UNIVERSIDAD DE MURCIA

TRABAJO DE FIN DE MASTER EN NUTRICION, TECNOLOGIA Y SEGURIDAD ALIMENTARIA

Quality control in milk and different types of cheeses

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ANEXO

Dña. María Laura Castelli, estudiante del Master en Nutrición, Tecnología y Seguridad Alimentaria de la Facultad de Veterinaria de la Universidad de Murcia, declaro:

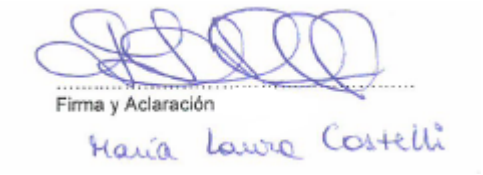
Que el Trabajo de Fin de Master que presento para su exposición y defensa titulado:

Quality control in milk and different types of cheeses

Y cuya tutora es la Dra. María Belén López Morales

Es original y todas las fuentes utilizadas para su realización han sido debidamente citadas en la misma.

Murcia, 11 de Septiembre de 2017



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1. ABSTRACT

Dairy and milk consumption are frequently included as important elements in a healthy and balanced diet. It is the first food for mammals and provides all the necessary energy and nutrients to ensure proper growth and development. Nevertheless, raw milk is an ideal culture medium for microorganisms. Because the microbial load of milk may hold spoilage and/or health risks, the manufacture of milk and milk products is subject to very stringent rules.

The aim of this project is to evaluate the quality controls in raw milk and cheeses elaborated in Central Quesera Montesinos S.L. Microbiological and physico-chemical controls were carried out in Fresh Cheese, Soft/Semi-hard and hard cheese, Processed Cheese, Curd/Ricotta and Acid cheeses.

In addition, different feeding strategies used in cow and goat milk to improve the fatty acid profile in milk and fresh cheeses were evaluates. Dietary manipulation was done by feeding goat cattle with diets supplemented with extruded flaxseed and fish oil. In cows, the study was carried out with diets supplemented with soybean and linseed oil. Control milk and cheeses fatty acid profile was analyzed and compared with milk and chesses obtained after supplementation in both cases. The results for the products analyzed were in concordance with the microbiological and physico-chemistry mandatory quality parameters.

Respect to the investigation about the improvements in the different diets, the fatty acid profile obtained was healthier because of polyunsaturated, CLA, ω-6 and ω-3 fatty acid concentrations which increased. In the other hand, saturated acids and ω-6/ω-3 ratio were reduced.

2. INTRODUCTION

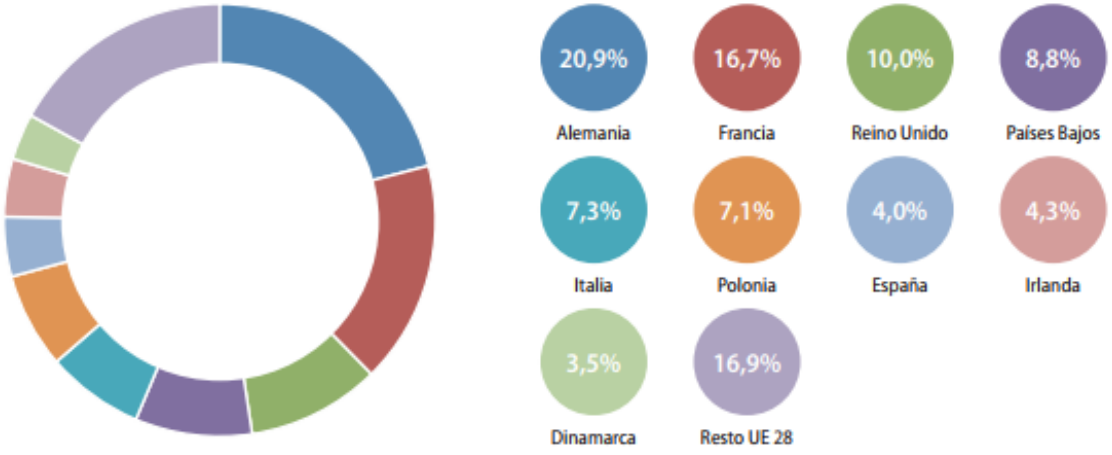
Milk production is a dynamic and growing industry that is fundamental to the wellbeing of hundreds of millions of people worldwide and is an important part of the economy in many countries.

2.1 Agro food industry in Spain

The Agro food industry in Spain is one of the most important industrial sectors. Especially Dairy Industry is a strategic area in this country because of its economic relevance, contribution and development of the rural population. Traditionally the agro food industry had been near to agricultural areas, however the migration to urban areas have changed the consumption patterns. As consequence, less developed Spanish regions were losing a significant part of the value added of the derived agricultural products. This situation has been changing since Spain joined the European Economic Community (EEC) in 1986. This period has also been characterized by a general economic development. More resources have been devoted to promote rural development (Gil and Pérez y Pérez, 1992).

Actually the dairy industry is the second important sector in Spain. Today, Spain is the 7th country producing cow milk (4% of the total production, Fig. 1), reached the 2nd place regarding to goat milk production (22% of the total production) and has the 1st position with 17% of the total production in relation to sheep milk (InLac, El Sector Lácteo en España. Datos de producción, industria y consumo (2008-2015)).

Figure 1 *– Cow´s production in the EEC*

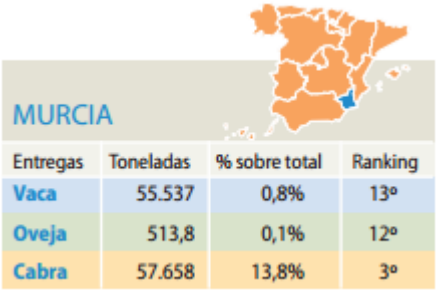


2.2 Agro food industry in Murcia

Agro food sector in Murcia is very important for the regional economy. According to the Agriculture Minister, Murcia is the 2nd Spanish province with the highest exportation rate in agrofood and beverages products (Informe sectorial. El sector agroalimentario en la Región de Murcia, 2016).

