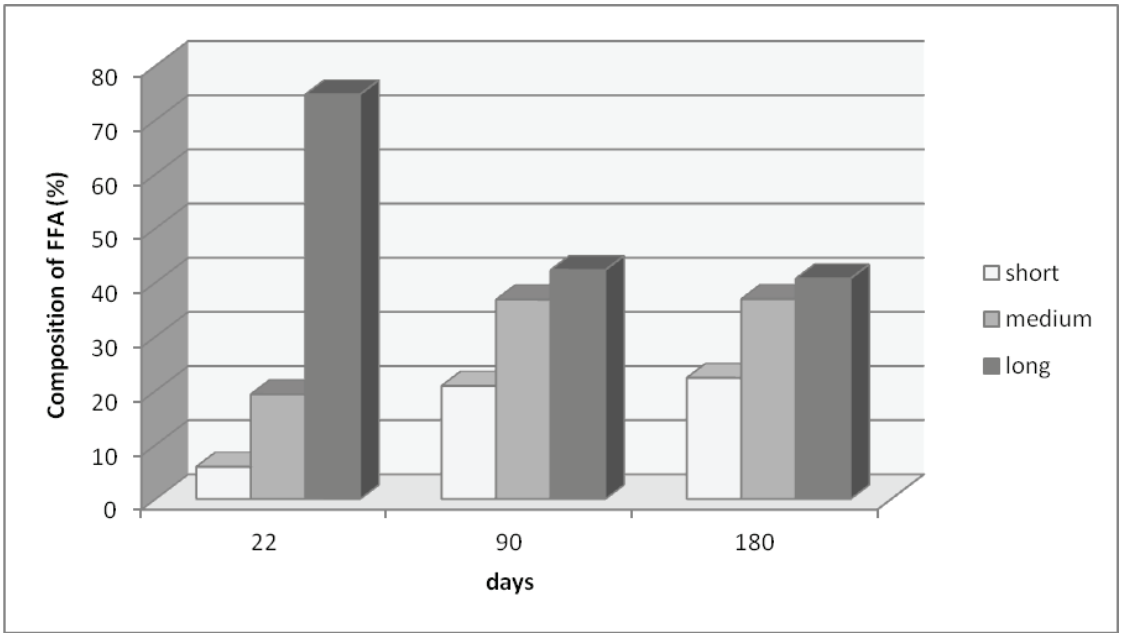

Figure 1. Percentage of short (C4-C8), medium (C10-C14) and long (C16-C18:3) chain FFA of Xinotyri cheese after 22, 90, 180 days of ripening

Xinotyri cheese underwent a very slight proteolysis during ripening; generally the WSN %TN and TCA-SN %TN contents remained stable, while the PTA-SN %TN content increased a little. Both β- and α_s1-caseins were slightly degraded until the age of 22 days and then remained stable, indicating that primary proteolysis during storage was very limited (Figure 2). RP-HPLC elution profiles of the WSE of cheese revealed that new peaks appeared while others decreased or disappeared at different ripening stages (results not shown).

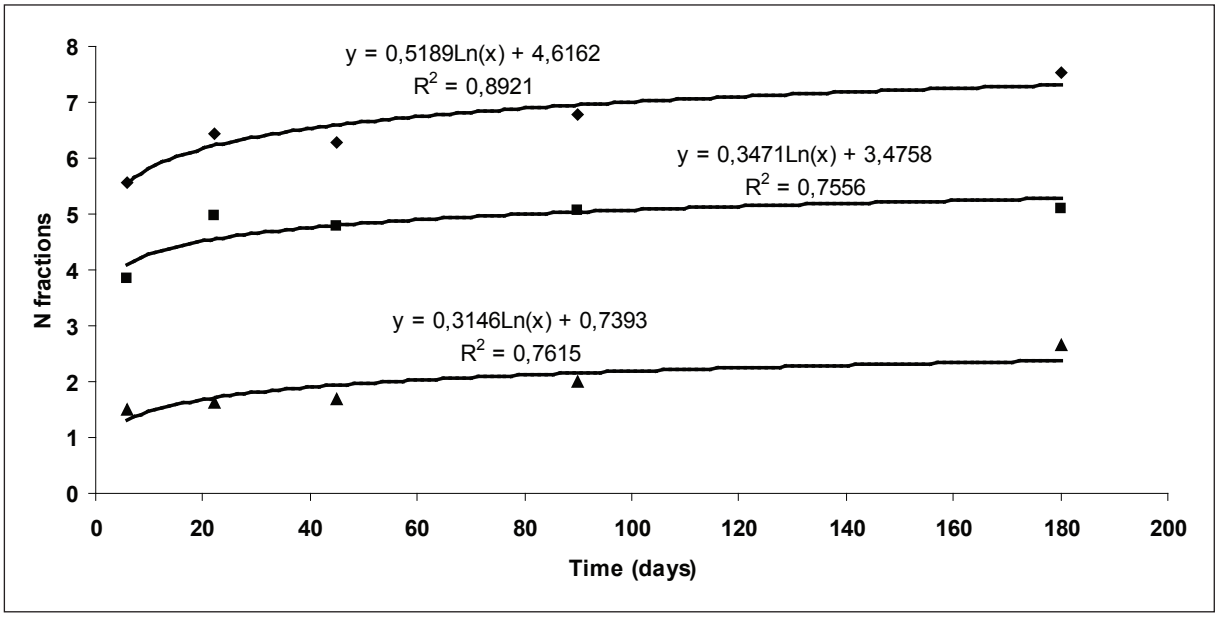

Figure 2. Changes of different parameters of proteolysis of Xinotyri cheese: WSN water-soluble nitrogen; TCA-SN trichloracetic acid- soluble nitrogen; PTA-SN phosphotungstic acid-soluble nitrogen

A total of 114 volatile compounds were detected. The most abundant group isolated at day 90 were alcohols followed by acids, ketones and esters (Figure 3).

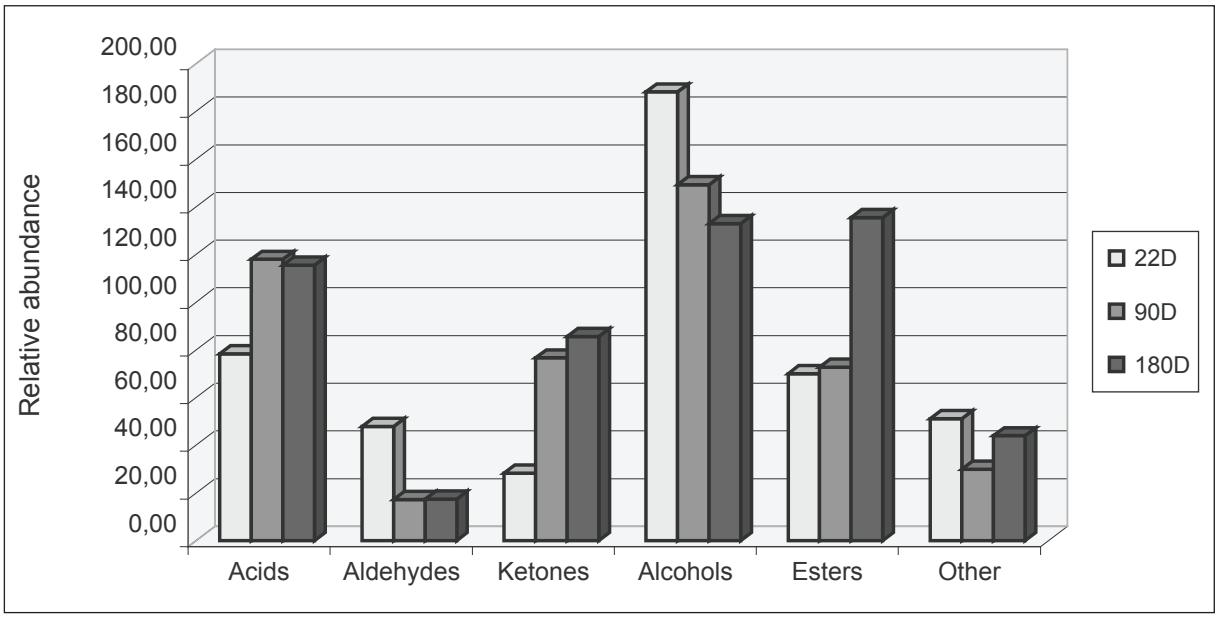

Figure 3. Evolution of volatile compounds grouped by their chemical nature during ripening

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3.9. (S3.25) Composition and Sensory Properties of Feta Cheese Made by Calf or Artisanal Kids and Lambs Rennet and Stored in Wooden Barrels or Tin Vessels

E. Kondyli[1], E. Pappa[1]

Summary

Feta cheeses ripened and stored in wooden barrels had lower moisture contents during aging, higher fat contents at 120 days and lower salt contents at 60 days of ripening than Feta cheeses stored in tins, regardless of the rennet type. Proteolysis of cheeses was affected neither by the type of rennet nor by the type of package. No differences were observed for the scores of appearance and body-texture among the cheeses.. Feta cheese made with artisanal rennet from kids and lambs abomasa, ripened and stored in wooden barrels received higher flavour and total scores than the other cheeses at 60 and 120 days of storage but at the age of 180 days no significant differences for the above attributes were observed among cheeses.

1. Introduction

Feta cheese is the most important white brined protected denomination of origin (PDO) Greek cheese much appreciated for its characteristic flavour by the consumers. The traditional technology and the new trends in Feta cheese manufacture are described by Anifantakis (1). During recent decades some convenient modifications have been applied to the cheesemaking procedure with respect to the basic traditional technology. Industrialized manufacture of Feta cheese includes milk pasteurization, addition of standardized calf rennet, which is free from lipolytic activity, and packaging in rectangular tins filled with brine. Moderate heat treatment of milk and artisanal rennet from kids and lambs abomasa which, apart from the milk clotting proteinases, also contains pregastric esterases with lipolytic activity, are usually used in traditionally made Feta (1, 2). Traditional Feta is also packaged in wooden barrels with or without addition of brine (1, 3). Nowadays there is a trend towards many dairy products obtained by traditional methods of production having greater acceptance by consumers than the industrialised products (4). Indeed traditionally made Feta is considered more tasteful than the industrially made Feta (5) by most consumers.

The aim of this study was to compare the composition, proteolysis and sensory properties of Feta cheeses made either with calf rennet or with artisanal rennet from kids and lambs abomasa and ripened and stored either in wooden barrels or in tin vessels.

2. Materials and Methods

Three cheesemaking trials were carried out in a local cheese factory (KARALIS SA, Arta, Greece) on a 50 kg scale for Feta ripened and stored in wooden barrels and a 17 kg scale for Feta ripened and stored in tin vessels, according to the procedure described by Anonymou, (3). Fresh raw ewe milk standardised to 6% fat content and pasteurised at 72° C for 15 sec, was divided into two portions. After cooling (35° C) the same normal starter culture at a rate of 0.75% and different types of rennet were added to each milk portion. Calf rennet (CR) was added in the first portion and artisanal rennet from kids and lambs abomasa (AR) in the second, in equivalent quantities so as the coagulation could be achieved in about 50 min. The two cheese curds were cut into small pieces and transferred into rectangular and cylindrical moulds. After draining the curd from rectangular moulds was put into tin vessels (T) and the curd from the cylindrical moulds into wooden barrels (W). Granular recrystallised NaCl equivalent to 2.5% of cheese weight was

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added and after one day the aqueous phase was removed and replaced by 7% NaCl solution in a ratio of brine volume to cheese weight of 1:4 for cheese in tin vessels and 1:6 for cheese in wooden barrels. The tin vessels and wooden barrels were sealed and left for ripening (16- 18° C) for about 15 days and then transferred into the storage rooms (3- 4° C) for up to six months.

The above cheesemaking procedure resulted in four cheeses, that is, Feta cheese made with calf rennet, ripened and stored in wooden barrels (CRW cheese), Feta made with calf rennet, ripened and stored in tin vessels (CRT cheese), Feta made with traditional artisanal rennet, ripened and stored in wooden barrels (ARW) and Feta made with traditional artisanal rennet, ripened and stored in tin vessels (ART cheese).

The composition and sensory evaluation of cheeses were determined according to Katsiari et al. (6). Proteolysis was evaluated according to Pappa et al. (7) .

Two ways analysis of variance (ANOVA) was used to test the influence of rennet and package type on the composition, proteolysis and sensory properties of cheeses, using the software Statgraphics (Statistical Graphics Corp. Rockville, MD, USA) statistical program.

3. Results and discussion

The moisture, fat and salt contents and pH of Feta cheeses are presented in Table 1. Moisture contents of cheeses were significantly affected by the type of package (wooden barrel or tin vessel), that is, cheeses ripened and stored in wooden barrels had lower moisture content than cheeses ripened and stored in tin vessels, regardless of the type of rennet during storage. The fat contents of cheeses were also significantly affected by the package type, that is, higher fat contents were found in Feta cheeses ripened and stored in wooden barrels than those in tin vessels regardless of the rennet type, at 20, 60 and 120 days of storage. Significantly higher values for the moisture in milk solids non fat (MSNF) were observed in cheeses ripened in tin vessels than those in wooden barrels at the age of 20, 60 and 120 days, while no significant differences were observed for the fat in dry matter (FDM) among the cheeses. The salt content was significantly higher in Feta stored in tin vessels than in wooden barrels at 20 and 60 days of ripening regardless of the rennet type. At the age of 120 and 180 days no significant differences were observed for the salt content among the cheeses.

Table 1: Composition of Feta cheese made by calf (CR) or traditional artisanal rennet from kids and lambs abomasa (AR) and ripened and stored in wooden barrels (W) or tin vessels (T)

Age of cheese (days)	Type of package	Moisture%		Fat %		MSNF		FDM		Salt%		S/M		pH	
		CR	TR	CR	TR	CR	TR	CR	TR	CR	TR	CR	TR	CR	TR
2	W	58.66^{a1}	60.34^{a2}	20.25^{a1}	19.5^{a1}	73.55^{a1}	74.96^{a2}	48.86^{a1}	49.16^{a1}	1.67^{a1}	1.41^{b1}	2.85^{a1}	2.34^{a1}	5.04^{a1}	4.94^{a1}
	T	58.66^{a1}	60.34^{a2}	20.25^{a1}	19.5^{a1}	73.55^{a1}	74.96^{a2}	48.86^{a1}	49.16^{a1}	1.67^{a1}	1.41^{a1}	2.85^{a1}	2.34^{a1}	5.04^{a1}	4.94^{a1}
20	W	55.50^{a1}	56.50^{a1}	22,42^{b1}	22.25^{b1}	71.54^{a1}	72.67^{a1}	50.38^{a1}	51.15^{a1}	1,84^{a1}	1,95^{a1}	3.31^{a1}	3.45^{a1}	4,51^{a1}	4,66^{a1}
	T	58.07^{b1}	57.60^{b1}	21.05^{a1}	21,62^{a1}	73.55^{b1}	73.49^{b1}	50.20^{a1}	50.99^{a1}	2,45^{b1}	2,63^{b1}	4.22^{b1}	4.56^{b1}	4,62^{a1}	4,59^{a1}
60	W	55,29^{a1}	56,49^{a1}	23,25^{b1}	23,00^{b1}	72.04^{a1}	73.36^{a1}	52.00^{a1}	52.86^{a1}	1,94^{a1}	2.19^{a1}	3.51^{a1}	3.88^{a1}	4,43^{a1}	4,45^{a1}
	T	57,29^{b1}	57,91^{b1}	22,42^{a1}	22,17^{a1}	73.85^{b1}	74.41^{b1}	52.49^{a1}	52.67^{a1}	2,72^{b1}	2,73^{b1}	4.75^{b1}	4.71^{b1}	4,43^{a1}	4,44^{a1}
120	W	54,59^{a1}	55.39^{a1}	23.75^{b1}	23.50^{b1}	71.59^{a1}	72.41^{a1}	52.30^{a1}	52.68^{a1}	2,58^{a1}	2,56^{a1}	4.73^{a1}	4.62^{a1}	4,41^{a1}	4,39^{a1}
	T	56,97^{b1}	57,54^{b1}	22,50^{a1}	22,50^{a1}	73.51^{b1}	74.24^{b1}	52.29^{a1}	52.99^{a1}	2,82^{a1}	2,89^{a1}	4.95^{a1}	5.02^{a1}	4,34^{a1}	4,34^{a1}
180	W	55.17^{a1}	56.70^{a1}	23.60^{a1}	23.00^{a1}	72.21^{a1}	73.63^{a1}	52.64^{a1}	53.11^{a1}	2.70^{a1}	2.77^{a1}	4.89^{a1}	4.88^{a1}	4.48^{b1}	4.45^{b1}
	T	56.91^{b1}	57.12^{b1}	22.50^{a1}	22.50^{a1}	73.41^{a1}	73.70^{a1}	52.21^{a1}	52.47^{a1}	2.83^{a1}	2.98^{a1}	4.97^{a1}	5.22^{a1}	4.38^{a1}	4.35^{a1}

^{a,b} Means in the same column at the same age with different letters differ significantly (P<0.05).
^{1,2} Means in the same row at the same age with different numbers differ significantly (P<0.05).

The rate and extent of proteolysis were monitored by measuring the levels of water soluble nitrogen (WSN), the nitrogen soluble in 12% trichloroacetic acid (TCA-SN) and in 5% phospho-tungstic acid (PTA-SN). No significant differences were observed for the above indexes among the cheeses (results not shown). It seems that proteolysis of the cheeses was affected neither by the type of packaging nor by the type of rennet.

The results of the sensory evaluation of cheeses are shown in Table 2. The appearance and body-texture of all cheeses were very good at all sampling ages with no significant differences among the cheeses. The rennet and the package type significantly affected the flavour scores of the cheeses Table (2). Feta cheese made with artisanal rennet and ripened and stored in wooden barrels received the highest scores for flavour among cheeses, at 60 and 120 days of storage.

Table 2: Sensory evaluation of Feta cheese made by calf (CR) or traditional artisanal rennet (AR) and ripened and stored in wooden barrels (W) or tin vessels (T)

Age of cheese (days)	Type of packaging	Appearance (10)*		Body-texture (40)		Flavour (50)		Total (100)	
		CR	AR	CR	AR	CR	AR	CR	AR
60	W	8.9	9.2	35.3	35.6	42.7a2	45.0^{b1}	86.9^{a2}	89.8^{b1}
	T	9.1	9.1	35.2	34.8	44.3b2	42.3^{a1}	88.6^{b2}	86.2^{a1}
120	W	8.9	9.1	35.2	35.8	42.8a2	46.0^{b1}	86.5^{a2}	90.3^{b1}
	T	8.9	9.0	35.4	34.7	44.5b2	42.3^{a1}	88.9^{b2}	86.0^{a1}
180	W	8.7	8.8	35.3	35.3	42.9	40.6	87.1	84.7
	T	9.0	9.0	35.5	35.8	43.4	42.5	87.9	87.3

[a,b] Means in the same column at the same age without a letter or bearing a common letter did not differ significantly ($P>0.05$).
[1,2] Means in the same row at the same age without a letter or bearing a common letter did not differ significantly ($P>0.05$).
* Numbers in parentheses are the maximum attainable scores.

4. Conclusions

The package type significantly affected the moisture content of cheeses during storage, the fat content at the age of 120 days and the salt content at the age of 60 days. Proteolysis of cheeses was affected neither by the type of rennet nor by the type of packaging material. Also no significant differences were observed in the appearance and body-texture scores among the cheese during aging. The flavour scores of cheeses were significantly affected by the type of rennet and the type of package and Feta cheese made with artisanal rennet, ripened and stored in wooden barrels received higher flavour and total scores than the other cheeses at 60 and 120 days of storage.

Acknowledgments

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3.10. (S3.29) Mattonella Cheese: Production Process and Microbiological Quality

V. Mariano[1], S. Gradassi[1], P. De Santis[2], E. Vergari[1], A. Nardi[1]

Summary

The objective of this study was to follow the production process and the bacteriological condition and to characterize the lactic flora of Mattonella cheese, a typical small scale dairy product of the Italian Maremma territory, made of goat milk. Five batches were followed and sampled along the production process. No pathogenic bacteria such as *Listeria* spp. and *Salmonella* spp. were isolated. *Staphylococcus* spp. were present in low concentrations (max= 8,2 x10^3 cfu.g^{-1}) during the first phases of cheese processing and decreased close to the minimum detectable limit (<1x10^2 cfu.g^{-1}) during the transformation process. Sulfite-reducting clostridia were isolated throughout the batches examined. Sample trends for mesophilic lactococci and lactobacilli, thermophilic lactococci and total mesophilic count were examined throughout the processing phases. *Lactococcus* spp. flora, when typified by PCR and DGGE, were identified as *Lactobacillus paracasei paracasei*.

1. Introduction

The purpose of Reg. CE 509/2006 and 510/2006 is to guarantee and enhance traditional productions. Goat milk production in Italy is high with 48520 tonnes/year [5] and destined mainly to traditional dairy industry. To assure the authenticity of DOP, IGP and STG productions it is necessary to distinguish these products. Dairy products are characterized from typical lactic flora naturally present in the primary product and/or in the production environment [2]. The aim of this preliminary study was to follow the production process and the bacteriological conditions of a typical small scale Maremma dairy products, such as Mattonella cheese, in order to identify the characteristics and safety of the traditional process. Mattonella is a rectangular, surface mould goat milk cheese obtained through the addition of *Penicillium candidum*.

2. Materials and Methods

Five batches of Mattonella cheese were sampled together with environmental samples and followed along the production process. Samples were taken from curd, from the product into the moulds (T0), after the transfer into the ripening room (T1= 1st day), at the middle (T2= 7th day) and at the end of the ripening period (T3=15th day). Two batches of Mattonella were also sampled after the drying period at T4 (35th day) and T5 (60th day). On each sample the hygiene of the production process and the bacteriological quality was tested for: *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, sulfite-reducting clostridia and total bacterial count. To characterize the product, cheese was examined for: *Lactobacillus* spp., thermophilic and mesophilic lactococci. Environmental samples from the working table, ripening rooms and the working tools utilized (containers, moulds, agitator) were taken along the productive process for pathogens bacteria. *Lactococcus* spp. flora was also characterized by PCR and DGGE reaction.

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3. Results

The productive processes of Mattonella utilizes pasteurized goat milk, which, together with lactic starter culture, salt and rennet, is heated at 30°C. The cutting of the curd is made using traditional manual method. Once transferred into the moulds, it is then stored in the ripening room for two months at a temperature around 10°-12°C. No pathogenic bacteria such as *Listeria* spp. and *Salmonella* spp. were isolated. Curd examination was negative for sulfite-reducting clostridia and *Staphylococcus aureus*. *Staphylococcus aureus* were isolated in two batches of cheese, but they were detected in low concentration (max = $8,2x10^3$ cfu.g^{-1}) during the first steps of cheese processing and they decreased along the transformation process, reaching a level close to the minimum detectable limit at the last sampling (<$1x10^2$ cfu.g^{-1}). Sulfite-reducting clostridia were isolated throughout the batches of cheese examined without showing any particular trend during the production process. Curd lactobacilli and lactococci count are reported in Table1. The average trend for each processing phase of cheese samples for mesophilic bacteria is reported in Figure 1 while the one for thermophilic bacteria is reported in Figure 2. *Lactococcus* spp. flora, typified by PCR, were identified as Lactobacillus paracasei paracasei.

Table 1: Curd lactococci, lactobacilli and mesophilic count per batch

Batch	Thermophilic lactococci (cfu/g)	Mesophilic lactococci (cfu/g)	Mesophilic lactobacilli (cfu/g)	Total mesophilic count (cfu/g)
1	<$1,0x10^4$	$3,1x10^8$	$1,5x10^8$	$1,7x10^7$
2	$5,2x10^2$	$3,1x10^7$	$2,2x10^7$	$3,2x10^7$
3	$6,9x10^2$	$1,0x10^7$	$2,1x10^7$	$3,0x10^7$
4	$8,3x10^3$	$1,3x10^8$	$4,0x10^7$	$5,0x10^7$
5	$1,5x10^3$	$3,1x10^{10}$	$3,0x10^{10}$	$5,5x10^{10}$

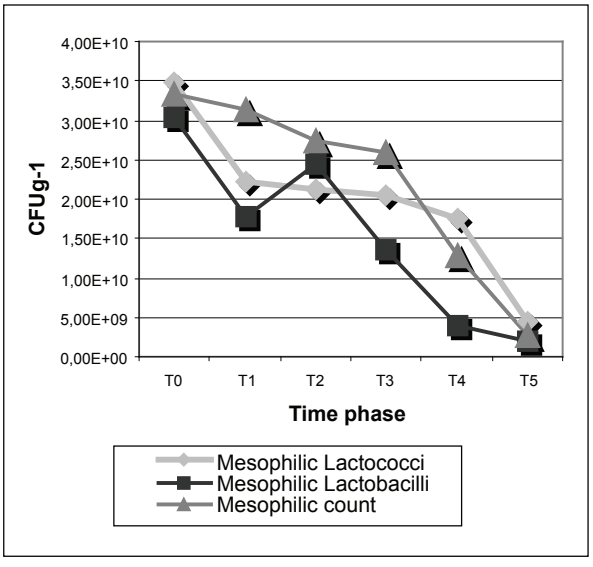

Figure 1. Average trend of Mattonella mesophilic bacteria count for processing phase

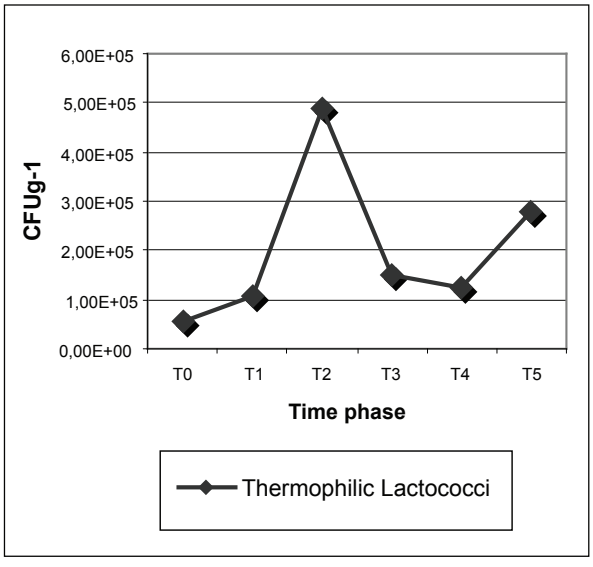

Figure 2. Average trend of Mattonella thermophilic lactococci for processing phase

4. Discussion and Conclusions

The study allowed to follow and describe the productive process of Mattonella cheese, a small scale traditional dairy products. No pathogenic bacteria such as *Listeria* spp. and *Salmonella* spp. were isolated in the cheese, nor in their environment, revealing high hygiene levels in the processing procedures. The presence and inconstant trend of sulphite-reducting clostridia, which did not cause any organoleptic alteration of the products, requires further study in order to better understand the conditions of the production process. *Staphylococcus aureus*, isolated in two batches of Mattonella cheese was detected in low concentrations (max = $8{,}2x10^3$ cfu.g^{-1} for Mattonella) during the first steps of cheese processing; moreover they decreased during the transformation process, reaching a level close to the minimum detectable limit at the final sampling (<$1x10^2$ cfu.g^{-1}) for Mattonella and $5.9x10^2$ cfu.g^{-1}. Thus the antagonist activity of lactic flora seem to be enough to ensure a good microbiological quality of the products at the end of the process, since the level of coagulase positive staphylococci is lower than the limit imposed by Council Regulation (EC) No 2073/2005. Previous studies have demonstrated the bacteriocin activity of *Lactobacillus paracasei* subsp. paracasei [1, 3]. Mesophilic lactobacilli, as well as thermophilic and mesophilic lactococci, in Mattonella cheese maintain high concentrations, respectively around 10^{10},10^5 and 10^{10} for all the sampling period. The high concentration of this bacteria is fundamental for the acidification of the product, which in turn contributes to the demineralization of casein, and therefore to good cheese processing. Furthermore, mesophilic lactococci and lactobacilli seem to have a proteolytic activity, which contributes to the organoleptic characteristics of the product [4]. These data collected will help to provide a basis for risk assessment that evaluate the microbiological safety of traditional and farmstead cheeses in Grosseto.

Aknowledgments

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3.11. (S3.30) Raviggiolo Cheese: Production Process and Microbiological Quality

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Abstract

The aim of this study was to follow the production process and the the bacteriological conditions and to characterize a typical small scale Maremma dairy product, such as Raviggiolo cheese which is a soft cheese with a short shelf life (4 days). Five batches of each product were followed and sampled during the production process. No pathogenic bacteria such as *Listeria* spp. and *Salmonella* spp. were isolated. *Staphylococcus* spp. were present in low concentrations (max=4 $x10^3$ cfu g^{-1}) during the first phases of cheese processing and decreased close to the minimum detectable limit ($5,9x10^2$ cfu g^{-1}) during the production process. Sulfite-reductive clostridia were isolated throughout the batches examined. Sample trends for mesophilic lactococci and lactobacilli, thermophilic lactococci and total mesophilic count were examined throughout the processing phases. *Lactococcus* spp. flora, typified by PCR and DGGE, resulted to be constituted of *Lactococcus lactis* and *L. delbrueckii* subsp. *bulgaricus*.

