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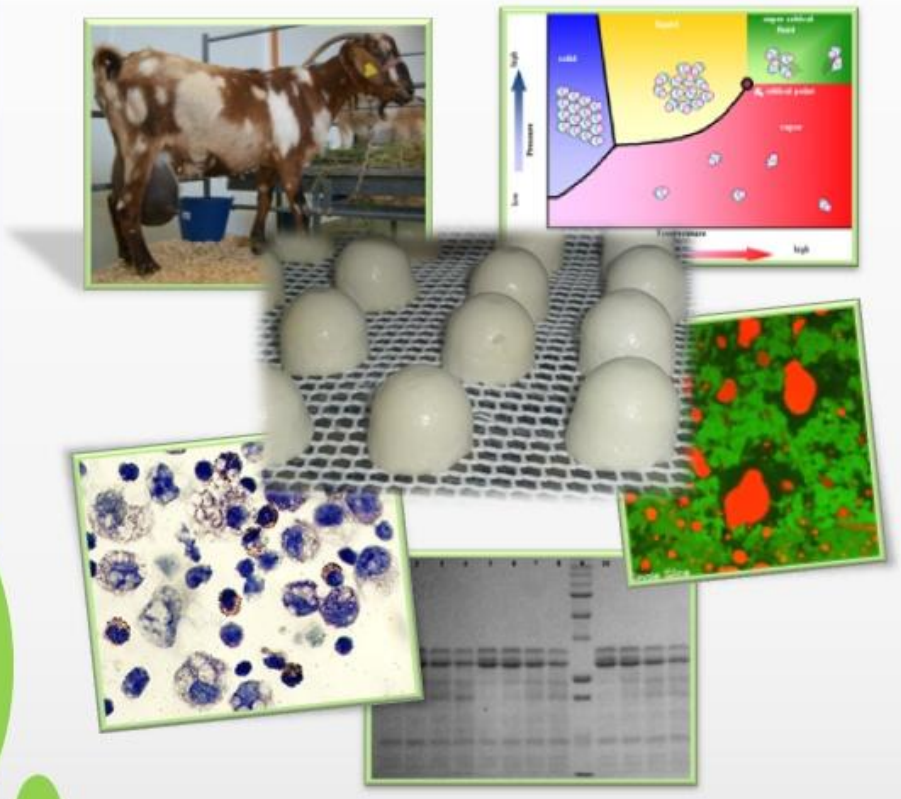

TESIS DOCTORAL

CÉLULAS SOMÁTICAS Y EXTRACCIÓN CON CO_2 SUPERCRÍTICO: PERFECCIONAMIENTO PARA LA PRODUCCIÓN DE QUESO DE CABRA BAJO EN GRASA

Davinia Sánchez Macías

Las Palmas de Gran Canaria, enero 2012

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Anexo II

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

Departamento: Instituto Universitario de Sanidad Animal y Seguridad Alimentaria

Programa de Doctorado: Sanidad Animal

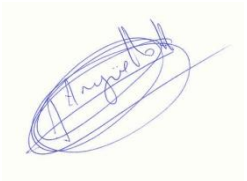
Título de la Tesis

"CÉLULAS SOMÁTICAS Y EXTRACCIÓN CON CO_2 SUPERCRÍTICO: PERFECCIONAMIENTO PARA LA PRODUCCIÓN DE QUESO DE CABRA BAJO EN GRASA".

Tesis Doctoral presentada por *Doña Davinia Sánchez Macías*

Dirigida por **el Dr. D. Anastasio Argüello Henríquez**

El Director, **La Doctoranda,**



Las Palmas de Gran Canaria, a 18 de octubre de 2011

ANASTASIO ARGÜELLO HENRÍQUEZ, PROFESOR TITULAR DE UNIVERSIDAD EN EL DEPARTAMENTO DE PATOLOGÍA ANIMAL, PRODUCCIÓN ANIMAL, BROMATOLOGÍA Y TECNOLOGÍA DE LOS ALIMENTOS DE LA FACULTAD DE VETERINARIA DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

INFORMA:

Que Davinia Sánchez Macías, Licenciada en Biología, ha realizado bajo mi dirección y asesoramiento el presente trabajo titulado **"CÉLULAS SOMÁTICAS Y EXTRACCIÓN CON CO_2 SUPERCRÍTICO: PERFECCIONAMIENTO PARA LA PRODUCCIÓN DE QUESO DE CABRA BAJO EN GRASA"** considerando que reúne las condiciones y calidad científica para optar al grado de Doctor en Veterinaria.

Las Palmas de Gran Canaria, octubre 2011

Fdo. Anastasio Argüello Henríquez

Agradecimientos

A medida que iba escribiendo esta Tesis Doctoral, en cada página, en cada frase y en cada palabra, me acordaba de alguien a quien me decía a mí misma: "no te olvides de agradecerle lo que ha hecho por ti". Son tantas las personas que, directa o indirectamente, me han echado una mano, me han dado un consejo o simplemente me ha dicho "venga, que tú puedes", que seguramente se me van quedar muchas atrás. Estos cuatro años que han pasado están llenos de momentos, de todos los colores y sabores, que han marcado mi vida, y de los que no me quiero olvidar, por que son las cosas que hacen crecer a uno. Sobre todo, me quedo con aquellos momentos que han pasado a "mi Historia" como los más anecdóticos, y que nombraré algunos de ellos, simplemente por el hecho de que queden grabados para siempre en algún lado.

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A Marichu y a Sergio, los grandes del queso en Canarias: sin ustedes esta tesis no hubiera sido posible. Gracias por enseñarme los entresijos de los quesos, los sabores, colores, aromas y texturas. Con ustedes empecé a valorar este maravilloso producto y a darme cuenta que no es simplemente "queso": es cultura y tradición. Y de igual manera, con la cabra: gracias Capote por enseñarme a descalificar animales supernumerarios y con despigmentaciones, y sobre todo por esos momentos de risas inteligentes que sólo tú eres capaz de crear. A Rafael Ginés, por esa inestimable ayuda para preparar las catas y las clases de análisis sensorial, muchas gracias por todo.

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que sólo los padres entienden. Soy consciente de que sin ustedes, no lo hubiera conseguido. A mis GRANDES hermanos Pepe, Pili y Melina, por creer en mí, cuidarme y por darme lo que necesitaba durante toda mi vida: el cariño y el empuje para avanzar y crecer. A todos mis sobrinos, por ser tan cariñosos, simpáticos y llenarme de vida y besos, así como regalarme esas caritas cuando daban un biberón a un baifo. Ustedes son mi familia y los que estarán conmigo pase lo que pase. A mi gran amiga Lorena, porque me has acompañado allá a donde fuera, eres un sol en el camino.

Dejo a mucha gente atrás, la lista es interminable: profesores, investigadores, compañeros, amigos, etc., etc. Pero los que me conocen, saben que en el fondo les doy las gracias de todo corazón, por la cosa más tonta que hayan hecho por mí, incluido un consejo o una broma.

Y como punto final, quisiera dedicar esta tesis a la memoria de mi abuelo Paye, conocido por todos como "Juan Sánchez, el que vendía naranjas y hacía queso". Ya sabía yo que la venita del queso me tenía que salir de algún lado. Y seguro que si mi abuelo pudiera hablarme, me diría: "¿tantos años estudiando para hacer queso como yo?". Y yo le contestaría: "para entenderlo Paye, para entenderlo…".

–

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Capítulo I: Contenido de la Tesis Doctoral

1. Estructura, enfoque y objetivos principales

El presente trabajo de investigación está centrado en la producción de quesos de leche de cabra bajos en grasa y la búsqueda de alternativas para mejorarlos. Los temas tratados al inicio de este manuscrito, que pueden parecer inconexos entre sí, como puede ser la implicación de las células somáticas y los quesos desnatados, se han combinado para intentar encontrar nuevas formas de abordar los defectos encontrados en los quesos bajos en grasa. Así, este trabajo de investigación se ha tratado como un manuscrito científico subdividido en capítulos, los cuales corresponden a una introducción divulgativa, los artículos científicos con cada uno de los experimentos realizados, algunos ya publicados y otros bajo revisión, y un capítulo final de conclusiones.

Este proyecto de investigación comenzó con la idea general de ampliar el mercado actual del queso de cabra, tan importante en Canarias, a partir de la elaboración de un queso artesano bajo en grasa. Los resultados preliminares nos mostraron una serie de defectos en este tipo de quesos, por lo que decidimos ahondar más y describir las características físico-químicas, bioquímicas y sensoriales de los mismos, con la idea de entender desde la base qué es lo que pasa cuando se elabora queso con leche de cabra baja en grasa.

Existen muchos trabajos en la literatura sobre quesos desnatados elaborados a partir de leche de vaca, pocos de oveja y muy escasos de cabra. Hasta ahora, muchas de las prácticas usadas para mejorar los quesos desnatados han tenido poco éxito, por lo que se hace necesario buscar alternativas a su producción. Esta tesis doctoral se ha centrado en dos alternativas totalmente diferentes e independientes una de la otra, y de los procesos convencionales hasta ahora usados en la industria, para mejorar y producir quesos de cabra bajos en grasa: la aceleración de la maduración a partir de las células somáticas de la leche y la extracción de grasa con fluidos supercríticos.

El capítulo II se ha centrado, de una manera muy simple y amena, en una introducción divulgativa, sin perder el carácter de revisión bibliográfica, que introduce al lector en el sector lechero caprino, los quesos de cabra, los quesos desnatados, las células somáticas y los fluidos supercríticos. Se ha evitado la extensión que normalmente se desarrolla en otras tesis doctorales, por el hecho de que en los posteriores capítulos hay una introducción más específica e integrada según el tema tratado y una discusión relacionada con los resultados obtenidos de cada uno de los trabajos experimentales.

Los siguientes capítulos corresponden a cada uno de los trabajos experimentales relacionados con esta tesis, y la idea general y los objetivos principales son los siguientes:

Capítulo IV: se elaboraron quesos de formato pequeño (300 gramos) a partir de leche cruda de cabra entera, semidesnatada y desnatada, siguiendo los procedimientos artesanales que normalmente llevan a cabo los queseros artesanos. Estos quesos se dejaron madurar hasta 28 días a 12ºC y 75-80% de humedad relativa. Los objetivos principales eran la caracterización de las propiedades físico-químicas (composición química, pH, densidad, etc.) de la leche y suero resultante de la elaboración, y la composición química, pH, textura instrumental y color de los quesos a 1, 7, 14 y 28 días de maduración.

Capítulo V: en este apartado se profundizó a nivel bioquímico en los quesos descritos en el capítulo IV. Los objetivos de este trabajo experimental fueron realizar un perfil de proteínas de los quesos de cabra con distinto contenido graso y evaluar la proteólisis y lipólisis durante los 28 días de maduración.

Capítulo VI: para evaluar sensorialmente las posibles diferencias de los quesos medidas a nivel instrumental, este capítulo se ha centrado en el análisis sensorial de los quesos de leche cruda de cabra descritos en el capítulo IV. Para alcanzar los objetivos principales de este estudio, nos basamos en pruebas de diferenciación y aceptación por parte de consumidores, y perfiles sensoriales por parte de jueces expertos, de los quesos enteros (realizados con leche

entera), reducidos (realizados con leche semidesnatada) y bajos en grasa a 1, 7, 14 y 28 días de maduración.