Regarding milk production, Murcia reached the 3nd place goat milk production which represented 13.8% of the Spanish production. About sheep and cow milk, Murcia produced 0.1% and 0.8% and represented the 12th and 13th Spain place respectively (Fig. 2) (InLac, El Sector Lácteo en España. Datos de producción, industria y consumo (2008-2015)).

Figure 2 *– Murcia´s production according the total production in Spain Community*



Entregas	Toneladas	% sobre total	Ranking
Vaca	55.537	0,8%	13º
Oveja	513,8	0,1%	12º
Cabra	57.658	13,8%	3º

Source: FEGA. Vacuno: Declaraciones obligatorias del sector vacuno de leche. 2015. Ovino y caprino: Datos de las declaraciones obligatorias del sector lácteo ovino/caprino. 2015.

Agro food industry in Jumilla

Jumilla is an historic city lies in the Altiplano of the Murcia Region, with an average altitude of 600 m and is the third largest municipal area in Spain. The land is arid and mountainous, of steppe and fields of rosemary, where goat herds have historically found an unmatched habitat to produce milk of exceptional quality.

Species well adapted to this environmental conditions, such as Murciano-Granadina goat breed. In general goat cattle was used for milk production mainly destined to the production of fresh milk and D.O.P: cheeses in Jumilla.

2.3 Central Quesera Montesinos S.L.

In 1978, Central Quesera Montesinos S.L. begins its activity like a small factory of fresh traditional cheeses. Actually the factory has 240 employees approximately including plant, offices and laboratory. The factory has a surface of 20000 m^2 and is structured in six lines of production, physically separated and interconnected. Each of them is equipped with the most modern technologies of production and has his industrial director and supervised by specialized technicians. The daily volume of production is 120000 liters of milk including cow, goat and sheep milk. The main activity is cheese production dividing in the following lines of production:

Fresh Cheese

1- Cow Fresh cheese 100%
2- Goat Fresh cheese 100%
3- Fresh cheese (goat and cow mix at different percentages)

Soft cheese/ Semi-hard cheese / hard cheese:

1- Tronchón
2- Murcia Al Vino cheese PDO
3- Murcia Hard PDO

Processed Cheese

Curd/Ricotta

Acid cheeses

1- Fresh (without moho)
2- Ripened (white moho in surface)

Every process is subjected to a rigorous control and analysis system according to the Spanish regulation. The microbiological and physicochemical assays were done in the Central Quesera Montesinos S.L laboratory. Around 20-30 samples were analyzed every day.

Good manufacturing practices were controlled in plant, too. For example:

- Environmental conditions in order to prevent contamination,
- Manufacturing processes are controlled,

- Operators are trained to carry out documental procedures, were controlled by plant supervisor.

Another important area in this dairy industry is the research and development laboratory. Central Quesera Montesinos S.L. has been involving in different projects related with functional food, according the new demands.

About quality area, this industry carries out internal audits in the different lines production. In other hand and since 2004, the manufacturing processes have annual evaluated with food safety and quality audits which conform to international standards under the BRC (British Retail Consortium), having obtained and maintained the maximum certification level available (Category AA).

3. STATE OF ART

According to CODEX STAN 206-1999 milk is the mammary normal secretion of dairy animals obtained by means of one or more milkings without any type of addition or extraction, destined to the consumption of liquid milk or to subsequent production.

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder (Tolle A., 1980). Microbial contamination can generally occur from three main sources such as; within the udder, exterior of the udder and surface of milk handling and storage equipment (Bramley AJ and McKinnon CH, 1990). Good milk hygiene practices such as maintaining clean and healthy cows, keeping a clean milking environment free of dust and mud, avoid milking if the farmer is suffering from communicable diseases like diarrhea or typhoid, not mixing colostrum and fore milk, washing hands with soap and clean water before milking, washing the udder with warm water and drying the udder with a clean dry cloth and use of clean containers for milking, will improve the quality of raw milk (Isha M. et al., 2006).

In the last years, milk quality has obtained very importance, due to milk is usually get contaminated by microorganisms. Bacterial contamination represents a risk to human health, by the possible presence of pathogens and their corresponding

toxins in addition to altering the properties physicochemical of milk (Lopez, 2013). The quality of milk may be evaluated by measuring the parameters that indicate both its suitability for consumption or processing into dairy products and the health status of the cow or herd producing the milk (Leitner et al., 2015).

Raw milks with high levels of somatic cells and bacteria are associated with increased enzyme activity that can result in product defects. Use of raw milk with somatic cell counts >100,000 cells/mL has been shown to reduce cheese yields, and higher levels, generally >400,000 cells/mL, have been associated with textural and flavor defects in cheese and other products (Murphy S.C., et al, 2016).

Dairy farmers are frequently offered monetary premium incentives to provide high quality milk to processors. These incentives are most often based on raw milk somatic cell and bacteria count levels well below the regulatory public health based limits. Justification for these incentive payments can be based on improved processed product quality and manufacturing efficiencies that provide the processor with a return on their investment for high-quality raw milk (Murphy S.C., et al, 2016). Because of that, the physicochemical and microbiological composition are direct related with the price. This double criterion of payment is the most used actually in the dairy industry (Lopez, 2013).

According to national legislation: Real Decreto 492/1985, chapter 3: Payment for milk quality: total fat, protein and dry matter must be analyzed in cow milk samples. Regarding to sheep and goat milk, Real Decreto 752/2001 established that for the quality milk payment, different physicochemical and sanity controls must be done. Total fat, protein, dry matter, somatic cells count and microorganisms account a 30 °C were analyzed, too.