1. Introduction

The objective of the European food safety policy is to ensure a high level of human health protection while also taking into account territorial diversity such as traditional food. The objectives of Council Regulation (EC) No 509 and 510 of 26 March 2006 are the enhancement and guarantee of traditional productions [2] In order to recognize traditional cultures and their products, there are three different kinds of EU warranty labels in Italy: DOP, IGP and STG. Italian traditional dairy products are exported all over the world. Italy is the largest EU exporter of cheese, exporting 1.116.397 tonnes/year - 2009 data [3]. There are studies concerning cheese quality of the best known cheeses but there are none on most of the small scale products produced and consumed only in some particular territories, sometimes because of their short shelf-life, sometimes because no marketing strategies are implemented by small scale dairies. Italy is the third European country for ovine milk production, producing about 580.000 tonnes of ovine milk per year [3], mainly produced by semi-extensive farms [1]. Tuscany is the 4th Italian Region for ovine milk production [6] and 50% of its ovine farms are located in Central Maremma. This area is the homeland of about 227.000 sheep, collected in 1383 farms [1], mostly devoted to the production of ovine milk. Thus, many of the dairy products made with ovine milk are known only locally and their characteristics have not been investigated yet. The aim of this study was to follow the production process, the bacteriological conditions and to characterize a typical small scale Maremma dairy product, such as Raviggiolo cheese, which is a soft cheese with a short shelf life (4 days) obtained from breaking the curd of ovine milk, typically served on fern leaves.

2. Material and Methods

Five batches of the product have been followed along the production process, noting environmental conditions, timing and temperature. Samples were taken from milk, from the product into the moulds (T0), twice during the shelf life of the product (T1=3rd day; T2=5th day) and some days after the shelf life (T3=8th day). Each sample was tested for total bacteria count,

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Salmonella spp., *Listeria monocytogenes*, *Staphylococcus aureus*, sulfite-reducting *Clostridium* spp., *Lactobacillus* spp., thermophilic and mesophilic lactococci. Environmental samples from the working surface, refrigerator and the working tools (containers, moulds, agitator) were taken during the production process for pathogenic bacteria. *Lactococcus* spp. flora was also characterized by PCR-DGGE methods.

3. Results

The production processes of Raviggiolo utilizes ovine raw milk to which is added a mesophilic starter culture, salt and rennet. It is heated at 15°-20°C for the curd formation. The soft cheese obtained from the curd breaking is transferred into the moulds and then stored in the refrigerator at 4°C. No pathogenic bacteria such as *Listeria* spp. and *Salmonella* spp. were isolated in either cheese, nor in their environment. Raviggiolo milk lactic flora results are summarized in Table 1. The average trend for each processing phase of cheese samples for thermophilic lactococci, mesophilic lactococci and lactobacilli is reported in Figure 1, while the trend for total mesophilic bacteria count is reported in Figure 2. *Staphylococcus aureus* were isolated all over the batches, but they were detected in low concentrations (max= $4{,}3x10^3$ UFC g^{-1}) during the first phases of cheese processing. They decreased even more during the production process, reaching a level close to the minimum detectable limit at the final sampling $5{,}9x10^2$ UFC g^{-1} (T3). Sulfite-reducting clostridia were isolated throughout the batches of both cheeses examined without showing any particular trend during the production process. Lactic flora, typified by PCR-DGGE, resulted to be constituted of *L. lactis* and *L. delbrueckii* subsp. *bulgaricus*.

Table 1: Raviggiolo milk lactic flora of the batches examined

Batch	Thermophilic lactococci cfu/g	Mesophilic lactococci cfu/g	Mesophilic lactobacilli cfu/g	Total mesophilic count cfu/g
1	$<1{,}0x10^2$	$9{,}0x10^4$	$1{,}8x10^3$	$1{,}0x10^6$
2	*	*	*	*
3	$9{,}7x10^1$	$1{,}7x10^4$	$8{,}8x10^2$	*
4	$<1{,}0x10^2$	$3{,}0x10^2$	$1{,}2x10^3$	*
5	$5{,}5x10^2$	$<1{,}0x10^2$	$3{,}4x10^2$	$7{,}5x10^5$

*Could not be examined

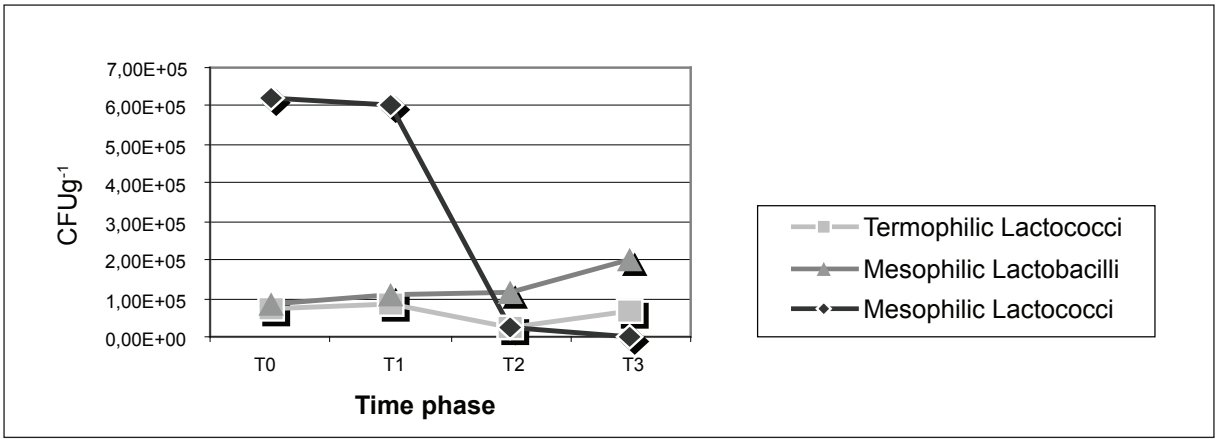

Figure 1. Average trend of Raviggiolo mesophilic lactobacilli, thermophilic and mesophilic lactococci count for each processing phase

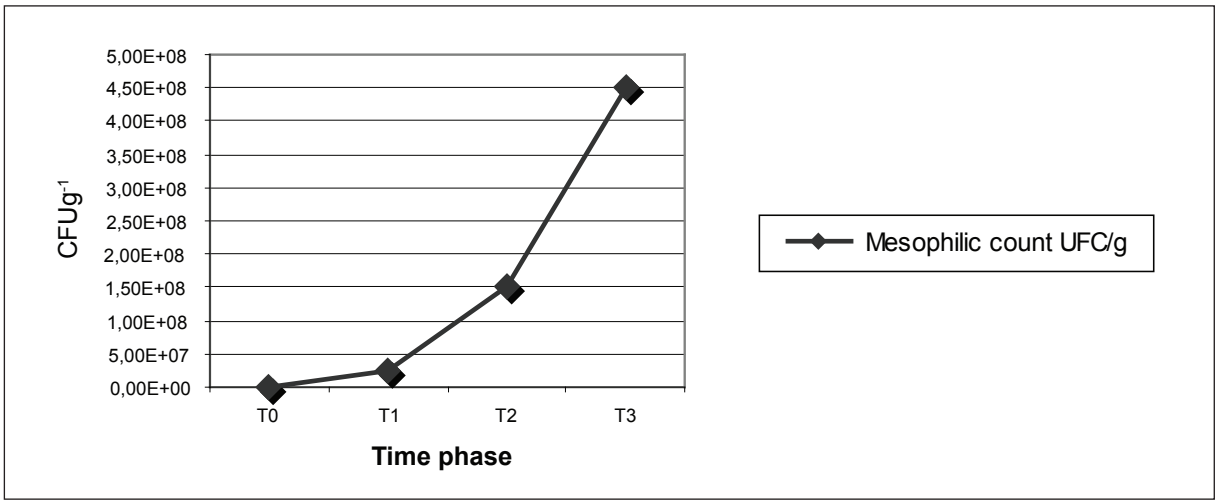

Figure 2. Average trend of Raviggiolo total mesophilic count for each processing phase

4. Discussion and conclusions

No pathogenic bacteria such as *Listeria* spp. and *Salmonella* spp. were isolated in cheese, nor in their environment, revealing high hygiene levels in the processing procedures. The presence and variable trend of sulphite-reducting clostridia, which did not cause any organoleptic change in the products, require further study in order to better understand the conditions of the production process. *Staphylococcus aureus* was found in all the Raviggiolo batches but in low concentrations (max= $4{,}3x10^3$ cfu g^{-1}) during the first steps of cheese processing; moreover they decreased during the production process, reaching a level close to the minimum detectable limit at the final sampling $5{,}9x10^2$ cfu g^{-1} (T3). The use of raw milk for the production of Raviggiolo provides a good chance for contamination by pathogens and survival throughout manufacture and storage as well. Nevertheless, the antagonistic activity of the lactic flora seems to be enough to ensure good microbiological quality of the products at the end of the process, since the level of *coagulase positive staphylococci* is lower than the limit imposed by Council Regulation (EC) No 2073/2005. Previous studies have demonstrated the bacteriocin activity of *Lactococcus lactis* [5] and Lactococcus delbrueckii [4]. Raviggiolo cheese revealed a high concentration of mesophilic lactobacilli, as well as thermophilic and mesophilic lactococci, respectively around 10^4,10^4 and 10^5 until the 4^{th} day. After this time a decrease is noted in mesophilic lactobacilli, as well as thermophilic and mesophilic lactococci, along with a relevant increase in the total mesophilic count; a four day shelf life of the product is therefore to be considered as appropriate. The data collected will help risk assessment that evaluates the microbiological safety of traditional and farmstead cheeses in Grosseto.

Aknowledgments

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3.12. (S3.31) Influence of Salting Method on The Mineral Content of Ovine Halloumi Cheese

F. Karali[1], E. Moschopoulou[1], S. Kaminarides[1]

Summary

Halloumi cheese was dry-salted or salted in brine under various conditions. The results obtained showed that the sodium content increased with increasing brine concentration and brining time, while the rate of increase was affected by the brining temperature. The concentrations of calcium, magnesium, phosphorus and, in particular, potassium in the cheese were also affected by the method of salting. Cheeses that were dry-salted exhibited higher levels of calcium, phosphorus, magnesium and potassium than cheeses salted in brine. In addition, the concentrations of calcium, phosphorus, magnesium and potassium decreased, as brining time increased.

1. Introduction

The salt content of a cheese depends on several parameters, such as the method of salting (e.g. dry or brine salting), the temperature, the cheese type and size etc., and affects not only the flavour but also most of the biochemical characteristics of the cheese. The aim of this study was to evaluate the effect of various methods of salting on the sodium, potassium, calcium, magnesium and phosphorus content of ovine Halloumi cheese.

2. Material and methods

Halloumi cheese was manufactured according to the technology described by Anifantakis and Kaminarides [1] and was salted in 2 ways: a) by dry-salting or b) in brine at three concentrations (7%, 10% and 13% NaCl) and at two temperatures (4°C and 20°C) for 3, 6, 24 and 48 hours. The dry-salted cheeses were analyzed after 24 h, while the control cheese was left unsalted. The salt content was determined by a potentiometric titration method [2], the Ca, Mg, K and Na contents were determined by Atomic Absorption Spectrometry (AAS) method [3] and the P content was determined by a spectrometric method [4].

3. Results

The sodium content of cheese salted in brine increased with the brine concentration and the brining time, while the rate of increase was affected mainly by the brining temperature (Figure 1a,b).

In contrast, the increase of the duration of brining caused a reduction in the concentrations of calcium, phosphorus, magnesium and potassium (Figure 2).

A comparison of the results obtained from the different methods of salting for 24 h, showed that the sodium content was lower in dry salted cheeses than in cheeses salted in brine (Figure 3a), whereas the reverse was observed for potassium (Figure 3b), calcium (Figure 3c), phosphorus (Figure 3d) and magnesium (Figure 3e).

4. Conclusion

From the obtained results it is clear that the method and conditions of salting significantly influenced the mineral content of ovine Halloumi cheese.

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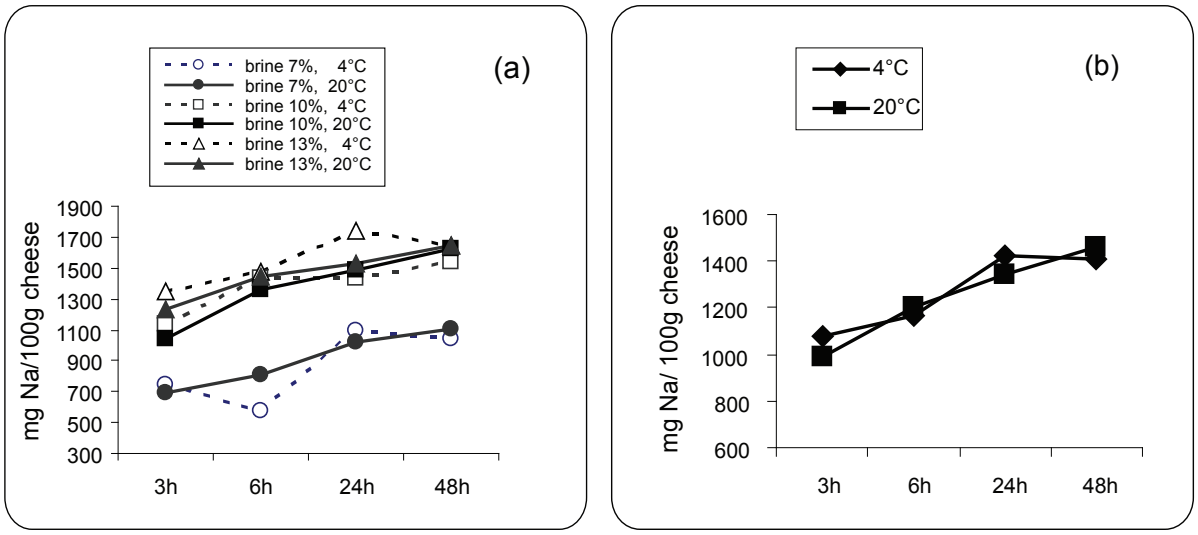

Figure 1. Influence of brine concentration and temperature (a) and time and temperature during brining (b) on the sodium content of Halloumi cheese

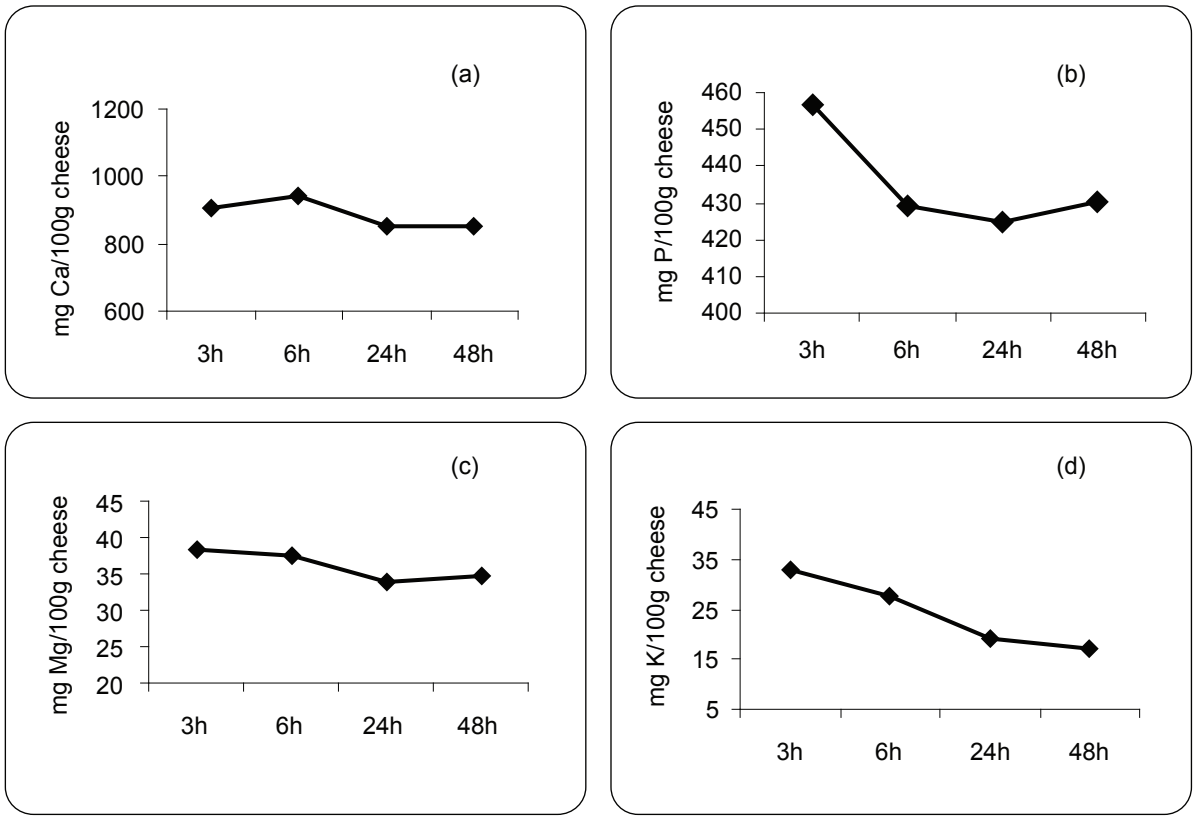

Figure 2. Influence of the duration of brining on calcium (a), phosphorus (b), magnesium (c) and potassium (d) contents of Halloumi cheese

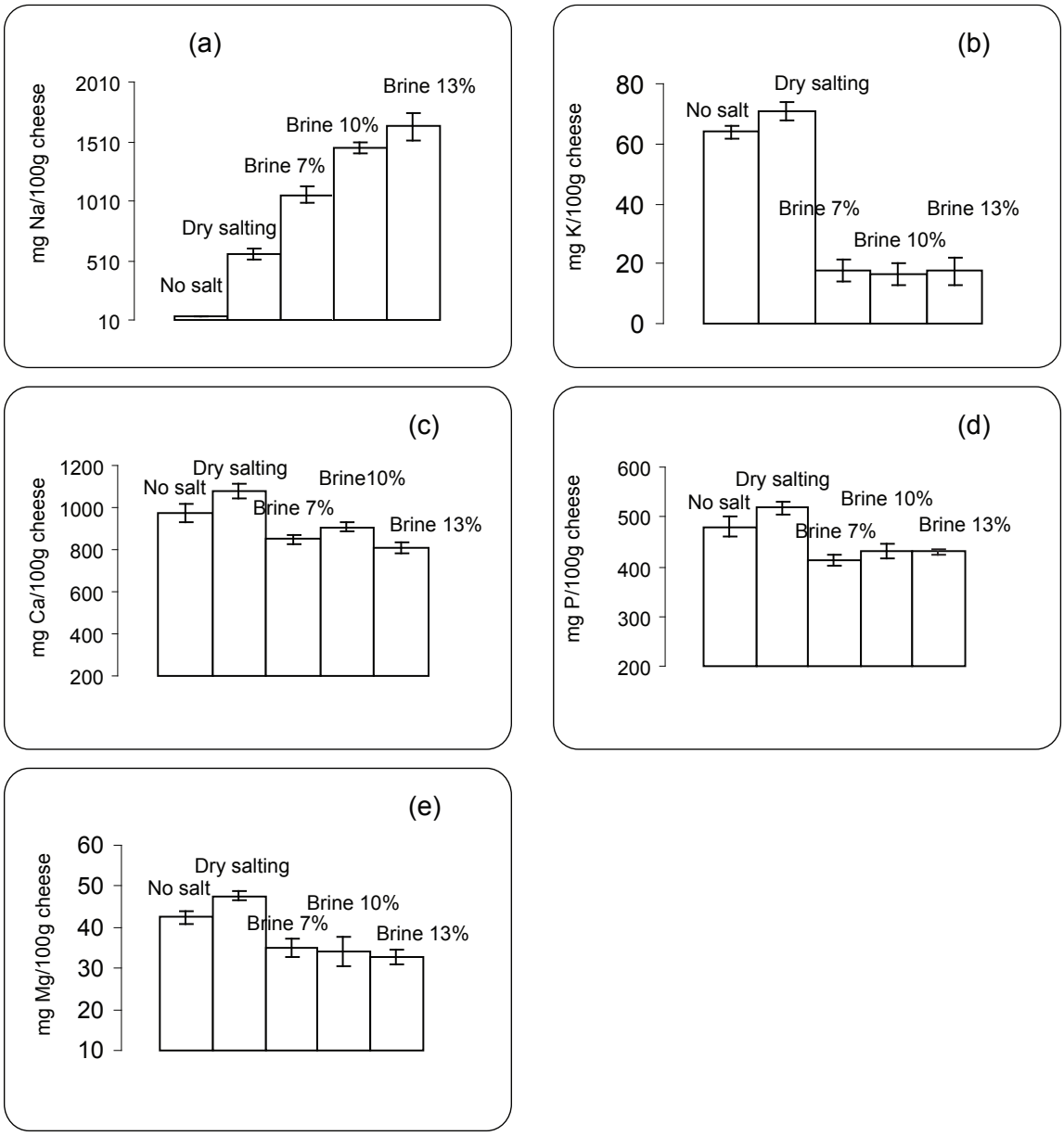

Figure 3. The effect of dry and 7-13% brine salting for 24 h on the sodium (a), potassium (b), calcium (c), phosphorus (d) and magnesium (e) content of Halloumi cheese

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3.13. (S3.34) Characteristics of Sjenica Artisanal Goat Brined Cheeses during Ripening

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Summary

Traditional cheeses from raw goat's milk have a long tradition of production in some regions of Serbia, such as Sjenica mountain region. The aim of this study was to investigate the ripening process of traditionally produced brined goat cheeses, which could allow standardization of production as well as potential preservation of the Serbian dairy heritage.

The brined cheeses, according to fat in dry matter and moisture in non fat solids (MNFS), belong to full fat and soft cheese category. Autochthonous microflora of lactic acid bacteria was composed of enterococci, thermophilic and mesophilic lactobacilli as well as lactococci. Complexity of the autochthonous microflora as well as high level of proteolysis contributed to the formation of specific sensory properties of cheese. Cheeses, at the end of ripening, can be characterized by a mild, aromatic taste as well as a smooth structure.

1. Introduction

White brined cheeses are the most widely produced and consumed cheeses in Serbia (Dozet et al., 2006). *Sjenica* region is one of the most popular parts of Serbia for the cheese production, especially of these kinds of cheeses. They are usually produced from cow`s milk (Jovanović et al., 2006), but they are also made from ewe`s and goat's milk. Production of goat milk as well as goat milk cheeses is still at the low level compared to the production of cow's milk products but in recent years it has shown a constant increasing trend. Goat milk brined cheeses are usually made in small scale dairy plants and households according to the traditional method. Compared to research about the white brined cheeses made from cow's milk, investigations of composition and properties of those from goat's milk are still very rare. The aim of this study was to investigate the ripening process traditionally produced brined goat cheeses which could allow standardization of the production procedure as well as potential preservation of the Serbian dairy heritage.

2. Material and methods

The white brined cheeses were made from fresh goat`s milk which was heated at 32°C. Then calf rennet was added and the coagulation took place in 45 min. Once curdling was completed, the coagulum was cut into small pieces (2–5 cm) and stirred three times for 5 min. in the course of 30 min. The cheese mass was carefully transferred from cheese vats into the mold with cheese cloths. After about 1h of draining (without pressing), the pressure was applied (max. 3kg.cm^{-1}) for 3h. Then, the cheese curds were cut into pieces of 10x10x7cm with knife. The curd blocks were dry salted with 2.4% NaCl. The ripening was in brine (8%) at 120C during 60 days of ripening. The cheeses were sampled and analyzed after 1, 7, 21, 35 and 60 days of ripening.

Cheese samples were analyzed in duplicate for dry matter (DM), fat (MF), total protein (TP) and salt content (NaCl) by standard methods.

Proteolysis was also studied by means of water-soluble nitrogen (WSN) according to the method of Kuchroo and Fox, 1982, and phosphotungstic acid (5%) soluble nitrogen (PTAN),

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according to (Stadhouders, 1960), both expressed as a percentage of the total nitrogen matter (WSN/TN and PTAN/TN). Autochthonous microflora of lactic acid bacteria was determined on appropriate selective media as follows: lactococci on M17agar, aerobic conditions at 30 and 44°C/48h; lactobacilli on MRS agar, anaerobic Gas Pack (BBL, Germany), at 30 and 44°C/48h; Citrate +: on LD agar+ 10g/l Ca citrate, aerobic conditions at 300C; Enterococci: SLANETZ and BARTLEY (Merck, Germany), aerobic conditions at 37°C.

SDS PAG electrophoresis of cheese samples was performed according to Laemmli (1970) method,using a vertical slab unit TV200YK (Consort, Belgium) with 100x200x1mm slabs, Tris-glycine electrode buffer, constant current of 80 mA, a max. voltage of 300V for 4 h, with 4% stacking gel (pH 6.8), and 12% separating gel (pH 8.9).

Data were analysed using STATISTICA 6.0 (StatSoft, USA) data analysis software. LSD test was used to determine differences among cheeses at a 0.05 statistical level.

3. Results and discussion

The brined cheeses, according to fat in dry matter and moisture in fat free content, belong to full fat and soft cheese category (Table 1). Compositional parameters of cheeses were slightly changed especially at the beginning of ripening. pH values of cheeses were decreased from 5.40, immediately after production, to the 4.65 that corresponds to pH of brined cheese group (data not shown).

Table 1: Composition of Sjenica goat brined cheeses

Days of ripening	Dry Matter (%)	Milk Fat (%)	Fat in Dry Matter (%)	Total Protein (%)	Moisture in Non Fat Solids (%)
1	40.57±0.38^{a}	24.50±0.50^{a}	60.38±0.68^{a}	10.95±0.19^{a}	78.71±0.08^{a}
7	41.49±0.35^{b}	22.17±0.29^{b}	53.43±1.05^{b}	11.61±0.23^{b}	75.17±0.67^{b}
21	45.76±0.32^{c}	25.17±0.29^{c}	55.00±0.83^{c}	12.93±0.31^{c}	72.48±0.57^{c}
35	45.93±0.14^{c}	26.17±0.29^{d}	56.97±0.60^{d}	13.48±0.24^{d}	73.23±0.29^{c}
60	45.40±0.22^{c}	25.67±0.29^{d}	56.54±0.37^{d}	13.12±0.10^{d}	73.46±0.05^{c}

*Means in each column with the same letter did not differ significantly (P > 0.05)

Autochthonous microflora of lactic acid bacteria was composed of enterococci, termophilic and mesophilic lactobacilli as well as cocci (Fig 1.). All of those microorganisms were present in high numbers (10^{7} - 10^{9} cfu/g) during ripening. Complexity of the autochthonous microflora contributed to the formation of specific and unique sensory properties of cheese.

The most important biochemical change during ripening of the brined cheeses was the extent of proteolysis. The water-soluble and PTA-soluble nitrogen to total nitrogen ratio increased significantly during ripening from 10 to 16% and 0.45 to 2.25%, respectively (Fig. 2a). From the evolution of the different nitrogen fractions (Fig. 2b), it is possible to conclude that Sjenica goat brined cheese do not undergoes intense proteolysis.

Cheeses, at the end of ripening, can be characterized by a mild, aromatic taste as well as a by smooth structure.