Capítulo VII: se elaboraron quesos, de formato miniatura (8-10 gramos), de leche de cabra cruda y pasteurizada a la que se le añadieron células somáticas obtenidas de leche de cabra sana, con el objeto de estudiar la capacidad potencial de las células en aceleración de la maduración. Los objetivos de este experimento fueron caracterizar el perfil de proteínas, proteólisis y lipólisis, y efectos en el color, de los quesos elaborados con leche cruda y pasteurizada, con 3 niveles de células somáticas (200.000, 1.000.000, y 2.500.000 células/ml), madurados 1 y 7 días.

Capítulo VIII: en este capítulo, se sigue un procedimiento parecido al capítulo anterior, pero esta vez se añade el factor grasa de la leche. Los objetivos fueron estudiar la posible aceleración de la proteólisis y lipólisis midiendo el perfil proteico y nivel de ácidos grasos libres en quesos elaborados a partir de leche cruda o pasteurizada, con 2 niveles de células somáticas (200.000 y 2.000.000 células/ml), leche entera o desnatada, madurados 1 y 7 días.

Capítulo IX: se estudió la utilización de la tecnología de los fluidos supercríticos para la extracción de grasa de los quesos de cabra elaborados con leche entera después de su maduración variando la presión. En este capítulo, los objetivos fueron estudiar los efectos de 4 presiones diferentes (100, 200, 300 y 400 bares) de tratamiento sobre las composición química, perfil de lípidos polares, microbiología y microestructura de dos quesos de leche de cabra curados 5 meses: Majorero y queso tipo Gouda de cabra.

Capítulo X: conclusiones obtenidas de la presente Tesis Doctoral.

2. Focus of the work and main targets

The present research work is focused in the production of goat cheese low in fat, especially in the looking for alternative to improve them. The topics at the beginning of this manuscript could seem disjointed each others, as somatic cells and low fat cheese. But they have been combined in such a way that might make sense, with the objective to find new methods to avoid the defects founds in low fat cheese. This work is a research manuscript divided in chapters, which correspond to a divulgative introduction, the scientific papers, and conclusions.

This project began with the main objective to develop an artisan low fat goat cheese, focusing in amplifying of the market in Canary Islands. The preliminary results showed some atypical properties in cheeses. Because of this, we decided to deep and describe the physicochemical, biochemical and sensory characteristics of these cheeses lower in fat, to enable an understanding and provide a baseline of the effect of milk fat reduction on goat cheese.

In the literature there is amply information about low fat cheese made with cow milk, not too much from sheep milk and very scarce from goat milk. So far, the conventional practices used to improved low fat cheese have had not too much success. It makes necessary to look for alternative to produced them.

This Ph.D. thesis is focused in two different alternatives, independent each other, to the conventional processes and methods used in the industry. The aim is to produce and improve low fat goat cheese using somatic cells from goat milk to accelerate the ripening or the supercritical fluid extraction technology to get goat cheese lower in fat after ripening.

The Chapter II is dedicated, in a very simple and readable way, to a divulgative introduction. Without losing the scientific revision propose, the reader is introduced in the caprine world, goat milk and cheese, low fat cheese, somatic cells topic, and supercritical fluid extraction. The extended concept of bibliographic revision was avoid due to in the next

chapters there is an introduction more specific for each experiment and there is a section where the main results are discussed comparing with other works.

The next chapters correspond to each experimental work relationed with this thesis. The main objectives are as follow:

Chapter IV: small cheeses (300 grams) were made with full-fat, reduced-fat and low-fat raw goat milk using the artisan method. The cheeses were ripened during 28 days at 12ºC and 75-80% relative moisture. The aims were to characterize the physicochemical milk and whey properties (gross composition, pH, density, etc.), and the chemical composition, pH, instrumental texture and color of the cheese ripened at 1, 7, 14 and 28 days.

Chapter V: the biochemical profiles of the cheese described in the chapter IV were studied. The objectives of this study were to characterize the protein profile of the cheeses and quantified the proteolysis and lipolysis during 28 days of ripening.

Chapter VI: to evaluate in a sensorial way the differences found with the instrumental analysis, this chapter was focused in the sensory analysis of the cheese described in the chapter IV. The main objective was to evaluate the sensory differences based in triangular test for differentiation, acceptation test for consumers, and sensory profile elaborated by expert judges, among the full-fat, reduced-fat, a-nd low-fat cheese at 1, 7, 14 and 28 days of ripening.

Chapter VII: Miniature cheeses (8-10 grams) were elaborated with raw or pasteurized milk and with different levels of added somatic cells from health goats, to evaluate the potential of somatic cells in the accelerated ripening. The aims of this experiment were to characterized the protein profile, quantified the proteolysis and lipolysis, and evaluate the effects on instrumental color of cheese elaborated with three different somatic cells levels (200,000, 1,000,000, or 2,500,000 cells/ml), raw or pasteurized goat milk, and ripened at 1 and 7 days.

Chapter VIII: in this chapter is described a similar procedure than in the chapter VII, but in this case, the parameter "fat content in milk" was introduced, with the proposal of

improve the biochemical defects found in low-fat goat cheese. The main objectives of this experiment were to characterize the protein profile, and quantified the proteolysis and lipolysis in cheeses elaborated with raw or pasteurized milk, two levels of somatic cells (200,000 or 2,000,000 cells/ml), and full-fat or low-fat goat milk, during 7 days of ripening.

Chapter IX: in this chapter, the supercritical fluid technology was used to extract fat from full-fat goat cheese after ripening varying the pressure of the treatment. The aims were evaluated the effect of four different pressures (100, 200, 300, and 400 bars) on the chemical composition, polar lipids profile, microbiology quality and microstructure of two varieties of goat cheese (Majorero and Gouda goat cheese) ripened during 5 months.

Chapters X: conclusions of this PhD thesis.

Capítulo II: Introducción Divulgativa

1. La leche de cabra
2. El queso de cabra
3. Las células somáticas
4. Quesos bajos en grasa
5. Los fluidos supercríticos

Capítulo II: Introducción Divulgativa

1. La leche de cabra

En los países desarrollados más del 95% de los productos lácteos consumidos son derivados de la leche de vaca. Una excepción a esta regla incluye los países de la cuenca Mediterránea, donde la leche de cabra y oveja, así como sus respectivos productos derivados, forman una parte fundamental del patrimonio cultural (Michaelidou, 2008). La leche de cabra comprende sólo el 1,98% de la producción mundial, siendo el área Mediterránea uno de los mayores productores (18%) después de India (22%).

De acuerdo a la FAO (Food and Agriculture Organization) de las Naciones Unidas (FAOSTAT, 2010), la población mundial de caprinos de aptitud lechera alcanzó 179 millones de cabezas en el 2008, y la producción de leche de cabra sobrepasó las 15,2 millones de toneladas, representando un incremento significante de 15 y 17 %, respectivamente, comparado con hace una década. Hay que destacar que la producción total de leche en los países en vías de desarrollo no incluye las cantidades desconocidas utilizadas para en autoconsumo, procesamiento directo de los ganaderos y las vendidas a la industria láctea para ser introducidas en el mercado organizado (Pirisi et al, 2007). Dubeuf y Boyazoglu (2009) sugirieron que el incremento en la población caprina durante los últimos 25 años significa que las cabras no son sólo "las vacas de los pobres", y que el creciente interés en sus productos lácteos a nivel mundial significa que la presente situación favorable se consolidará para explotar el comportamiento de los consumidores y todas las oportunidades de mercado potenciales (Dubeuf, 2005).

1.1. Situación actual de la leche de cabra en España y Canarias

La población caprina nacional, comprende el 22,0% del censo comunitario después de Grecia (39,8%), con un total de 2.906.517 cabezas en el 2009. En la Figura 1 se puede

observar la distribución del censo caprino en la Unión Europea en el 2008. Sin embargo, la evolución del censo en España ha sufrido oscilaciones significativas, a consecuencia, entre otros, de la variabilidad de los precios de la leche de cabra, principal producción caprina en nuestro país. Las principales zonas productoras son: Andalucía, Castilla-La Mancha, Canarias y Extremadura (Figura 2, MARM, 2009).

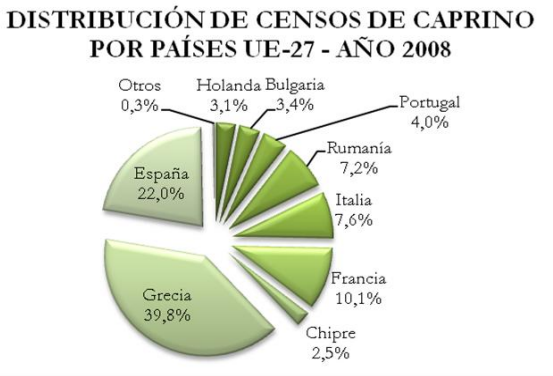

Figura 1. Fuente EUROSTAT. 2010.

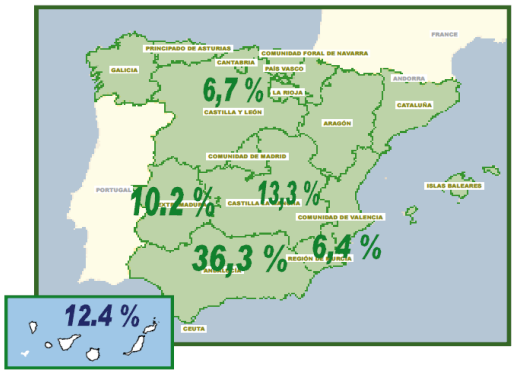

Figura 2. Principales áreas de producción caprina en España. 2009.

La producción lechera en España cifrada, según los datos oficiales (MARM, 2009), en 490 millones de litros en 2008 (incluyendo autoconsumo y comercialización) compite con la de los productores más dinámicos de nuestro entorno, Francia y Holanda, en la producción de quesos frescos y curados.

1.2. Interés renovado y características generales

De acuerdo a Haenlein (2004) y Park y Haenlein (2007), hay tres principales razones por las cuales se demanda leche de cabra: incremento del consumo en el hogar debido al crecimiento de la población humana, interés en los productos lácteos de cabra y por propósitos médicos. Nutricionalmente, la leche de cabra es una fuente de proteínas de alto valor biológico y ácidos grasos esenciales, además de minerales y vitamina A. Es de gran

importancia para los infantes por su alto valor nutricional, hipoalergenicidad, así como por su alta digestibilidad debido a los pequeños glóbulos de grasa. Ribeiro y Ribeiro (2010) y Silanikove *et al.* (2010) han reforzado las propiedades saludables de la leche de cabra y sus productos derivados en amplias revisiones, justificando su alta calidad y beneficios de su consumo. Mowlen (2005) reportó que la población no está especialmente preocupada sobre el costo de los productos en el mercado si al consumir productos derivados de las cabras puede obtener beneficios para la salud. De acuerdo a recientes trabajos de investigación, los productos derivados de la leche de cabra y oveja proveen una alternativa rentable a éstos de leche de vaca debido a su específica composición, calidad sensorial y atributos saludables (Haenlein, 2004; Raynal-Ljutovac *et al.*, 2008). Actualmente también existen revisiones que han profundizado en las características físico-químicas (Park *et al.*, 2007), reológicas (Park, 2007) e higiénico-sanitarias (Raynal-Ljutovac *et al.*, 2007) de la leche de cabra.