Other important factor in quality milk is the fatty acid composition. Milk and dairy products are one of the main sources of lipids in our diet, although the major fatty acids in milk are saturated, leading to an unfavorable opinion in consumers' minds as regard the consumption of fat from dairy sources. This has encouraged the dairy industry to develop products with an enhanced content of omega-3 fatty

acids, conjugated linoleic acid (CLA) and other components that are claimed to promote human health (Zhang et al., 2006).

Some authors studied the fatty acid profile in different type of milk and they observed that it was changed because of the age, stage of lactation, and season effects. For example, Lock and Garnsworthy (2003) concluded that milk fat produced during the summer contained greater amounts of short-chain fatty acids at the expense of medium-chain fatty acids indicating that fresh grass may alter the pattern of fatty acids produced de novo in the mammary gland. Results suggest that fresh grass promotes the synthesis of CLA in the dairy cow through an increase in Δ9-desaturase activity in the mammary gland and possibly other unknown factors, when fresh grass was fed, with increasing levels of buffer feeding given as the summer progressed.

Fatty acid profile may be improved through supplementation, too. Numerous studies have shown that modification of the basal diet of cattle and, especially, lipid supplementation are the most common procedure used to modify the fatty acid profile of milk (Lock A. and. Bauman D. E., 2004; Shingfield et al., 2008), although any supplementation must take into account the constraints necessary to avoid negative effects on rumen fermentations and milk production (Gonthier et al., 2005). For example, when the diets was supplemented with fish oil or linsed oil the content of Polyunsaturated Fatty Acid (PUFA), Conjugated Linoleic Acid (CLA) and ω-6/ω-3 ratio were raised and Saturated Fatty Acid (SFA) was decreased (AbuGhazaleh A. A. and Holmes L. D., 2007; Dhiman T.R., et. al, 2000).

CLA is a heterogeneous group of positional and geometric isomers of linoleic acid predominantly found in milk, milk products, meat and meat products of ruminants (Benjamin S. and Spener F., 2009). CLA are consumed in low levels, but they are important because of their beneficial effects. The different CLA isomers are associated with several health benefits, such as anticarcinogenic, antioxidant, inmune modulation, antidiabetic and antitherosclerosis properties (Benjamin S. and Spener F., 2009). Isomers C18:2 10t,12c, and C18:2 9c,11t are considered to be the most important isomers of CLA with biological activity.

About ω-3, it is known that the western diets are deficient in this fatty acids, and have an excessive concentration of ω-6 fatty acids, compared to the diet followed by the prehistory´s men. High value of this ratio (ω-6/ω-3) promotes many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, while the increase of ω-3 has a protective effect (Simopoulos, 2008). This author recommend that the ideal ratio between fatty acids to prevent cardiovascular disease should be equal to or less than 4:1.

Regarding to saturated fatty acid, a high concentration of it may be associated with obesity and coronary heart disease, while polyunsaturated fatty acids have a positive effect on human health (Boutoial et al., 2013).

4. OBJECTIVE

The aim of this thesis is to describe the quality controls carried out in milk and cheeses elaborated in Central Quesera Montesinos S.L. and also different feeding strategies used in cow and goat to improve milk and cheeses fatty acid profile.

The aim of this work is divided in three different sections:

1. **Describe the quality controls (Microbiological and physicochemical controls) carried out in raw milk**
2. **Describe the quality controls (Microbiological and physicochemical controls) carried out in cheeses**
3. **Describe different development strategies used to improve the fatty acid profile in milk and fresh cheeses by dietary manipulation**

   3.1 Study the FA profile in goat milk with high levels in PUFA and CLA obtained by dietary manipulation.

   The objective was studied the fatty acid profile in goat milk elaborated with control and treatment diet. Dietary manipulation was done by feeding goat cattle with diets supplemented with extruded flaxseed and fish oil. Control milk was analyzed and compared with milk obtained after supplementation.

3.2 Study the FA profile in goat cheese Murciano-Granadina with high levels in PUFA and CLA obtained by dietary manipulation

The objective was studied the fatty acid profile in goat cheeses elaborated with high levels in PUFA and CLA milk, obtained by dietary manipulation.

3.3 Study the FA profile in cow milk with high levels in CLA obtained by dietary manipulation

The objective was studied the CLA content in milk obtained by supplementation in cattle. Dietary manipulation was done by feeding cattle with diets supplemented with soybean and linseed oil.

3.4 Study the FA profile in soft cheese elaborated with cow milk with high levels in CLA obtained by dietary manipulation

The objective was studied the CLA content in soft cheese habitually consumed in Argentina.

5. MATERIALS AND METHODS

Central Quesera Montesinos S.L. elaborated between 7-8 Fresh Cheeses batches, 6-7 Curd batches and 5-6 Processed Cheese batches, daily. During the week, this industry is elaborating other types of cheese depending on the market demand. Because of that, 20-30 samples were analyzed every day. One sample from each batch was analyzed in the same day of production. The milk samples used for the different bath were studied, too.

5.1- Milk analyses

5.1.1- Microbiological tests

The presence of *Enterobacteriaceae ssp.* microorganism in milk was analyzed according with the COMMISSION REGULATION (EC) No 2073/2005: Chapter 2, using TEMPO EB method, from bioMérieux, France.

5.1.1.1- Enterobacteriaceae ssp.

The *Enterobacteriaceae ssp.* family includes important food spoilage agents and certain intestinal pathogens such as *Salmonella spp., Shigella spp.*

TEMPO EB has an *Enterobacteriaceae ssp.* count. Confirmation is included in 22 hours, compared to 72 hours for the reference method EN-ISO 21528-2 which requires a two-step confirmation.