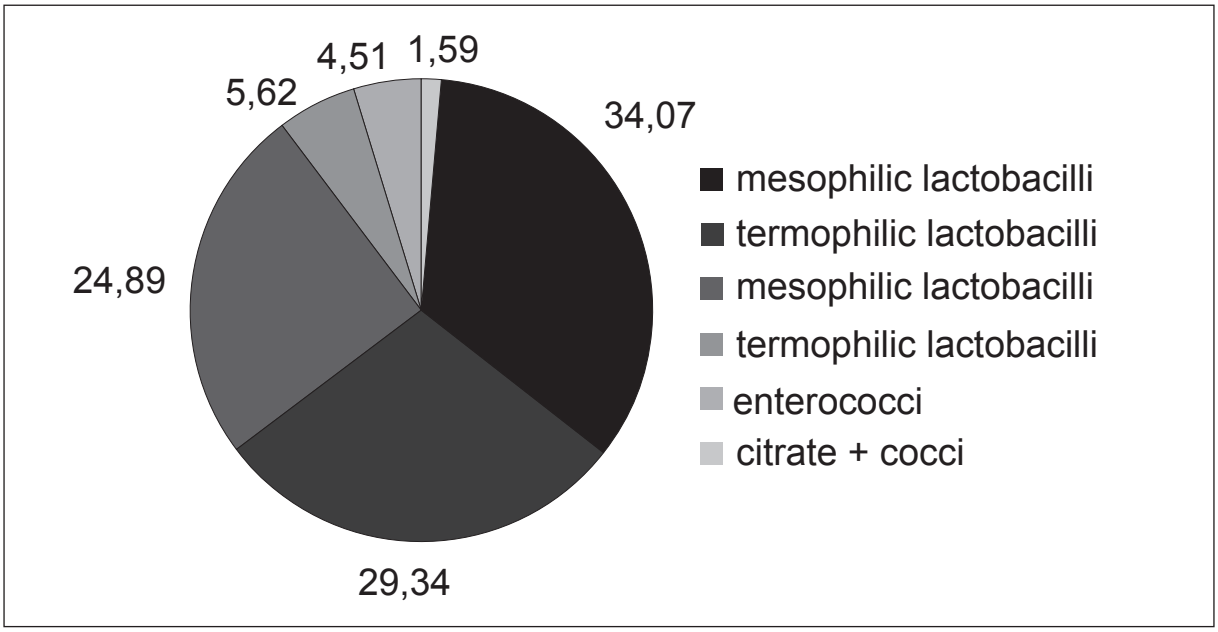

Figure 1. Microflora of Sjenica goat brined cheeses

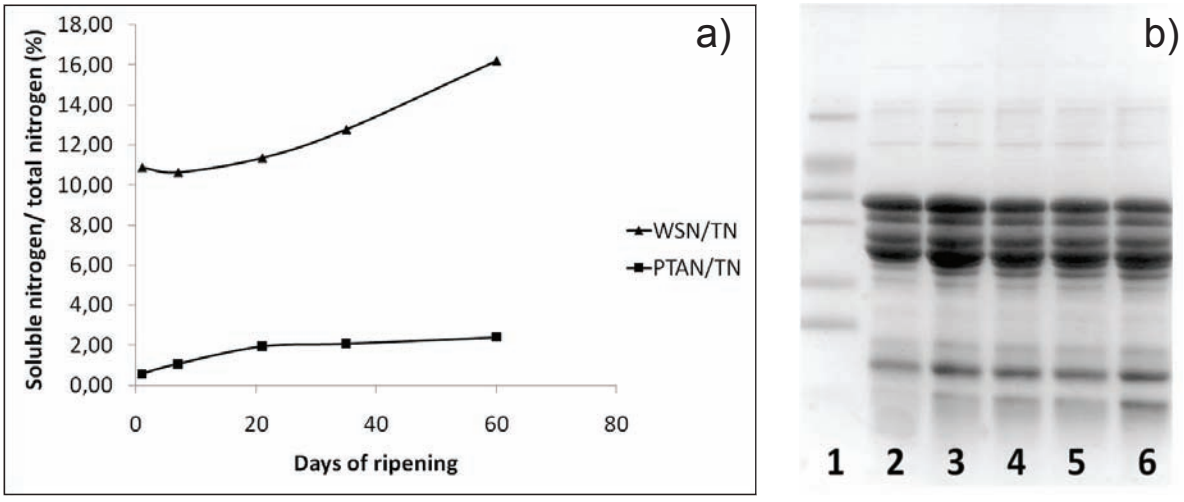

Figure 2. a) The changes of WSN/TN (water soluble nitrogen/total nitrogen) and PTAN (phosphotungstic acid-soluble nitrogen) of Sjenica goat brined cheese during ripening; **b)** SDS gel electrophoretograms of caseins and their degradation products of cheese during 60 days (1-standard molecular weight, 2-6 lines: cheese after 1, 7, 21, 35 and 60 days of ripening)

4. Conclusion

Standardization of production procedures and the potential protection of designation origin may contribute to the recognition of these as a brand in the Sjenica region as well as in all Serbia.

Acknowledgment

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3.14. (S3.35) Effect of Commercial and Potential Probiotics on The Characteristics of Soft Goat Cheeses

Z. Radulovic[1], J. Miocinovic[1], M. Petrusic[1], N. Mirkovic[1], D. Paunovic[1], T. Petrovic[1]

Summary

The aim of this study was to investigate the survival of commercial and potential probiotic bacteria and their effect on the chemical composition and sensory quality of soft goat cheeses. Three variants of soft goat cheeses were produced: 1. control cheese (starter CHN 11; Chr. Hansen, Denmark); 2. starter + autochthonous potential probiotic *Lactobacillus plantarum* 564; 3. starter + commercial probiotic Lactobacillus acidophilus LA-5 (Chr. Hansen, Denmark). The survival of potential and commercial probiotic bacteria, chemical composition, pH values and sensory evaluation were examined during storage. *Lb. plantarum* 564 and *Lb. acidophilus* LA-5 counts were at the level of $>10^7$ cfu.g^{-1}. The chemical composition and pH values of cheeses with probiotic bacteria did not differ significantly from the control variant. High viability of probiotic bacteria and acceptable cheese sensory properties indicate that these probiotic bacteria can be successfully used in the production of soft goat cheeses.

1. Introduction

Probiotic bacteria are living microorganisms which have a beneficial effect on human health. Effects on human health include an enhanced immune response, reduction of serum cholesterol, vitamin synthesis, anticarcinogenic activity and antibacterial activity [1]. The use of goat's milk in combination with probiotic bacterial strains in cheese production represents one of the technology options for manufacturing new functional dairy products. Considering that the cheeses has been shown as a very good carrier product for delivering probiotic bacteria, it is important to conduct more studies of using potential probiotic bacteria in soft cheeses made from goat milk and their effect on final product characteristics of the cheeses. The objective of this study was to determine viability and survival of potential and commercial probiotic bacteria in soft cheeses made from goat milk during 56 days of storage, and their effect on chemical properties and sensory quality of cheeses.

2. Materials and Methods

Milk was pasteurized at 85°C for 10 min, cooled to 30°C and divided into three equal parts. Control cheese (C1) was produced using commercial starter culture CHN 11. Cheese C2 contained starter culture + potential probiotic strain *Lb. plantarum* 564 (isolated from white brined cheese), from the strain collection of the Department for Food Microbiology, Faculty of Agriculture, University of Belgrade. *Lb. plantarum* 564 was tested for its probiotic potential properties by Radulović et al. (2010). Test cheese C3 contained starter culture + commercial probiotic strain *Lb. acidophilus* LA-5. All three batches were inoculated with starter cultures (1mL/100mL) and potential and commercial probiotic strains were added in order to achieve 7 log cfu.g^{-1}. Solution of rennet (Sacco Clarifici, Italy) was added in concentration of 0.5g/100 L^{-1} to each batch. Fermentation lasted for 15 hours. After fermentation, cheeses were cut into small cubes, placed in sterilized cotton cheesecloth, and drained for 10 hours at 15°C. Then, the cheeses were salted with 1% of NaCl and packed in individual plastic cups. Cheeses were stored under refrigeration at 4°C for 56 days.

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Potential probiotic strain *Lb. plantarum* 564 was enumerated on MRS agar (Oxoid, CM 361) and incubated anaerobically (Gas Pak, BBL, Germany) for 48 h at 37°C. Commercial probiotic strain *Lb. acidophilus* LA-5 was enumerated on MRSiM agar with addition of maltose dilution (200g/L) and incubated anaerobically (Gas Pak, BBL, Germany) at 37°C for 48 h. Cheese samples were analyzed for the determination of total solid, fat and nitrogen content according to the IDF standard methods (1982, 1986, 2002). The pH value of cheese slurry was measured by a pH meter (Consort, Belgium). Five panel members evaluated cheese for exterior and interior appearance, body and texture, and flavour (odour and taste) using a 5-point scale, with 1 being the worst and 5 the best quality. Depending on the importance of attributes, they were multiplied by 3, 7 and 10, respectively. The total sensory quality (100) was expressed as a percentage of the maximum quality.

3. Results and discussion

The viability of potential probiotic *Lb. plantarum* 564 and commercial probiotic *Lb. acidophilus* LA-5 are shown in Figure 1. *Lb. plantarum* 564 counts in probiotic cheese C2 was 7.68 log cfu g^{-1} at the first day of storage and after 56 day of storage it was 7.02 log cfu g^{-1}. Number of *Lb. acidophilus* LA-05 in cheese C3 was 8.01 log cfu g^{-1} on first day, and 7.04 log cfu g^{-1} after 56 day of storage.

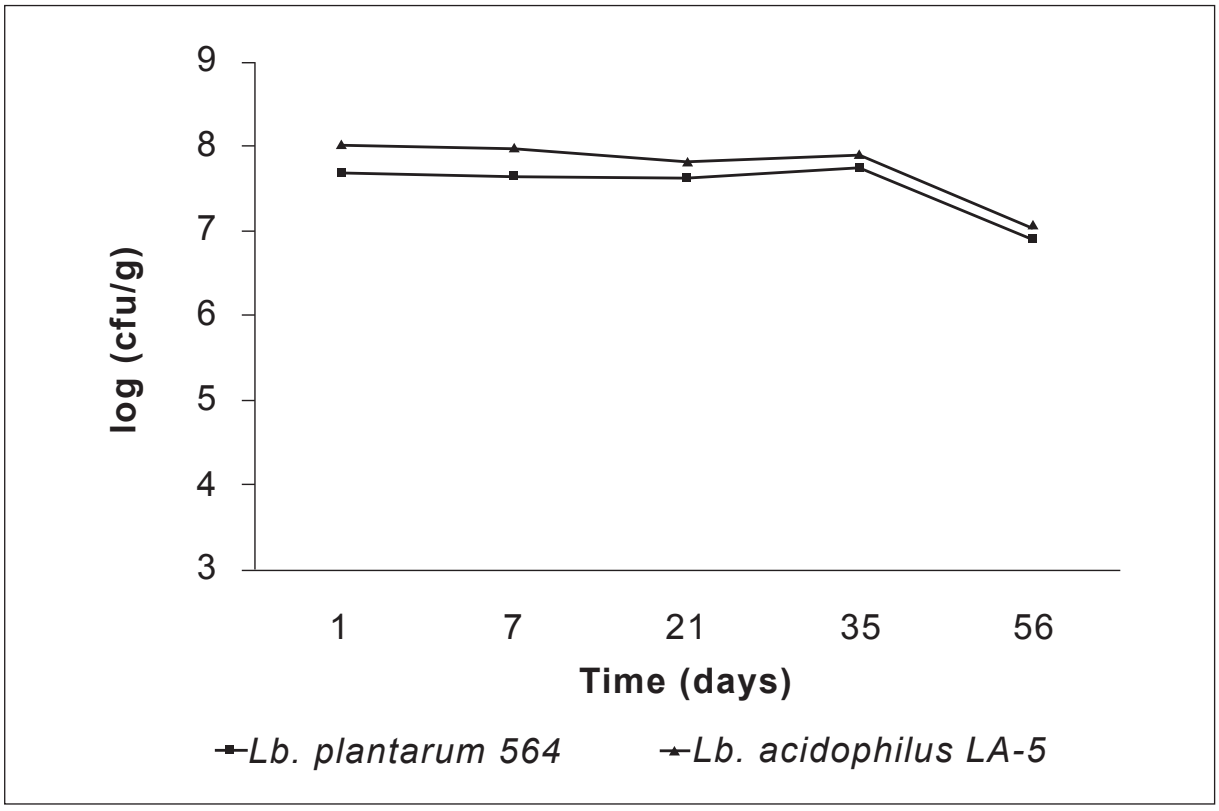

Figure 1. Viability of potential and commercial probiotic strains in cheeses

These results are similar to those obtained by Kılıç et al. (2009) who found viability of 10^8 cfu/g in a Turkish Beyaz cheese produced with potential probiotic strains after 90 days of ripening.

Results of chemical composition for C1, C2 and C3 cheeses during storage are summarized in Table 1.

Table 1: Chemical composition of cheeses during storage

Parameters	Dry matter (%)	Fat (%)	Protein (%)
Day 7			
Variant C1	25.37	10.50	7.7555
Variant C2	25,22	11,00	7,28
Variant C3	24.89	9.50	7.75
Day 21			
Variant C1	25.24	12.50	7.0339
Variant C2	25,08	12,00	7,49
Variant C3	24.89	12.00	7.65

After 7 and 21 days of storage, all variants of cheeses had similar dry mater content (24-25%). In the beginning there were some differences in fat content, but after 21 days, all variant of cheeses had the same content (12%). Protein content in cheeses was similar after 7 days of storage, but after 21 days, variant C1 (7.03) had the lowest protein content. Initially pH ranged from 4.07-4.16. At the end of storage, cheeses reached their minimum pH values of 3.75-3.81. There is no significant difference in chemical composition between control cheese (C1) and cheeses with added probiotic strains (C2 and C3).

Results of sensory evaluation are shown in table 2.

Table 2: The sensory evaluation of cheeses

Sensory attributes*		Exterior and Interior Appearance		Body and texture		Flavour and odour		% Maximum quality
Cheeses	Time (days)	R*	W*	R*	W*	R*	W*	
Cheese 1	7	4.9	14.70	4.9	34.30	4.5	45.00	94.00
	21	5.0	15.00	5.0	35.00	4.2	42.00	92.00
Cheese 2	7	4.88	14.63	5.0	35.00	4.75	47.50	97.13
	21	5.0	15.00	5.0	35.00	4.9	49.00	99.00
Cheese 3	7	4.88	14.63	4.88	34.13	3.75	37.50	86.25
	21	5.00	15.00	4.8	33.60	2.8	28.00	76.60

*Sensory attributes: Appearance 0-15; Body and texture 0-30; Flavour and odour 0-45

At 7 days of storage, all cheese samples were graded high for every parameter of sensory quality. After 21 days of storage, all variants of cheeses were similarly graded for exterior and interior appearance and for body and texture, but there were large difference in flavour grades. Cheese C2 with potential probiotic *Lb. plantarum* 564 was similar graded as control variant, while the variant with commercial probiotic *Lb. acidophilus* LA-5 had lower grades for flavour (2.8).

4. Conclusion

Based on the results of the study, it can be concluded that potential and commercial probiotic strains can be successfully used as adjunct cultures in production of soft cheeses from goat's milk. Potential probioitc strains *Lb. plantarum* 564 and commercial probiotic *Lb. acidophilus*

LA-5 maintained viability of > 7 log cfu.g^{-1} during storage period. Chemical composition of cheeses showed no significant difference between control cheese and probiotic cheeses. Cheese with potential probiotic strain *Lb.plantarum* 564 had better sensory quality than cheeses with commercial probiotic strain *Lb. acidophilus* LA-5. These results indicate that potential probiotic strain *Lb. plantarum* 564 is suitable for the production of soft goat cheeses as a new functional product.

Acknowledgments

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3.15. (S3.43) Lactic Acid Bacteria Isolated from Artisanal Sheep Kashkaval Cheese

Z. Radulović[1], N. Mirković[2], M. Petrušić[1], M. Barać[1], D. Paunović[1], D. Obradović[1]

Summary

Kashkaval is hard cheese, traditionally made from sheep milk, without the addition of starter cultures. A total of 36 autochthonous lactic acid bacteria from 9 samples of Kashkaval were isolated. All isolates were tested for their Gram reaction, catalase test, acidification ability, growth ability in 20, 40, 65 g NaCl/l, proteolytic ability to hydrolyze β-casein by SDS PAGE electrophoresis and biochemical characterization using API 50 CHL and API RAPID ID 32 Strep. Results showed that all catalase negative strains, 16 of them were Gram positive bacilli and 20 strains were Gram positive cocci. Thirteen strains were very good acid producers, up to pH 4.12-4.89, while 23 strains reduced pH poorly to 5.10-6.48 after 24 hours of fermentation. All isolated lactic acid bacteria grew in the presence of 20 and 40 g NaCl/l, while 21 isolates grew in the presence of 65 g NaCl/l. Some of isolates very extensively degraded β-casein, in contrast to others.

1. Introduction

Kaskaval is one of the most popular pasta filata cheese types and is produce in almost all Mediterranean and some Eastern and Central European countries (7). Kashkaval cheese is traditionally produced from raw ewe's milk (7), but recently cow's and sometimes goat's milk are also used (3). Raw milk is used without the addition of starter cultures, which means that present natural microflora has major role in fermentation and ripening. Autochthonous microflora represents a significant pool of different species of lactic acid bacteria (LAB) and by selection strains could be obtained for industrial implantation (1). Generally, Kashkaval cheese has good quality with recognizable sensory characteristics, but question of these characteristics remaining consistent is very important.

2. Materials and Methods

Isolation was conducted from 9 samples of Kashkaval cheeses. Cheese samples, 20 g, were homogenized with 180 ml of 2% sodium citrate solution in Stomacher 400 (Seward, UK). *Lactobacillus* strains were isolated on MRS medium (Oxoid M 361) incubated at 37°C for mesophilic and 43°C for thermophilic bacteria under anaerobic conditions (GasPack System, BBL) for 48 hours. *Lactococcus* strains were isolated on M17 medium (Oxoid CM 785) and incubated at 30°C under aerobic conditions for 48 h. Isolated strains were tested for Gram reaction, catalase test 0.3% (v/v) H_2O_2 and cell shape by microscopy of overnight cultures. Growth ability at different NaCl concentrations, 2%, 4%, 6.5% (w/v) NaCl was determined. Acidogenic activity was determined by inoculation 1% (v/v) broth cultures in 10% (w/v) reconstituised skim milk (RSM) and incubation overnight. pH values were measured after 2, 4, 6, 8 and 24 h using pH-meter (InoLab., pH 720, Made in Germany). Proteolytic ability of isolates to hydrolyze β-casein was examined using SDS-PAGE electrophoresis (LKB 2001-001, Sweden) and Sigma Gel tm (Jandel Corporation, Germany) software was used for analyses (6). Selected strains were identified with API 50 CHL (bioMerieux, France), for rod shaped bacteria and API RAPID ID 32 Strep for coccus shaped bacteria.

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3. Results and Discussion

From 9 cheese samples 36 autochthonous LAB were isolated on selective media. Sixteen of them were Gram positive and catalase negative rods, while 20 were Gram positive and catalase negative cocci.

Table 1: Autochthonous bacteria isolated from Kashkaval cheese

Genera	Thermophilic		Mesophilic	
	No. Isolates	Percent (%)	No. Isolates	Percent (%)
Lactococci	0	0	8	22.2
Streptococci	4	11.1	0	0
Enterococci	8	22.3	0	0
Lactobacilli	9	25.9	7	18.5
Total	21	59.3	15	40.7

In Kashkaval cheese, cocci were determined as dominant bacteria (55.6%), including *Enterococcus* sp. (22.3%), *Lactococcus* sp. (22.2%) and *Streptococcus* sp. (11.1%), and yet the presence of lactobacilli was significant (44.4%).

Traditionally, Kashkaval cheese ripens in highly concentrated brine, 10% (w/v) NaCl, so it can be expected that high number of isolated autochthonous LAB are tolerant to high concentration of NaCl (5). *Lactococcus* sp. can not grow in the presence of 6.5% NaCl, but according to these results, some wild *Lactococcus* strains can tolerate this concentration, so they are often present in artisanal cheeses (8). *Lactococcus* and *Lactobacillus* strains, can grow in the presence 2% and 4% but only half of them growth in the presence of 6.5% (w/v) NaCl. *Enteroccus* strains, grow in the presence 2%, 4% and 6.5% (w/v). Results are shown in Table 2.

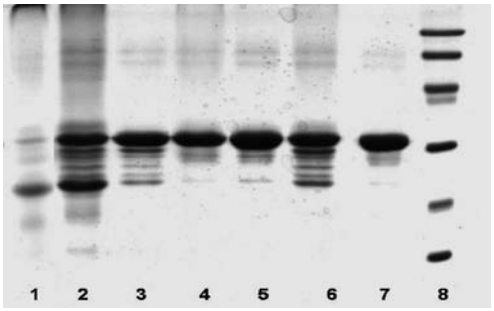
The ability to produce acid rapidly is probably the most important property of starter bacteria. The majority of *Lactococcus* sp. isolates, all *Enterococcus* sp. and *Streptococcus* sp. were good acid producers. *Lactobacillus* sp. isolates showed very different acid production ability. Production of lactic acid reduces the pH which in turn enables expulsion of whey from the curd, has impact on cheese texture and reduces the microbial spoilage of the cheese (2). Results of acidification are shown in the Table 2.

Table 2: Acidogenic activity and growth on different salt concentrations

Isolates from cheeses	Total no. tested	Kashkaval cheese					
		% NaCl			pH		
		2	4	6.5	<4.6	<5.2	<6.0
Lactococcus sp	8	8	8	4	4	4	0
Streptococcus sp	4	4	4	1	4	0	0
Enterococcus sp.	8	8	8	8	8	0	0
Lactobacillus sp	16	16	16	8	3	2	2
Total	36	36	36	21	15	3	2

Biochemical characterization of selected strains was performed with API RAPID ID32 and resulted in the identification of 4 species of lactococci: *Lactococcuus lactis* ssp *lactis* 5 isolates (13.8%), *Lactococcus lactis* ssp *cremoris* 3 isloates (8.3%), *Streptococcus thermophilus* 4 isolates (11.1%) and *Enterococcus* sp. 8 isolates (22.2%). Characterization of lactobacilli by API 50 CHL resulted in the identification of 5 species: *Lactobacillus delbrueckii* ssp *delbrueckii* 4 isolates (11.1%), *Lactobacillus casei* 3 isolates (8.3%), *Lactobacillus plantarum* 4 isolates (11.1%), *Lactobacillus fermentum* 3 isolates (8.3%) and Lactobacillus curvatus 2 isolates (5.5%).

Proteolytic activity is very important attribute of starter bacteria, because proteolysis is important in development of flavor in cheese (4). LAB are weekly proteolytic but possess very comprehensive proteinase/peptidase system capable of hydrolyzing casein derived peptides to small peptides and amino acids.



1, 2, 3, 4, 5, 6 – isolates of lactococci; 7 – pure β-casein; 8 – standard mol. weight

Figure 1. Proteolytic degradation of β-casein by autochthonous lactococci isolates

Results showed that isolates of lactococci degraded β-casein in various ways (Figure 1). There was considerable variation in proteolytic activity within some bacterial groups from the same cheese, but also there was limited variation in the proteolytic activity of the lactobacilli or lactococci (2). The reason is not clear.

4. Conclusion

Mesophilic lactic acid bacteria and *Lactobacillus* sp. constitute the predominant natural lactic acid microflora of Kashkaval cheeses in Serbia. Considerable variation was found in characteristics of isolated LAB, concerning their ability to produce acid, tolerance to salt and proteolytic activity. Screening autochthonous lactic acid bacteria for these important attributes, after additional testing, e.g. bacteriocins production and phage typing, enables the production of useful starters for industrial production of cheeses.

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3.16. (S3.45) Consumer Preferences of Cheeses from The Canary Islands Made With Traditional Kid Rennet Pastes

M. Fresno[1], S. Álvarez[1], M. de Renobales[2]

Summary

Preferences of 118 non-expert tasters and 24 expert panellists were analyzed. Cheeses were made using 2 different traditional kid rennet pastes: 1) rennet prepared from abomasa full of milk at collection time (TAR); 2) rennet prepared from emptied and washed abomasa which were then refilled with goat's raw milk (AAR). Recombinant chymosin was used in control cheese fabrications (CC). According to the expert panel, the intensity of traditional characteristics (piquant odour with rancid notes and rennet taste) of cheeses made with TAR rennet was higher than those of cheeses made with AAR rennet, but these characteristics were not detected in CC cheeses. AAR cheeses were preferred by 45% of all panellists (expert and non-expert), whereas 31% of them preferred TAR cheeses and only 24% preferred CC cheeses. However, expert panellists selected TAR and AAR cheeses with the same frequency, and none of them preferred CC cheeses. A majority (76.1 %) of consumers chose cheeses made with rennet pastes.

1. Introduction

In the past, cheese makers have used rennet paste preparations for curdling milk that were produced by macerating the stomachs from suckling ruminants according to various local uses. Because rennet preparation is cumbersome and time-consuming, nowadays most of the cheeses are made with commercial rennet and these artisan pastes are used only in some ovine and caprine raw milk cheeses [1, 2, 3], In contrast, recombinant chymosin is widely used, even in farmhouse cheeses, resulting in the loss of differentiating characteristics of cheeses with protected denomination of origin (PDO). Sensory characteristics of these cheeses made with traditional kid rennet paste should correspond to the traditional product. However, in spite of technical aspects, cheese makers need to take consumers' preferences into consideration, even if they differ from those of expert taste panels.

This paper analyses the preferences of non-expert consumers and expert panellists about cheeses made with traditional kid rennet pastes compared with cheeses made with recombinant chymosin.

2. Material and methods

Cheeses were made in the traditional way using 2 different traditional rennet pastes: 1) traditional artisan rennet prepared from abomasa full of milk at collection time (TAR); 2) adapted artisan rennet prepared from emptied and washed abomasa which were refilled with goat's raw milk (AAR). Recombinant chymosin was used in control cheese fabrications (CC). All rennet solutions were prepared to have the same rennet strength and added to the vats in order to complete clotting in 30-35 min at 30 ± 1 ºC [4]. Cheeses were ripened for 90 days.

Sensory profile analysis was carried out by a panel of 7 formally trained and highly experienced judges. Odour and flavour attributes were as described by [5] Berodier et al. (1996), and texture followed the guidelines published by [6] Lavanchy et al. (1999) both adapted to goat cheeses by [7] Fresno and Álvarez (2007). For the preference test, each participant (n=142; expert and non-expert) had to choose only one of the 3 different cheese samples presented. The non-expert panel (n= 118) comprised individuals who consume cheese regularly. It was

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divided into 2 groups: 44 university students under 25 years of age, and 74 consumers over 25 years old. Twenty-four expert judges also participated in this preference test following an adaptation of [8] UNE 87-005-92.

3. Results and discussion

According to the expert panel (n=7), sensory results for cheese description profile were:

- Odour and taste intensity: TAR > AAR>CC.
- Rennet odour and taste: TAR>AAR
- Piquant odour and taste: TAR
- Rancid notes in odour and taste: TAR
- Butter odour and taste: AAR
- Fermented fruits: CC
- Pungent in taste: TAR > AAR>CC.

Authors agree that rennet pastes prepared from suckling animals slaughtered right after suckling have significantly higher pregastric lipase activity than those prepared from animals sacrificed with empty stomachs [1, 3]. The increase in lipolysis yields cheeses with higher intensity scores for strong sensory attributes such as pungent and "natural rennet" flavour [1, 9]. This is most likely the explanation for TAR cheeses receiving higher intensity scores in traditional characteristics (piquant odour with rancid notes and rennet taste) as compared to cheeses made with AAR rennet. Those characteristics were not detected in CC cheeses.

Table 1 shows the preference test results. AAR cheeses were preferred by 45% of all panellists, whereas 31% preferred TAR cheeses, and only 24% preferred CC cheeses. However, expert panellists selected TAR and AAR cheeses with the same frequency, and none of them preferred CC cheeses. But non-expert student panellists preferred AAR and CC cheese with the same frequency, whereas older panellists preferred TAR and AAR cheeses, although almost 19% of them chose CC cheeses. It was clear that 76.1% of all panellists (expert and non expert) preferred cheeses made with artisan rennet pastes.

Table 1: Preference test results for cheeses made with different coagulants

Judges	CC	TAR	AAR
Non-expert university students under 25 years of age (n=44)	20	4	20
Non-expert consumers over 25 years of age (n=74)	14	30	30
Trained judges (n=24)	-	10	14
Total (n=142)	34	44	64
Judges who selected each cheese (%)	23.94	30.99	45.07

CC: control cheese made with recombinant rennet; TAR: traditional kid artisan rennet prepared from abomasa full of ingested milk; AAR: adapted kid artisan rennet prepared from emptied abomasa refilled with raw goat's milk.

4. Conclusion

TAR cheeses obtained higher scores in strong attributes: odour and flavour intensity, rennet and pungent odour and flavour, while recombinant rennet cheeses did not present these attributes. Expert judges and older consumers preferred cheeses made with TAR rennet and university students preferred AAR and recombinant rennet cheeses because they considered TAR cheeses to be too strong. TAR cheeses can be a good choice for consumers that like strong traditional cheeses, and AAR cheeses can be a good solution for consumers that like milder traditional tastes.

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3.17. (S3.46) Influence of By-Product Diets on Textural and Colour Properties of Artisanal Goat Cheeses

S. Álvarez[1], P. Méndez[1], T. Pérez[2], M. Fresno[1]

Summary

The objective of this experiment was to analyze the effect of fermented silage diets using agroindustrial by-products on different physicochemical goat cheese properties. Three experimental groups were used: DT was fed a standard diet (mixture of cereal grain and straw), while DB1 and DB2 were supplemented with different increasing amounts of fermented feed: 18% and 36% of DM respectively. A Texture Profile Analysis (TPA) and colour profile was carried on fresh (7 d) and semihard (45 d) raw milk goat cheeses.

Diet showed higher effect on fresh cheeses than on semihard cheeses. Five of six textural parameters presented significant differences. No statistical effect was detected on elasticity. DT fresh cheeses showed lower fracturability, hardness and gumminess scores while they were more cohesive and adhesive. Diet affected ($p<0.001$) all colour parameters in fresh and semihard cheeses. DB1 and DB2 cheeses presented similar colour characteristics in fresh cheeses but not in 45 day cheeses.