1.3. Propiedades físico-químicas

En términos generales, la leche de cabra difiere de la de vaca en tener una mayor digestibilidad, alcalinidad y efecto tampón. Tiene mayor densidad, viscosidad, acidez y un menor índice refractivo y punto de congelación.

Las micelas de caseína de la leche de cabra contienen más calcio y fósforo, y están menos solvatadas e hidratadas. Son menos estables al calor y pierden β-caseína más rápidamente que las micelas de la leche de vaca. El tiempo de coagulación es más corto que para la leche de vaca (Raynal y Remeuf, 2000) y la firmeza de la cuajada es mucho menor, incluso equiparando el mismo contenido de caseínas en ambas leches (Storry *et al.*, 1983).

Los lípidos se presentan en forma de glóbulos de grasa, los cuales abundan característicamente en tamaños menores de 3,5 μm de diámetro. Este hecho es una gran ventaja que le confiere a la leche de cabra mayor digestibilidad y un metabolismo lipídico más eficiente comparado con la leche de vaca (Park, 1994).

1.4. Proteínas

En cuanto a las proteínas, éstas se dividen principalmente en la leche como caseínas y proteínas séricas, aunque se pueden encontrar otras proteínas minoritarias, como inmunoglobulinas, lactoferrina, transferrina, ferritina, peptona proteasa, prolactina, etc. El contenido total de proteínas es uno de los principales criterios de calidad usados como sistema de pago de la leche de cabra en muchos países (Raynal-Ljutovac *et al.*, 2005; Pirisi *et al.*, 2007).

En general, la leche de cabra no contiene o contiene relativamente poca α_{S1}-caseína, mientras que la β-caseína es la principal caseína (Tziboula-Clarke, 2003). La proporción de las 4 caseínas mayoritarias en la leche de cabra está determinada por polimorfismos genéticos, pero en general el orden de proporción es β-caseína > α_{S2}-caseína > α_{S1}-caseína > κ-caseína. De media, la α_{S1}-caseína representa el 10% del total de las caseínas, variando de 0 a 25% (Boulanger *et al.*, 1984; Ciafarone y Addeo, 1984), dependiendo del genotipo del animal. Las razas caprinas canarias (Majorera, Tinerfeña y, especialmente, Palmera) representan un caso particular donde el 60% de los loci de los alelos de la α_{S1}-caseína caprina son A y B (Jordana *et al.*, 1996), por lo que esta caseína es relativamente abundante en la leche y quesos elaborados con estas razas.

Existe una amplia revisión (Park *et al.*, 2007) sobre péptidos bioactivos, proteínas menores y componentes nitrogenados no proteicos derivados de la leche de cabra, pero no serán objeto de esta tesis.

1.5. Lípidos

El contenido de grasa es el componente más variable cuantitativa y cualitativamente en la leche, dependiendo del estado de lactación, estación, raza, genotipo y alimentación, entre otros factores. La leche de cabra presenta una mayor cantidad de ácidos grasos insaturados y de cadena corta (Park, 2006) comparado con la leche de vaca y oveja. Los triglicéridos constituyen la mayor parte de los lípidos presentes en la leche de cabra (cerca del 98%), así

como también se pueden encontrar otros lípidos simples (diglicéridos, monoglicéridos, ésteres de colesterol), complejos (fosfolípidos) y componentes liposolubles (esteroles, ésteres de colesterol, hidrocarburos) (Haenlein y Wendorff, 2006; Park, 2006). De las especies moleculares que componen los lípidos de la leche de cabra, cinco de ellos forman más del 75% (Tabla 1): C10:0, C14:0, C16:0, C18:0 y el ácido graso insaturado C18:1; y respecto a los niveles de ácidos grasos de cadena media y corta de alto valor (C6:0, C8:0, C10:0 y C12:0, caproico, caprílico, cáprico y láurico, respectivamente) son significativamente mayores que en la leche de vaca (Alonso *et al.*, 1999; Chilliard *et al.*, 2006). Se ha determinado, usando más de una técnica cromatográfica, que los ácidos grasos más importantes que forman los triglicéridos, en términos cuantitativos, son aquellos de cadena media (C8:0, C10:0 y C12:0) y el C18:1 (Fontecha *et al.*, 2000).

En la leche de cabra se han cuantificado 36 ácidos grasos de cadena ramificada (Alonso *et al.*, 1999). No se han encontrado apreciables diferencia en el mecanismo de secreción de los glóbulos de grasa en cabra, oveja y vaca, y el perfil de fosfolípidos es bastante similar al de la membrana plasmática, lo que corresponde al 0,8% del total de lípidos en la leche. La estructura y la composición de la membrana es similar en las tres especies, representando sobre el 1% del volumen total del glóbulo de grasa (Scolozzi *et al.*, 2003).

1.6. Carbohidratos

La lactosa en el carbohidrato por excelencia en la leche de cabra, vaca y oveja, y en cabra se suele encontrar sobre 0,2-0,5% menos que en la leche de vaca. Otros carbohidratos presentes en la leche de cabra son los oligosacáridos, glicopéptidos, glicoproteínas y nucleótidos (Larson y Smith, 1974), pero sus funciones han sido muy poco estudiadas.

Tabla 1. Valores medios y rangos en porcentaje de los principales ácidos grasos encontrados en los lípidos de la leche de cabra (Alonso *et al.*, 1999).

Ácido graso	Media %	Mínimo-Máximo
C4:0	2,18	1,97–2,44
C6:0	2,39	2,03–2,70
C8:0	2,73	2,28–3,04
C10:0	9,97	8,85–11,0
C10:1	0,24	0,19–0,38
C12:0	4,99	3,87–6,18
C12:1	0,19	0,10–0,40
C13:0	0,15	0,06–0,28
C14:0	9,81	7,71–11,2
iso-C15:0	0,13	0,12–0,15
anteiso-C15:0	0,21	0,17–0,24
C14:1	0,18	0,17–0,20
C15:0	0,71	0,46–0,85
iso-C16:0	0,24	0,17–0,40
C16:0	28,2	23,2–34,8
iso-C17:0	0,35	0,24–0,52
anteiso-C17:0	0,42	0,30–0,50
C16:1	1,59	1,00–2,70
C17:0	0,72	0,52–0,90
C17:1	0,39	0,24–0,48
C18:0	8,88	5,77–13,2
C18:1 total	19,30	15,4–27,7
C18:2 total	3,19	2,49–4,34
C20:0	0,15	0,08–0,35
C18:3	0,42	0,19–0,87
C18:2 conjugado total	0,70	0,32–1,17

1.7. Vitaminas, minerales y otros componentes

El contenido de macrominerales en la leche de cabra es mucho mayor que el de la leche humana, con cuatro y seis veces más calcio y fósforo, respectivamente. Comparativamente, la leche de cabra contiene más calcio, fósforo, potasio, magnesio y cloro, y

menos sodio y azufre que la leche de vaca (Haenlein y Caccese, 1984; Park y Chukwu, 1988; Chandan *et al.*, 1992). Debido a que las cabras convierten todo el β-caroteno en vitamina A en la leche, la leche de cabra presenta más vitamina A y es mucho más blanca que la leche de vaca. También contiene más tiamina, riboflavina, niacina y vitamina C que la leche de vaca (Park *et al.*, 2007).

2. El queso de cabra

Se entiende por queso el producto fresco o madurado, sólido o semisólido, obtenido de la leche, de la leche total o parcialmente desnatada, de la nata, del suero de mantequilla o de una mezcla de algunos o de todos estos productos, coagulados total o parcialmente por la acción del cuajo u otros coagulantes apropiados, antes del desuerado o después de la eliminación parcial de la parte acuosa, con o sin hidrólisis previa de la lactosa, siempre que la relación entre la caseína y las proteínas séricas sea igual o superior a la de la leche (RD 1113/2009, del 29 de Septiembre).

Existen numerosas variedades de queso de cabra en el mundo, las cuales dependen de la localización de producción, composición de la leche y técnicas de manufactura. Así mismo, la diversidad de los mismos se atribuyen principalmente a los cambios producidos durante la maduración, los cuales son también influenciados por otro tipo de aditivos o cultivos microbianos que se añaden a la cuajada durante el procesado (Park, 2001).

2.1. Queso de cabra en España

La leche de cabra que se obtiene en España se destina casi en su totalidad a la fabricación de queso, cuajada y otros productos fermentados. La proporción de consumo de leche líquida respecto al total sigue siendo muy baja y está limitada a determinadas zonas de demanda tradicional. Ramírez *et al.* (2009) identifica un total de 28 quesos puros de leche de cabra y 21 de mezcla con leche de oveja y/o vaca en España.

La producción de queso puro de cabra en España se ha ido incrementando de manera gradual en los últimos años, pasando de 14.000 toneladas en el año 2005 a 21.600 toneladas en 2007. En el año 2008 sin embargo se registró un ligero descenso en la producción situándose esta en 20.400 toneladas según los últimos datos oficiales provisionales. A partir de estos datos, aplicando un coeficiente de transformación de 10 (litros de leche necesarios para fabricar un kg de queso puro de cabra) únicamente el 40% de la leche de cabra recogida en España se destina a la fabricación de queso puro de cabra, siendo el resto de la leche destinada a quesos de mezcla, otros productos lácteos o exportada a otros países (MARM, 2011).

2.2. Importancia cultural y económica del queso en Canarias

El queso en Canarias forma parte del patrimonio cultural del Archipiélago Canario, resultado del esfuerzo y labor de los ganaderos y maestros queseros de las islas, los cuales aseguran la calidad y adaptación de los productos y procesos a las nuevas necesidades de los consumidores.

La mayoría de los quesos producidos en Canarias se elaboran, principalmente, con leche de cabra y se destina al mercado interior. Sin embargo, la singularidad y calidad de los mismos ha aumentado su importancia y valor en el mercado nacional e internacional. En las Islas Canarias se producen más de 91.000 toneladas de leche de cabra cada año (MARM, 2010) para ser usadas en la elaboración de queso, la mayor parte de los cuales se elaboran con leche cruda usando métodos tradicionales y son consumidos tras breves periodos de maduración (7 días) (Fresno *et al.*, 2008). La economía agrícola se ha beneficiado en los recientes años por el mayor interés en los quesos de cabra, lo cual puede ser factible para muchos ganaderos que producen queso de alta calidad a pequeña escala. Actualmente existe un interés en preservar la producción tradicional de quesos. La variedad de los quesos en Canarias está vinculada a las características climáticas y paisajísticas de las islas, a las excelentes razas autóctonas productoras de leche de alta calidad y las aptitudes tradicionales en su elaboración. Así como la

riqueza genética caprina y forrajera, las Islas Canarias tienen una excepcional situación sanitaria debido al estar oficialmente libres de Brucelosis Caprina y Ovina, lo cual permite a aproximadamente 500 productores artesanos la venta de quesos de leche cruda con menos de 60 días de maduración (Fresno y Álvarez, 2007). En la comunidad autónoma de Canarias destaca la elaboración de dos quesos puros de leche de cabra que poseen una Denominación de Origen Protegida, Majorero y Palmero, y un queso de mezcla de oveja con leche de vaca y/o cabra, el "Queso Flor de Guía y Queso de Guía" que tiene concedida la Protección Nacional Transitoria a la Denominación de Origen Protegida, aunque en este último caso, la leche de cabra podrá ser utilizada en un 10% como máximo.