Method

One milliliter of milk was transferred to TEMPO EB medium vials, which were previously reconstituted with 3 ml of sterile distilled water to achieve a final volume of 4 ml. It is corresponded to a 1/4 dilution of the sample. These dilutions were automatically transferred to the card, containing 48 wells of three sizes using the TEMPO filler. The total volume of the card was larger than the volume of diluted samples. Gaseous headspace was therefore formed in each filled well. These headspaces ensured that the contents of the wells were kept separated from each other. As soon as filling was complete, the transfer tube of the cards was cut and the cards were hermetically sealed. After 24 h incubation at 35°C, readings were automatically performed by the TEMPO reader. Once the readings were complete, results were automatically analyzed by the system software which determined which wells were positive. The number of positive wells obtained, in relation to the volume of the wells and the dilution of the samples, allowed enumeration of the results in CFU/g for the original samples, using the MPN tables.

5.1.2 - Physicochemical composition

To make cheeses is important to know the protein percentage in different milks because its percentage affects the quality of the final product. All milk bathes used for the cheese elaboration were analyzed, daily.

Method

MilkoScan™ FT1 (FOSS, Denmark) gives information on physicochemical composition such as fat, protein, dry matter and lactose.

5.1.3 - Alkaline Phosphatase (ALP) determination

ALP is an enzyme naturally present in all raw milks, which is used as an indicator of proper milk pasteurization. Complete pasteurization will inactivate the enzyme to below levels which are detectable by conventional methods.

Because the heat stability of ALP is greater than that of pathogens which may be present in milk, the enzyme serves as an indicator of product safety. However, the failure to detect ALP activity does not guarantee that the product is pathogen free.

Method

One of the classic test for the determination of alkaline phosphatase activity is the Lactognost Test, from Heyl. The enzyme cleaves a phosphate group from the substrate, disodium phenyl phosphate, liberating phenol which then reacts with a color producing compound to give a blue color.

5.2 - Cheese analyses

5.2.1 - Microbiological tests

Salmonella ssp. and *Lysteria monocytogenes* were analyzed in cheese samples, according with the COMMISSION REGULATION (EC) No 2073/2005: Chapter 1. Food safety on microbiological criteria for foodstuffs.

This microorganism were analyzed using the immunoassay VIDAS® method from bioMérieux, France. It uses ELFA (Enzyme Linked Fluorescent Assay) technology and it requires a one-point recalibration once every 14 or 28 days depending on the assay. VIDAS® included ready-to-use reagents and the protocol was carried out according to the procedures recommended by the manufacturer.

5.2.1.1- Salmonella ssp.

Method

Representative 25 g cheese samples were homogenized with 250 ml peptone medium in a Stomacher, SMASHER^{TM} from bioMérieux (France) for 30 seconds, and incubated at 41 °C for 18-24 hours. Then, 500 µl homogenate is collocated in its respective cartridge and hot at 131 °C for 5 minutes. For last, it is cold during 10 minutes and it is ready for its analyses in the VIDAS instrument. The results are available within 50 minutes.

5.2.1.2- Lysteria monocytogenes

Method

Representative 25 g cheese samples were homogenized with 250 ml LMX medium in a Stomacher, SMASHER^{TM} from bioMérieux (France) for 30 seconds, and incubated at 37 °C for 26-30 hours. Then, 250 µl homogenate is collocated in its respective cartridge and hot at 131 °C for 5 minutes. For last, it is cold during 10 minutes and it is ready for its analyses in the VIDAS instrument. The results are available within 80 minutes.

Figure 3 *- VIDAS® instrument*

Increasing emphasis on a total quality approach in food production, HACCP plans and Risk Assessment procedures enhance the role that quality indicators such as Total Viable Count, *Coliforms, Escherichia coli* and *Enterobacteriaceae ssp.* have in monitoring the hygienic and commercial quality of food. The presence of this

microorganisms were analyzed in cheeses, according with the COMMISSION REGULATION (EC) No 2073/2005 Chapter 2. Process Hygiene Criteria for foodstuffs. *Enterobacteriaceae ssp.* was analyzed in milk samples using TEMPO method from bioMérieux, France.

5.2.1.3- Coliforms ssp.

TEMPO CC (Coliforms Count) enumerates Coliforms according to the BAM definition: Gram-negative, facultative anaerobic rod shaped bacteria that ferments lactose to produce acid and gas (within 48 h at 35° C) in food products within 24 hours.

Sample preparation

Representative 25 g cheese samples were homogenized with 250 ml peptone medium in a Stomacher for 30 seconds.

Method

One milliliter aliquots of the primary dilutions of samples were transferred to TEMPO CC medium vials, which were previously reconstituted with 3 ml of sterile distilled water to achieve a final volume of 4 ml. The 4 ml of inoculated medium obtained corresponds to a 1/40 dilution of the sample. These dilutions were automatically transferred to the card, containing 48 wells of three sizes using the TEMPO filler. The total volume of the card was larger than the volume of diluted samples. Gaseous headspaces were therefore formed in each filled well. These headspaces ensured that the contents of the wells were kept separated from each other. As soon as filling was complete, the transfer tube of the cards was cut and the cards were hermetically sealed. After 24 h incubation at 30°C, readings were automatically performed by the TEMPO reader. Once the readings were complete, results were automatically analyzed by the system software which determined which wells were positive. The number of positive wells obtained, in relation to the volume of the wells and the dilution of the samples, allowed enumeration of the results in CFU/g for the original samples, using the MPN tables.

5.2.1.4- E.Coli

TEMPO EC (*Escherichia Coli*) enumerates *E. coli* according to the BAM definition. *E. coli* is a member of the family *Enterobacteriaceae ssp.*, which includes many genera, including known pathogens such as *Salmonella ssp.*, *Shigella ssp.*, and *Yersinia*. Although most strains of *E. coli* are not regarded as pathogens, they can be opportunistic pathogens that cause infections in immunocompromised hosts. There are also pathogenic strains of *E. coli* that when ingested, causes gastrointestinal illness in healthy humans.

The enumeration of *E. coli* with TEMPO EC system only takes 22-27 hours compared to several days with the reference method ISO 16649-1:2013 and ISO 16649-2:2001.

Sample preparation

Representative 25 g cheese samples were homogenized with 250 ml peptone medium in a Stomacher for 30 seconds.