1. Introduction

The use of agro-industrial by-products in animal nutrition can be adopted as a strategy to reduce feeding costs and also to cope with the need to recycle waste material which is costly to dispose of. The traditional problems usually encountered with by-products are seasonality of supply that is often accented by their high moisture content. Hence, they easily spoil creating a nuisance and are often wasted. Ensiling by-products is the most suitable method for their conservation for a long period. Silage may be defined as moist forage in the absence of air and preserved by fermentation [1]. Fermentation is carried out by bacteria acting on plant carbohydrates in the chopped forage. The bacteria feed on the carbohydrates in the forage and rapidly produce volatile acids and lactic acid. When the production of this acid reaches a certain level, it prevents further bacterial action; resulting in the preserved feed we call silage.

The objective of this experiment was to analyze the effect of fermented silage diets using agroindustrial by-products on instrumental texture and colour goat cheese properties.

2. Material and methods

42 goats belonging to the Canarian breeds were divided into three experimental groups. Each group received a different diet: group DT was fed a standard diet (mixture of cereal grain and straw), while groups DB1 and DB2 were supplemented with different increasing amounts of fermented feed based on agroindustrial by-products (brewer's grain and bran): 18% and 36% of DM respectively. Cheeses, to evaluate diet effect, were made according previous methodology [2]. A Texture Profile Analysis (TPA) was carried on fresh (7 d) and semihard (45 d) raw milk goat cheeses determining fracturability, hardness, cohesiveness, adhesiveness, elasticity and gumminess (Texturometer TA-Xt2i, Stable Micro Systems, Surrey, UK). Inside cheese colour was also measured using a colorimeter, measuring Lightness (L), Croma (C*), Hue angle (H*), a and b parameters (Colorimeter CR 300, Minolta, Osaka, Japan). Statistical methodologies were made with SPSS 15.0.

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3. Results and discussion

Diet showed higher effect on fresh cheeses than on semihard cheeses (Table 1 and 2). In 7d cheeses five of six textural parameters presented significant differences ($p<0.01$). No statistical effect was detected on elasticity. DT fresh cheeses showed lower fracturability, hardness and gumminess scores while they were more cohesive and adhesive.

Table 1: Texture parameters determined by TPA of fresh cheeses

Texture	Diet		
	DT	DB1	DB2
Fracturability	7.52±3.45^{a}	28.84±9.39^{b}	22.36±8.12^{b}
Hardness	15.57±1.85^{a}	40.27±8.99^{b}	31.03±13.97^{b}
Cohesiveness	0.22±0.03^{a}	0.15±0.03^{b}	0.13±0.03^{b}
Adhesiveness	1.62±1.88^{a}	0.31±0.71^{ab}	0.10±0.12^{b}
Elasticity	87.70±7.66	81.92±11.14	87.14±5.01
Gumminess	305.80±50.03^{a}	514.19±191.41^{b}	367.23±199.11^{ab}

Different letters in the same row mean significant differences ^{a,b}=$p<0.001$

With the ripening process hardness, fracturability and gumminess increased, while elasticity decreased. As it is common in Canarian cheeses [3], cohesiveness and adhesiveness did not change as cheeses matured.

Table 2: Texture parameters determined by TPA of semihard cheeses

Texture	Diet		
	DT	DB1	DB2
Fracturability	67.63±17.95	65.17±18.24	81.53±18.88
Hardness	139.70±33.41^{a}	145.92±40.96^{ab}	181.26±23.10^{b}
Cohesiveness	0.14±0.04	0.14±0.02	0.15±0.01
Adhesiveness	0.64±0.53^{ab}	0.93±0.87^{b}	0.10±0.17^{a}
Elasticity	55.38±20.06	64.26±10.55	62.90±3.91
Gumminess	1006.93±362.52^{a}	1275.23±408.47^{a}	1707.12±306.80^{b}

Different letters in the same row mean significant differences ^{a,b}=$p<0.001$

Diet affected ($p<0.001$) all colour parameters in fresh and semihard cheeses (Tables 3 and 4). DB1 and DB2 cheeses presented similar colour characteristics in fresh cheeses but not in 45 day cheeses. In reference to lightness values, cheeses from DB2 group were the lightest, but once completed the ripening process they became the darkest. Differences were also found in Croma values where the most intensive colour cheeses were DT cheeses and DB1 the least. Hue Angle values showed that standard diet cheeses were the least yellow and DB1 the most.

Table 3: Inside colour values of fresh cheeses

Colour	Diet		
	DT	DB1	DB2
L	90.87±0.64[a]	90.62±0.74[a]	91.55±0.44[b]
C	6.77±0.35[a]	5.90±0.45[b]	5.83±0.41[b]
H	61.50±2.60[a]	50.58±7.50[b]	49.18±7.16[b]
a	3.17±0.12[a]	3.68±0.25[b]	3.73±0.24[b]
b	5.98±0.45[a]	4.56±0.83[b]	4.44±0.72[b]

Different letters in the same row mean significant differences [a,b]=p<0.001

Table 4: Inside colour values of semihard cheeses

Colour	Diet		
	DT	DB1	DB2
L	84.50±3.51[a]	85.82±3.08[a]	77.14±4.26[b]
C	8.28±0.60[a]	6.84±0.66[c]	7.65±0.74[b]
H	77.84±1.62[b]	70.61±6.46[a]	74.44±4.98[ab]
a	1.73±0.20[a]	2.19±0.47[b]	2.01±0.50[ab]
b	8.09±0.61[a]	6.43±0.87[c]	7.35±0.84[b]

Different letters in the same row mean significant differences [a,b]=p<0.001

In conclusion, diet affected instrumental texture and colour parameters.

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3.18. (S3.47) Sensory Characteristics of Goat Cheeses: The Use of Agroindustrial By-Products in The Diet

S. Álvarez[1], P. Méndez[1], T. Pérez[2], M. Fresno[1]

Summary

The main objective of this experiment is the evaluation of some agroindustrial by-products (beer and flour industry by-products) as silage forages for feeding goats. Three experimental groups were used: A first group, DT, was fed a standard diet (mixture of cereal grain and straw), while Groups DB1 and DB2 were supplemented with different increasing amounts of fermented feed: 18% and 36% of DM respectively. The organoleptic profile of manufactured cheese (7 and 45 days) was determined: texture, odour and flavour parameters were evaluated by an expert panel.

The cheeses studied were different when analyzing their organoleptic profile. DB1 and DB2 fresh cheeses were more elastic ($p<0.001$) and showed higher granularity scores but had lower solubility and adhesivity than DT fresh cheeses. The odour and flavour intensity and persistence were higher in DB1 and DB2 cheeses. All the experimental cheeses showed notes of goat milk, whey and cream. Six of the nine texture parameters were affected by diet in semihard cheeses. None of the cheeses studied showed odour and flavours that do not correspond with the typical characteristics of Canarian goat raw milk cheeses.

1. Introduction

Agro-industrial by-products can be an important source in animal feeding and can minimize two important problems in the Canary Islands: the high cost of Canarian goat industry now linked to imported and expensive products and the environmental risk that may result from the uncontrolled dumping of by-products. High-moisture agro-industrial by-products are often of high nutritional value. In industrial countries there are well-developed technologies for recovering by-products and converting them into protein-rich meals and/or energy-rich concentrates. Ensiling by-products is a simple and low-cost option which can preserve feeds that are seasonally abundant for later feeding during periods of feed shortage. Ensiling can also render some previously unpalatable products useful to livestock by changing the chemical nature of the feed [1]. The brewer's residues: extracted malt or spent grain, contains 75 - 80% water when filtered off. Wet spent grain spoils rather quickly and should be used fresh or stored out of contact with air. Wet spent grain can be ensiled alone or in association with other ingredients, for example, with flour industry by-products. The latter has the advantage to absorb the juice from spent grain and consequently to limit losses during fermentation.

The main objective of this experiment is the evaluation of some agroindustrial by-products (beer and flour industry by-products) as silage forages for feeding goats.

2. Material and methods

In this study 42 goats belonging to the Canarian breeds were divided into three experimental groups. Each group received a different diet: group DT was fed a standard diet (mixture of cereal grain and straw), while in groups DB1 and DB2 concentrate was partially substituted with different amounts of fermented feed based on agroindustrial by-products (brewer's grain and bran): 18% and 36% of DM respectively. Cheeses were made according previous methodology [2], to evaluate the effect of diet. The organoleptic profile of cheeses (7 and 45 days) was determined: texture, odour and flavour parameters were evaluated by an expert panel following the methodology described by (3) Fresno and Álvarez (2007). Statistical methodologies were made with SPSS 15.0.

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3. Results and discussion

Fresh cheeses were quite different when analyzing their texture profile (Table 1). DB1 and DB2 fresh cheeses were more elastic ($p<0.001$) and showed higher granulosity scores but had lower solubility and adhesivity than DT fresh cheeses. Fresh cheeses showed similar firmness, friability and mouth moisture scores.

Table 1: Texture sensory evaluation of fresh cheeses. DT standard diet (mixture of cereal grain and straw); DB1 concentrate partially substituted with fermented brewer's grain and bran 18% DM; DB2 concentrate partially substituted with fermented brewer's grain and bran 36% DM

	Diet		
	DT	DB1	DB2
Rugosity	3,14±0,24^{a}	2,15±0,27^{b}	2,21±0,26^{b}
Superficial Moisture	5,28±0,27^{a}	5,80±0,28^{b}	5,85±0,28^{b}
Springiness	1,79±0,30^{a}	3,43±0,45^{b}	3,50±0,40^{b}
Firmness	2,57±0,45	2,71±0,39	2,75±0,40
Friability	2,00±0,30	2,00±0,30	2,00±0,30
Adhesivity	4,30±0,27^{a}	2,36±0,24^{b}	2,36±0,24^{b}
Solubility	4,00±0,30^{a}	3,00±0,30^{b}	3,00±0,30^{b}
Mouth moisture	4,14±0,24	4,14±0,24	4,14±0,24
Granulosity	1,71±0,30^{a}	3,00±0,29^{b}	3,00±0,29^{b}

Different letters in the same row mean significant differences ^{a,b}=$p<0.001$

Six of the nine texture parameters were affected by diet in semihard cheeses (Table 2). DB2 cheeses where the most different, presenting lower superficial moisture and elasticity scores while showed higher firmness, solubility and granularity ($p<0.001$). DT and DB1 cheeses were quite similar with no differences in all textural parameters. As more silage is added to the diets differences in cheese texture are extended. In opposite to fresh cheeses where DB1 and DB2 were a homogeneous group, in semihard cheeses DT and DB1 showed the same textural sensory profile. All experimental cheeses presented typical texture characteristics of Canarian goat cheeses [3].

Table 2: Texture sensory evaluation of semihard cheeses. DT standard diet (mixture of cereal grain and straw); DB1 concentrate partially substituted with fermented brewer's grain and bran 18% DM; DB2 concentrate partially substituted with fermented brewer's grain and bran 36% DM

	Diet		
	DT	DB1	DB2
Rugosity	3,80±0,27	3,80±0,27	3,80±0,27
Superficial moisture	3,00±0,19^{a}	3,10±0,20^{a}	2,28±0,30^{b}
Springiness	3,14±0,24^{a}	3,14±0,24^{a}	2,50±0,29^{b}
Firmness	4,14±0,24^{a}	4,20±0,20^{a}	5,14±0,25^{b}
Friability	4,14±0,24	4,20±0,20	4,00±0,41
Adhesivity	3,14±0,24	3,14±0,24	3,50±0,40
Solubility	4,57±0,18^{a}	4,65±0,20^{a}	4,15±0,25^{b}
Mouth moisture	3,21±0,26^{a}	3,25±0,30^{a}	2,86±0,24^{b}
Granulosity	3,20±0,27^{a}	3,30±0,30^{a}	2,14±0,25^{b}

Different letters in the same row mean significant differences ^{a,b}=$p<0.001$

Diet also affected five odour, flavour and taste parameters in fresh cheeses. The odour intensity, flavour intensity and persistence were higher in cheeses made with milk from goats fed fermented products. All the experimental cheeses showed notes of goat milk, whey and cream (data not shown). DT group cheeses were sweeter and less acidic. As is normal in fresh cheeses, bitterness, astringency or trigeminal sensations were not detected.

Table 3: Odour, flavour and taste evaluation of fresh cheeses. DT standard diet (mixture of cereal grain and straw); DB1 concentrate partially substituted with fermented brewer's grain and bran 18% DM; DB2 concentrate partially substituted with fermented brewer's grain and bran 36% DM

	Diet		
	DT	DB1	DB2
Odour intensity	1,36±0,24^{a}	1,79±0,27^{b}	1,85±0,30^{b}
Flavour intensity	1,79±0,27^{a}	2,14±0,24^{b}	2,20±0,24^{b}
Saltiness	3,14±0,24	3,14±0,24	3,14±0,24
Sweetness	1,43±0,45^{a}	0,64±0,25^{b}	0^{c}
Acidity	1,64±0,38^{a}	2,07±0,35^{a}	2,64±0,38^{b}
Persistence	1,79±0,26^{a}	2,30±0,28^{b}	2,40±0,30^{b}

Different letters in the same row mean significant differences ^{a,b}=p<0.001

The sensory profile showing odour, flavour and taste characteristics of semihard cheeses is presented in Table 4. As was expected odour and flavour intensity increased compared to fresh cheeses. DB1 presented the highest intensities (p<0.001) while DT cheeses the lowest. The persistence value behaved differently, the mouthfeel of DB1 cheeses disappeared before. The only parameter that showed no significant difference was saltiness, all cheeses showed a proper degree of salt, slightly below average. It is important to remark that dried and fermented fruit descriptors appeared in DB1 and DB2 cheeses with the ripening process. None of the cheeses analysed showed textures, odours or flavours that do not correspond with the typical characteristics of Canarian goat raw milk cheeses [3].

Table 4: Odour, flavour and taste evaluation of semihard cheeses DT standard diet (mixture of cereal grain and straw); DB1 concentrate partially substituted with fermented brewer's grain and bran 18% DM; DB2 concentrate partially substituted with fermented brewer's grain and bran 36% DM

	Diet		
	DT	DB1	DB2
Odour intensity	2,86±0,24^{a}	4,21±0,27^{b}	3,79±0,27^{c}
Flavour intensity	3,36±0,38^{a}	4,99±0,26^{b}	4,50±0,41^{c}
Saltiness	3,50±0,15	3,50±0,15	3,50±0,15
Sweetness	0^{a}	1,71±0,27^{b}	0,71±0,30^{c}
Acidity	3,50±0,41^{a}	2,79±0,27^{b}	1,14±0,24^{c}
Spicy	1,14±0,24	1,50±0,55	1,36±0,30
Astringency	0^{a}	0^{a}	1,00±0,08^{b}
Persistence	4,14±0,25^{a}	3,14±0,30^{b}	4,14±0,25^{a}

Different letters in the same row mean significant differences ^{a,b}=p<0.001

As a conclusion DB1 and DB2 cheeses showed traditional characteristics. This result may encourage the use of agroindustrial byproducts in the diets of dairy ruminants.

Acknowledgments

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3.19. (S3.55) Implications of Farm Environment Microflora and Milk Production Practices for Acidifying Ability of Raw Goat Milk in Lactic Cheese-Making

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Summary

The control of acidification step in lactic cheese-making based on whey as a starter culture and raw milk depends on the acidifying ability of milk microflora. This acidifying ability was studied, in relation with the acidifying ability of two microflora reservoirs: the milking machine (MM) and the teat's skin. Twenty farms were visited twice, to collect information about production practices and samples from the considered reservoirs. The pH measured show that microflora from the teats and from milking machine can settle and have an acidifying activity in the milk. Among the two reservoirs investigated, milking machine contributes the most to the raw milk acidifying ability. The most influential practices on the acidifying capacity of the milking machine reservoir are the design, the maintenance of the milking parlour and the milking machine. Further investigations are necessary to make practical recommendations for the farmers.

1. Introduction

This study was part of a three-year program dealing with the sustainability of the use of natural whey starter for farmhouse lactic goat cheeses. Quality of whey depends on the control of acidification step, itself linked with the acidifying ability of milk microflora. This acidifying ability was studied, in relation to the acidifying ability of two microflora reservoirs: the milking machine (MM) and the teats' skin. The influence of some farm management practices on these reservoirs was also investigated.

2. Materials and methods

This study was carried out at 20 goat cheese dairy farms in the Rhône-Alpes region in France. It was important for the study for the farms chosen to have comparable milking systems, but different milk production practices. Also farms employing extreme practices resulting in sub-standard levels of hygiene were eliminated as previous studies have shown that the levels of flora in these farms are generally higher, thus increasing the risk of the uncontrolled development of spoilage flora and even potentially pathogenic flora [2]. Two visits were conducted, between March and May 2009, with an interval of six weeks between the two visits. During the visits, the farms were given a comprehensive questionnaire on their milk production practices and samples were collected for each reservoir. For each sample, 20 ml of milk (UHT milk that had passed through the milking machine (MM), composite milk used to smear the teats, milk from one milking before inoculation) was placed to incubate in five sterile test tubes at 22°C and pH measurements were taken at 0, 8, 24, 32 and 48 hours. In parallel, the enumeration of microflora and identifications were made (results not presented here). The MPLS (Multiblock Partial Least Square) statistical method used, was programmed in the Scilab® software (INRIA, France). This method is used to explain a given data block (Y) in relation to several other blocks (X) based on the hierarchical structure of the data blocks studied. This method provides three types of result: BIP (Block Importance for the Projection): contribution of an X block to explain

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a Y block; VIP (Variable Importance for the Projection): contribution of each variable in the explanatory blocks to explain a given Y block; regression coefficients between each Y block variable and each block X variable. The MPLS method allows us to study on the one hand the relation between the acidification capacity of the reservoirs and the milk collected and on the other hand between the milk production practices and the acidification capacity of the flora in the reservoir.

3. Results and discussion

Acidification capacity of milk and reservoirs.

The microflora in the reservoirs is thus capable of settling in the milk and producing an acidifying effect (table 1). The pH measurements of the samples of milk inoculated by the microflora in the MM and the teats explained the 80% plus variability of the pH measurements of the milk collected.

Table 1: Acidification characteristics of the reservoir samples

	Milk collected(n=40)	Milking machine milk (n=40)	Composite milk (n=40)
Average pH at 48 hours (standard deviation)	4.58 (0.39)	4.58 (0.37)	4.88 (0.52)
Average pH reduction (standard deviation)	-2.17 (0.38)	-2.16 (0.38)	-1.80 (0.51)
% samples attaining a pH level≤4.6 at 48 hours (standard deviation)	72.50%	70.00%	42.50%

Between the two reservoirs, the milking machine showed a preponderant importance: the pH measurements of the samples of UHT milk having passed through the MM explained the 68% plus pH levels of the milk collected (table 2). This confirms the importance of the milking machine in the acidification capacity of milk [1].

Table 2: Influence of each "pH of reservoir" on the "pH of milk collected" (block importance for the collection, BIP)

	Milk collected	Confidence interval of 95% (BIP under null hypothesis: 50%)	
MM	68.93%	56.87%	80.99%
Teats	31.07%	19.02%	43.13%

Impact of milk production practices on the acidification capacity of flora in the milking machine.

The explanatory power of the model is lower than previously shown (24.84%), which is insufficient to be able to provide practical recommendations for producers but may be useful for constructing hypotheses. Observing the contribution of each block of practices to the block of pH measurements does not allow us to confirm that any of them has significantly higher or lower importance than the others. Examining the values of the VIP showed that the variable concerning general dust accumulation after milking is considered significantly more important than the others, as is the age of the teat cup liners, the existence or not of a waiting area, the percentage of plastic in the milking machine and the dates when liners last changed. It was

observed that the majority of the variables concerning the cleaning practices used on the MM are significantly less important than the others, while previous studies showed they had an impact on the acidifying flora in the milk [5, 6]. It is probable that the cleaning practices have less of an impact in our study due to their lesser variability, in line with the criteria used to select the farms for the study. Moreover, in previous studies, direct relations were sought, descriptively without statistical modeling, between the milk collected and combinations of practices involving other actions than cleaning.

An examination of the regression coefficients showed that the most important variables are related to the design of the milking parlour and the design and maintenance of the milking machine. The results are consistent with previous studies [1, 2, 5] or could be explained [3, 4]. Although we were unable to measure their impact on the pH data block in their ensemble, some cleaning practices were nevertheless in line with the pH levels at end of acidification. A positive influence of prewashing was noted. The lower delta pH indicating more thorough rinsing is commensurate with the higher acidifying capacity of the milk, which is similar to what was observed [2]. Regarding the milking practices, UHT milk that passes through the milking machine is more acidifying when more air enters when the claws are attached.

4. Conclusion

The microflora in the reservoirs studied (milking machines, teats) is capable of settling in the milk and producing an acidifying effect. Applying an innovative data analysis method, we confirmed that the milking machine has an important impact on the acidifying capacity of the milk collected. The most influential practices on the acidifying capacity of the milking machine reservoir are the design, the maintenance of the milking parlour and the milking machine. It is therefore difficult with the current data in hand to recommend more sophisticated management controls to farmers wishing to maintain a healthy balance between preserving the beneficial flora and reducing the spoilage flora.

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3.20. (S3.62) Traditional Cheeses: Effect of Cheesemaking Technology on The Physicochemical Composition and Mineral Contents

A. Nega[1], C. Kehagias[1], G. Moatsou[2]

Summary

The objective of the present study was to determine the major minerals of various ovine/caprine Greek cheese types in relation to physicochemical composition and to investigate the influence of cheesemaking technology on them. Forty eight cheese market samples, belonging to various cheese groups, i.e. brined, gruyère, hard cheese, and pasta filata types, were analyzed. Ca, P and Mg contents varied from 318 to 963, from 248 to 623 and from 22 to 59 mg·100 g^{-1}, respectively following the variability in the ash content, i.e. from 4.08 to 7.58 g·100 g^{-1}. The results show how the cheesemaking technology is reflected, not only in the gross composition, but also in the mineral content of various cheese varieties. Especially, pH, calcium and moisture were strongly correlated and they can serve as identity indices for each cheese group. In fact, mineral contents and both Ca:P and Ca:Protein ratios were indicative of each cheese type.

1. Introduction

One of the major characteristics of Greek dairy sector is the production of various cheese types in different regions of the country. A great part of them are cheeses of Protected Designation of Origin [3]. They are mainly produced from ovine milk or from its mixtures with caprine milk. These two kinds of milk correspond to the 60% of the total milk production of the country. They are produced mainly from animals of autochthonous breeds and their exploitation through cheese production is an ancient practice. In fact, only 15% of the cheese production in the country is manufactured from bovine milk. Cheese characteristics are determined by the cheesemilk composition, cheesemaking technology and ripening conditions [1]. The way of cutting and the thermal treatment of the cheesecurd affect curd moisture and control acid development and pH that along with the salt determine the environment, in which the biochemical reactions involved in cheese ripening also have an influence. Thermal treatment of the cheese curd also affects the numbers and the kinds of bacteria as well as the enzymatic activities in the curd, these two being the most important ripening factors in cheese [1, 5]. Cheese pH, concentration of minerals and the ripening changes particular for each cheese variety affect both cheese flavour and texture [6]. The aim of the present study was to determine the major minerals of various Greek cheese types, to relate them with the physicochemical composition reported by Nega & Moatsou [7] and to examine the influence of cheesemaking technology on them.

2. Materials and Methods

Samples of 48 commercial cheeses belonging to various cheese groups, i.e. brined-, Gruyère, hard cheese, and pasta filata types were analyzed (Table 1). The determination of physicochemical characteristics have been published by Nega & Moatsou [8]. The estimation of Ca, Mg, K and Na in the ash fraction of cheeses was carried out by means of the atomic absorption spectrometric method [3]. For the stock dilution 40 mg of cheese ash was dissolved in 1 ml nitric acid 25% (v/v) and then the final volume was made up to 100 ml with ultra pure water HPLC grade. Various quantities of the stock ash dilution were further diluted in ultra pure water after the addition of 10 ml lanthanum trichloride solution (1% in 25% nitric acid). AAS analyses were carried out in the above-mentioned dilutions and quantification was based on the respective reference curves.

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Phosphorus was also determined in the ash fraction of cheeses using molecular absorption spectrometry [4].

Table 1: Samples of traditional Greek cheese varieties analyzed in the present study

Code	Cheese variety	Cheese type	Number of samples
B1	Feta	Brined	19
B2	Kalathaki Limnou	Brined	3
B3	Sfela	Brined	3
G1	Graviera Kritis	Gruyére	6
H1	Kefalograviera	Hard	5
H2	Ladotyri Mytilinis	Hard	3
P1	Kasseri	Pasta filata	9

3. Results and Discussion

The major mineral constituents of the ash fraction of the studied cheese samples are presented in Table 2. The Na content was as expected high in the cheeses with high salt content, confirmed also by the high correlation coefficient (R=0.951) between Na and NaCl content of cheeses [8]. It is evident that acidic cheeses, i.e. B1 and B2 had the lowest calcium, phosphorus and magnesium contents due to the effect of intense curd acidification on CCP solubilisation and concomitant loss of minerals in the whey. High values of these minerals were observed in cheeses with pH >5.5. Calcium concentration was correlated strongly positively with cheese pH (R=0.845) and negatively (R=-0.902) with cheese moisture [7]. The pH and therefore calcium content configure for the most part the particular textural characteristics of each cheese category [5, 6].

According to Lucey and Fox [6], cheeses can be classified on the basis of Ca:PO_4 ratio. They report that this ratio tends to decrease as the overall mineral content of cheese decreases, because during cheesemaking calcium is lost in the whey more rapidly than phosphorus as whey pH decreases. This is in accordance with the low Ca:P ratios of B1 and B2 acidic cheese varieties (1.28 and 1.19 respectively) and the high ratios of hard-type and Gruyère-type cheese (ranged from 1.34 to 1.55). The same was true for the Ca:protein ratio, which is also indicative of cheesemaking conditions, i.e. acid development and pH at drainage, as suggested by the same authors. The ratio Ca:protein was low, i.e. lower than 0.021, in acidic high moisture cheeses, whereas high ratios were observed in high pH cheeses, i.e. >0.029 [7].

Table 2: Major minerals of traditional Greek cheeses, in mg×100 g^{-1} (mean ±SD)

Cheese code*	Ca	P	Mg	K	Na
B1	318.2 ± 99.1	248.1 ±82.7	22.3 ±8.4	69.8 ±28.2	939.8 ±429.3
B2	320.9 ± 82.8	269.6 ±39.0	24.7 ±3.0	63.7 ±16.0	1371.3±267.8
B3	621.0 ±34.6	458.8 ±11.3	43.2 ±3.8	65.8 ±7.1	2296 ±418.6
G1	963.2 ±104.8	623.9 ±61.9	57.8 ±6.1	94.8 ±17.9	642.0 ±185.8
H1	850.6 ± 91.6	574.1±126.6	55.8 ±3.2	80.9 ±9.6	1509.6±216.2
H2	894.5 ±64.9	575.5 ±61.8	59.0 ±6.1	77.8 ±16.1	803.0 ±137.7
P1	858.2 ±76.7	560.5 ±36.5	48.0 ±4.0	74.1 ±13.0	599.0 ±266.7

*For cheese codes see Table 1

4. Conclusions

Cheeses of the present study can serve as an example of how the cheesemaking technology is depicted on the mineral content of various cheese varieties. Especially, pH, calcium and moisture of the cheeses are strongly inter-correlated and they could serve as identity indices of each cheese variety. In fact, mineral contents both Ca:P and Ca:Protein ratios are indicative of each cheese type. These physicochemical characteristics configure the environment in which the various ripening factors are brought into action during cheese maturation and storage.

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3.21. (S3.64) Use of a Plasminogen Activator in Kefalotyri Cheese Manufacture

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Summary

Urokinase was added to milk for Kefalotyri cheese manufacture to examine the possibility of accelerating proteolysis during ripening by conversion of indigenous plasminogen to plasmin. Cheeses were analyzed for their composition and plasmin activity, plasminogen level, proteolysis and casein hydrolysis after 1, 9, 60 and 120 days of ripening. The addition of urokinase to the milk significantly increased plasmin activity in the cheese with concomitant decreases in plasminogen level and plasminogen:plasmin ratio. Activation of plasminogen to plasmin occurred principally during 1st day of manufacture presenting a progressive increase up to 120 days of ripening. The increased plasmin activity in cheese caused increased production of γ-caseins and N-fractions during ripening indicating that the addition of urokinase to cheesemilk accelerated proteolysis in Kefalotyri cheese.

1. Introduction

Kefalotyri is a Greek cheese made from ewes'' milk or a mixture of ewes'' and goats'' milk. The cheese is scalded at 45 oC and ripened for 90 to 120 days. Plasmin has potential role in cheese ripening, as it contributes to proteolysis, especially in high temperature-cooked varieties. In the present study a plasminogen activator (urokinase) was added to ewe milk in order to accelerate proteolysis in Kefalotyri cheese. Ewe milk with low somatic cell count was chosen and the plasminolytic system and proteolytic profile of the resulting cheese during ripening were studied.

2. Materials and methods

Urokinase (Sigma Chemical Co., St. Louis, MO) was added to cheesemilk after pasteurization at a level of 0.5 Units/l and Kefalotyri cheese (cheese C), was manufactured according to the traditional protocol described by Anifantakis, 1991 [2]. Two control cheeses, one supplemented with 80 mM plasmin inhibitor 6-aminohexanoic acid (AHA, Sigma Chemical Co., St. Louis, MO) (cheese A) and the other without additives (cheese B), were also manufactured at the same time with the same procedure.