2.3. Composición química

La composición química del queso de cabra está directamente relacionada con la de la leche de partida, lo que no implica que ambos tengan la misma composición, la cual a su vez dependerá de la raza caprina y su genética (Soryal *et al.*, 2005), dieta de los animales (Álvarez *et al.*, 2007) y estado de lactación (Zeng *et al.*, 2007), entre otros factores. Durante el proceso de elaboración, algunos compuestos sufren una transformación y otros se pierden en el suero. Además, los componentes del queso sufrirán cambios a lo largo de la maduración, debido a factores químicos y físicos, propios del queso y del ambiente. La composición del queso también depende del tipo de queso (fresco, curado, cremoso, duro, coagulación ácida o enzimática, etc), tal como se puede observar en la Tabla 2 recogida por Raynal-Ljutovac *et al.* (2008), donde resume la composición química de algunos quesos de cabra. Por tanto, dar una visión general sobre la composición química del queso de cabra es, cuanto menos, complicada. Sin embargo, según el Real Decreto 1113/2006, donde se aprueban las normas de calidad para quesos y quesos fundidos en España, sólo se recoge una clasificación de quesos en cuanto a su composición química basada en el contenido de grasa sobre la materia seca. Así, se pueden denominar los quesos de la siguiente manera:

Extragraso: el que contenga un mínimo de 60%.

Graso: el que contenga un mínimo de 45 y menos de 60%.

Semigraso: el que contenga un mínimo de 25 y menos de 45%.

Semidesnatado: el que contenga un mínimo de 10 y menos de 25%.

Desnatado: el que contenga menos de 10%.

Así mismo, el Real Decreto prohíbe expresamente el uso de grasas, proteínas o ambas distintas a las de la leche, así como que el extracto seco lácteo inferior al 15% en el queso.

Tabla 2. Composición química de algunos quesos de cabra. Adaptado de Raynal-Ljutovac *et al.* (2008).

Producto	**Tipo**	**País**	**Materia seca, %**	**Grasa %**	**Proteína %**	**Cenizas %**	**Referencias**
Tipo Manchego	Fresco	España	59	34	22	4	Cabezas *et al.* (2005)
	Semicurado	España	65	36	23	5	"
	Curado	España	71	36	25	4	"
Tipo Cheddar		USA	58	27	30		Park (2000)
Tipo Cheddar	Duro, 6m.	USA	62	28	22		Fekadu *et al.* (2005)
Tipo Colby	Pasta lavada, 6m.	USA	54	21	18		"
Láctico	Láctico cremoso	USA	34	16	13		Soryal *et al.* (2005)
Láctico	Láctico cremoso	USA	40	23	19		Park (2000)
Servilletta	Ligeramente prensado	España	38	18	14		Sendra and Saldo (2004)
Domiati	Fresco y cremoso	USA	38	15	12		Soryal *et al.* (2004)
Cacioricotta	Fresco, 7d.	Italia	47	17	17		Albenzio *et al.* (2006)
Apulian Cacioricotta	Fresco cremoso, cuajo enzim.	Italia	36	17	14	1.7	Pasqualone *et al.* (2003)
Ricotta	Queso de suero, 1d.	Italia	31	21	7	0.9	Pizzillo *et al.* (2005)
Brocciu	Queso de suero	Francia	29	17	6		Guerrini *et al.* (1997)
	Queso de suero mezcla	Francia	25	13	6		"

Vadeteja	27 d.	España	73	43	26	4.5	Carballo *et al.* (1994)
Armada	Queso prensado, 120d.	España	79	57	37	3	Fresno *et al.* (1996)
Ibores	Prensado, 6m.	España	59	31	23		Mas *et al.* (2002)
QuesoTravnicki	Blanco en salmuera, 30d.	Bosnia	53	29	20		Saric *et al.* (2002)
Tipo Feta	21d.	S. Africa	41	17	15		Pitso and Bester (2000)
Prensado	Prensado, 3m.	Grecia	69	38			Kondyli and Katsiari (2001)
Prensado	Prensado, 45d.	España	67	37			Trujilllo *et al.* (1999)
Mato	Fresco	España	33	16	12	1.5	Capellas *et al.* (2001)
Tipo Paneer	Fresco	India	53		20	1.9	Agnihotri and Pal (1996)
Rocamadour	Láctico cremoso	Francia	43	23			Lucas *et al.* (2006a)
Ste Maure 21d	Láctico cremoso	Francia	49	27	12		Pierre *et al.* (1999)
Ste Maure 58d	Láctico cremoso	Francia	66	36	16	.	"
Valencay	Láctico cremoso	Francia	40	20	16	2	Hosono and Sawada (1995)
Crottin de Chavignol	Láctico cremoso	Francia	41	23	15	2.6	Hosono and Shirota (1994)
Queso fresco	15d.	España	44	22	16	2.7	Martin Hernandez *et al.* (1992a)
Pasta lavada	60d.	España	55	30	20	3.7	"
Majorero	90 d.	España	61	32	22	4.5	"
Majorero	2d.	España	52	19.73	19.5		Álvarez *et al.* (2007)
Majorero	15d.	España	55	23	21		"
Majorero	60d.	España	58	28	22		"
Bastelicaccia	Cremoso, 30d.	Francia	60	33			Casalta *et al.* (2001)
Cendrat del Montsec	Mezcla de cuajos, 63d.	España	51	31	18	2.2	Carretero *et al.* (1992)
Láctico	Madurado	Francia	51–58	28–32	17–22		Favier and Dorsainvil (1987) and CIQUAL *et al.* (2002)
Láctico	Fresco	Francia	15	6.1	4.7		"
Cremoso	Madurado	Francia	35	18	11		"

En un estudio de evaluación nutricional de más de 30 variedades de quesos de cabra comerciales en Estados Unidos, se observó que la mayor parte de ellos mostraban un mayor

contenido en humedad y proteína, y menor contenido graso que su correspondiente o similar tipo hecho con leche de vaca (Park, 1990).

2.4. Color

El color, definido por la norma CIE (Commission International d´Eclairage) incluye los siguientes parámetros o planos cromáticos de coordenadas (Fig. 3), donde a* representa en sus valores positivos tonos rojos y negativos en tonos verdes y la b* representa en sus valores positivos tonos amarillos y negativos en tonos azules, situándose perpendicular a ellos el eje L (luminosidad) de acuerdo con Wyszecki y Stiles (1982). Por último, el valor de C* (Chroma o cromatismo) representa el módulo o longitud del vector determinado por las coordenadas a* y b* y el valor de Hº (ángulo Hue) la rotación del mismo sobre el eje x.

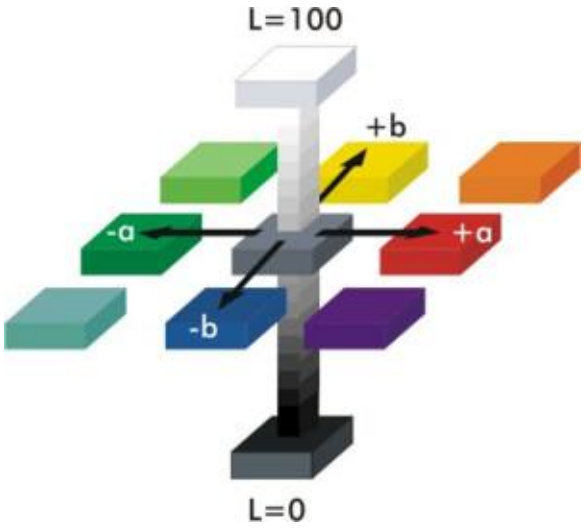

Figura 3. Representación espacial de los parámetros L, a* y b*.

En un material sólido como el queso, la luz penetra las capas superficiales y es dispersada por los glóbulos grasos (Lemay *et al.*, 1994) y por poros formados por el suero (Paulson *et al.*, 1998). La luminosidad disminuye con la maduración (Khosrowshahi *et al.*, 2006), el suero difunde a través de los poros del queso hacia la salmuera o al exterior,

acompañado por una pérdida de humedad. Por tanto, el área antes ocupada por estos centros dispersores de luz disminuye, con la consiguiente disminución de la luminosidad de los quesos. De acuerdo a Rohm y Jaros (1996) y Fresno *et al.* (2006), durante la maduración del queso ocurren modificaciones bioquímicas con las cuales se adquieren los aromas y sabores, así como que se alcanza unos colores más oscuros e incrementa el ángulo hue. Como norma general, y como ya se explicó anteriormente, las cabras transforman prácticamente todo el β-caroteno en vitamina A, de manera que la leche, y por tanto los quesos, de cabra son más blancos que la leche y los quesos de vaca.

2.5. Maduración

Durante la distribución y almacenamiento de los quesos ocurren numerosos cambios bioquímicos y físicos debido a la maduración y degradación de los componentes presentes en el mismo. La proteólisis y lipólisis son los dos principales procesos. La calidad de un queso está altamente influenciada por los niveles de péptidos, amino ácidos y ácidos grasos libres como resultados de estos procesos bioquímicos, aunque también existen cambios en el pH y la calidad microbiológica, aunque este último parámetro no será tratado en este manuscrito.

2.5.1. pH

En los quesos de cabra de Canarias, típicamente de coagulación enzimática, el pH es de aproximadamente 6.6 el día 1 de maduración (Fresno y Álvarez, 2007), lo que está dentro del rango reportado por Martín-Hernández y Ramos (1984) y Juárez *et al.* (1991) para quesos de cabra de España, lo cual también ha sido observado por Álvarez *et al.* (2007) en quesos Majoreros. Durante los siguientes días y semanas de maduración, el pH en el queso disminuye por la acción de las bacterias ácidos lácticas, bien propias de la leche o añadidas como iniciadores, al convertir la lactosa retenida en la cuajada en ácido láctico, para luego

incrementarse ligeramente debido a la liberación de aminoácidos básicos, metabolismo del lactato y formación de iones NH_3 (Alais, 1985).

2.5.2. Proteólisis

La proteólisis es probablemente el evento bioquímico más importante en la maduración de un queso, ya que provee cambios en el sabor y la textura. Debido a las diferencias de composición de los tipos de caseínas entre la leche de cabra y de vaca, tal como se explicó anteriormente, es de esperar que los quesos de ambas especies, en cuanto a la maduración y propiedades proteolíticas, sean significantemente diferentes (Park, 2001). Existen al menos 5 agentes proteolíticos involucrados en la maduración del queso: las enzimas endógenas de la leche, enzimas coagulantes, las bacterias iniciadoras, iniciadores secundarios como bacterias propiónicas o mohos, y bacterias no iniciadoras que oportunistamente forman parte de queso (Fox, 1989).