Method

The procedure is the same that the one used for the c*oliforms* analysis, but the incubation temperature is 37°C.

5.2.1.5- Enterobacteriaceae ssp.

Sample preparation

Representative 25 g cheese samples were homogenized with 250 ml peptone medium in a Stomacher for 30 seconds.

Method

The procedure is the same that the one used for the c*oliforms* analysis, but the incubation temperature is 35°C in this case.

Figure 4 *– TEMPO instrument*

5.3. Improve the profile of Fatty Acid (FA) composition in milk and cheeses by dietary manipulation. Comparison the results between diets.

5.3.1 Study the FA profile in goat milk with high levels in PUFA and CLA obtained by dietary manipulation.

Dietary manipulation was done by feeding 323 goats with diets supplemented with extruded flaxseed and fish oil. Control milk was obtained of the rest of the goat cattle.

Method

5.3.1.1 - Fat extraction

10 g of sample (liquid milk) was weighed and transferred to extraction tube. Then, 1.25 ml of ammonia sp. gr. 0.91 (or an equivalent volume of a more concentrated ammonia solution may be used) was added, mixed and shacked thoroughly. Then 10 ml ethyl alcohol was added and mixed again. 25 ml of diethyl ether (peroxide free) was added and shacked vigorously for about a minute. For last, 25 ml petroleum ether was added and shacked vigorously again for about half a minute. In this moment the upper ethereal layer was separated completely and it was decanted off into a suitable vessel.

The aqueous face was extracted with 15 ml of ether for three times. The ethereal extract was added to the same container and evaporated off completely. Then, the sample was dried in an air oven at 102 ± 2°C for two hours, cooled in a desiccator and weighed. This process was repeated until the difference between two successive weights does not exceed 1 mg.

5.3.1.2 - Percentage of fat in milk

$$\text{Fat \% (w/w)} = \frac{\text{Weight of Extracted Fat}}{\text{Weight of milk}} \text{X100}$$

5.3.1.3 – Fatty Acid Methyl Esters (FAME´s) analysis

The esterification of total fatty acid composition were performed according Method ISO 15884-IDF 182. The fatty acid methyl esters (FAME) profile was analyzed using a gas chromatograph equipped with flame ionization detector (FID) capillary split injection system and autoinjector. Fatty acid were separated using a capillary column (WCOT Fused Silica Coating Selecting FAME; dimensions: 100 m x 0.25 mm ID). The injector and detector temperatures were set to be 250 and 255°C. The analysis was made using ramp of temperature. The initial column temperature was held at 70° C for 1 minute after injection and finally, the temperature was raised to 225°C. Hydrogen was used as carrier gas.

5.3.1.4 - Identification of FAME´s

Individual FAME were identified by order elution and comparison their retention times with those of the corresponding peaks in the standard solution (SUPELCOTM 37 Component FAME Mix).

5.3.1.5 - Quantification of FAME´s

Individual FAME were quantified using internal standard (undecanoic acid methyl ester: Sigma U 0250). The percentages of particular FA were calculated based on their areas. The results were expressed in percentage (%) of total peaks area.

5.3.2 Study the FA profile in fresh goat cheese Murciano-Granadina with high levels in PUFA and CLA obtained by dietary manipulation

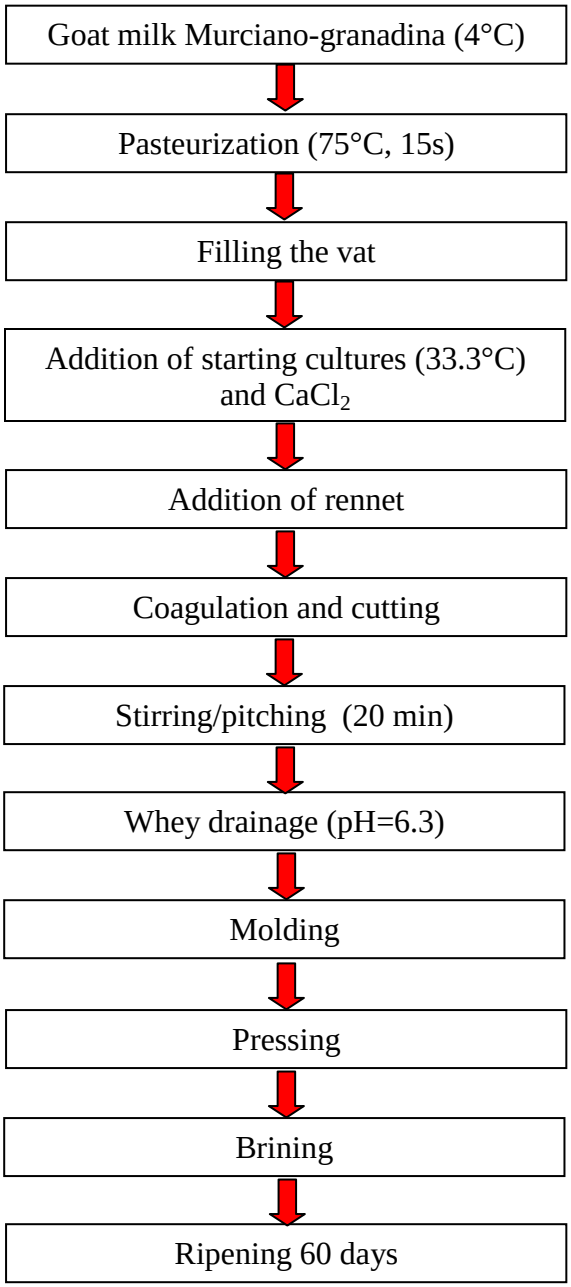
Functional Cheeses were elaborated to industrial scale after the selection of the better characteristics. The objective was comparing the different treatments:

Treatment 1: control cheese, elaborated with goat milk without supplementation

Treatment 2: cheeses elaborated with goat milk supplementation

Cheese were prepared in the Central Quesera Montesinos S.L. according to the Figure 5.