Cheese milk was analyzed for chemical composition, somatic cell count (SCC) and total mesophilic microflora (TBC) according to IDF standards [7, 9, 5].

Cheeses were analyzed at 1, 9, 60 and 120 days for protein [6], moisture [8], fat [10], salt content [4], pH, total nitrogen (TN%) and nitrogen fractions (N-fractions):nitrogen soluble at pH 4.4 (pH 4.4-SN) % and nitrogen soluble fraction at 12% TCA (TCA-SN) % [6]. The proteolytic profile of cheeses was examined by urea-PAGE [1].

Plasmin (PL) and plasminogen (PG) activity in milk was determined as described by Korycka-Dahl et al. (1983) and Politis et al. (1989b) [11, 13] and in cheeses according to Farkye et al. (1992) [3].

For statistical analysis the software Statgraphics Plus for Windows v.5.2 (Manugistics Inc., Rockville, Maryland 20852, USA) was used.

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3. Results and discussion

3.1. Chemical composition

Chemical composition showed no significant difference between experimental and control cheeses on the day of manufacture. The mean values were: fat in dry matter 47.4%, protein 23.3%, moisture 40.8%, salt in moisture 3.8% and pH 5.36.

3.2. Plasminolytic system

PL activity in cheeses C during ripening increased with concomitant decreases in the level of PG and PG:PL ratio (Table 1) indicating increased conversion of PG to PL. Most activation of the PG to PL by urokinase occurred on the 1st day of manufacture. This activation was also apparent in cheeses B, suggesting the presence of PG activators in Kefalotyri cheese matrix, while in cheeses A it was low. After day 1, PG activation was decreased up to 120 days but the conversion rate of PG to PL was greater in the experimental cheeses C (Figure 1).

Table 1: N-fractions on dry matter (%), PL (U/g)[1], PG (U/g)[1] and PG/PL during ripening of Kefalotyri cheese. Values are means of triplicate analyses of three trials

		Cheeses code[2]		
Item	Time of ripening (days)	A	B	C
Total Nitrogen (TN) (%)	1	6.12^{a}	6.25^{a}	6.13^{a}
	9	6.03^{a}	6.10^{a}	6.05^{a}
	60	6.02^{a}	6.07^{a}	6.04^{a}
	120	5.91^{a}	6.04^{a}	5.77^{a}
pH 4.4-soluble nitrogen SN (%) TN	1	5.88^{a}	5.92^{a}	6.36^{a}
	9	7.67^{a}	9.01^{b}	9.78^{b}
	60	10.13^{a}	13.18^{ab}	13.59^{b}
	120	20.29^{a}	23.17^{b}	25.29^{c}
12% trichloracetic acid-soluble nitrogen TCA-SN %TN	1	3.32^{a}	2.61^{a}	2.96^{a}
	9	5.17^{a}	5.79^{a}	6.00^{a}
	60	5.99^{a}	7.94^{a}	8.82^{a}
	120	11.05^{a}	13.05^{ab}	15.45^{b}
PG (U/g)	1	92.24^{a}	93.63^{a}	91.92^{a}
	9	91.00^{a}	89.70^{a}	89.42^{a}
	60	87.88^{a}	86.98^{a}	85.72^{a}
	120	85.13^{a}	83.77^{a}	82.36^{a}
PL(U/g)	1	14.70^{a}	24.21^{b}	24.10^{b}
	9	15.14^{a}	26.35^{bc}	27.32^{c}
	60	15.60^{a}	28.24^{b}	31.10^{c}
	120	16.72^{a}	30.90^{b}	35.04^{c}
PG /PL	1	6.30^{a}	3.93^{b}	3.80^{b}
	9	6.01^{a}	3.47b^{c}	3.32^{c}
	60	5.67^{a}	3.13^{b}	2.80^{c}
	120	5.13^{a}	2.76^{b}	2.37^{c}

^{a,b,c} Means within the same row with a different superscript differ significantly (Duncan test, $P<0.05$)

1 Plasmin activity expressed as Units/g (U/g)

2 Cheese A: Kefalotyri cheese made from ewe milk supplemented with plasmin inhibitor; Cheese B: Kefalotyri cheese made from ewe milk without additives; Cheese C: Kefalotyri cheese made from ewe milk supplemented with 0.5 Units/l urokinase

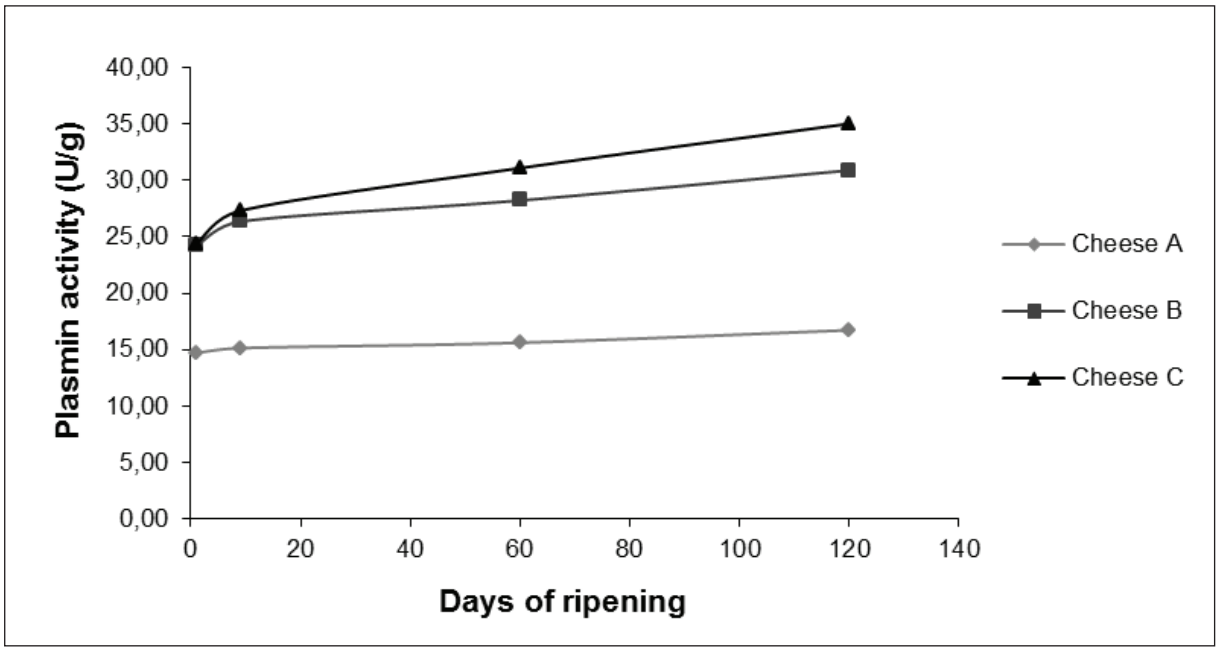

Figure 1. Plasmin activity expressed as Units/g (U/g) in Kefalotyri cheeses during ripening. Values are means of triplicate analyses of three trials. Cheese codes are presented in Table 1

3.3. Proteolysis

The evolution of the N-fractions during ripening are reported on Table 1. The total nitrogen in dry matter was not significantly different for cheeses A, B and C throughout ripening. Percentage pH 4.4-SN expressed on total nitrogen (pH 4.4-SN/TN%) (maturation index) and on dry matter and 12% TCA-SN expressed on total nitrogen (TCA-SN/TN%) and on dry matter, were higher in experimental cheese C, especially at the end of ripening, compared to the control cheeses. Control cheese A, had the lowest maturation index. The increased production of nitrogen fractions in cheese C during ripening is ascribed to the proteolytic process accelerated by the increased PL activity observed. Our findings are consistent with the observations of Farkye et al. [3] and McSweeney et al. [12].

3.4. Casein Hydrolysis

Plasmin cleaves β- and α_s- caseins into γ-casein fractions and several proteose-peptones. In our study a breakdown of αs- and β- caseins occurred in cheeses throughout maturation especially after 9 days of ripening, while more marked bands ascribed to γ-caseins were observed (Figure 2). The bands were more intense for cheese C, followed by those of cheeses B and A. These results were in good agreement with PL activity levels (Table 1). Inhibitor added in cheese A prevented, while urokinase in cheese C accelerated, the hydrolysis of caseins by plasmin.

4. Conclusion

Urokinase successfully activated PG in Kefalotyri cheese to PL, as indicated by increase in PL activity and concomitant decrease in PG level. Plasminogen activation occurred principally during 1st day of cheese making and then presented a progressive less increase up to 120 days of ripening. The resulting elevated plasmin activity led to accelerated proteolysis in Kefalotyri cheese as apparent from the levels of N-fractions and casein profiles of the cheese.

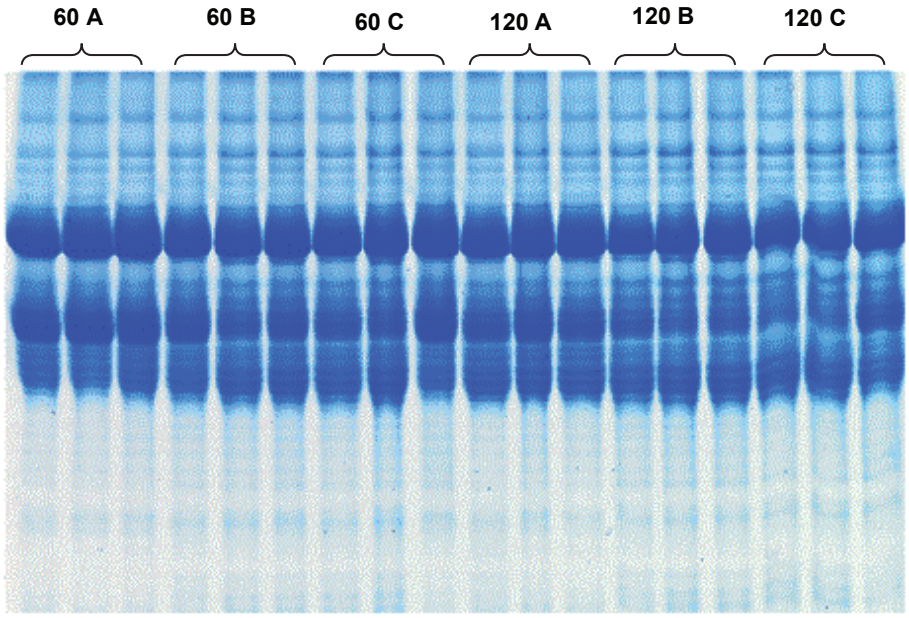

Figure 2. Urea-PAGE electrophoretograms of Kefalotyri cheese during ripening. Triplicates of each cheese sample were analyzed in each gel. Lanes 1-3, 4-6, 7-9: cheeses A, B and C at 60days of ripening; Lanes 10-12, 13-15, 16-18: cheeses A, B and C at 120days of ripening; Cheese codes are presented in Table 1

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3.22. (S3.65) Microbial Flora Associated with the Sardinian "Semicotto Caprino" goat cheese made from raw milk

F. Fancello[1], N.P. Mangia[1], M.A.Murgia[1], E.Floris[2], P. Deiana[1]

Summary

The microbial diversity of "semicotto caprino" cheese made from raw goat milk and without the use of any starter culture has been studied by the classical culturing method, and also by culture independent methods (16S rDNA-PCR TTGE). Raw milk, curd and cheese samples from four different farms were aseptically sampled at 3, 10, 20, 30, 60 and 90 days of ripening and investigated. The microbial counts showed that mesophilic lactobacilli and lactococci were the dominant flora throughout the entire ripening. The TTGE analysis of twelve different cheese batches showed that the microbial consortium associated with the "semicotto caprino" during the whole ripening was dominated by *Lactococcus lactis* and *Lactobacillus* spp.

1. Introduction

Dairy goat breeding has recently gained particular attention in Italy, especially because of the strong interest of consumers in the different traditional fresh and ripened raw milk cheeses. Goat breeding is relevant in Sardinia even though its potential is currently not fully expressed. This is due to a lack of local valorization of both goat milk and milk products such as ripened and fresh cheese and yogurt. The main milk product manufactured in Sardinia from goat milk is the "Semicotto Caprino" cheese, a semi-cooked cheese produced using non-pasteurized and pasteurized milk from the mixture of the evening and the morning milking. The natural microbial flora present in the raw milk can make a large contribution to the properties of raw milk cheeses, such as aroma and flavour, especially through their enzymatic action on cheese proteins and fatty acids. For this reason a full understanding of the microbial evolution in the cheese during ripening is crucial to improve cheese quality. Therefore, the aim of this work was to study the evolution of the microbial diversity within the "Semicotto Caprino" cheese during the ripening by standard culture dependent (microbial counts) and culture independent methods (16S rDNA-PCR TTGE profile).

2. Materials and Methods

The samples of "Semicotto Caprino" goat cheese analyzed in this study were obtained from three different batches made in four different cheese farmhouses. Raw milk, curd and cheese were aseptically sampled at 3, 10, 20, 30, 60, 90 days of ripening. Dilution of samples was made in a ¼ strength sterile ringer solution. Appropriate dilutions were inoculated onto the relevant plates. The following media and incubation conditions were used to enumerate microbial counts of samples. Total mesophilic counts (TMC) were determined on PCA (Oxoid) after incubation at 30°C for 3 days, lactococci were enumerated on M17 agar (Oxoid) after incubation at 22°C for 2 days, mesophilic and thermophilic lactobacilli were counted on MRS agar (Oxoid) after incubation at 30°C and 45°C, respectively, for 2 days, enterococci were counted on Slanetz & Bartley agar (Oxoid) after incubation in anaerobically condition at 37°C for 2 days, staphylococci coagulase positive and coagulase negative were counted in Baird Parker medium (Oxoid) supplemented with egg yolk tellurite emulsion (Oxoid) after incubation at 37°C for 2 days. Presumptive colonies of coagulase positive staphylococci were tested for confirmation with StaphyTest (Oxoid) and Coagulase Test (Microbiol). Enterobacteria were counted in Violet Red Bile Glucose Agar (Oxoid), after incubation at 37°C for 24 h, propionibacteria were counted on Yeast

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Extract Sodium Lactate agar Medium after incubation at 30°C for 7 days, *Pseudomonas* spp. were counted in PSA agar medium (Microbiol) after incubation at 22°C for 2 days, yeasts were enumerated on oxytetracycline glucose yeast extract agar (OGYE-Agar) and the plates were incubated at 22°C for 5 days. Results were expressed as log of colony forming units (cfu) per ml or gram of sample.

DNA extraction, PCR amplification of the V3 variable region of the 16s rDNA gene and TTGE analysis were performed as previously described [1], with the following minor differences: gels were stained with SBYR safe (Invitrogen), no identification ladder made up of reference species was utilized, bands were excised from gels after staining, eluted overnight in 50 µl of sterile water, re-amplified with the same primers used for 16S rDNA PCR without the GC-clamp and then sequenced.

3. Results and discussion

The evolution of the microbial flora based on three batches per farm were analyzed throughout the ripening. In Table 1 we report the composition and evolution of the average number of the different microbial groups. The microbial composition of the raw goat milk collected from different farms is highly variable, as highlighted by the high standard deviation values. Enterobacteria, *Pseudomonas* spp, mesophilic and thermophilic lactobacilli were the most variable populations. Similar results were obtained by other authors for raw goat and cow milk, Foschino et al. [2], Delbes et al. [3] respectively. On the contrary, the counts of the different microbial populations were rather similar between batches of the same farm, except for the enterobacteria group which was variable. This was most likely due to the different hygienic and sanitary conditions in the farms. Counts of total mesophilic bacteria reached the highest value at day three (9.8 log cfu/g of cheese), as did counts of mesophilic lactobacilli and lactococci, with maximal populations around 9.3 log cfu/g of cheese: these numbers tend to decrease by one logarithm unit towards the end of ripening. A similar pattern was followed by coagulase negative staphylococci. Coagulase positive staphylococci were detected in low numbers in milk and tended to disappear after 3 days of ripening. The number of propionibacteria increased from the first day and reached a peak after 20 days of ripening whereas yeast counts reached a maximum of 5.9 log cfu/g of cheese at 30 days of ripening.

Table 1: Average microbial count (in Log cfu/ml or g) and standard deviation of diverse microbial groups along manufacturing and ripening stage of 12 batches made in four different farmhouses (three batches per farmhouse)

Microbial Groups	Raw milk	Curd	C3	C10	C20	C30	C60	C90
TMC	6,29 ± 0,94	7,31 ± 0,22	9,68 ± 0,10	9,40 ± 0,16	9,10 ± 0,24	9,06 ± 0,06	8,66 ± 0,29	8,65 ± 0,32
Mesophilic lactobacilli	5,18 ± 1,27	6,33 ± 0,50	9,31 ± 0,08	9,16 ± 0,06	9,00 ± 0,32	8,84 ± 0,08	8,40 ± 0,46	8,47 ± 0,14
Thermophilic lactobacilli	3,29 ± 1,27	4,91 ± 1,10	7,35 ± 0,84	7,29 ± 0,62	7,46 ± 0,65	7,35 ± 0,56	7,49 ± 0,61	7,68 ± 0,54
Enterococci	3,22 ± 1,08	4,73 ± 0,43	7,63 ± 0,43	7,72 ± 0,39	7,69 ± 0,51	7,90 ± 0,38	7,87 ± 0,41	7,86 ± 0,36
Lactococci	6,08 ± 0,83	6,91 ± 0,43	9,39 ± 0,03	9,24 ± 0,09	9,05 ± 0,21	8,81 ± 0,21	8,66 ± 0,09	8,35 ± 0,06
Enterobacteria	3,11 ± 1,97	4,59 ± 2,71	6,57 ± 1,92	5,56 ± 1,58	5,55 ± 0,46	3,72 ± 0,56	1,02 ± 1,76	1,02 ± 1,76
Propionibacteria	3,87 ± 0,54	4,38 ± 0,14	5,57 ± 0,39	5,30 ± 0,60	5,38 ± 1,51	4,75 ± 2,40	3,78 ± 1,76	3,52 ± 3,06
CNS	4,13 ± 1,05	4,67 ± 1,25	6,10 ± 0,58	6,73 ± 0,37	6,32 ± 0,41	6,50 ± 0,40	6,34 ± 0,60	6,52 ± 0,50
CPS	1,78 ± 0,74	2,09 ± 0,88	0,43 ± 0,74	0,47 ± 0,81	0,44 ± 0,76	0,64 ± 1,11	0,00 ± 0,00	0,00 ± 0,00
Pseudomonas spp.	3,15 ± 2,74	4,38 ± 1,52	3,29 ± 0,85	1,71 ± 1,48	1,29 ± 1,55	0,97 ± 0,89	0,54 ± 0,47	0,33 ± 0,57
Yeast	3,08 ± 0,78	3,79 ± 0,57	5,28 ± 1,74	5,82 ± 0,47	5,84 ± 0,14	5,90 ± 1,15	4,85 ± 0,55	4,84 ± 0,91

TMC: Total mesophilic count; CNS: Coagulase Negative Staphylococci; CPS: Coagulase Positive Staphylococci; C3, C10, C20, C30, C60, C90: cheese at the 3, 10, 20, 30, 60, 90 days of ripening.

Samples of raw milk, curd and cheese at 3, 10, 20, 30, 60, and 90 days of ripening of the 12 batches were analyzed by TTGE. As an example, the fingerprint obtained using the V3 variable region of the 16S rDNA for one of the batches is presented in figure 1. The electrophoresis profiles of the raw milk showed the presence of three principal bands which were identified as *Lactococcus lactis* (band m), *Enterococcus faecalis* (band g) and *Escherichia coli* (band l). During the ripening, the most prominent band in all samples was that of *Lactococcus lactis*. The intensity of the band, as seen in the figure 1, increased throughout the ripening period. The prevalence of recovery of this species is mostly in agreement with previous findings [4,5]. Two other bands were also detected during ripening, one faint band in all samples corresponding to *Lactobacillus plantarum*, and an intense one corresponding to *Enterococcus faecium*. Other bands detected only in some samples were identified as *Leuconostoc mesenteroides*, *Staphylococcus xilosus* and *Hafnia alvei*. An intense band identified as *Lactobacillus casei* was only detected at 90 days of ripening. This species together with *L. plantarum* and others LAB species belonging to non-starter lactic acid bacteria (NSLAB) can play an important role in the formation of the aroma and taste of cheese.

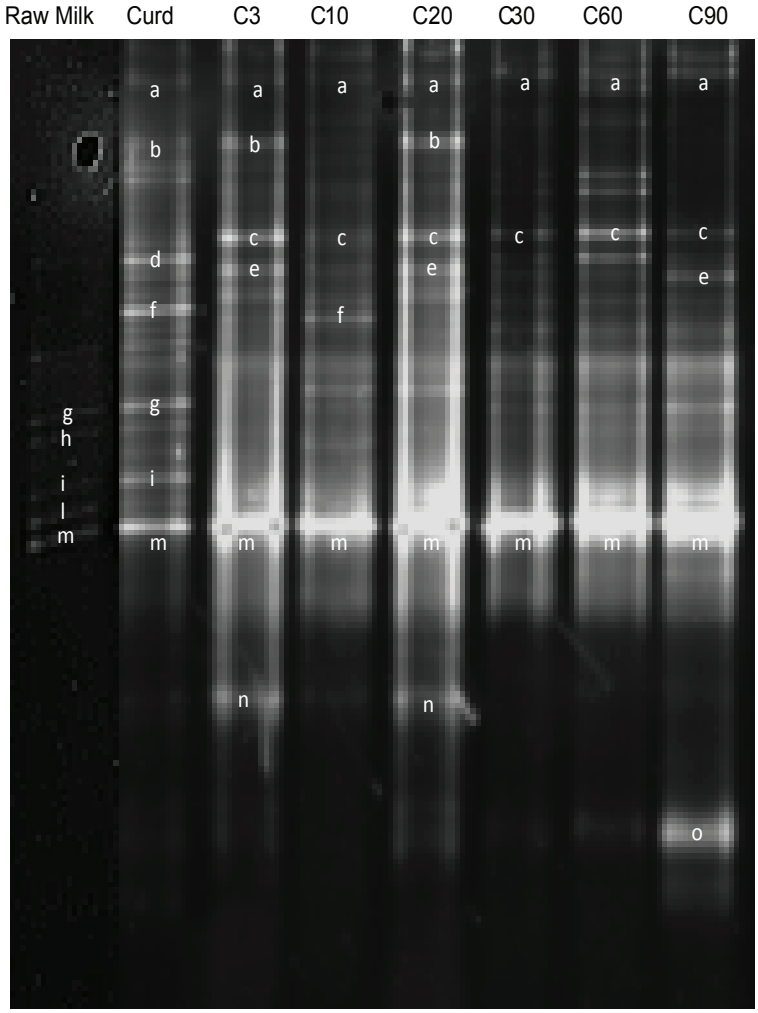

Figure 1. TTGE analysis of V3 16s rDNA fragments from samples of one batch of "Semicotto Caprino" goat cheese at various stages of manufacturing and ripening. Bands indicated by letters were selected for sequencing. **A**, *Lactobacillus plantarum*; **b**, *Leuconostoc mesenteroides*; **c**, *Enterococcus faecium*; **d** and **e**, sequencing failure; **f**, *Staphylococcus xilosus*; **g**, *Enterococcus faecalis*; **h** and **i**, sequencing failure; **l**, *Escherichia coli*; **m** *Lactococcus lactis*; **n**, *Hafnia alvei*; **o**, *Lactobacillus casei*

4. Conclusion

This study provides a complete overview of the composition of the microbial flora in "Semicotto Caprino" cheese obtained using a polyphasic approach combining culture-dependent and independent methods. Lactic acid bacterial species were the predominant species during ripening and among this group Lactococcus lactis was the most abundant species.

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3.23. (S3.71) Detection of Caprine Milk in Ovine Milk

S. Alexandraki[1], G. Moatsou[1]

Summary

The aim of the present study was the determination of the percentage of bovine, ovine and caprine milk in mixtures. The para-κ-casein fraction was prepared by rennet treatment of the above milk mixtures. Analysis of the curds was carried out by means of cation-exchange HPLC. Para-κ-casein retention times were verified, using hydrolysates of crude preparation of κ-casein from the three kinds of milk. The quantitative determination of the composition of the mixtures was successful, based on the area of the para-κ-casein chromatographic peaks which were standardized taking into account the differences in the protein content of the milks. The results showed that para-κ-caseins of the three different types of milk were clearly separated. Although the differences between caprine and ovine para-κ-casein are limited, an average difference of 1.6 minutes between the two retention times with low standard deviations (less than 4% of the average) was observed.

1. Introduction

The problem of adding bovine milk or proteins to cheesemilk has been addressed in detail by the official method of isoelectric focusing of γ-casein. However, the discrimination between the proteins of goat and sheep milk is difficult, since they have very few differences. Also, it is known that the application of isoelectric focusing for the separation of their para-κ-caseins does not give safe results due to the occurrence of multiple overlapping zones [2]. Thus, the present study was designed to identify and quantify the type of milk in mixtures based on para-kappa casein, using an easily applicable chromatographic method [1, 3].

2. Materials and Methods

Four milk mixtures were prepared in triplicate (w/w, Table 1).

Table 1: Codes, composition and reconstitution concentrations of experimental milk mixtures

Code	Composition	Concentration of reconstitution g/40 ml
M2	70% ovine + 30% caprine milk	4.16
M3	50% ovine + 50% caprine milk	4.00
M4	40% ovine + 40% caprine + 20% bovine milk	3.84
M5	50% ovine + 50% bovine milk	3.80

The para-κ-casein fraction was prepared by rennet treatment of the above milk mixtures as described below. Lyophilized mixtures of milk were reconstituted in 40 ml of distilled water followed by good stirring and heating at 35oC. At this temperature an aqueous solution of rennet 5% (800 μl) was added. After incubation at 37oC for 1 h, the curd was cut and placed in a fabric filter to drain. The next day, 5 g of the curd was dissolved in 50 ml trisodium citrate buffer 0.1 M, pH 6.6 with continuous stirring and mild heating until the solution was clear. Subsequently,

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casein was precipitated by adjusting the pH of the sample to 4.6 with HCl. After centrifugation, the supernatant was rejected and 2 g of the sediment dissolved in 14 ml of the following buffer: 5 M urea, 10 mM malonic acid, pH 6.0 adjusted using NaOH 1 N. In the sample 210 µl mercaptoethanol was added followed by dilution with intense stirring and heating. When the sample was fully dissolved, the pH was adjusted to 6.0 with NaOH. The last step of this procedure was the filtration of the sample through 0.45 mm filter .

Analysis of the curds was carried out by means of cation-exchange HPLC using a Macrosphere Ion Exchange Column WCX 7 µm (Alltech Associates, Inc., IL 60015). The results were processed by MILLENIUM v.2.15 (1994, WATERS) software. The following buffers were used: A, 5 M urea, 10 mM malonic acid, pH 6.0 using NaOH 1 N; B, 5 M urea, 10 mM malonic acid, 0.5 M NaCl, pH 6.0 using NaOH 1 N. The elution was at room temperature at 1 ml/min using solvents A and B as follows: 100% A for 5 min, 0-80% B in 20 min, 80-100% B in 6 min, 100 % B for 7 min, 0-100% A in 1 min and finally 100% A for 21 min. The volume of the sample was 130 ml. The eluate was monitored at 280 nm. All assays were in triplicate. Para-κ-casein retention times were verified, using hydrolysates of crude preparation of κ-casein from the three kinds of milk. The quantitative determination of the composition of the mixtures was based on the area of the para-κ-casein chromatographic peaks, which were standardized taking into account the differences in the protein content of the milk used.

3. Results

The results showed that para-κ-casein of the three different types of milk were clearly separated (Figure 1). Although the differences between caprine and ovine para-κ-casein are limited, an average difference of 1.6 minutes between the two retention times with low deviations (less than 4% of the average) was observed (Table 2). The results of the quantitative determination of the composition of the mixtures are presented (Table 3).

Table 2: Para-κ-caseins retention times (min) of the three kinds of milk

Ovine	Caprine	Bovine
18.726 ± 0.801	17.065 ± 0.410	25.014 ± 0.920

Table 3: Identification of ovine, caprine and bovine para-κ-casein in standard mixtures of ovine (O), caprine (C) and bovine (B) milk (averages of 3 experimental preparations ± SD)

Code / composition of mixtures	Para-κ-casein peak area ($\times 10^6$)		
	Ovine	Caprine	Bovine
M2: 70%O + 30%C	70.7 ± 3.3	29.3 ± 3.3	-
M3: 50%O + 50%C	51.9 ± 9.1	48.1 ± 9.1	-
M4: 40%O + 40%C + 20%B	42.0 ± 4.5	41.9 ± 5.1	16.1 ± 0.6
M5: 50%O + 50B	53.8 ± 7.6	-	46.2 ± 7.6

4. Conclusion

In conclusion, para-κ-casein is a reliable indicator for determining the type of milk. The method used was effective for the separation of para-κ-caseins in sheep and goat milk mixtures, although they differ only in 5 amino acids. The advantages of this method include the speed and the relatively simple analytical equipment required. In addition, cow's milk can be detected simultaneously. Finally, it should be noted that the column was very stable throughout the analysis, and no problems were noticed in respect to the system pressure and the pH of the eluates.