La proteólisis se puede dividir en tres estados dependiendo del momento de la elaboración del queso: a) antes de la elaboración, ya que existe muchas proteinasas endógenas de la leche, de las que la plasmina es la principal, e hidroliza preferentemente β-caseína, b) durante la elaboración, por ejemplo en caso de la coagulación con enzimas, las cuales hidrolizan la κ-caseína formándose principalmente la para-κ-caseína y facilitando la agregación del resto de la caseínas, y c) después de la elaboración, durante la maduración, donde las enzimas retenidas en la cuajada y las enzimas procedentes de los microorganismos y otras células contribuyen a la formación del aroma y cambios en la matriz del queso (Park, 2001). Trujillo *et al.* (1997) describió que el orden de susceptibilidad de las caseínas de quesos elaborados con leche de cabra a la actividad del cuajo animal es α_{S1}-caseína > β-caseína > α_{S2}-caseína.

La proteólisis durante la maduración de quesos se pueden dividir en dos grandes fases (Park, 2001). En la primera fase, durante las primeras dos semanas de maduración, la matriz

del queso se vuelve más cremosa, con una textura más homogénea. La matriz de caseína es considerablemente debilitada por la proteólisis de sobre el 20% en un solo corte de la α_{S1}-caseína para producir el péptido α_{S1}-I por acción del coagulante (descrito en queso Cheddar de leche de vaca, Creamer y Olson, 1982) y una acción menos extensiva sobre la β-caseína, ya que la acción del coagulante residual es más intensa que la actividad de la plasmina (Grappin *et al.*, 1985; Fox, 1993; McSweeney y Sousa, 2000). En la segunda fase, ocurre un cambio más gradual, sobre todo en la textura, por la degradación del resto de la α_{S1}-caseína durante un tiempo más prolongado de maduración.

2.5.3. Lipólisis

La composición e integridad de la fracción lipídica es importante en la calidad final de un queso. La lipólisis resulta en la formación de ácidos grasos libres, especialmente aquellos de cadena corta y media que contribuyen al aroma del queso y también sirven como sustrato en otras reacciones, produciéndose productos finales altamente aromáticos (Collins *et al.*, 2003). El nivel de lipólisis varía considerablemente de una variedad a otra de queso. Especialmente en algunas variedades, como en los quesos italianos y azules, los ácidos grasos libres son los mayores contribuyentes al desarrollo de sus aromas característicos (Fenelon y Guinee, 2000). Raynal-Ljutovac *et al.* (2005) han observado que la lipólisis en los quesos durante la maduración tiene un mayor efecto que el nivel de lipólisis inicial de la leche. La lipólisis en los quesos se debe a la presencia de enzimas lipolíticas, las cuales hidrolizan la unión éster de ácido graso y la molécula de glicerol del triglicérido a lo largo de la maduración, dando como resultado ácidos grasos libres y mono- y diglicéridos (Deeth y Touch, 2000). Las lipasas que intervienen en el queso provienen de las mismas 5 fuentes que la de las proteinasas discutidas anteriormente (McSweeney y Sousa, 2000).

Las peculiaridades de los ácidos grasos de la leche de cabra y su sistema lipolítico juegan un papel muy importante en el desarrollo del aroma del queso. La actividad de la lipasa

lipoproteica, aunque menor que en la leche de vaca, está más unida a los glóbulos de grasa (Chilliard *et al.*, 1984) y está más correlacionada con la lipólisis espontánea de la leche de cabra y, posiblemente, en el queso.

3. Células somáticas en la leche de cabra

3.1. ¿Qué son las células somáticas?

Las células somáticas están presentes en la leche de todos los mamíferos, no tienen capacidad para multiplicarse y provienen del propio animal. Según su origen, se clasifican en dos grandes grupos: células de origen sanguíneo y células epiteliales. Las células de origen sanguíneo son leucocitos: macrófagos, polimorfonucleares (PMN) y linfocitos (Sordillo y Streicher, 2002). Normalmente estas células se encuentran en la glándula mamaria sana, aunque puede considerarse un indicador de inflamación y/o infección debido a que en estas situaciones se produce un incremento en el trasvase de leucocitos a la leche, especialmente PMN. Las células epiteliales provienen de la descamación del epitelio alveolar y de los conductos de la glándula mamaria.

En la leche también podemos encontrar material extracelular membranoso, restos nucleares y fragmentos celulares (Gonzalo *et al.*, 1998), llamados partículas citoplasmáticas, originados a partir de la porción distal de las células secretoras alveolares. Son abundantes cuando la secreción de leche es apocrina y muy escasos cuando la secreción es merocrina (Fig. 4). La producción de leche de cabra es altamente apocrina y las partículas citoplasmáticas de tamaño similar a las células somáticas son constituyentes normales de la leche de esta especie (Dulin *et al.*, 1983).

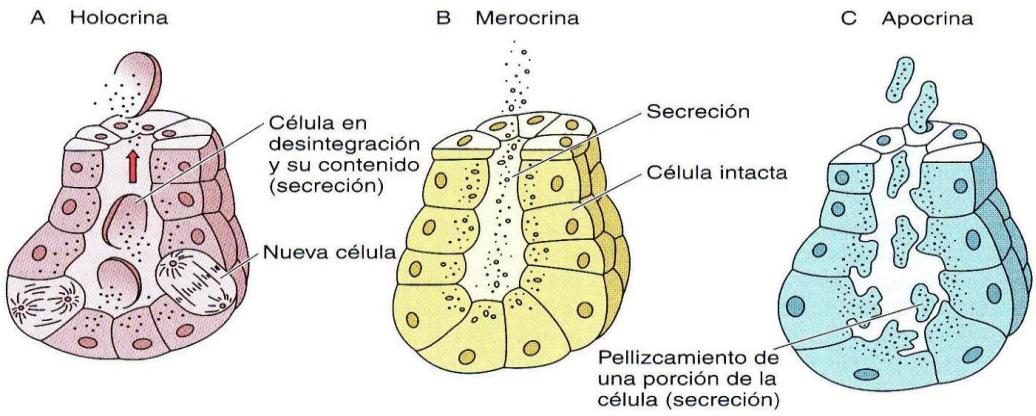

Figura 4. Esquema general de los tipos de secresión holocrina, merocrina y apocrina (http://lahistotecadenico.blogspot.com/2010/11/tejido-epitelial-glandular.html).

En ausencia de infección, y en comparación con la leche de oveja y vaca, la leche de cabra presenta tres características relacionadas con los elementos celulares: elevado contenido de partículas citoplasmáticas (Paape y Capuco, 1997), elevado porcentaje de PMN (Paape *et al.*, 2001), tanto en glándulas mamarias infectadas como no infectadas, y un mayor recuento de células somáticas (RCS) (Dulin *et al.*, 1983; Contreras *et al.*, 1997; Paape *et al.*, 2007), con hasta 5 millones de células somáticas por mL en ausencia de infección (Tabla 3).

Tabla 3. Recuento de células somáticas (RCS) y partículas citoplasmáticas por ml, y porcentaje de células epiteliales, polimorfonucleares (PMN), linfocitos y macrófagos en la leche de distintas especies (Boutinaud y Jammes, 2002; Paape *et al.*, 2001).

Especie	RCS x 1000/mL	Part. Citopl. x 1000/mL	Cels. Epit. %	PMN %	Linfocitos %	Macrófagos %
Humano	9	90	50-90	6	5-9	8
Bovino	75	No detect.	Muy bajo	5-20	20-30	61
Ovino	110	15	Muy bajo	22	10-25	70
Caprino	1100	128	10-20	45-75	3-10	10-35
Porcino	1	---	60-90	5-10	15-25	5-10

La alta variación del RCS en la leche de cabra puede ser causada por infección, pero también por razones fisiológicas (Dulin *et al.*, 1983; Haenlein y Hickley, 1995; Paape *et al.*, 2001; Paape *et al.*, 2007; Fernández *et al.*, 2009). El estado y número de lactaciones en particular (Dulin *et al.*, 1983; Luengo *et al.*, 2004; Paape *et al.*, 2007), así como el celo (McDouglas y Voermans, 2002; Barth y Aulrich, 2007; Moroni *et al.*, 2007) tienen un mayor efecto en el número de células en la leche.

El Amine (2009) ha confirmado la existencia de elevaciones bruscas y transitorias del recuento de células somáticas de naturaleza no infecciosa en el ganado caprino, multiplicándose al menos por 2,5 de un día para otro, en un rango de 0,7 a 15 millones de células por mL en ambas glándulas, encontrándose tanto en cabras infectadas como no. El mismo autor también reportó que el celo y el estrés puntual y extremo provoca importantes incrementos transitorios del RCS en la leche, y que ciertos datos apuntan a que los recuentos en el tanque aumentan con las cabras multíparas. Estos incrementos transitorios del RCS no se explicaron por los cambios en la producción de leche. Sin embargo, se han observado incrementos del RCS junto con una disminución de la producción de leche durante el celo (McDouglas and Voermans, 2002).

En las ubres sanas de cabras, el RCS incrementa progresivamente durante la lactación (De Cremoux, 1995), hay fluctuaciones de un día para otro (Zeng *et al.*, 1997) e incluso dentro del mismo día. El apurado durante o después del ordeño puede interferir en el RCS (Haenlein, 2002). Por tanto, la aplicación de un criterio para la evaluación de la calidad de la leche y para la detección de mastitis está sin resolver.

3.2. Criterios de calidad y situación actual

En muchos países se han establecido unos criterios de calidad para la leche de acuerdo a los requerimientos higiénicos, tecnológicos y sensoriales. Estos criterios de calidad forman parte de un sistema de pago que, a su vez, asegurará la calidad de los productos finales

(Raynal-Ljutovac *et al.*, 2005). En los Estados Unidos, el límite legal de RCS establecido en leche por la FDA (Food and Drug Administration) es de 750.000 y 1.000.000 células por mL en vacas y cabras, respectivamente. En la Unión Europea (Directica 92/46 ECC Counsil, 1992) el límite legal para vacas en 400.000 células por mL y no hay límite para cabras y ovejas, tal como viene recogido en el paquete de higiene, en los Reglamentos (CE) nº 852/2004 del Parlamento Europeo y del Consejo, de 29 de abril, y 853/2004 del Parlamento Europeo y del Consejo, de 29 de abril, que establecen los criterios generales y específicos de higiene que deben cumplir los productos alimenticios, y en particular de la leche cruda de oveja y cabra.

Algunos autores (Paape *et al.*, 2007; Raynal-Ljutovac *et al.*, 2007) han informado que los ganaderos de cabras de Norte América tienen dificultades para mantener el RCS en la leche de tanque por debajo del límite establecido de 1 millón de células somáticas por mL. Muchas granjas lecheras eliminan la leche que excede de los límites de células somáticas para el consumo o la elaboración de queso, lo cual incrementa las pérdidas económicas.