Figure 5 *– Flow sheet used for functional cheeses elaboration*



Method

5.3.2.1 - Fat extraction

The cheese samples were analyzed for total fat. In this case the method used was ISO 3433:2008. Van Gulik method is an empirical procedure which, when applied to a cheese, gives a value for fat content, expressed in grams per 100 g of cheese. After dissolving the proteins by adding sulfuric acid, the fat in the product is separated by centrifuging it in a Van Gulik butyrometer. The separation is enhanced by the addition of isoamilyc alcohol.

5.3.2.2 - Percentage of fat in cheeses

The fat content of cheese is read directly in a Van Gulik butyrometer.

5.3.2.3 - FA analysis: See *5.3.1.3*

5.3.2.4 - Identification of FAME´s: See *5.3.1.4*

5.3.2.5 - Quantification of FAME´s: See *5.3.1.5*

5.3.3 Study the FA profile in cow milk with high levels in CLA obtained by dietary manipulation

Dietary manipulation was done by feeding cattle with diets supplemented with soybean and linseed oil. At the beginning of the experiment, control milk was obtained and after 45 days manipulation diet, supplemented milk. It was carried out with 20 multiparous Holando Argentino cows.

Method

5.3.3.1 - Fat extraction

The fat was extracted from cheese and milk using surfactant solution in similar volumes. The surfactant solution is composed by 12 ml Triton X-100, 50 ml isopropyl alcohol, 2.5 g urea, 25 g of sodium hexametaphosphate and distilled water (amount required to make 500 ml of solution). The extraction is carried out in an oven at a temperature of 90° C. The upper layer of phase fat is removed from the aqueous layer and transferred to a storage vial.

5.3.3.2 - FA analysis: See *5.3.1.3*

5.3.3.3 - Identification of FAME´s: See *5.3.1.4*

5.3.3.4 - Quantification of FAME´s

The percentages of particular FA were calculated based on their areas. The results were expressed in percentage (%) of total peaks area.

5.3.4 Study the FA profile in soft cheese elaborated with cow milk with high levels in CLA obtained by dietary manipulation

Two elaborations of soft cheeses with control and supplemented milk were elaborated. Eight pieces of soft cheese, were made with 40 liters in each elaboration. Cheese were prepared in the Development and Application Laboratory of INTI's Dairy Center (PTM, Buenos Aires, Argentina) according to the standard procedure (Fig 6).

Method

5.3.4.1 - Fat extraction: See 5.3.3.1

5.3.4.2 - FA analysis: See 5.3.1.3

5.3.4.3 - Identification of FAME´s: See 5.3.1.4

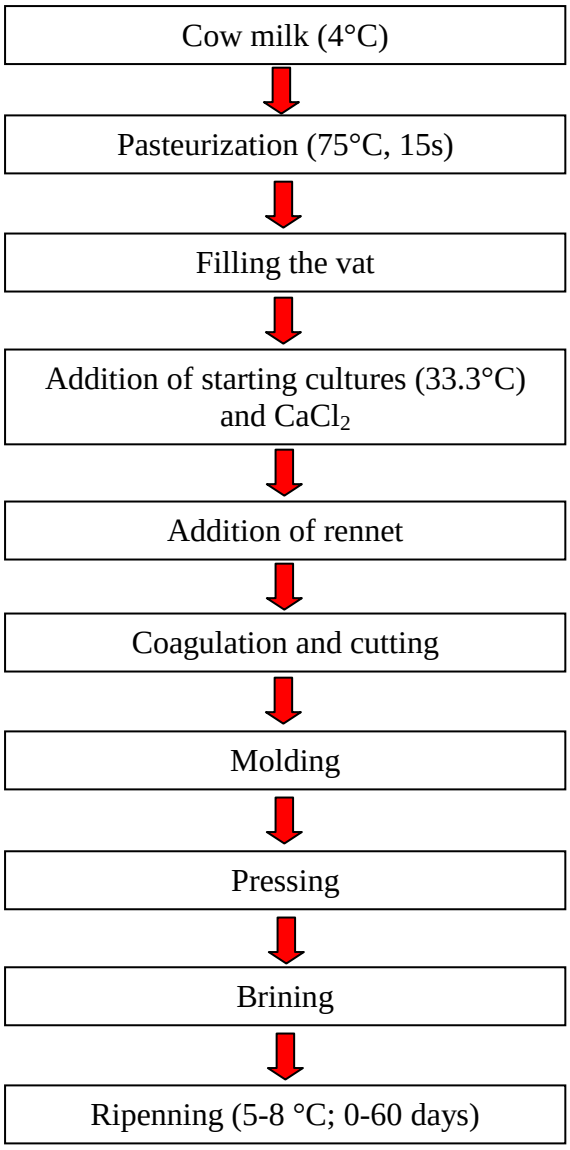
5.3.4.4 - Quantification of FAME´s: See 5.3.3.4

5.4. Statistical analysis

Statistical analysis for **3.1** and **3.2** were performed using Minitab v15.0 (Addlink Scientific Software SL, Barcelona, Spain). Analysis of variance (ANOVA) was used to determine the significant differences between the different treatments.

Statistical analysis for **3.3** and **3.4** were made using the computer program "InfoStat" version 2008. Differences between treatment groups were determined, using the Student's paired samples T-test.

Figure 6 *– Flow sheet used for functional soft cow cheeses elaboration*



6. RESULTS AND DISCUSSION

6. 1. Milk analyses

6.1.1- Microbiological tests

Table 1 shows the sampling plan and the limits for different microorganisms analyzed in pasteurized milk samples according with the COMMISSION REGULATION (EC) No 2073/2005.

Table 1 *– Microbiological results in milk*

Food	Microorganism	Sampling plan		Limits		Reference Method
		n (1)	c (2)	m	M	
Pasteurized milk	*Enterobacteriaceae ssp.*	5	2	1 ufc/ml	5 ufc/ml	ISO 21528-1

(1) n = number of samples for batch

(2) c = number of samples with values between m and M.