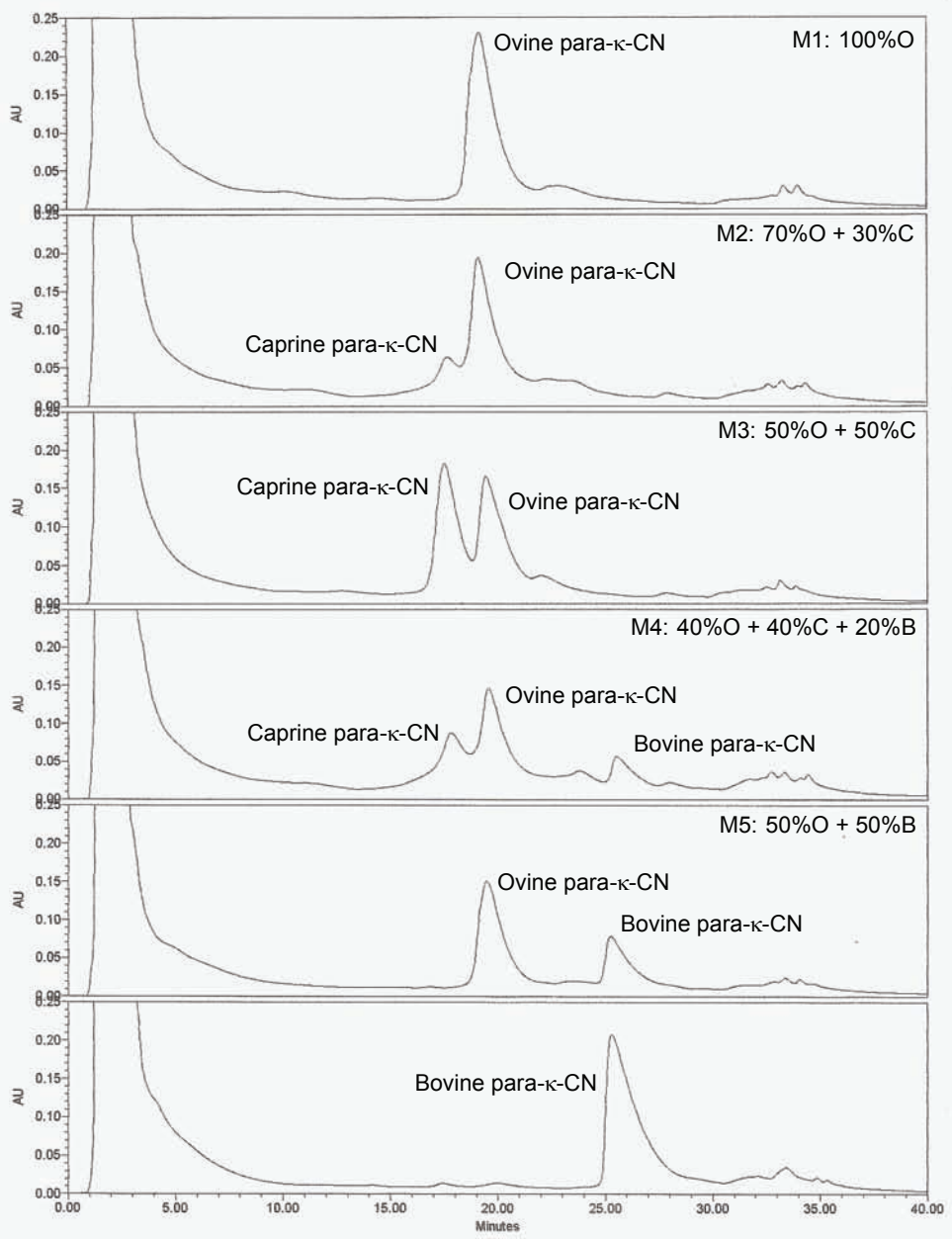

Figure 1. Chromatographic profiles of the paracasein fraction of standard mixtures of ovine (O), caprine (C) and bovine (B) milk

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Session 4: Characteristics and Nutritional Value Of Ewe, Goat and Other Non-Cow Milk and Milk Products

Posters

4.1. (S4.1) Origin and Control of The Typical Goat Flavour: Example of French Cheeses

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Summary

Goat milk cheeses are characterized by their unique sensorial properties and especially their typical goat flavor. Compounds such as the 4 ethyl octanoic acid (thresholds, concentration in milk according to lactation stages and diets), the impact of technological steps of cheese making and the "quality" of the lipolysis are presented as the main contributors of goat flavour. Emphasis is placed on the optimum ratio between 4 ethyloctanoic and other free fatty acids to induce a favorable typical goat flavor. The results concerning the impact of surface ripening strains (*Penicillium camemberti, Geotrichum candidum, yeasts*…) in relation to the technological parameters and biochemical composition of cheeses are also discussed. Finally, the most recent studies carried out using an edible lactic cheese model for rapid screening of the ripening strains for their aromatic potentiality are presented.

1. Introduction

Goat milk cheeses are characterized by their typical goat flavour. One of the most influential compounds is 4 ethyl C8 acid with very low perception thresholds. Parameters controlling its release in goat milk cheeses are complex and were studied at Actilait. These parameters are the impact of technological steps, the ripening strains but also its total content in milk according to lactation stage and feeding of the goats.

2. Perception thresholds

Typical goat flavour is mainly linked to specific free fatty acids: caproïc (C6:0), caprylic (C8:0) and capric (C10:0) acids but the main marker is 4-ethyloctanoïc acid (4-Ethyl C8) [1, 2] with very low threshold values (table 1).

Table 1: Some perception thresholds according to Le Quéré et al (1996)

Free Fatty acids	Perception thresholdin water (µg/l)	Perception thresholdin oil (µg/l)
C8	5,8 - 19	10 - 350
4-methyl C8	0,02	-
4-ethyl C8	0,0018	0,006 – 2,4

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In order to evaluate the threshold level for 4 ethyl octanoïc acid in dairy matrices, the latter was added to cow milk products free of this compound: soft white cheese and UHT skim milk (Jaubert and Gaborit 1997, unpublished data). The required concentration to obtain a score of 5/10 for goat flavour (sensory analysis carried out with a trained panel) varied from about 1 ppm in skimmed milk to more than 10 ppm in soft white cheese with 40% fat in dry matter (Figure 1).

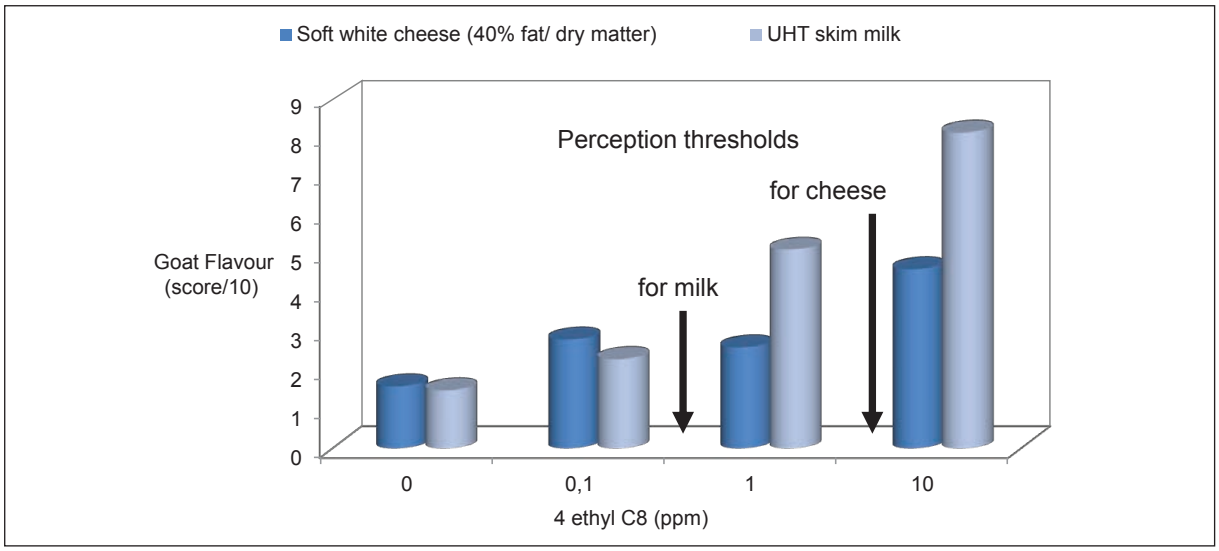

Figure 1. Perception levels in milk products according to Jaubert and Gaborit (unpublished)

3. Impact of cheese making parameters

In milk and dairy products, fatty acids are included in triacyglycerol molecules; the main condition for flavour perception is the release of these fatty acids from the triacyglycerol molecule by the lipases. It can be either lipase from milk (LPL) or from microorganisms. Their activities vary according to physiological stage, genetic, sanitary status, etc… and can be amplified by milk processing (homogenisation, cold storage…). Concerning cheeses, lipase activity is attributed mainly to the surface mold and yeast ripening strains, especially for the French soft surface mold ripened cheeses. The main ripening strains encountered on French soft cheeses made with goat milk are yeasts, *Geotrichum candidum* and *Penicillium*. The specific lipolytic behaviour of ripening strains and the predominant role for goat flavour development of *G candidum* in ripened goat cheeses in comparison to *Penicillium camemberti* was shown [3] (Figure 2), by specifically releasing 4 ethyl octanoic acid (more than 0,25‰ of free fatty acids for *G. candidum* against about 0,15‰ for *P camemberti*).

The analysis of the free fatty acids [4] showed that goat flavour intensity was directly correlated to the concentration of 4 ethyl octanoïc acid up to a level of lipolysis 10 g Oleic Acid per 100 g Fat, equivalent to a concentration of total free fatty acids of 20 g/kg (Figure 3). Beyond this threshold, defects occurred such as rancid-lipolysed flavour at the expense of goat flavour. The expression of the aromatic potential of these ripening strains also depends on technological conditions of manufacture such as moisture of non fat cheese [3] and therefore on the type of cheese, lactic cheese or camembert type cheese (Figure 2).

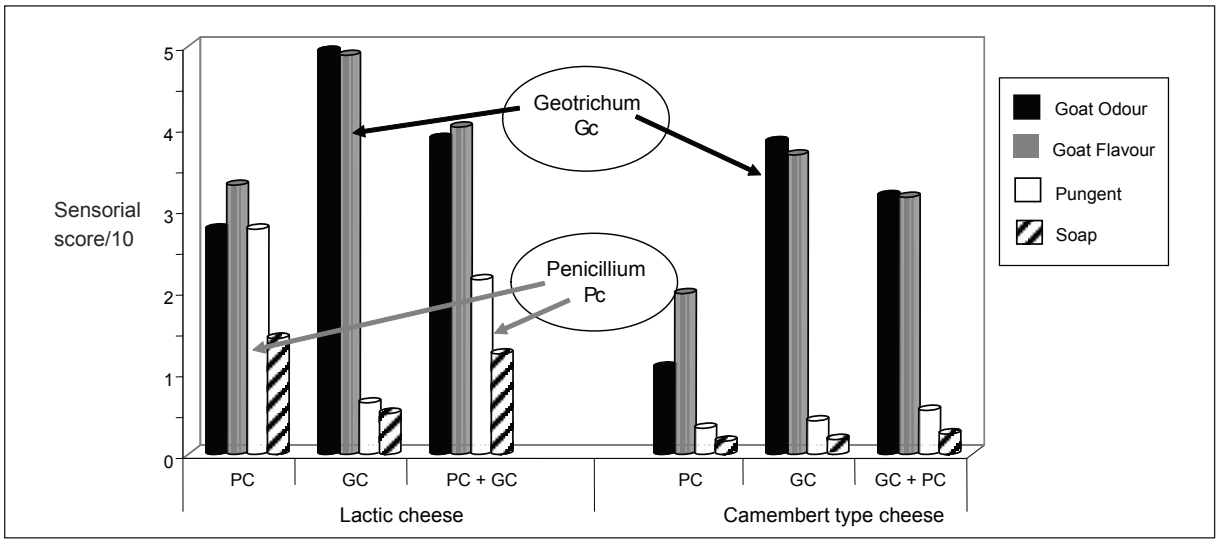

Figure 2. Influence of the cheese making (lactic or camembert type cheese) and ripening strains (Penicillium camemberti and Geotrichum candidum) on the sensorial characteristics of cheeses

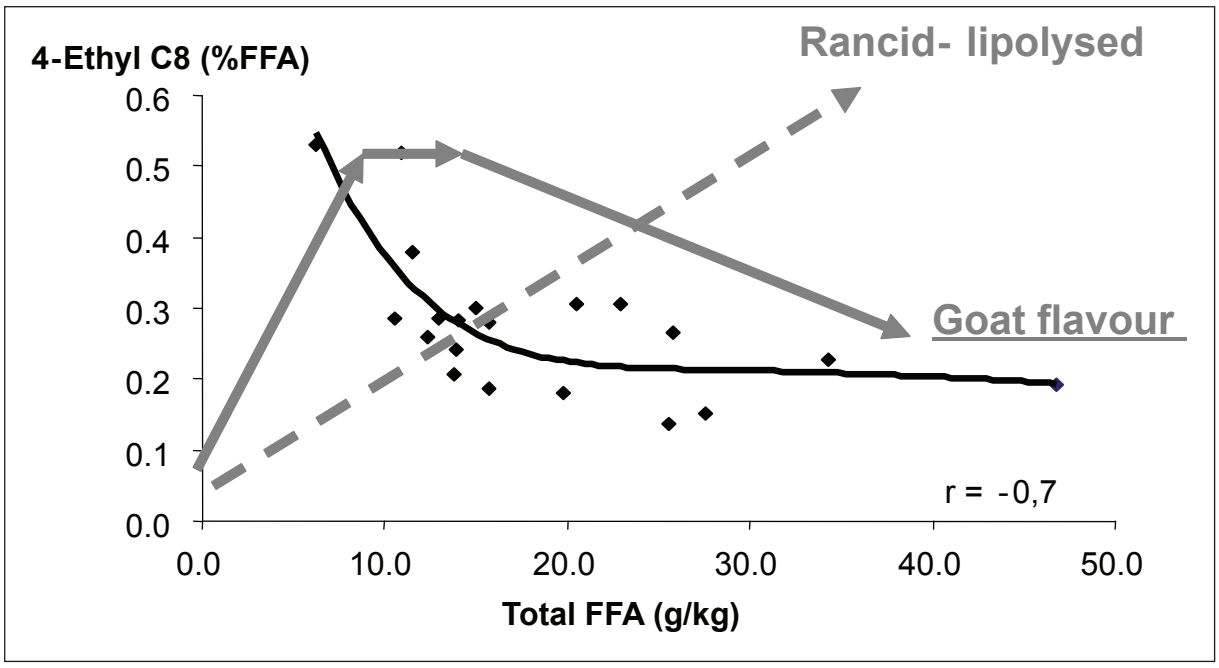

Figure 3. Influence of the proportion of free 4 ethyl C8 acid on the sensorial characteristics of cheeses

4. Origin of 4 ethyl octanoic acid

Finally, with the aim of better understanding and controlling goat flavour, the total content of 4 ethyl C8 acid present in goat milk was recently studied by Actilait. The minimum values were observed at the beginning of lactation (Figure 4) whatever the goat feed (Dehydrated Hay, Corn Silage or Pasture). These data complete those of Lamberet et al. [5] who also studied levels of total 4 ethyl octanoic acid in goat milks, not according to the lactation stage but according to the alpha s1-casein genotype (0.019 % and 0.015 % of 4 ethylC8 for FF and AA genotypes respectively).

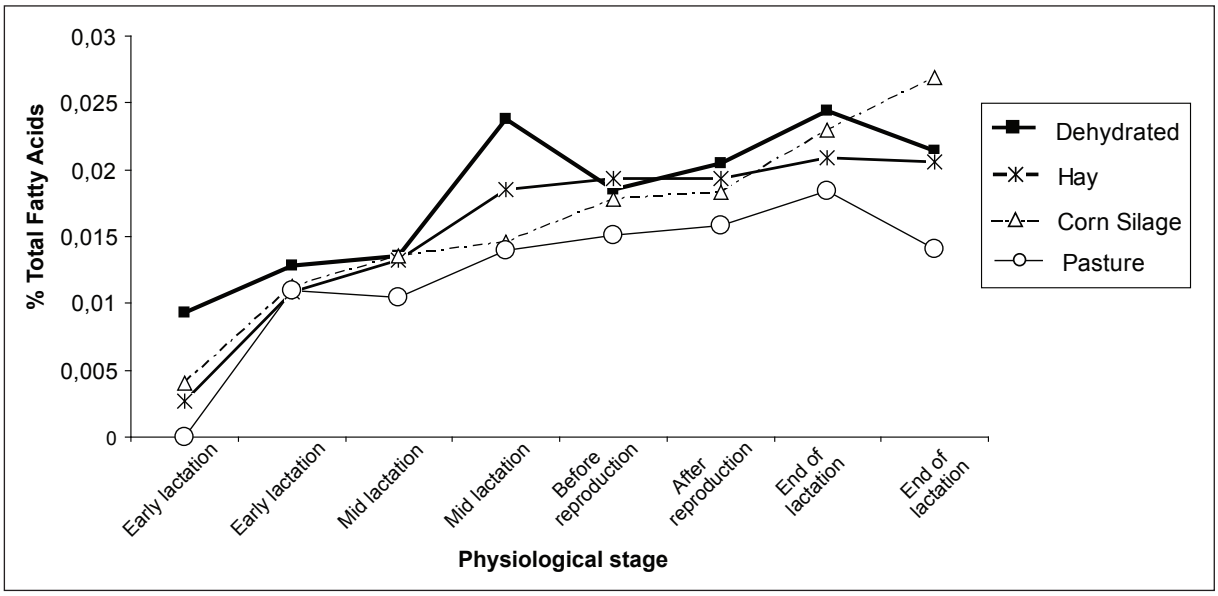

Figure 4. Variability of total 4 ethylC8 content in milk according to lactation stage and goat feeding (4 herds per group)

5. Conclusion and perspectives

All these studies help towards understanding goat flavour of cheeses but further work is needed to evaluate the possible synergy between total 4EthylC8 in milk and its release by the lipase of technological yeast and mold strains. Also, it is important to understand the 4 ethyl octanoic acid origin to understand the factors acting on its biosynthesis better. Reliable data are also needed concerning the exact position of 4ethyl C8 on triacylglycerol (variability) and the link with specificity of the lipases.

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4.2. (S4.2) Study of Sensorial Characteristics of Ripening Strain on a New Edible Goat Milk Model Cheese

P. Gaborit[1], K. Raynal-Ljutovac[1]

Abstract

The flavor of soft surface-ripened goat milk cheeses is related to the activity of ripening strains. In order to evaluate rapidly, effectively and without significant cost the aromatic potential of ripening strains an edible experimental lactic cheese was set up, taking into account goat cheese specificities (goat milkfat and lactic technology),. Lactic curd, prepared with standardized pasteurized goat milk, was frozen or freeze dried and stored at -20°C. Frozen, reconstituted freeze dried curd and fresh control curds were ripened with *Penicillium* or *Geotrichum*, with or without yeasts, in Petri dishes at 12°C (= model cheeses) and compared to classical cheeses. Model cheeses enabled a ripening process twice as fast as that observed during cheese making with similar biochemical (proteolysis and lipolysis) and sensory patterns. A rapid screening of genus, species and strains specificities was possible, especially concerning goaty descriptors but also yeast and mould morphology in the mature cheese.

1. Introduction

Flavor of soft surface-ripened goat milk cheeses is mainly linked to the activity of their ripening strains. Many models, based on cow milk components, have been used or developed to study strains or specific compounds [1-4] but these models do not meet our requirements in respect to goat milkfat in the raw material, lactic cheese composition and edible product. Therefore, in order to evaluate rapidly, effectively and with minimal costs the aromatic potential of ripening strains, an edible experimental lactic cheese was set up.

2. Material and methods

After lactic coagulation of standardised pasteurized bulk goat milk, curd was either frozen or freeze dried and then stored at -20°C. The model cheese, made with frozen curd or reconstituted from freeze dried curd to get the same characteristics as the control (43% dry matter for fresh curd) and all were salted at 1.3 %. Model cheeses inoculated with ripening strains were poured into large Petri dishes (140 mm diameter with a non hermetical lid) and ripened at 12°C (Figure 1). All the trials were compared to classic lactic buchette-type (small log-shaped) cheeses ripened with the same strains. The strains used in the study were commercial strains of Geotrichum candidum (Gc), Penicillium camemberti (Pc), Debaryomyces hansenii (Dh) and Kluyveromyces lactis (Kl). At this stage only mixtures (two strains) were studied that are related to the cheese making process:

2 cocktails with Pc as the main strain (Pc at 2 doses/ 100 kg of curd + Gc at 1 dose/ 100 kg of curd): PC Neige + GEO17 and PC02 + GCM33 (Choozit - Danisco, Dangé St Romain, France)

2 cocktails with Gc as the main strain (Gc at 2 doses/ 100kg of curd + yeast at 1 dose/ 100 kg of curd): GCD (Cargill, La Ferté-sous-Jouarre, France) + DhR (Cargill) and GEO18 (Danisco) + KL71 (Danisco).

The ripening tests made on reconstituted model cheeses, including visual development, sensorial analysis, microbiological and biochemical characterisations were conducted on curds stored 0, 1, 3, 6 and 9 months at -20°C.

Total solids (TS) were assessed [5]. Total nitrogen (TN), non casein nitrogen (NCN) and Non Protein Nitrogen (NPN) were prepared according to [6] and analysed according to Kjeldhal

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method. Fat was measured according to [7] and lipolysis according to a procedure adapted from [8]. pH was also measured.

The total bacteria [9] and yeast and moulds [10] were assessed. The absence of the main pathogens *E. coli*, *L. monocytogenes*, *Salmonella* and *S. aureus* was checked before each sensorial analysis.

Sensorial analysis was performed with 12 trained panelists of the sensorial department of ENILIA (Surgères, France), specialised for goat milks products, who evaluated the samples according to [11].

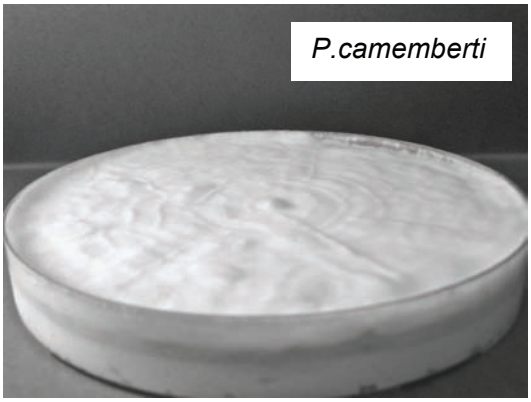

Figure 1. Example of model cheese in Petri dish (140 mm diameter) ripened with *Penicillium camemberti*

3. Results and discussion

Lipolysis and proteolysis levels observed for fresh curd and stored frozen and freeze dried curds indicated a faster expression of enzymatic potential of the surface ripening strains on the model cheese, compared to a classical buchette-type lactic cheese making. Actually, at 12 and 26 days after the inoculation of ripening strains, the proteolysis and lipolysis levels of the model cheese corresponded to those observed in ½ shelf life cheeses (about 20-25 days after inoculation = average consumption stage) and at the end of cheese shelf-life (40-45 days after inoculation), respectively. For instance, average lipolysis levels were about 5,5 g oleic acid/ 100 g fat for both *Geotrichum candidum* strains grown 12 days on freeze dried curd (whatever the cold storage time before use) and Gc grown 20 days on buchette-type cheese. The early expression of the ripening strains can be related to a large contact area of the ripening strain surface with its substrate in the Petri dish and to the very low dehydration throughout the ripening (very low increase in dry matter, data not shown).

For each cocktail of strains, the overall intensities of odor and flavor were very similar for all the tested curds, either cold stored or fresh curd. As for the biochemical characteristics, the intensities of odor and flavor descriptors at day 12 and 26 were close to those of a ½ shelf life (Figure 2) and an end-shelf life cheese respectively.

A significant differentiation was observed not only between genera but also between strains of ripening microorganisms, concerning both expression of their potential enzymatic proteolysis and lipolysis and their sensory specificities (Figure 3). For example, G2 strain specifically released more free 4-ethyl octanoïc acid (0,14 ‰ of total free fatty acids) than G1 (0,03 ‰). This was related to the sensory profile meaning a higher intensity of the goaty descriptors for G2.

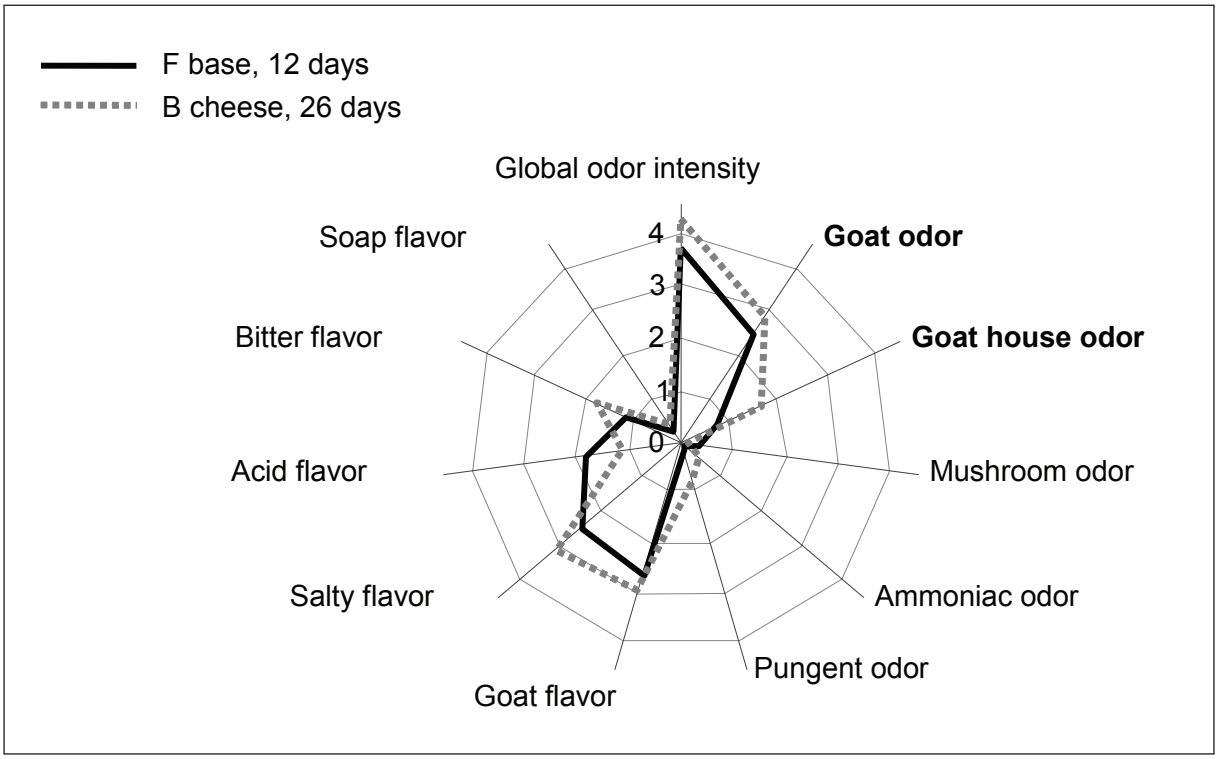

Figure 2. Sensory profiles obtained using a mixture of *Geotrichum candidum* + yeast grown on freeze (F) dried curd in Petri dish (solid line) and lactic buchette-type (B) cheese (dotted line)

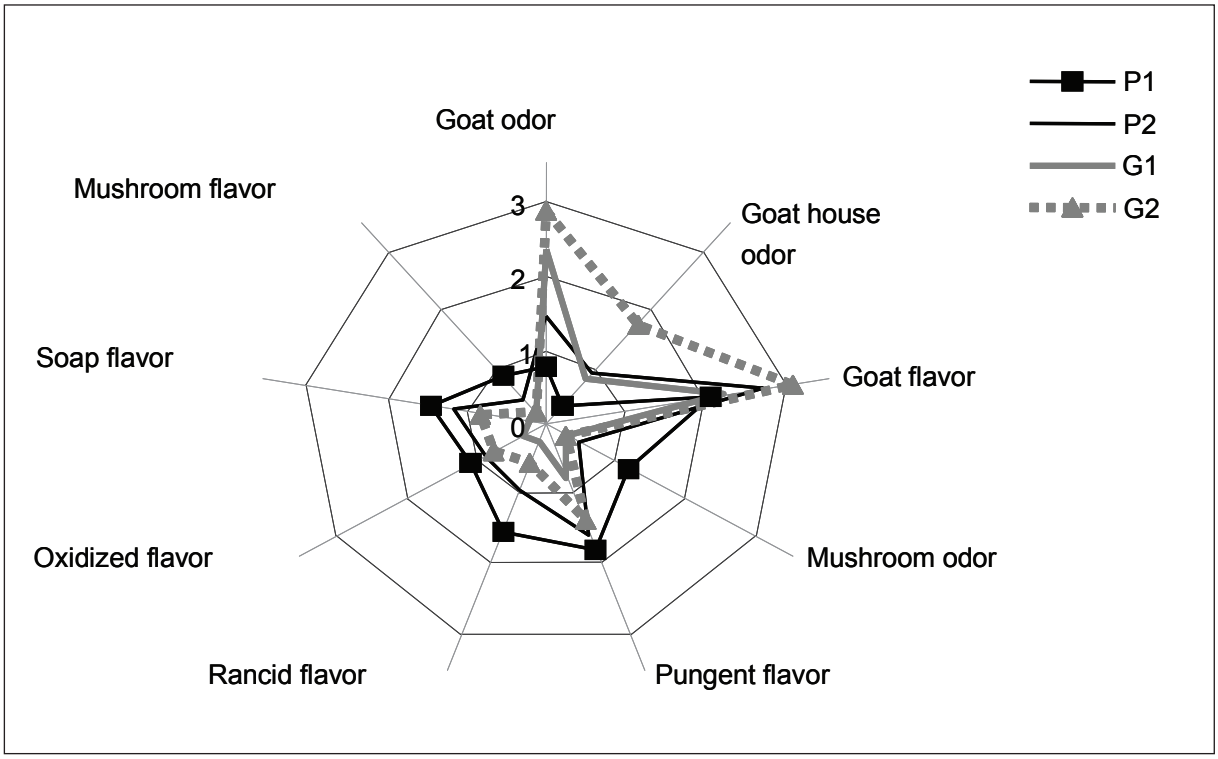

Figure 3. Sensorial profiles of strains of *Penicillium camemberti* (P1 and P2) or *Geotrichum candidum* (G1 and G2) (Petri dish) at day 12 and after a 9 month cold storage of freeze dried curd

4. Conclusion

Owing to this innovative work in goat milk cheese field, surface ripening strains can be rapidly evaluated for their flavour and odour but also for the yeast and mould morphology in the ripened lactic goat milk cheeses. Depending on the ripening time, the model cheese may also amplify the sensory characteristics of the strains, which enable the anticipation of their behaviour when technological parameters are changed (ripening temperature, dry matter and salt content). This model could be appropriate for the screening of strains that are not used nowadays but which could be of technological interest.