En España, ya hay algunas industrias queseras que están pagando la leche de cabra a los ganaderos según su composición química básica (grasa y proteína) así como en función de la calidad higiénico-sanitaria (microbiología, recuento de células somáticas), pudiendo aplicarse primas o penalizaciones, tal como se recoge en la homologación de contrato-tipo de suministro de leche de cabra con destino a su transformación en productos lácteos (Orden ARM/2387/2010, de 1 de Septiembre).

3.3. Mastitis e inflamación de la ubre

Aunque el objeto de este estudio es independiente a los procesos infecciosos e inflamación de la ubre, hay que destacar algunos mecanismos que ocurren durante la inflamación, y que darían respuesta a los cambios producidos en la leche y los productos derivados de la misma que acompañan al incremento del recuento de las células somáticas en estos casos. Esta aclaración es importante por el hecho de que muchos estudios y

experimentos se han realizado basando las comparaciones a partir de leche sana y mamítica, o agrupación de animales en función de su nivel de células somáticas, sin tener en cuenta que existen otros parámetros que cambian en la leche, algunos de los cuales se conocen y se pueden medir, y otros, en cambio, quizás no conozcamos.

Durante el proceso inflamatorio de la glándula mamaria hay 3 mecanismos involucrados en los cambios de la composición de la leche (Le Roux *et al.*, 2003): a) disminución de la síntesis de leche, b) incremento en la permeabilidad y c) un incremento en la actividad proteolítica.

Las células epiteliales pierden capacidad de síntesis láctea y/o reorientan su metabolismo celular. Silanikove *et al.* (2000) demostró en vacas que un fragmento de la β-caseína, resultado de la proteólisis en animales con mamitis, está involucrado en la disminución de la secreción de leche, quizás al bloquear las bombas de potasio en la parte apical de la células epiteliales, disminuyendo la secreción de lactosa y, por tanto, del rendimiento lechero, ya que la lactosa actúa como un osmorregulador en la producción láctea.

En la leche de vaca, cuando hay una infección, los macrófagos se encargan de reclutar los PMN desde el torrente sanguíneo. Este fenómeno permite la transferencia de componentes entre la sangre y la leche en ambas direcciones debido a cambios en la permeabilidad de la membrana y espacios intersticiales, por lo que las concentraciones de muchos de estos componentes cambian en ambos compartimentos. Hay que tener en cuenta que los factores quimiotácticos que atraen a los PMN a la leche en las glándulas sanas son distintos de aquellos que los atraen a las glándulas infectadas (Manlongat *et al.*, 1998), de manera que es posible que las células difieran entre sí y tengan distinta actividad enzimática según el estado de salud de la ubre. Mehrzad *et al.* (2004) observó que, infectando vacas con Escherichia coli, inducía el trasvase de PMN, la mayor parte de los cuales eran jóvenes e inmaduros. Estos PMN presentaban una baja autoinducción de la apoptosis y, consecuentemente, sobreviven más tiempo que aquellos PMN maduros que ya estaban en la

leche y que no son muy eficientes en su función (Van Merris *et al.*, 2002: Burvenich *et al.*, 2003). Mehrzad *et al.* (2009) también observaron en animales sanos que la capacidad de los PMN para fagocitar Staphilococcus aureus era mucho mayor en sangre y en vacas primíparas que en leche y vacas multíparas.

La principal enzima de la leche es la plasmina, la cual contribuye a la proteólisis primaria de muchos quesos. Se ha encontrado que la leche de vaca mamítica o con altos recuentos de células somáticas presentan una mayor actividad de esta enzima (Le Roux *et al.*, 1995). Estudios in vitro han reportado la capacidad caseinolítica de algunas proteasas aisladas de PMN: colagenasa, que hidroliza la β-caseína (Gilles y Keil, 1976); catepsina D, que hidroliza β-caseína, α_{S1}-caseína, α_{S2}-caseína y κ-caseína (Larsen *et al.*, 1996; McSweeney *et al.*, 1993); catepsina G, que hidroliza β-caseína y α_{S1}-caseína (Considine *et al.*, 2002); y elastasa, que hidroliza β-caseína y α_{S1}-caseína (Considine *et al.*, 1999; Considine *et al.*, 2000).

El estado de madurez de los PMN está ligado a una diferente expresión de proteasas localizadas en distintos tipo de gránulos dentro de las células (Borregaard *et al.*, 1993), así como que no se puede decir que las células recuperadas de la leche sana tengan el mismo perfil proteolítico que las células provenientes de leche mamítica, tal como se señaló anteriormente. Las serin-proteasas, como por ejemplo la elastasa y catepsina G, son expresadas en estados tempranos de maduración, de promielocitos hasta metamielocitos, y muy a bajo nivel en PMN maduros (Takeuchi *et al.*, 1987); las metalo-proteasas, como la colagenasa, se expresan en los estadíos más avanzados de los PMN, como metamielocitos y células segmentadas (Borregaard *et al.*, 1993); y las proteasas ácidas, como las catepsinas B, C, D, H y L están localizadas en los lisosomas en un ambiente ácido, y son expresadas en ambos PMN maduros e inmaduros (Jain, 1993).

3.4. Efectos de las células somáticas en la leche

Existen numerosos estudios que han demostrado que el incremento del RCS en la leche de vaca produce una disminución en la producción de leche y afecta a la vida media de la leche destinada al consumidor (Politis and Ng-Kwai-Hang, 1988; Ma *et al.*, 2000; Erden *et al.*, 2010). El principal componente de las pérdidas económicas asociado con el RCS es la reducción del rendimiento lechero en cabras con mastitis (Huijps *et al.*, 2008). Zeng y Escobar (1996) siguieron el rendimiento lechero durante una lactación de 15 cabras alpinas sin ningún signo de mastitis durante el estudio, y encontraron una correlación negativa entre el RCS y la producción de leche a nivel individual. Hay que tener en cuenta que durante la lactación ocurren cambios en el rendimiento lechero, los cuales pueden resultar en un efecto de concentración de las células somáticas (Paape *et al.*, 2007). Por tanto, la disminución del rendimiento lechero podría no deberse a un incremento en el RCS, sino lo contrario. Sin embargo, el aumento brusco del RCS al final de la lactación puede ser debido no sólo a un descenso de la producción láctea, ya que algunos autores (Manlongat *et al.*, 1998) han reportado una mayor transferencia de PMN a la leche debido a una mayor actividad de factores quimiotácticos, los cuales podrían tener una función en la involución de la glándula mamaria.

Pellegrini *et al.* (1997), Othmane *et al.* (2002) y Nudda *et al.* (2003), entre otros autores, reportaron que en la leche de oveja con mayores recuentos de células somáticas mostraban menor rendimiento lechero que aquellas con menor recuento. También mencionaron que la leche de oveja con alto RCS era acompañada por un incremento en proteínas séricas, lo que resultaba en una pérdida de rendimiento quesero.

En la Tabla 4 están resumidos algunos de los cambios asociados al incremento del RCS en la leche de cabra.

Tabla 4. Resumen de los cambios en la composición de la leche de cabra asociado a un incremento de células somáticas (=, no varía; ↓, disminuye; ↑, aumenta) (adaptado de Raynal-Ljutovac *et al.*, 2007).

Parámetro	**Efecto**	**Referencia**
pH	=	Pasquini *et al.* (1996) y Jaubert *et al.* (1996b)
Sólidos totales	=	Pasquini *et al.* (1996) y Jaubert *et al.* (1996b)
Lactosa	=	Pasquini *et al.* (1996)
	↓	Jaubert *et al.* (1996b) y Zeng y Escobar (1996a)
Grasa	=	Pasquini *et al.* (1996), Baudry *et al.* (1997) y Ying *et al.* (2002)
	↓	Pisoni *et al.*, 2004a y Pisoni *et al.*, 2004b
Proteínas totales	↑	Pizzillo *et al.* (1996) y Ying *et al.* (2002)
	↓	Pisoni *et al.*, (2004a) yPisoni *et al.* (2004b)
Caseínas	=	Pizzillo *et al.* (1996)
Proteínas séricas	↑	Leitner *et al.* (2004b), Pasquini *et al.* (1996), Le Mens *et al.* (1996), Jaubert *et al.* (1996b), Morgan y Gaspard (1999)
Inmunoglobulina G	↑	Jaubert *et al.* (1996b) y Morgan y Gaspard (1999)
Sodio	↑	Morgan y Gaspard (1999)
Cloro	↑	Morgan y Gaspard (1999)
Potasio	↑	Ying *et al.* (2002)

En cuanto a la proteólisis en la leche asociada al RCS, Skeie (2007) sugirió que la leche de vaca debería tener bajo recuento de células somáticas ya que las proteasas de las células somáticas atacan las caseínas y reducen el rendimiento quesero. Verdi y Barbano (1991) demostraron que la actividad proteolítica a pH 6,6 era significantemente mayor en células somáticas aisladas de la leche de vaca mamítica que de los leucocitos aislados de la sangre de un toro, sugiriendo que este incremento puede ser debido a una mayor proporción de células activadas en animales enfermos que animales sanos. En este caso, no sólo variaron los animales, sino también el estado de salud, además del género de los animales a partir de los cuales se obtuvieron las células, así como la proporción de cada población celular, por lo que es difícil obtener unas conclusiones objetivas.

Duranti y Casoli (1991) observaron una disminución del contenido de α-caseínas y β-caseína asociado con un incremento de células somáticas en la leche de oveja; Bianchi *et al.* (2004) encontraron que las caseínas estaban sujetas a hidrólisis, lo cual resultaba en una disminución de β-caseína e incremento de γ-caseína, así como mayores índices proteolíticos en la leche de oveja proveniente de ubres infectadas comparadas con la de ubres sanas.

Respecto a la leche de cabra, Politis *et al.* (1994) demostraron que la células somáticas contienen un tipo de activador del plasminógeno, y que las actividades de la plasmina y estos activadores son mayores en la leche de cabra que en la de oveja y vaca, incrementándose con mayores niveles de RCS (Leitner *et al.*, 2004) en aquellos cuarterones de cabras naturalmente infectados comparados con sus respectivos paralelos no infectados.

Existe un desacuerdo en cuanto al efecto de las células somáticas sobre la actividad lipolítica. Jaubert *et al.* (1996) observó una correlación positiva entre la intensidad de sabor de cabra, lipólisis y RCS en la leche de cabra. Se ha sugerido que las células podrían ser responsables de suficiente lipólisis de los triglicéridos de los glóbulos de grasa, proveyendo defectos de sabor en la leche vaca (Azzara y Dimick, en leche mamítica; 1985; y Ma *et al.*, 2000, en leche mamítica; Santos *et al.*, 2003, en leche agrupada según el recuento de células somáticas). Por el contrario, otros estudios no han encontrado ninguna relación entre el recuento células somáticas y el nivel de lipólisis (Lee *et al.*, 1980; Cartier y Chilliard, 1990).

La lipólisis es un proceso que sólo ocurre en la leche de vaca cuando la membrana de los glóbulos de grasa se rompe y la grasa es accesible a las lipasas, como la lipasa lipoproteica, la cual en la leche de vaca está asociada a la fracción de caseína de la leche. Azzara y Dimick (1985) encontraron que los macrófagos de leche mamítica de vaca secretaban enzimas lipolíticas las cuales se unían a la membrana del glóbulo de grasa y la dañaban, exponiendo así los lípidos a la degradación por la lipasa lipoproteica.