When the number of samples with values between m and M is higher than c the batch is rejected. When the result for one sample is higher than M the batch is rejected.

Enterobacteriaceae provide a good indicator of overall Good Manufacture Practice (GMP) (Baylis C. et al., 2011). Dirt (organic matter) may stay of the surfaces and it is very important to remove it. In the cleanliness multiple factors intervene, from the quality of the used water, the quantity and quality of the detergents, the type of dirt, the mechanical components that we use, the temperature, and up to the time of action. Because of that is very important to have a cleanliness and disinfection plans in the different industries.

6.1.2 - Physicochemical composition

MilkoScan is particularly useful for liquid samples, for example, raw milk testing, milk and cream standardization for consistent final products. Sometimes the protein contain in the milk is corrected according the final product to elaborated.

The information about physicochemical composition is important for to know some adulteration and to help safeguard quality, too. In general, cow milk contained: fat (3 to 4 %), protein (3.5 %) and lactose (5 %), but the gross chemical composition of cow milk varies depending on the breed. Sheep milk has higher fat and protein contents than goat and cow milk and also generally has a higher lactose content than cow, buffalo and goat milks. The high protein and overall solid contents of sheep milk make it appropriate for making yoghurt and cheeses such as pecorino, Caciocavallo and feta (http://www.fao.org/agriculture/dairy-gateway/milk-and-milk-products/milk-composition/en/#.WbEw77JJbIU)

6.1.3 - Alkaline Phosphatase (ALP) determination

Pasteurization of fluid milk has been routine in a lot of country. Pasteurization is very effective against bacterial organisms such as *Salmonella ssp., Lysteria monocitogenes*, and Escherichia coli, so foodborne outbreaks associated with these organisms in pasteurized milk or milk products are rare, and when they do occur, are typically the result of improper pasteurization techniques or postpasteurization contamination (Van Kessel J. S. et al., 2004).

This assay (ALP) is very important an indication of the safety of a dairy product, measuring the effectiveness of pasteurization. The test is based on the detection of the phosphatase enzyme, a constituent of raw milk which is destroyed by pasteurizing. If pasteurization is faulty, some phosphatase remains and can be detected producing a blue color. It is detected visually. In this case, the milk is discarded for production of cheeses.

6.2. Cheese analyses

6.2.1 - Microbiological tests

Lysteria monocytogenes, *Salmonella ssp.*, and pathogenic *E. coli* are frequently isolated from dairy cattle and from various locations within dairy farm environments such as water, feed, manure, and bird droppings. Most *E. coli* are commensal intestinal organisms that do not cause disease, but a small percentage of *E. coli* are enteropathogenic (Van Kessel J. S. et al., 2004). Although the prevalence of *Lysteria monocytogenes* and *Salmonella ssp.* was low, these pathogens represent a potential risk to consumers of raw milk and raw milk products. Because of that is very important verified the presence of them.

Table 2 shows the sampling plan and the limits for different microorganisms analyzed in cheeses samples according with the COMMISSION REGULATION (EC) No 2073/2005.

Table 2 *– Microbiological results in cheeses*

Food	Microorganism	Sampling plan		Limits		Reference
		n (1)	c (2)	m	M	
Cheese	*Salmonella ssp.*	5	0	No present in 25 g sample		UNE-EN/ISO 6579
	Lysteria monocitogenes	5	0	No present in 25 g sample		UNE-EN/ISO 11290-1
	E. Coli	5	2	100 ufc/g	1000 ufc/g	ISO 16649-1 o ISO 16649-2
	Enterobacteriaceae ssp.	5	2	1 ufc/ml	5 ufc/ml	ISO 21528-1

(1) n = number of samples for batch

(2) c = number of samples with values between m and M.

When the number of samples with values between m and M is higher than c the batch is rejected. When the result for one sample is higher than M the batch is rejected.

6.3. Improve the profile of fatty acids (FA) composition in milk and cheeses by dietary manipulation

6.3.1 Study the FA profile in goat milk with high levels in PUFA and CLA obtained by dietary manipulation.

Significant differences occurred between control and treatment (extruded flaxseed and fish oil) milk for FA groups studied (Table 3). The highly increments of PUFA (63.97%), CLA (19.53%), ω-3 (285.34%) and ω-6 (33.52%) between control and treatment cheeses can be explain because linseed oil is rich in C18:0, C18:1 and C18:3 FA, which can contribute to reduced SFA an increased concentration of C18:1 FA, MUFA and PUFA concentrations in milk compared with control diet (M.C. Fuentes et al, 2008).

Table 3 *- Comparison between different FA groups in control and treatment goat milk*

	Supplemented goat milk	Control goat milk	Differences (%)
∑ **SFA**	71.16±0.73^{b}	73.74±1.30^{a}	-3.50
∑ **MUFA**	21.25±0.67^{a}	21.44±1.11^{a}	
∑ **PUFA**	6.77±0.30^{b}	4.13±0.20^{a}	63.97
CLA	0.82±0.06^{a}	0.68±0.03^{b}	19.53
ω-3	1.41±0.09^{a}	0.37±0.05^{b}	285.34
ω-6	4.69±0.22^{a}	3.51±0.16^{b}	33.52
ω-6/ ω-3	3.34±0.14^{b}	9.76±1.16^{a}	-65.76

SFA: Saturated FA. MUFA: Monounsaturated FA. PUFA: Polyunsaturated FA
CLA: Conjugated Linoleic Acid
Means with different letters indicate differences (P<0.05) between columns

6.3.2 Study the FA profile in goat cheese Murciano-Granadina with high levels in PUFA and CLA obtained by dietary manipulation

The results are similar to the milk analyzed (see 6.3.1). The statistical analysis applied indicates a significant increase of PUFA (43.47%), CLA (37.93%), ω-3 (172.17%) and ω-6 (25.78%) between control and treatment cheeses (Table 4). It showed that FA was transferred from milk to cheese in a high concentration.