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4.3. (S4.6) The Nutritional Value of Trahanas : A Fermented Milk-Cereal Traditional Greek Food

A. Georgala[1], E. Anastasaki[1], D. Xitos[1], D. Kapogiannis[1], T. Malouxos[2], T. Massouras[1], I. Kandarakis[+1]

Summary

Trahanas is one of the most popular fermented milk-cereal products in Greece which is produced during summer mainly from whole fresh ewes', goats' milk or a mixture of them. Acidified (sour) milk or sweet milk is used and the final products are called sour or sweet trahanas, respectively. Sometimes instead of milk a pulp of vegetables is used and the product is called nistisimos trahanas.

40 trahanas samples were examined for their gross composition, fatty acid and mineral composition. The most significant differences were found to be in the amount of total saturated fatty acids (TSFAs) and total unsaturated fatty acids (TUFAs) and the mineral calcium.

1. Introduction

Traditional dried fermented milk/cereal foods are widely used in the diet of people in the Middle East, Asia, Africa and some parts of Europe. These products have high nutritive value and interesting organoleptic characteristics. There are similar products with different names such as 'Kishk' in Egypt, Syria, Lebanon and Jordan, 'Kushuk' in Iraq, 'Tarhana' in Turkey, 'Trahanas' in Cyprus and Greece, 'Tarhonya/Talkuna' in Hungary and Finland [1, 3, 4]. Trahanas is one of the most popular fermented milk-cereal products of Greece which is produced during summer mainly from whole fresh ewes', goats' milk or a mixture of them. Sometimes instead of milk a pulp of vegetables is used and the product taken is called nistisimos trahanas. For the production of trahanas fresh milk is allowed to be acidified for some days either spontaneously or by adding a culture of yoghurt. It is stirred every day until it reaches the desired acidity. Then it is heated and some ground wheat and salt are added gradually. The mixture is heated thereafter to the boiling point and cooled down. The paste taken is cut in finger sized pieces and subsequently sun-dried. When it is dry enough, it is stored in a cool place. Sometimes instead of sour milk, sweet milk is used for the production of sweet trahana [1, 3, 5]. In this study the gross, fatty acid and mineral composition of 40 trahanas samples was studied.

2. Materials and Methods

40 trahanas samples purchased from local market, dairy companies or home made were studied. The methods used were : 1. Moisture content : drying of samples at 105°C for 24 h. 2. Ash content : samples were ashed at 550 ± 5°C for 6 hours. 3. Total crude fat content : Soxhlet method. 4. Salt content : BSI method [2]. 5. Dietary fiber : Fibertec System. 6. Protein content : Kjeldhal method. 7. Fatty acid content : Gas chromatography (GC) after methylation of the lipid extraction taken by the Sohxlet method. 8. Minerals : in ash using atomic absorption.

3. Results and Discussion

The mean composition of 'nistisimos' trahanas was: moisture 11.1, total solids 88.9, total carbohydrates 70.5 g/100g product, protein 11.9, fat 2.9, dietary fiber 1.4, ash 2.5, salt 1.6

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g/100g dry matter while for the 'sour' product, 'sour with eggs' and 'sweet' trahanas it was : moisture 9.8, total solids 90.2, total carbohydrates 67.6 g/100 g product, protein 15.3, fat 3.9, dietary fiber 1.5, ash 3.2 and salt 1.9 g/100 g dry matter (Table 1). The variation in moisture and fat content is probably due to the ingredients used in the formulation and the production technology for trahanas. The mean protein content of trahanas made with milk was higher than that of nistisimos trahanas due to the presence of milk. The highest ash content was found in sour trahanas made with eggs. The different flours usually used for trahanas making are mainly responsible for the great variation in ash content. The variation in salt content was mainly due to the fact that different amounts of salt are added by trahana makers. The variation in dietary fiber content was due to the fact that different kinds of wheat flour are used for its production. Wheat germ and wheat flour are known to be good sources of carbohydrates: that is a reason for the high concentration of carbohydrates in trahanas samples.

In trahanas made with milk the TSFAs were the 58.4% and the TUFAs the 41.7, % w/w. In trahanas made without milk (nistisimos trahanas) the TSFAs were the 25.9% and the TUFAs the 74.1, % w/w. Nistisimos trahanas had the highest amount of total unsaturated fatty acids (74.1%) (Table 1). In trahanas made with milk the mean mineral composition composition was: calcium 96.0, magnesium 64.7, potassium 304.2, natrium 1510.0 mg/100 g dry matter, manganese 1112.0, copper 352.0, lead 12.1 μg/100 g dry matter, while in trahanas made without milk it was : calcium 51.2, magnesium 70.8, potassium 319.4, natrium 1230,0, mg/100 g dry matter, manganese 829.0, copper 465.5, lead 6.0, μg/100 g dry matter. The lowest amount of calcium was observed in nistisimos trahanas due to the absence of milk (Table 1).

Table 1: Composition of different Greek Trahanas samples

	Types of Trahanas							
	Trahanas without milk		Trahanas made with milk					
	'Nistisimos trahanas'		'Sour trahanas without eggs'		'Sour trahanas with eggs'		'Sweet trahanas'	
Chemical composition	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Moisture (%)	11.1	1.6	10.7	2.2	9.2	1.4	9.6	2.2
Total Solids (%)	88.9	1.6	89.3	2.2	90.8	1.4	90.4	2.2
Protein[a]	11.9	0.7	15.3	1.1	15.3	1.1	15.3	2.2
Fat[a]	2.9	2.6	4.3	2.0	3.1	0.9	4.4	3.0
Total Carbohydrates[b]	70.5	2.1	65.2	4.0	69.4	3.1	68.0	5.9
Dietary Fiber[a]	1.4	0.5	1.4	0.6	1.0	0.2	2.1	0.8
Ash[a]	2.5	1.1	4.1	1.6	2.6	1.0	2.9	1.0
Salt[a]	1.6	1.1	2.6	1.5	1.7	1.1	1.4	0.9
Fatty acids (% w/w)								
SFA	25.9	6.64	63.4	0.9	54.7	0.4	57.0	2.1
MUFA	24.3	6.40	22.1	1.3	24.9	0.7	23.3	2.1
PUFA	49.8	2.34	14.5	1.0	20.4	0.7	19.8	3.0
UFA (MUFA+PUFA)	74.1	5.0	36.6	1.0	45.3	0.5	43.1	2.5
Minerals								
Calcium (Ca) *	51.2	28.5	118.6	49.3	78.3	9.1	91.2	46.2
Magnesium (Mg) *	70.8	55.4	60.2	20.4	45.4	15.5	88.4	44.3
Potassium (K) *	319.4	68.0	328.9	115.9	235.4	41.2	348.4	159.4
Sodium (Na) *	1230	931	2358	1577	1017	795.7	1154	910.3
Maganese (Mn) **	829	458.4	1238	619.8	559.1	206.4	1539	657.4

a : Values were g/100g dry matter, b : carbohydrates = Total solids - (Protein + Fat + Ash), SFA : Saturated Fatty Acids, MUFA : Mono-Unsaturated Fatty Acids, PUFA : Poly Unsaturated Fatty Acids, * Values in mg/100g dry matter, ** Values in μg/100g dry matter.

4. Conclusion

Between trahanas made without milk and trahanas made with milk the most significant differences were found to be in the amount of saturated and unsaturated fatty acids and the mineral calcium. Trahanas is a very nutritive food as it is a good source of proteins, minerals and other components that is why it is widely used for feeding people.

Acknowledgments

This paper is dedicated to the memory of Professor Kandarakis+. Authors are grateful to Professor Anifantakis for his contribution to this work.

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4.4. (S4.10) Enhancing Proteolytic Activity, Nutritional and Physiological Benefits of Caprine Yogurt

D.M. Beshkova[1], E.D. Simova[2], Zh.P. Dimitrov[3], Zh.I. Simov[2]

Summary

The proteolytic systems play an essential role in nitrogen metabolism of lactic acid bacteria in milk. A novel approach for enhancing the nutritional and physiological benefits of caprine yogurt involves effective use of the proteolytic potential of the *Lactobacillus bulgaricus* by including in the fermentation process a probiotic and bacteriocinogenic culture with high lytic effect against the starter proteolytic. The strain *Lactobacillus bulgaricus* P1 had a highly developed proteolytic system – a broad spectrum of strong amino-, di- and tripepsidases, oligopeptidase and strong proline-specific peptidases. Co-cultivation of a yogurt culture with a probiotic bacteriocinogenic culture in caprine milk offers a possibility of producing alternative probiotic yogurt.

1. Introduction

Caprine milk is beneficial to health, however, its physico-chemical properties are not fully exploited for manufacturing foods with enhanced nutritional and medicinal properties. The nutritional advantages of caprine milk over cow's and sheep's milk are due to the specificity and higher level of short-chain fatty acids within the lipids. Caprine milk contains higher levels of caproic, caprylic, capric, medium chain fatty acids and medium chain triglycerides that could be used to treat some metabolic disorders [1].

Caprine milk proteins are also more easily digestible, and the released amino acids are absorbed more efficiently than those from cows' milk. The anti-allergic properties of caprine milk are due to the inability of milk proteins to pass through the walls of the digestive tract in native and undigested state. Because of this property, caprine milk can be used as an alternative therapeutic food in the diet of people suffering from allergies to cow's milk and also in patients with ulcers and ulcerative colitis. [2, 3].

The use of caprine milk for fermented dairy products by combining selected starter cultures with active peptidase systems, able to produce antimicrobial substances, is an innovative direction in the production of new products.

Here we report on enhancing the proteolytic activity and the nutritional composition of fermented caprine milk. Our goal is to improve proteolysis in caprine milk by the lytic action of bacteriocinogenic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* BB18 on the proteolytic strain *Lactobacillus delbrueckii* ssp. bulgaricus P1.

2. Material and methods

Samples of goat milk were collected from 3 goat herds in an ecological region in Rodophi Mountains.

The strains *Lactobacillus bulgaricus* P1 (*Lb. bulgaricus* P1), and *Streptococcus thermophilus* TEP4 (*S. thermophilus* TEP4) have been isolated from authentic home made fermented milks, produced in ecological regions in Rodophi Mountains, and the strain Lactobacillus bulgaricus BB18 (Lb. bulgaricus BB18) isolated from kefir grains. Strain Lb. bulgaricus P1 shows strong intracellular peptidase activities [4]. Strain Lb. bulgaricus BB18 is an active producer of bacteriocins [5].

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Each strain in the bacterial starter was cultivated in autoclaved caprine milk, inoculated with 1% of each single-strain culture. The cultures were incubated until coagulation of milk at 37 °C as follows: *S. thermophilus* TEP4 for 4.5 h; Lb. bulgaricus P1 for 5.5 h; *Lb. bulgaricus* BB18 at 37 °C for 9 h;

The yogurt starters were prepared as follows: Starter 1 (for yogurt 1) — A 2% (v/v) individual inoculum of yogurt strains Lb. bulgaricus P1 + S. thermophilus TEP4 (1:1 v/v) was inoculated in autoclaved (115 °C for 15 min) caprine milk and incubated at 37 °C for 5.5 h; Starter 2 (for yogurt 2) — the autoclaved caprine milk was inoculated with 2% (v/v) of individual strains *Lb. bulgaricus* P1 + *S. thermophilus* TEP4 + *Lb. bulgaricus* BB18 in ratio 1:3:1 (v/v/v) and incubated at 37 °C for 9 h.

Yogurt 1 and yogurt 2 were cultivated in a 6 litre MBR bioreactor (MBR AG Ltd, Zurich, Switzerland) with repeated agitation, without oxygen, to pH 5.1 for 5.5 h and 9 h, respectively, portioned in sterile packages for yogurt and after that stored at 6 °C about 168 h.

All methodology for determination of specific hydrolase activities was described by Simova & Beshkova [4].

All the experiments were carried out in three independent experiments and the results are shown as mean ± SD. Statistical analysis was undertaken using the software SigmaPlot 2001.

3. Results and discussion

The intracellular peptidases present in *Lb. bulgaricus* P1 and *S. thermophilus* TEP4 are presented in Table 1. It was found that the growth phase significantly ($P < 0.05$) affects the peptidase activities in cell free extracts of *Lb. bulgaricus* P1 and *S. thermophilus* TEP4. The highest activities were recorded when the cells were harvested at the end of the late log phase of both strains (Table 1).

The results point to a highly complex peptidase system of *Lb. bulgaricus* P1, including high total aminopeptidase activity with significant specificity to substrates containing Lis, Leu and Arg; strong dipeptidases (Leu-Tyr, Leu-Gly, Leu-Leu and Ala-His); strong proline-specific peptidases (PepX-X-prolyl-dipeptidyl aminopeptidase, PepI, PepQ-prolidase and Pro-Ile hydrolase).

S. thermophilus TEP4 had significantly lower aminopeptidases, dipeptidases and proline-specific peptidases (Table 1) than those of Lb. bulgaricus P1. The most remarkable difference between the specific peptidase activities of both strains was found in the hydrolysis of the Glu-Glu substrate. It is probably the glutamyl-aminopeptidase (RerA) that is responsible for this activity of *S. thermophilus* TEP4. This enzyme is probably less present in the cells of *Lb. bulgaricus* P1.

In the cell-free extracts of both strains, there was oligopeptidase (PepF) able to hydrolyse the long peptide bradykinin. The Pro7-Phe8 bond in bradykinin was efficiently hydrolysed by the oligopeptidase of *Lb. bulgaricus* P1, which indicates the activity of this enzyme in hydrolysing prolyl peptide bonds. The oligopeptidase of *S. thermophilus* TEP4 has about 3.5 times weaker activity than that of *Lb. bulgaricus* P1 (Table 1).

The bacteriocinogenic strain *Lb. bulgaricus* BB18 showed the presence of peptidases with a significantly lower peptidase activity than that of *Lb. bulgaricus* P1 (data not presented). The aminopeptidases of *Lb. bulgaricus* BB18 showed no capacity for active hydrolysis of substrates. The dipeptidases showed 2-4 times lower activity, and the proline-specific peptidases PepI, PepX, PepQ and Pro-Ile hydrolase revealed about 1.5-3 times lower hydrolase activity than that of *Lb. bulgaricus* P1.

The results suggest that during the fermentation starter culture 2 actively uses the amino acids released from *Lb. bulgaricus* P1 + *Lb. bulgaricus* BB18 for growth and accumulation of cell mass (data not presented). During fermentation until coagulation of milk in yogurt 2 the accumulated free amino acids were around 3.6 times more. The bacteriocins produced during fermentation provoked the lysis of the *Lb. bulgaricus* P1 cells and supported the natural cell lysis, occurring mainly during cooling. Thus, the lytic effect of bacteriocin BB18 raised the level of free amino acids in the yoghurt until the beginning of cooling. The lysis of the *Lb. bulgaricus* P1 cells and the release of free amino acids continued during the cooling after 24 h up to 168 h (data not presented).

Table 1: Specific hydrolase activities in cell-free extracts of *Lb. bulgaricus* P1 and *S. thermophilus* TEP4 from mid log phase, late log phase and stationary phase in caprine milk

Substrate/ peptidases	*Lb. bulgaricus* P1			*S. thermophilus* TEP4		
	Mid-log phase	Late-log phase	Stationary phase	Mid-log фаза	Late-log phase	Stationary phase
Aminopeptidase Lis-pNa[a]	147.15*±1.08	368.96±10.33	226.40±2.05	13.10*±1.71	29.33±4.71	24.12±3.05
Leu-pNa[a]	229.06±6.45	393.08±4.42	198.32±8.30	8.12±0.57	20.11±2.03	17.90±1.01
Met-pNa[a]	26.63±1.05	96.87±2.38	29.27±3.07	4.83±0.19	16.23±3.47	10.21±1.63
Ala-pNa[a]	131.44±0.76	144.65±10.78	49.40±5.48	21.11±3.33	45.04±1.02	17.83±9.21
Arg-pNa[a]	198.96±10.12	296.74±2.52	136.20±11.28	23.04±4.03	40.10±2.47	20.61±4.31
Glu-pNa[a]	9.74±1,71	11.45±3.10	10.94±2.97	31.28±4.72	58.43±3.21	43.72±3.38
Dipeptidase Leu-Gly[b]	80.97±9.05	280.80±9.44	158.21±1.53	107.25±1.78	78.34±7.78	49.06±8.09
Ala-His[b]	111.04±6.48	260.42±1.38	169.16±4.05	67.30±1.12	80.73±6.63	36.02±4.08
Leu-Tyr[b]	246.13±4.22	697.48±20.68	298.94±4.18	58.40±0.70	66.40±4.10	38.70±2.40
Glu-Glu[b]	22.10±0.76	29.77±0.36	27.89±0.44	167.23±3.70	315.61±8.70	248.25±6.40
Leu-Leu[b]	102.30±4.03	298.03±6.05	206.22±4.25	66.12±4.28	82.70±6.24	73.40±5.70
Tripeptidase Leu-Leu-Leu[c]	116.41±17.02	98.96±7.53	90.48±9.73	31.42±0.34	47.70±1.74	28.72±0.47
Proline-specific peptidase Pro-pNa[a]	152.10±7.60	346.26±10.87	203.12±7.49	27.12±6.20	62.10±4.07	37.44±11.12
Ala-Pro-pNa[a]	192.20±4.03	388.11±8.07	301.06±7.02	86.57±8.21	188.43±7.37	147.65±10.12
Leu-Prob	106.78±13.44	265.29±3.34	208.78±9.06	20.34±0.37	48.26±2.10	22.60±1.70
Pro-Ileb	118.27±9.03	240.21±6.20	149.89±1.12	113.23±4.20	221.17±6.11	71.10±2.02
Pro-Gly-Gly[c]	0±0.0	0.14±0.20	0.21±0.08	0.09±0.04	0.14±0.05	0.17±0.11
Arg-Pro-Proc	9.67±0.79	29.07±3.40	15.43±3.76	4.16±0.06	16.33±0.08	14 .23±0.07
Pro-Leu-Gly-Gly[c]	3.94±0.43	5.15±0.48	4.10±0.38	0.18±0.04	0.25±0.07	0.17±0.09
Oligopeptidase Bradykinin[d]	0.48±0.06	0.88±0.12	0.53±0.05	0.18±0.06	0.28±0.06	0.13±0.08

* Values are the means±SD of triplicate analysis of CFEs prepared in independent triplicate; [a]Specific activity expressed as μmoles pNa mg protein^{-1} min^{-1}; [b]Specific activity expressed as μmoles amino acid mg protein^{-1}min^{-1}; [c] Specific activity expressed as nmoles amino acid mg protein^{-1} min^{-1}; dSpecific activity expressed in μmoles peptide mg protein^{-1} min^{-1}

5. Conclusion

The reported results indicate for the first time a possible mechanism for enhancing proteolysis, and hence the nutritional value and health benefits of caprine milk. The caprine milk fermented with yogurt culture + bacteriocinogenic culture enters into the group of products with great prospects in the future with regard to their functional and therapeutic properties.

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4.5. (S4.11) Biochemical Characteristics of Yogurt Beverage From Donkey Milk

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Summary

Donkey milk was supplied from the region of Northern Greece. The yogurt beverage was obtained by a technology developed by the research team, using a symbiotic starter culture for Bulgarian yogurt from selected probiotic strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* BB18 and *Streptococcus thermophilus*. The beverage was stored for 15 days at +4 °C – +6 °C. The biotransformation of casein and milk fat was studied during refrigerated storage of the yogurt beverage by determining the free amino acids, peptide fractions and content of fatty acids. During refrigerated storage, the content of free amino acids in the yogurt beverage increased to 632.50 $mg.kg^{-1}$ on day 15. A significant increase was found in the proportion of hydrophilic peptides – up to 59.9% on day 15. The yogurt beverage from donkey milk is characterized by higher biological value, and high concentrations of monounsaturated and polyunsaturated fatty acids.

1. Introduction

In recent years there has been a growing interest in donkey milk due to the fact that its composition is similar to that of breast milk [1]. Donkey milk can be used as a good substitute for cow's milk for infants and children with severe allergy to cow's milk proteins [2].

According to Guo et al. [3], donkey milk is poor in proteins and milk fat, but rich in lactose, making it closer to breast milk and mare's milk. It is characterized as albumin milk with low casein content and a higher content of whey proteins, β-LG and lysozyme. Donkey milk contains higher levels of the eight essential amino acids, higher levels of Ser, Glu, Arg and Val, and lower levels of Cys. Because of that composition, donkey milk exhibits unique nutritional characteristics and can be potentially used as a new dietary and functional substitute for breast milk. Pediatric studies point to an increased interest in the consumption of donkey milk in the form of various dairy products, mainly fermented probiotic milks [4].

The purpose of this study was based on our preliminary studies on the composition and technological properties of donkey milk in the region of northern Greece, aimed to obtain a yogurt beverage using symbiotic starter cultures for Bulgarian yogurt from a selected probiotic strain *Lb. bulgaricus* BB18 and *S. thermophilus*, and to trace the biochemical characteristics of the beverage by determining the fatty acid composition and the products of biotransformation of casein during cold storage of the yogurt beverage.

2. Materials and methods

In laboratory tests, donkey milk produced in the region of Northern Greece was used with the following chemical composition (g l^{-1}): total protein - 17.8, including casein -7.4, whey proteins - 10.4 solids - 89.8, milkfat - 5.6 lactose - 68.0, ash - 3.5, pH- 7.03.

A starter culture for Bulgarian yoghurt was used, consisting of *Lb. bulgaricus* BB18 and *S. thermophilus*. Strain *Lb. bulgaricus* BB18 was isolated from kefir grain [5]. It has a high and broad antimicrobial activity against pathogens [5].

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Milk in a quantity of 1 l was pasteurized at 68 °C for 5 min. After cooling to 42 °C, it was portioned into 100 ml in 200 ml sterile containers and inoculated with the liquid symbiotic starter culture in a quantity of 40 g l^{-1}. The fermentation was conducted at 42 °C until pH 4.6 was reached. After coagulation at pH 4.6, the resulting coagulum was stirred very well and cooled to 6 °C. At this temperature the yogurt beverage was stored for 15 days.

The content of free amino acids was determined by the Pico Taq method (Millipore, USA) with a HPLC system (Water Corp., Milford, MA, USA), described by Cohen et al. [6].

Peptide fractions were analyzed using a ProSphere C18 column, absorbance at 210 nm.

The fatty acid composition was analyzed on a gas chromatography column (Shimadzu GC 17A, Japan) using a capillary gas chromatography column (Bp-1.30 m and 0.32 mm).

Three independent experiments were carried out and the results are shown as mean ± SD. The statistical analysis was undertaken using the software SigmaPlot 2001.

3. Results and discussion

The physicochemical composition of the yogurt beverage from donkey milk is as follows (g kg^{-1}): solids, 90.1±0.29; proteins, 18.0±0.22; milkfat, 6.0±0.12; lactose, 62.5±0.58; ash, 3.8 ±0.18; pH - 4.61±0.05.

The concentration of free amino acids of the yogurt beverage from donkey milk is shown in Table 1.

Table 1: Content of free amino acids (mg kg^{-1}) in yogurt beverage from donkey milk during refrigerated storage at 6 °C

Amino acids, mg kg^{-1}	Refrigerated storage, d		
	1	5	15
Asp	18.3±7.93	21.6±5.61	13.2±4.77
Glu	23.5±1.97	33.4±1.08	39.5±1.85
Asn	9.4±0.27	14.1±0.19	12.3±0.08
Ser	51.2±1.01	64.6±1.13	69.8±1.20
Gln	30.3±1.1	40.3±0.93	45.3±3.63
Gly	8.5±0.38	15.0±6.93	12.4±3.4
His	11.4±3.57	19.1±4.77	35.0±3.54
Arg	29.8±21.04	38.6±26.07	47.1±12.63
Thr	13.2±9.93	18.7±7.05	14.3±4.77
Ala	12.1±4.77	16.4±6.89	24.3±8.77
Pro	38.7±26.77	40.8±11.56	45.9±27.07
Tyr	12.5±5.83	18.5±6.29	27.4±11.76
Val	20.3±5.62	24.1±9.46	32.1±10.31
Met	3.2±4.20	6.5±4.45	28.0±5.62
Cys	1.2±1.53	2.3±2.97	3.8±3.70
Ile	23.5±5.62	27.9±5.86	32.2±21.04
Leu	30.1±20.10	39.7±15.56	63.5±35.01
Phe	18.9±8.82	25.6±7.30	45.1±35.29
Trp	3.6±3.65	7.2±9.24	9.1±2.93
Lys	14.5±1.86	26.8±5.01	42.2±13.92
Total	**374.3±53.17**	**501.2±37.23**	**632.5±64.33**

On the first day of refrigeration, the total concentration of free amino acids found in the yogurt beverage was 374.3 ± 53.17 mg kg^{-1}. The highest concentrations were found for Ser, Pro, Gln, Leu and Glu. As a result of the proteolytic activity of the starter culture, a significant increase in the concentration of free amino acids was observed on day 5 (501.2 ± 37.23 mg kg^{-1}) and day 15 (632.5 ± 64.33 mg kg^{-1}), respectively. On day 15 of the refrigerated storage, a decrease in the concentration of Asp, Asn, Gly and Thr of the yogurt beverage was recorded.

The analysis of lipids in the yogurt beverage indicated the presence of 23 fatty acids (Table 2). The lipid fraction of the yogurt beverage is characterized by a high content of monounsaturated and polyunsaturated fatty acids, 33.67% and 20.48%, respectively, with higher concentrations of C16:1, C18:1, C18:2, C18:3.

Table 2: Content of fatty acids in yogurt beverage from donkey milk

Fatty acids	Contents, %
Saturated fatty acids	
C4:0	0.15±0.04
C6:0	0.52±0.12
C8:0	5.09±0. 82
C10:0	7.50±2.96
C12:0	5.30±1.02
C14:0	4.81±0.60
C15:0	0.40±0.15
C16:0	20.29±4.04
C17:0	0.23±0.14
C18:0	1.54±0.12
C20:0	trace
Total	**45.83±2.82**
Monounsaturated fatty acids	
C14:1	0.32±0.28
C16:1	6.78±2.82
C17:1	0.76±0.18
C18:1	25.52±1.16
C20:1	0.29±0.14
Total	**33.67±3.61**
Polyunsaturated fatty acids	
C18:2	11.14±1.0
C18:3	8.39±0.65
C18:4	0.30±0.15
C20:2	0.31±0.21
C20:3	0.15±0.10
C20:4	0.07±0.05
C20:5	0.12±0.07
Total	**20.48±3.0**

The concentration of hydrophilic and hydrophobic peptides and the total peptide area, expressed in meV, of the yogurt beverage are presented in Figure 1.