3.5. Efectos de las células somáticas en el queso

Raynal-Ljutovac *et al.* (2005) concluyeron que las células somáticas, aunque son un indicador de la calidad higiénica de la leche de cabra, no parecen necesariamente empeorar la elaboración de queso. Sin embargo, Raynal-Ljutovac *et al.* (2007) revisaron en profundidad los aspectos analíticos, sanitarios, productivos y tecnológicos de las células somáticas sobre la leche de cabra y oveja. La mayoría de los estudios realizados compararon la leche, las propiedades de coagulación o los quesos, a partir de diferentes grupos de cabras, incluyendo infectadas y no infectadas, o cabras agrupadas de acuerdo a su nivel de RCS. Algunos de estos trabajos también fueron comparados, difiriendo entre sí al utilizar diferentes procedimientos de elaboración de queso, leche cruda o pasteurizada, o coagulación enzimática y ácida. En la Tabla 5 se resumen algunos de los resultados de los trabajos sobre el queso de cabra anteriormente citados (adaptado de Raynal-Ljutovac *et al.*, 2007). Debido a estas diferencias, no parecen claros y objetivos los efectos que producen las células somáticas en las propiedades de la leche, calidad del queso, lipólisis y proteólisis.

Tabla 5. Resumen de algunos efectos del incremento del recuento de células somáticas en la leche de cabra sobre las propiedades de coagulación y producción de queso (adaptado de Raynal-Ljutovac *et al.*, 2007).

Parámetro	Efecto	Referencias
Propiedades de coagulación		
Tiempo de coagulación	↑	Pizzillo *et al.* (1996) y Le Mens *et al.* (1996)
	=	Jaubert *et al.* (1996)
Tiempo para adquirir firmeza	=	Jaubert *et al.* (1996)
Firmeza	=	Jaubert *et al.* (1996)
Propiedades del queso		
Rendimiento quesero	↓	Leitner *et al.* (2004)
	=	Queso cremoso: Morgan y Gaspard (1999) y Galina *et al.* (1996)

Humedad en queso	↓	Queso cremoso: Morgan y Gaspard (1999)
Proteólisis y lipólisis	↑	Queso fresco cremoso: Morgan y Gaspard (1999)
	=	Queso cremoso madurado: Morgan y Gaspard (1999)
Calidad sensorial	=	Queso cremoso: Morgan y Gaspard (1999) y Zeng y Escobar (1996)

Auldist *et al.* (1996) detectó que la leche de vaca con más de medio millón de células somáticas reducía el rendimiento quesero, ya que la leche estaba asociada a una mayor actividad proteolítica, menor concentración de grasa y caseínas, y un mayor contenido de proteínas séricas, especialmente albúmina e inmunoglobulinas.

Pirisi *et al.* (2000) y Vianna *et al.* (2008) han descrito que mayores niveles de células somáticas en la leche resultó en un incremento del pH del queso, mientras que otros autores (Jaeggi *et al.*, 2003) no observaron variaciones significativas. Marino *et al.* (2005) al añadir células somáticas aisladas de la leche de vaca mamítica a leche en buenas condiciones no encontraron diferencias significativas de pH entre los quesos.

Como ya se señaló anteriormente, las células somáticas son una fuente de enzimas lisosomales (Le Roux *et al.*, 2003), muchas de las cuales pasan a la leche y causan proteólisis y lipólisis más intensa (Le Roux *et al.*, 1995; Albenzio *et al.*, 2005). Muchas de estas enzimas han sido encontradas o medidas en fracciones de la leche más que en las propias células, lo que sugiere que existe una secreción o liberación desde las células, o que las células se lisan y liberan las enzimas intracelulares (Kelly y Fox, 2006). Además, hay que tener en cuenta que el incremento de las células somáticas, o su causa, normalmente viene acompañado por un desbalance de otros parámetros de la leche, por lo que es muy difícil hacer responsables a las células somáticas de los cambios en la leche y en el queso.

3.5.1. Proteólisis

Marino *et al.* (2005) recuperaron y aislaron células somáticas a partir de leche mamítica de vaca, las añadieron a leche de buena calidad higiénica, y después evaluaron su contribución a la proteólisis durante la maduración de queso Cheddar. Estos autores encontraron que añadiendo células somáticas de leche mamítica a leche sana de vaca resultó en una mayor degradación de α_{S1}-caseína en los quesos a los 15 días de maduración, indicando una acelerada proteólisis de esta caseína. Caroprese *et al.* (2007), por su parte, aislaron macrófagos a partir de leche de ovejas sanas y estudiaron su actividad sobre la proteólisis y actividad de la plasmina. Cuando se lisaron estos macrófagos de la leche de oveja y se incubaron con Na caseinato, tras 24 horas se observaron cambios porcentuales en la composición de las caseínas y sus productos de degradación: las α-caseínas disminuyeron sobre un 20%, mientras que para la β-caseína no se observó ninguna degradación apreciable.

Conney *et al.* (2000) sugirieron una influencia directa de las proteasas (en especial la catepsina D) de los PMN sobre la maduración de queso tipo suizo y sobre el alto nivel de proteólisis endógena de la leche de vaca con alto recuento de células somáticas y PMN. En este experimento, los autores tomaron leche (no especifican si era mamítica) con aproximadamente 2 millones de células por mL con más del 90% de PMN y la mezclaron con leche sana para obtener distintos porcentajes de recuento total y PMN. Kelly *et al.* (2000) sugirió que debería ligarse los incrementos en el contenido de PMN y no sólo el incremento del RCS al efecto en leche y derivados lácteos de vaca, ya que los PMN tienen distintos perfiles enzimáticos que los macrófagos. Y hay apreciar que, tal como se describió anteriormente, en la leche de cabra los PMN son los principales leucocitos tanto en leche mamítica como sana, por lo que las comparaciones con los resultados de experimentos con leche de vaca son ambiguos o no concluyentes.

Moslehishad y Ezzatpanah (2010) describieron que un mayor recuento de células somáticas en la leche de vaca afecta a la microestructura y propiedades de las micelas de

caseína, las cuales se presentaban con un menor tamaño y agregadas, debido a la hidrólisis de las partículas de caseína por un incremento de la actividad proteolítica de enzimas como la plasmina, proteasas neutras y ácidas, lo que las hace más susceptibles a la degradación.

3.5.2. Lipolisis

Chen *et al.* (2010) no encontró diferencias significativas, en cuanto a nivel de lipólisis, en quesos de cabra elaborados con leche de animales agrupados según el nivel de células somáticas, y resultados similares has sido descritos por Jaeggi *et al.* (2003) en quesos curados de leche de oveja. En ambos casos, la leche fue recolectada y agrupada de acuerdo al RCS, por lo que es posible que otros componentes de la leche estuvieran involucrados, restando objetividad al efecto de las células somáticas sobre la lipólisis en los quesos.

Colectivamente, se han reportados pocos estudios en la literatura sobre los efectos directos de las células somáticas de animales sanos en los quesos, y no se ha encontrado en la literatura ningún trabajo objetivo acerca de la contribución a la capacidad lipolítica de las células somáticas sobre el queso de cabra.

4. El queso bajo en grasa

4.1. Situación actual

Los lípidos son componentes vitales en nuestra dieta: son una fuente de energía y de ácidos grasos esenciales. Sin embargo, el consumo de grasa saturada está altamente correlacionado con un incremento de riesgo de obesidad, arterioesclerosis, enfermedades coronarias y elevación de la presión sanguínea (Watts *et al.*, 1996; Van Horn y Ernest, 2002). Aunque esta información hoy en día es controvertida (Astrup *et al.*, 2011), el consumo de comidas altamente procesadas con harinas, azúcares, grasas y aceites refinados se ha incrementado considerablemente y a menudo fallan en el contenido de nutrientes esenciales (Kant, 2000). Al mismo tiempo, la disminución de las tareas del hogar y avances en los medios

de transporte han incrementado la vida sedentaria en los últimos 30 años (Hill *et al.*, 2003). Fuertemente influenciado por el cambio de la conciencia de los consumidores, la industria alimentaria se ha visto presionada para reducir la cantidad de grasa, azúcar, colesterol, sal y otros componentes en la dieta.

El interés actual hacia las alimentos más saludables y la tendencia de los consumidores hacia comidas con menor contenido graso ha resultado en un creciente interés en los quesos bajos en grasa (Johansen *et al.*, 2011). Sin embargo, la percepción de los consumidores por esos productos no ha sido especialmente positiva, y el consumo aún es bajo debido a su sabor y textura inadecuados (Childs y Drake, 2009).

4.2. Características

4.2.1. Composición química

La regulación española requiere unos rangos de contenido de grasa sobre materia seca para la designación de queso graso (60-45%), semigraso (45-25%), semidesnatado (25-10%) y desnatado (<10%).

De acuerdo a la literatura (Bryant *et al.*, 1995; Rudan *et al.*, 1999; Lteif *et al.*, 2009), los quesos desnatados presentan mayor contenido de proteína y humedad que sus respectivos quesos grasos. La grasa y humedad actúan rellenando la matriz de caseína del queso. Cuando la grasa se reduce, la humedad no reemplaza en equivalencia la grasa eliminada (Rudan *et al.*, 1999), lo cual resulta en un mayor contenido de proteína a medida que el contenido graso disminuye en el queso, así como un menor contenido en humedad sobre sólidos no grasos.

4.2.2. Textura

La textura, especialmente en quesos, es uno de los atributos más importante que ayudan a determinar la identidad de un producto. En los quesos grasos, la grasa forma cavidades en la matriz proteica, dándole una estructura más abierta, mejorando la

masticabilidad del queso (Johnson *et al.*, 2009). La eliminación de la grasa de la leche y posterior elaboración de queso, da como resultado la formación de una red de caseína mucho más cerrada y compacta, lo que produce un queso mucho más firme (Aryana y Haque, 2001; Banks, 2004; Rahimi *et al.*, 2007), de mayor dureza (Küçüköner y Haque, 2006; Rogers *et al.*, 2009) y cohesividad (Bryant *et al.*, 1995; Rudan *et al.*, 1999; Sahan *et al.*, 2008).

4.2.3. Proteólisis

Algunos autores han descrito que reduciendo el contenido de grasa en quesos Cheddar (Fenelon y Guinee, 2000) y Mozzarella (Tunick *et al.*, 1995) elaborados con leche de vaca resultó en mayores niveles de α_{S1}-caseína y β-caseína durante la maduración. Este efecto podría ser debido a ciertos factores asociados a los quesos: la disminución del ratio de actividad de enzimas coagulantes y nivel de proteínas (Fenelon y Guinee, 2000) y al mayor pH en quesos desnatados, lo cual los hace menos favorables a la actividad proteolítica del cuajo residual (Tam y Whitaker, 1972; O'Keeffe *et al.*, 1976).

Dado que la proteólisis de las caseínas en el queso es inadecuada, el resultado es que el queso presenta una textura relativamente firme (Mistry *et al.*, 1996; Mistry, 2001), y otros defectos en otros parámetros texturales, tal como se ha descrito en el apartado anterior.