Regarding MUFA results, there were not any significant differences between control and treatment cheeses.

Table 4. *Comparison between different FA groups in cheeses (t=2 days)*

	Cheeses elaborated with supplemented milk	Cheeses elaborated with control milk	Differences (%)
∑ **SFA**	71.53±0.78^{b}	73.84±0.14^{a}	-3.12
∑ **MUFA**	21.52±0.68^{a}	21.29±0.16^{a}	
∑ **PUFA**	6.24±0.45^{a}	4.35±0.03^{b}	43.47
CLA	0.71±0.08^{a}	0.52±0.02^{b}	37.93
ω-3	1.23±0.16^{a}	0.45±0.00^{b}	172.17
ω-6	4.38±0.29^{a}	3.49±0.01^{b}	25.78
ω-6/ ω-3	3.59±0.31^{b}	7.70±0.04^{a}	-53.32

SFA: Saturated FA. MUFA: Monounsaturated FA. PUFA: Polyunsaturated FA
CLA: Conjugated Linoleic Acid
Means with different letters indicate differences (P<0.05) between columns

6.3.3 Study the FA profile in cow milk with high levels in CLA obtained by dietary manipulation

Significant differences were observed between control and supplemented cow milk for FA groups studied (α<0.05, Test T de Student). (Table 5). The high amount of 18:1 11t (VA); 18:2 9c,11t (RA) and ω-3 FA between control and supplemented milk can be explained by the fact that the FA profile depends on the source of lipids in the animal´s diet (Prandini et al, 2007). The C18:1 FA are derived from the partial biohydrogenation of the C18:2 and C18:3 FA that occurs in the rumen, and by the desaturation of stearic acid, which occurs in the mammary gland due to the action of the enzyme ∆-9-desaturase (Kennelly, 1996; Loor J.J. and Herbein, J.H., 2003; Dhiman et al., 2000).

Table 5 *- Comparison between different FA groups in control and treatment cow milk*

	Control Cow Milk		Supplemented Cow Milk	
	0 d	**SD**	**0 d**	**SD**
∑ SFA	60,96^{b}	0.21	59,60^{a}	0.36
∑ MUFA	29,20^{a}	0.08	28,37^{a}	0.09
C18:1 10t	1,43^{a}	0.05	0,61^{b}	0.02
C18:1 11t	2,52^{a}	0.02	5,43^{b}	0.07
∑ PUFA	3,55^{b}	0.06	2,74^{a}	0.07
w-6	3,01^{b}	0.05	1,89^{a}	0.06
w-3	0,54^{a}	0.01	0,85^{b}	0.01
w-6/w-3	5,61^{b}	0.04	2,24^{a}	0.04
∑ TransFA	4,79^{a}	0.10	6,97^{b}	0.19
C 18:2 9c11t (CLA)	1,50^{a}	0.01	2,32^{b}	0.02

SFA: Saturated FA. MUFA: Monounsaturated FA. PUFA: Polyunsaturated FA
CLA: Conjugated Linoleic Acid
Means with different letters indicate differences (P<0.05) between columns

6.3.4 Study the FA profile in soft cheese elaborated with cow milk with high levels in CLA obtained by dietary manipulation

Significant differences occurred between control and treatment cheeses for FA groups studied (α<0.05, Test T de Student) (Table 6). Including high amounts of PUFA in the diet can lead to the accumulation of trans FA (C18:1 trans-11, C18:2 trans-10, cis-12), known for their inhibitory effect on the de novo synthesis of milk FA (Bauman, D.E and Griinari, J.M., 2001).

Another highlight is the comparison between milks and just elaborated cheese FA profile. The FA composition results were similar, so the FA transfers from milk to cheese was high. These results are consistent with findings of other authors (Gagliostro et al, 2007).

***Table 6**: Comparison between different FA groups in control and treatment cow cheeses*

	Cheeses elaborated with control milk		**Cheeses elaborated with supplemented milk**	
	0 d	SD	0 d	SD
∑ SFA	60,74[b]	0.23	59,42[a]	0.09
∑ MUFA	29,25[a]	0.05	28,44[a]	0.04
C18:1 10t	1,44 [a]	0.03	0,64[b]	0.02
C18:1 11t	2,49[a]	0.01	5,48[b]	0.03
∑ PUFA	3,72[b]	0.17	2,64[a]	0.01
w-6	3,17[b]	0.15	1,78[a]	0.02
w-3	0,55[a]	0.02	0,86[b]	0.01
w-6/w-3	5,80[b]	0.11	2,08[a]	0.03
∑ TransFA	4,80[a]	0.05	7,12[b]	0.05
C 18:2 9c11t (CLA)	1,48[a]	0.02	2,37[b]	0.02

SFA: Saturated FA. MUFA: Monounsaturated FA. PUFA: Polyunsaturated FA

Means with different letters indicate differences (P<0.05) between columns

7. CONCLUSIONS

Because of milk was treated with different thermal treatments (pasteurized, sterilized milk, UHT) for sale or for production of dairy products, the quality control is essential for obtain products with the requirements established by the different lays and answering to the needs of the consumers.

According to the results, the products analyzed in Central Quesera Montesinos S.L. were in concordance with the microbiological and physico-chemical mandatory quality parameters.

About the investigation and development assay, supplementing goats diets with flax seeds and fish oil increased polyunsaturated, CLA, ω-6 and ω-3 fatty acid concentrations and reduced the saturated acids and ω-6/ω-3 ratio. Similar results were obtained supplementing cow diets with soybean and linseed oil. Because of that, milk and cheeses made from animals supplemented with these diets were considered healthier.

Respect the cheeses elaborated, the results indicates that normalized elaboration process has not a significant effect on the FA profile between milk and cheeses. It demonstrated that the pasteurized process do not affect the fatty acid profile.

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