A significant increase was found in the counts of hydrophilic peptides, soluble at pH 4.6, from 47% to 59.9% on day 5 and day 15 of the cold storage (+6 °C) of the yogurt beverage, respectively.

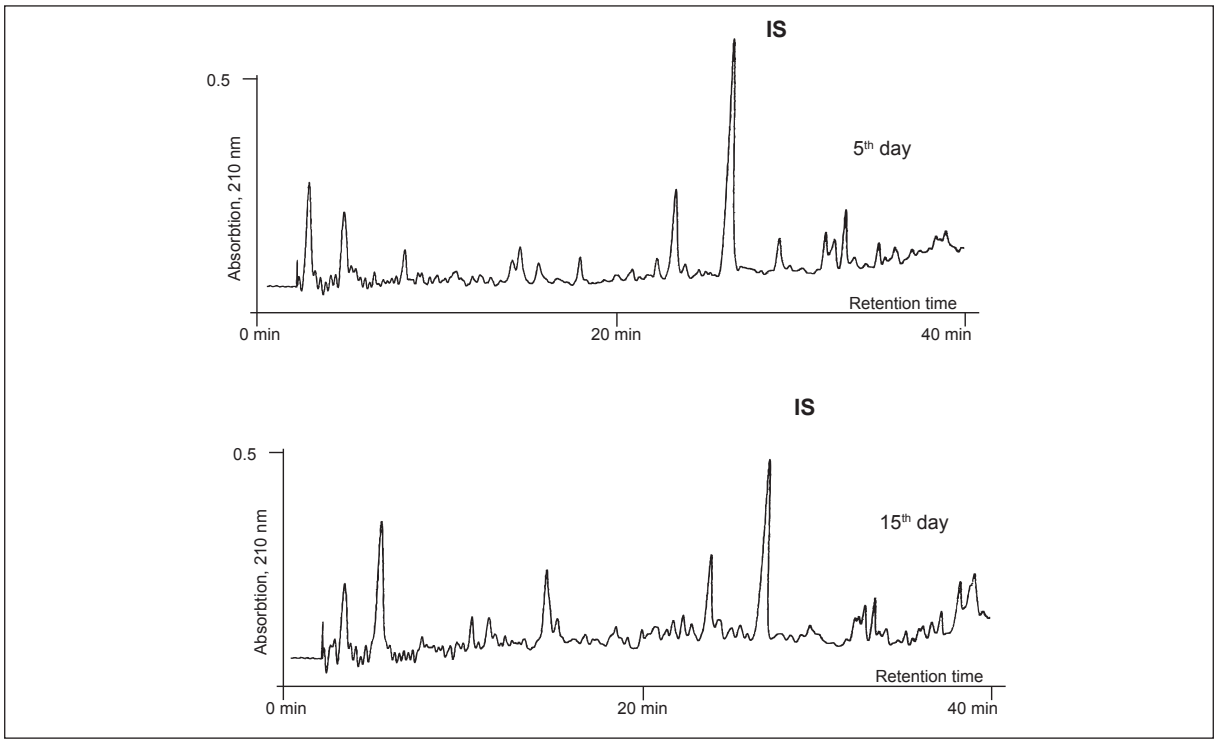

Figure 1. Chromatograms of soluble peptides at pH 4.6 with molecular mass lower than 10 kDa of yogurt beverage from donkey milk in cold storage

4. Conclusion

During the cold storage (6 °C for 15 days) of the yogurt beverage obtained from donkey milk, using a symbiotic starter culture for Bulgarian yoghurt, a high total concentration of free amino acids was found on day 15 - 632.5 mg kg^{-1}, with high concentrations of Ser, Pro, Gln, Leu and Glu. A significant increase from 47.9% to 59.9% was found in the concentration of hydrophilic peptides, soluble at pH 4.6, on day 5 and day 15, respectively. The analysis of lipids in the yogurt beverage indicated the presence of 23 fatty acids, including 45.83% of saturated, 33.67% of monounsaturated, and 20.48% of polyunsaturated acids.

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4.6. (S4.27) Temporal Variations of Conjugated Linoleic Acid (Cla) in Goat's Milk

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Summary

Diet is the most significant factor affecting the quality of ruminant milk fat and the content of beneficial fatty acids (FA) such as conjugated linoleic acid (CLA). However, other physiological factors, such as stage of lactation, that may significantly affect milk CLA content, have not been extensively investigated yet. The aim of this study was to evaluate temporal changes as lactation progresses of milk FA profile and CLA content of dairy goats fed a constant diet. Individual milk samples were collected at 60, 90, 120, 150 and 180 d of lactation from seven second parity Saanen dairy goats fed the same conventional diet throughout the duration of the trial. Milk fat cis-9, trans-11 CLA (rumenic acid; RA) increased by 61.3% as lactation progressed from 60 to 180 days. Milk trans-11 C18:1, the mammary precursor of RA, did not vary significantly while cis-9, cis-12 C18:2, the ruminal precursor of RA, increased with progressing lactation.

1. Introduction

A number of studies have investigated the effects of nutrition on the concentration of the *cis*-9, *trans*-11 CLA isomer (rumenic acid, RA) in ruminant milk, but very limited information is available on the effects of other factors that may affect milk CLA content, such as stage of lactation. Previous studies [1] with Saanen and Alpine goats fed the same conventional diet showed a significant increase in milk fat *cis*-9, *trans*-11 CLA (averaging 0.56 g/100 g FA methyl esters), by 62% and by 76% respectively, as lactation progressed. Similar temporal changes have been confirmed in studies on sheep [2, 3] and were observed also in dairy cows [4, 5]. The variation in milk CLA content observed has been suggested to be a possible result of changes in stearoyl-CoA desaturase (SCD) activity in the mammary gland, responsible for the synthesis of most of the *cis*-9, *trans*-11 CLA from its precursor vaccenic acid (VA) (*trans* -11 C 18:1) and of several MUFA (monounsaturated FA) from their saturated FA (fatty acids) counterparts.

The aim of this study was to evaluate temporal changes in milk fatty acid profile and CLA content and of various desaturase indices in dairy goats fed the same constant diet , during 4 months of lactation.

2. Material and methods

Seven second parity Saanen dairy goats, reared at the goat farm G.P. G. Cavalchini of the University of Milan, were used to determine differences in milk FA as lactation progressed from 60 to 180 days. Goats were housed in a free stall and fed the same total mixed ration for the entire period (table 1). Individual milk samples were collected at 60, 90, 120, 150 and 180 d of lactation. For FA analysis, total lipids were extracted from milk samples using a modified Folch procedure [6]. FAME (FA methyl esters) were prepared using sodium methoxide in methanol [7] and were separated by an automated gas chromatograph (GC autosystem XL, Perkin Elmer), equipped with a 100-m fused silica capillary column (0.25 mm i.d.) coated with 0.25 µm of CP-Sil 88 phase (Chrompack, Middelburg, The Netherlands). Desaturase indices (C14I, C16I, C18I and CLAI) were used as a proxy for SCD activity and were determined as follows: [product of SCD] / [product of SCD + substrate of SCD] for each FA pair and multiplied by 100 [8] . Data of milk FA composition and desaturase indices were analysed statistically as repeated measures by GLM of SAS [9].

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Table 1: Ingredients (DM basis) and fatty acid profile of the diet

Ingredients (% DM)	
Alfalfa hay	36.00
Beetpulp dry	14.80
Triticale silage	13.60
Corn	10.00
Wheat bran and cereal by-products	8.00
Barley	5.72
Field bean	4.60
Sunflower meal	3.75
Alfalfa meal	2.00
Molasses	0.88
Mineral premix	0.65
Fatty acid profile (% total FA)	
C12	0.15
C14	0.40
C16	17.75
C16:1	0.20
C18	3.35
C18:1, *cis* - 9	30.75
C18:2, *cis*-9, *cis*-12	36.05
C18:3, *cis*-9, *cis*-12, *cis*-15	8.20

3. Results and discussion

Temporal changes in milk FA profile occurred during lactation of dairy goats fed the same constant diet and are reported in table 2. Proportions (g/100 g FAME) of several *de novo* FA increased ($P<0.05$) as lactation progressed (C12:0 4.23 *vs* 5.81; C14:0 9.49 vs 11.92; C15:0 0.8 *vs* 1.22, respectively at 60 and 150 days) while the proportion of stearic acid decreased ($P<0.01$), averaging 14.06 and 9.64 g/100 g FAME, respectively at 60 and 180 days.

Milk fat *cis*-9, *trans*-11 CLA increased by 61.3% as lactation progressed from 60 to 180 days (0.62 *vs* 1 g/100 g FAME, $P<0.05$) in agreement with our previous results with different breeds of goats [1] . Milk *trans*-11 C18:1 (vaccenic acid), the mammary precursor of *cis*-9, *trans*-11 CLA, in the present study did not vary significantly during lactation, while *cis*-9, *cis*-12 C18:2 (linoleic acid), the ruminal precursor of *cis*-9, *trans*-11 CLA, increased with progressing lactation (3.46 *vs* 4.66 g/100 g FAME, respectively at 60 and 150 days, $P<0.05$).

Temporal changes in desaturase indices, which represent an estimation of SCD activity, occurred only for C18 index, which was higher at 180 day than at 60 days (70.03 *vs* 60.55, $P<0.01$)

Table 2: Temporal changes at 60, 90, 120, 150 and 180 days of lactation of goat's milk FA profile (g/100 g FAME) and SCD desaturase indices

FA and desaturase	60	90	120	150	180	SEM	P
6:0	2.15	1.75	1.97	1.68	2.01	0.19	0.42
8:0	2.75	2.57	2.55	2.4	2.74	0.2	0.73
10:0	9.51	9.58	9.44	10.02	10.06	0.71	0.95
12:0	4.23^{a}	4.34^{a}	4.55	5.81^{b}	5.33	0.45	0.10
14:0	9.49^{a}	9.56^{a}	10.16	11.92^{b}	10.78	0.62	0.07
14:1	0.26	0.3	0.35	0.34	0.41	0.08	0.73
15:0	0.8^{a}	1.02	1.09	1.22^{b}	0.91	0.12	0.18
16:0	26.93	27.42	27.49	30.83	28.15	1.5	0.44
16:1	0.99	1.11	1.03	1.16	1.08	0.06	0.47
17:0	0.86	1.02	0.9	0.97	0.89	0.07	0.43
17:1	0.29	0.33	0.33	0.24	0.23	0.06	0.47
18:0	14.06^{aA}	13.36^{a}	11.08	9.78^{b}	9.64^{bB}	1.08	0.02
18:1, *trans*-11 (VA)	0.78	0.76	1.16	1.11	1.19	0.31	0.76
18:1, *cis*-9	19.85	21.58	19.61	18.8	21.04	1.5	0.77
18:2, *cis*-9, *cis*-12	3.46^{a}	4.38	4.21	4.66^{b}	4.32	0.43	0.24
18:2, *cis*-9, *trans*-11 CLA	0.62^{a}	0.72	0.79	0.98^{b}	1.00^{b}	0.103	0.05
18:3, *cis*-9, *cis*-12, *cis*-15	0.35	0.42	0.36	0.34	0.31	0.05	0.66
20:0	0.3	0.32	0.25	0.25	0.26	0.03	0.34
Saturated FA	71.07	70.89	69.48	74.88	70.6	2.80	0.75
MUFAs	18.52	19.64	15.48	14.25	19.29	3.85	0.83
PUFAs	4.66	5.62	4.79	6.26	5.82	0.68	0.29
C14 desaturase index	2.53	2.95	3.35	2.84	3.68	0.70	0.8
C16 desaturase index	3.75	3.87	3.57	3.65	3.78	0.30	0.97
C18 desaturase index	60.55^{A}	63.21^{a}	63.29^{a}	64.59	70.03^{Bb}	1.70	0.01
CLA desaturase index	47.19	55.05	46.52	53.51	48.16	5.68	0.75

A,B $P<0.01$; a,b $P<0.05$

4. Conclusion

In conclusion, stage of lactation had an influence on *cis*-9, *trans*-11 CLA concentration in goat's milk. The increase by 61.3% in milk CLA content was not a result of dietary changes, since all goats received the same diet throughout the entire duration of the trial, nor did it correspond to a higher value of SCD desaturase indices (except for C18 index) . Since the greatest part of *cis*-9, *trans*-11 CLA originates from mammary synthesis by means of SCD activity, more studies are needed to explain this relationship completely.

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4.7. (S4.28) Fatty Acid Profile of Sarda and Maltese Goat Milk

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Summary

This study compared the fatty acid (FA) profile of milk produced by Sarda and Maltese goats. It was carried out utilizing one group of 13 Maltese goats and one group of 13 Sarda goats in two dairy farms (N=52). Individual milk samples were collected 4 times over one lactation period. FA were analyzed by gas chromatography utilizing 100 m Supelco SLB-IL111 capillary column, that provided the separation of most *trans*-fatty acids (*t*-FA) and conjugated linoleic acid (CLA) isomers. For each goat breed we evaluated the milk fat content in saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids (PUFA), plus the detailed composition in *t*-FA and CLA isomers. The n-3 polyunsaturated fatty acid docosahexaenoic acid (C22:6, DHA) content was higher in the milk of the Sarda breed while CLA, vaccenic acid (VA) and *t*-FA were higher in the milk of the Maltese breed, suggesting the influence of breed on milk FA profile.

1. Introduction

Milk of small dairy ruminants was proven to have more beneficial effect on consumer's health than cow's milk. In fact, human consumption of goat milk reduces total and LDL cholesterol plasma levels due to its high content of medium chain fatty acids (MCFA) which decrease cholesterol biosynthesis [1]. The high content of short chain fatty acids (SCFA), and of CLA, plus the favorable n-6/n-3 ratio, provide goat milk with a distinct advantage for human nutrition [2, 3]. The most abundant FAs in goat milk are 16:0, 18:1 n-9, 14:0 and 10:0 [4]. The lipid content of milk from healthy animals varies widely among different species, within the same species, and within breeds. The influence of breed on the Δ9-desaturase metabolic activity in the mammary gland results in differences in the metabolism of lipids and milk FA composition [5], especially for PUFA, 18:0 FA, and CLA.

Dairy and meat products from ruminants are a source of *t*-FAs [6]. The milk content in VA and CLA, including rumenic acid (*c*9,*t*11-18:2), is of special interest because of the reported beneficial physiological effects of these FAs [7]. The total trans MUFA (*t*-MUFA) content may represent up to 23% of total milk fat, considering all the 16:1, 18:1, 20:1, 22:1 and 24:1 *t*-FA isomers [8] that occur. The objective of the present study was to determine the influence of the breed on the milk FA composition and metabolic index for the Sarda and Maltese goat breeds farmed in Sardinia, under similar dietary conditions.

2. Materials and methods

Goat milk samples were collected from two dairy farms located in Sardinia. Thirteen Maltese and thirteen Sarda goats from each farm were sampled 4 times in the period from January to June. Samples were immediately cooled to 4 °C, transported to the laboratory within 12 hours, and kept frozen at −20 °C until analysis. The total fat content of milk was measured by infrared spectrometry using an Integrated Milk Testing Milkoscan FT 6000 (CombiFoss 6000 FC instrument, Denmark) following the method in FIL-IDF 141C:2000 . Fatty acids were extracted from milk samples applying a liquid/liquid extraction with chloroform–methanol–water, according to the Bligh and Dyer method (with modifications) [9]. The FA methylation was obtained using sodium

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methoxide in methanol following the procedure described by Cruz-Hernandez et al. [10]. Gas chromatographic separation of the FAs as fatty acid methyl esters (FAMEs) was achieved with a Shimadzu GC-2010 gas chromatograph (Shimadzu Italia, Milano) equipped with a flame ionization detector (FID), and a SLB-IL111 ionic liquid capillary column (100 m, 0.25 mm i.d., 0.2 µm film thickness, Supelco, Bellefonte, PA) [11]. The oven temperature was set at 80 °C for 2 min, then programmed at 15 °C/min to 162 °C, held constant per 18 min, raised at 5 °C/min to 186 °C and held constant 33 min. The injector was maintained at 250 °C and the detector at 280 °C. The injection port was operated in split injection with 50:1 split ratio, and typical injection volume was 1µL. The carrier gas was H_2 at 1.36 ml/min, while the gases for the detector were H_2 at 30 ml/min, ultrapure air at 400 ml/min, and make-up gas N2 at 30 ml/min. Tritridecanoin (13:0 triglyceride) was used as internal standard. Individual FA identification was carried out by retention time comparison with available FA reference standards, and available literature. The FA content was expressed as mg per gram of milk fat. Each analysis was performed in triplicate. Statistical analysis was performed using the general linear model (GLM) procedure of Statgraphics Centurion XVI (StatPoint Technologies, Warrenton, VA, USA).

3. Results and discussion

The data presented in this study are the average of 4 independent samples, expressed as mg per gram of fat. The milk fat content was higher for the Sarda goat breed (5.55 g per 100mL) than for the Maltese (4.57 g per 100mL) (P <0.05). The major FA components were 16:0, *c*9-18:1, 18:0, 10:0 and 14:0 (NS). Among PUFAs no significant difference was found between the two breeds, except for the DHA content (P <0.05). The content of DHA, the n-3 fatty acid most representative and concentrated in human tissues, and the metabolic index DHA/EPA (EPA, eicosapentaenoic acid) were higher in milk of Sarda goat breed (P <0.05). The concentration of DHA was respectively 0.51 mg per g in the Maltese milk fat and 0.63 mg per g in the Sarda milk fat. The content of total CLA was higher (P <0.001) in Maltese goat milk (7.02 mg per g of fat) compared to the one of Sarda goat milk (5.76 mg per g of fat). Among the CLA isomers investigated, *c*9,*t*11–18:2, *t*10,*t*12-18:2 and *t*9,*t*11-18:2 were present in higher amounts in the Maltese goat milk than in Sarda goat milk (P <0.05). The most abundant CLA isomer was rumenic acid, *c*9,*t*11–18:2 with a content of 5.8 mg per g of milk fat for the Maltese breed and 4.8 mg per g for the Sarda breed. Other CLA isomers measured in the milk of both breeds were *t*7,*c*9-18:2, *t*11,*c*13-18:2 and *t*9,*c*11-18:2. The content of *t*-FA was higher (P <0.01) in the milk of the Maltese breed (44.32 mg per g of fat) than in the milk of Sarda breed (37.34 mg per g of fat). The most abundant *t*-FA was VA with a content of 14.94 mg per g of fat for the Maltese breed and 11.36 mg per g of fat for the Sarda, respectively (P <0.05). The other *trans*-18:1 FAs were found in greater amounts in the milk of the Maltese breed than in Sarda breed, and consisted of *t*10-18:1, *t*13- and *t*14-18:1 (co-eluting), and *t*16-18:1 which co-elutes with *c*14-18:1 (P <0.05). The trans-16:1 FAs were higher in the milk fat of the Maltese breed (P <0.01) compared to Sarda; the sum of the *trans*-16:1 FAs was 5.04 mg per g of fat for the Maltese breed, compared to 4.57 mg per g of fat for the Sarda. In this study, the measured *trans*-16:1 FAs were: *t*6-16:1, *t*9-16:1 that elute with *ai*-17:0, *t*10-16:1; *t*11-16:1 that co-elutes with *c*7-16:1 and *t*12-16:1. There was no significant difference in the SCFA content (6:0–10:0 FAs) of both goat breeds. Among medium and long chain saturated fatty acids (12:0–24:0), only the 15:0 content was significantly higher in the milk fat of Maltese breed than in the milk of Sarda, with values of 9.48 and 8.51 mg per g of fat (P <0.05), respectively.

4. Conclusions

Sarda goat breed milk showed a nutritionally valuable FA profile with higher desiderable n-3 fatty acids (DHA) than that of the Maltese. This suggests that the activity of peroxisomal beta-oxidation of n-3 FAs, which participates in the formation of DHA from EPA, and it is also involved in the catabolism of pro inflammatory eicosanoids, is more effective for this breed than for the Maltese. The beneficial value of Sarda goat milk is also increased by the observed lower content in *t*-FAs. Maltese goat milk fat on the other hand showed a higher content in CLA and VA compared

to Sarda goats, which present well-known nutritional value and health benefits [7]. The results of this study showed that the goat breed significantly affected the FA composition of goat milk fat when goats receive an identical diet and are housed under the same conditions. Whether these differences are also nutritionally significant has to be investigated.

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4.8. (S4.36) Composition and Nutritional Value of Donkey Milk

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Summary

This study aims to assess the potentialities of donkey milk as functional food. Fat and total protein contents were low and milk protein fraction was rich in whey proteins. β-lactoglobulin and α-lactalbumin were the most important whey proteins detected. Furthermore, donkey milk was found to be richer in the proteose-peptone fraction than cow milk. The high content of lactose can be considered one of the characteristics of this milk. The glycemic index value and the Energy content indicated that donkey milk may be considered a low calorie product, potentially evaluable as an easily digestible and highly bio-available carbohydrate supplement.

1. Introduction

In recent years, interest in donkey milk was considerably increased due to its composition, which is very close to that of human milk, so much so that it may be considered a good substitute for dairy cow milk derivatives in feeding children with severe cow milk protein allergy Despite the number of studies on composition, dietary, therapeutic and functional properties of donkey milk [1, 2, 3, 4], its characterization in terms of glycemic index (GI) has never been investigated. Foods containing low GI carbohydrates improve diabetes management and blood cholesterol levels. On the other hand, foods containing high GI carbohydrates improve glycemia and help to refuel carbohydrate stores after physical activity [5]. The aim of this study was to assess the potentialities of donkey milk as functional food, with particular attention to the nutritional contribution of the lactose. For this purpose, the glycemic index and the evaluation of Energy content of donkey milk were carried out and compared with the values of other milks.

2. Material and methods

Two independent samplings were performed. For each trial, bulk milk samples were obtained from 10 Amiatina breed pluriparous donkeys in mid-stage of lactation. The milk samples were taken from the collection of two daily milkings and they were stored at 4 °C and analyzed within 24 hours. A portion of milk was for chemical composition analyses. The milk portion for the glycemic index determination was pasteurized at 70 °C for 30 s and then frozen in 400mL portions until the test.

The following analyses were performed: moisture [6], ash [7], fat [8], lactose [9]. Content in total protein, casein and the various non-casein proteins was determined according to Aschaffenburg and Drewry [10]. The whey protein profile was determined by the RP-HPLC method reported by De Noni et al. [11].

The determination of the glycemic index (GI) was performed on healthy, non-diabetic volunteers of normal weight (6 men and 5 women; mean age = 24.8 years; mean Body Mass Index = 22.2). They were involved in two tests, one test for the reference food (a glucose solution) and one for donkey milk. All tests were carried out with a minimum interval of 2 days. The volunteers submitted to testing fasted the evening before the day of analysis and samples were taken at 0, 15, 30, 60, 90, 120 minutes after the ingestion of food. During the trial, the volunteers were kept in a state of physical rest. As a reference, a glucose solution (25 g of pure glucose in approximately 347 mL of water) was used. The isoglucidic portion of donkey milk was equal

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to 347 mL. Blood samples were analyzed with a glucometer (G+meter, A. Menarini Diagnostics) daily used for monitoring of blood glucose in diabetic subjects. Data on GI were calculated according to the Tai method [12].

2. Results and discussion

The mean gross composition of the donkey milk samples is reported in Table 1. The major component of donkey milk was lactose, being present in an amount higher than 7%, whereas total protein, fat and ash contents were present at about 1.5%, 0.35% and 0.25%, respectively. These data are consistent with the values reported in the literature for donkey milk [1, 2]. As regards proteins, the total whey proteins accounted for about 64% of the total protein content. Donkey milk richer in whey protein fraction was also reported by other authors [2, 3].

Table 1: Chemical parameters of raw donkey milk

	g/100 g ± Standard Deviation
Dry matter	8.58 ± 0.01
Ash	0.34 ± 0.048
Lactose	7.16 ± 0.057
Fat	0.25 ± 0.040
Total protein	1.46 ± 0.022
Whey protein	0.93 ± 0.003
Casein	0.53 ± 0.006

Table 2 summarizes the whey protein distribution determined on the donkey milk samples. β-lactoglobulin and α-lactalbumin were the most important whey proteins, in agreement with the data reported by Vincenzetti et al. [2]. In comparison with cow milk, the donkey milk samples were characterized by a lower amount of β-lactoglobulin and a higher percentage of α-lactalbumin. Moreover, it is noteworthy that donkey milk was found to be richer in the proteose peptone fraction than cow milk. No data on the proteose peptone fraction in donkey milk have previously been reported in the literature. Therefore, the detection of the proteose peptone fraction in donkey milk may be considered as new information.

Table 2: Whey proteins distribution in raw donkey milk and in raw cow milk

	Raw donkey milk	Raw cow milk
α-lactoalbumin (mg/mL)	1.620 ± 0.09	1.093±0.042
Serum albumin (mg/mL)	0.014 ±0.0001	0.381±0.064
β-lactoglobulin (mg/mL)	3.324±0.0900	4.432±0.343
Proteose peptone (mg/mL)	2.270±0.0900	1.221±0.240

Table 3 reports the glycemic index (GI) and the Energy Value (EV) determined in the donkey milk sample. The mean value of donkey milk GI was equal to 89.25 (SD = 7.03). Therefore, donkey milk may be consider as a good source of quick energy. If compared with other types of milk, the GI value of donkey milk is much higher than the data for whole cow milk (31), skimmed cow milk (47) and soy milk (44) reported by Atkinson et al. [13]. The GI value of donkey milk is related to its compositional profile, which is poor in fat and protein, but rich in lactose, as previously discussed. In fact, it is known that the presence of lipids and proteins and fiber decreases the glucose absorption rate [14]. As regards the Energy, the values were calculated by appropriate conversion factors [15] taking into account the experimental data of composition for the donkey milk and the data reported in the literature for the other types of milk. It can be seen that donkey milk was characterized by a low value, which is similar to the value of soy milk, but much lower to that of cow milk. The percentage of Energy from lactose is 71.5% for donkey milk against 28.6% and 9.5% for cow milk and soy milk, respectively [16].

Table 3: Glycemic index and energy value of different types of milk

Milk	Glycemic index	Energy (kcal/kJ)[c]
Donkey	89.25 ± 7.03	36/151
Whole Cow	31[a]	64/287[b]
Skimmed Cow	47[a]	106/441[b]
Soy	44[a]	32/132[b]

[a] [13]
[b] [16]
[c] Energy conversion factors by [15].

3. Conclusion

Donkey milk was found to be poor in protein and fat and rich in lactose and with high value of GI. These data may suggest the use of donkey milk both as a quick source of energy and as a low-calorie carbohydrate beverage to restore carbohydrate stores after physical activity. In comparison with cow milk, donkey milk GI was estimated to be about three times greater, whereas the Energy intake was found to be much lower. The protein profile was characterized mainly by the whey protein fraction, in which a significant amount of proteose peptones was measured.

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IDF INTERNATIONAL SYMPOSIUM ON SHEEP, GOAT AND OTHER NON-COW MILK

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ABSTRACT

Developments in ewes milk, goat milk and other non-cow milk in many countries concerning production of milk, products made from it (cheese, yogurt, and many local products), microbiology, analysis, composition, technology, nutritional properties.

KEYWORDS: Donkey, Ewe, Goat, Milk, Cheese, Yogurt, Artisanal products, Biochemistry, Microbiology, Production, Technology, Analysis, Animal feed, Human nutrition

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- Reference lists at the end of the document to contain the following:
  * Names and initials of all authors;
  * Title of paper (or chapter, if the publication is a book);
  * If the publication is a journal, title of journal (abbreviated according to 'Bibliographic Guide for Editors and Authors', published by The American Chemical Society, Washington, DC), and volume number;
  * If the publication is a book, names of the publishers, city or town, and the names and initials of the editors;
  * If the publication is a thesis, name of the university and city or town;
  * Page number or number of pages, and date.

Example: 1 Singh, H. & Creamer, L.K. Aggregation & dissociation of milk protein complexes in heated reconstituted skim milks. J. Food Sci. 56:238-246 (1991).

Example: 2 Walstra, P. The role of proteins in the stabilization of emulsions. In: G.O. Phillips, D.J. Wedlock & P.A. William (Editors), Gums & Stabilizers in the Food Industry - 4. IRL Press, Oxford (1988).

Abstracts

An abstract not exceeding 150 words must be provided for each paper/chapter to be published..

Address

Authors & co-authors must indicate their full address (including e-mail address).

Conventions on spelling and editing

IDF's conventions on spelling and editing should be observed. See Annex 1.

ANNEX 1 **IDF CONVENTIONS ON SPELLING AND EDITING**

In the case of native English speakers the author's national conventions (British, American etc.) are respected for spelling, grammar etc. but errors will be corrected and explanation given where confusion might arise, for example, in the case of units with differing values (gallon) or words with significantly different meanings (billion).

" ..... Usually double quotes and not single quotes
? ! ..... 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,...
skimmilk ..... One word if adjective, two words if substantive
sulfuric, sulfite, sulfate ..... Not sulphuric, sulphite, sulphate (as agreed by IUPAC)
AOAC International ..... 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. Uthar[1] & B. Prof[2]
[1] University of .....
[2] Danish Dairy Board .....
IDF does not spell out international organizations

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