4.2.4. Lipólisis

En cuanto a la lipólisis en quesos desnatados, hay muy poca información en la literatura. En los quesos, a medida que el contenido de grasa es reducido, evidentemente el nivel de ácidos grasos libres también lo hace (Banks *et al.*, 1989; Aly, 1994; Kondyli *et al.*, 2002) cuando se expresa en relación al queso total o materia seca. Pero sería más conveniente expresar el nivel de ácidos grasos en relación a la materia grasa para estudiar el grado de lipólisis real. Dimos *et al.* (1996) observó que ciertos ácidos grasos expresados sobre materia grasa en quesos Cheddar reducidos en grasa aparecían en menor cantidad de lo esperado

comparado en su respectivo queso graso, y sugirieron que la lipólisis fue casi completamente suprimida. La explicación que estos autores dan es que la enzima lipasa lipoproteica se ve inhibida en parte o que la matriz proteica de los quesos protege la grasa y la hace menos accesible a la lipasa (Fox *et al.*, 1993). Sin embargo, habría que tener en cuenta otro proceso que está relacionado con la lipólisis: el catabolismo de los ácidos grasos libres. Existen reacciones metabólicas de degradación de los ácidos grasos libres en los quesos dando lugar a otros compuestos, muchos de los cuales son volátiles (Collins *et al.*, 2003). Es posible que el menor nivel de ácidos grasos libres encontrados en los quesos bajos en grasa se deba no a una inhibición de la lipólisis, sino al catabolismo de los mismos. De hecho, Drake *et al.* (2010), en un análisis exhaustivo de componentes aromáticos en quesos Cheddar grasos y bajos en grasa, tanto a nivel instrumental como sensorial, observaron grandes diferencias de concentración (desbalances entre ambos quesos) de estos componentes. Los mismos componentes fueron encontrados en todos los quesos, pero en distintas concentraciones, por lo que se sugiere que la bioquímica que ocurre en quesos grasos y bajos en grasa es diferente.

4.2.5. Microestructura

López *et al.* (2007) identificaron 4 formas de presentación de la grasa en el queso graso Emmental estudiado a nivel de microscopía laser confocal: a) Glóbulos de grasa intactos, los cuales son pequeños, esféricos y se dispersan a través del queso, b) glóbulos de grasa agregados, que aparece como un grupo de glóbulos circulares, c) glóbulos de grasa coalescentes, que se han unido y forman glóbulos esféricos pero más grandes que los típicos glóbulos de grasa, y c) grasa no globular o libre.

Gunasekaran y Ding (1999) y Guinee *et al.* (2000) examinaron las características de los glóbulos de grasa en quesos Cheddar y encontraron que a menor contenido graso en el queso, los glóbulos de grasa eran más pequeños, se encontraban en más cantidad y disminuía la unión o coalescencia de los glóbulos remanentes. Rogers *et al.* (2010), reportó que, según imágenes

con microscopía laser confocal, el tamaño de los glóbulos de grasa aumentaban al incrementar el contenido de grasa en el queso. Al mismo tiempo, todos estos autores coinciden en que la red proteica de los quesos bajos en grasa aparece mucho más intrincada.

4.2.6. Color

Los quesos elaborados con leche baja en grasa presentan una menor opacidad y parecen más traslúcidos que sus respectivos quesos grasos (Fife *et al.*, 1996; Merrill *et al.*, 1996). El queso mozzarella con 6 y 10% de contenido en grasa pareció tener un índice de color verde (a* negativo) mayor, lo cual ha sido atribuido a una disminución del porcentaje de grasa (Tunick *et al.*, 1993; Merril *et al.*, 1994; Rudan *et al.*, 1998a). Rudan *et al.* (1998b) observó que cuando añadía un sustituto de grasa, el queso se volvía más luminoso que su respectivo bajo en grasa. En general, poco se ha descrito acerca del color instrumental en quesos de cabra bajos en grasa, básicamente porque existe muy poca literatura acerca de este tipo de quesos.

4.2.7. Características sensoriales

El análisis sensorial es una herramienta de alto valor para entender los aspectos que diferencian los quesos (Foegeding y Drake, 2007). La textura, color, sabor, aroma y la apariencia visual son características que pueden ser usadas para definir la calidad sensorial (Di Monaco *et al.*, 2008).

Drake (2008) ha evaluado la aceptación por parte de los consumidores de los quesos bajos en grasa, y ha identificado varios inconvenientes como son la falta de aroma, textura gomosa y pegajosa, baja fundición con el calor, y percepción sensorial por parte de los consumidores como no natural y poca atractiva comparada con los sus respectivos quesos grasos. Childs y Drake (2009) demostraron que cuando el sabor y la textura son diferentes de la versión grasa del queso, incluso si las diferencias son muy pequeñas, los consumidores no lo aceptan. Sin embargo, los consumidores estarían dispuestos a sacrificar ligeramente el sabor y

la textura de estos quesos si estuvieran interesados en quesos bajos en grasa, aunque en realidad, los consumidores interesados en esta opción son pocos. Los menores niveles de ácidos grasos como el butanoico y hexanoico, y de metil-cetonas, así como el incremento de otros componentes, han sido atribuído al sabor atípico de, por ejemplo, queso Cheddar bajo en grasa (Carunchia-Whestine *et al.*, 2006). Drake *et al.* (2010) evaluaron el impacto de la reducción de la grasa sobre los componentes aromáticos a nivel sensorial, y observaron que al inicio de la maduración las diferencias ya eran evidentes entre los quesos grasos y los bajos en grasa, pero que éstas se acentuaron durante la maduración prolongada: algunos descriptores se presentaban con mayor intensidad en los quesos bajos en grasa, como rosal y chamuscado, los cuales no se encontraron en los quesos grasos.

No está muy claro aún que la falta de sabor en los quesos bajos en grasa sea debido a la falta de precursores de aromas derivados de la grasa, la falta de grasa como solvente de los componentes aromáticos o por las diferencias estructurales de los quesos reducidos en grasa, lo cual podría modificar el ratio de algunas reacciones enzimáticas que son esenciales para la formación de los componentes del aroma (Urbach, 1997). Siguiendo esta línea, Carunchia-Wheststine *et al.* (2006) realizaron análisis sensoriales e instrumentales para caracterizar los aromas encontrados en quesos grasos Cheddar, los mismos quesos reducidos en grasa usando un método de centrifugación y la grasa extraída tras el proceso. Los resultados de los análisis sensoriales e instrumentales resultaron en que los quesos reducidos en grasa tenían perfiles aromáticos similares a sus respectivos quesos grasos, y la grasa extraída fue caracterizada por una baja intensidad de aromas. De esta manera, se demuestra que la mayoría de los componentes aromáticos permanecen en el queso tras la maduración y no se eliminan con la grasa.

Por otro lado, Yee *et al.* (2007) observaron que, eliminando la grasa del queso Cheddar y Parmesano con CO_2 supercrítico tras la maduración, los resultantes quesos reducidos en grasa no diferían de sus respectivos quesos grasos en cuanto a componentes aromáticos

solubles en agua; sin embargo, se detectaron más componentes en los quesos después del tratamiento. Los test de preferencia a consumidores revelaron que existía una mayor preferencia por los quesos tratados con el fluido supercrítico que por su correspondiente queso sin tratar o queso comercial bajo en grasa.

4.3. Métodos utilizados para mejorar los quesos bajos en grasa

A medida que la grasa es eliminada de la leche, los procedimientos de elaboración de queso bajos en grasa deberían ser modificados para corregir los defectos asociados con la textura y el sabor.

Se han empleado numerosos métodos para modificar y mejorar la textura de los quesos bajos en grasa. La alteración de los procesos de elaboración (Mistry, 2001; Guinee y McSweeney, 2006), la inclusión de aditivos y reemplazantes de grasa, extensamente revisado por Larsen (2009), y el uso de bacterias iniciadoras que producen exopolisacáridos (Awad *et al.*, 2005; Dabour *et al.*, 2006; Jiménez-Guzmán *et al.*, 2009), entre otros, se han propuesto como mecanismos para mejorar la calidad textural de los quesos bajos en grasa. Pero en general, los quesos grasos fueron siempre mejor aceptados que sus respectivos bajos en grasa.

Drake y Swanson (1995) concluyeron que mientras los quesos Cheddar y Mozzarella bajos en grasa elaborados con leche ultrafiltrada mostraban un mayor incremento de humedad, éstos fallaban en cuanto a textura. En el mismo estudio también se hizo una comparación con quesos elaborados con leche homogeneizada y también se incrementó el contenido en humedad, mejoró la textura instrumental, pero seguían sin tener valor comercial. En otros estudio, en el que se homogeneizó la crema que posteriormente se añadió a la leche para elaborar quesos bajos en grasa, mejoraba la textura, el sabor y la apariencia (Madadlou *et al.*, 2007), pero las diferencias respecto al queso graso seguían siendo muy significativas.

Un mayor pH en el desuerado implica una mayor retención de calcio en la cuajada y menor de la quimosina en quesos bajos en grasa, lo que contribuye a una textura más firme

(Mistry, 2001). La preacidificación de la leche ha mostrado una mejora de la textura del queso Mozzarella bajo en grasa o Kashar reducido en grasa (Merrill *et al.*, 1994; Fife *et al.*, 1996; Metzger *et al.*, 2001a, b, y c; Kaceli *et al.*, 2006) al disminuir el contenido de calcio, resultando en quesos más cremosos y menos gomosos. Madadlou *et al.* (2005) demostró que doblando la concentración de cuajo mejoraba las propiedades reológicas e impresiones sensoriales, así como la capacidad de fundirse, en queso Blanco Iraní desnatado.

Reducir la temperatura y tiempo de cocción de la cuajada, acortar el tiempo de agitación, lavar la cuajada y obtener un mayor tamaño de grano de la misma pueden disminuir algunos de los defectos asociados a la textura al incrementar el contenido en humedad sobre sólidos no grasos (Banks *et al.*, 1989; Mistry, 2001; Johnson *et al.*, 2009). Sin embargo, ninguno de estos métodos han mejorado el queso lo suficiente como para generar un queso de calidad satisfactoria.

Los reemplazantes de grasa (a base de carbohidratos o proteínas) han mejorado las propiedades sensoriales y funcionales de quesos bajos en grasa por su efecto de retención de humedad y por dar sensación de lubricidad y cremosidad (Romeih *et al.*, 2002), pero sigue existiendo el problema de los aromas atípicos. Otras inclusiones en los quesos bajos en grasa que han resultado en alguna mejora en la textura incluye el β-glucano (Konuklar *et al.*, 2004; Vithanage *et al.*, 2008), la lecitina (Sipahioglu *et al.*, 1999), el polisacárido goma tragacanto (Rahimi *et al.*, 2007), el carragenato (Kavas *et al.*, 2004; Totosaus y Guemes-Vera, 2008), las pectinas (Liu *et al.*, 2008), los emulsionantes (Lobato-Calleros *et al.*, 2008) y las bacterias productoras de exopolisacáridos (Perry *et al.*, 1997; Broadbent *et al.*, 2001).

4.4. Maduración acelerada

Para obtener las características mínimas de calidad, tal como lo percibe un consumidor, un queso normalmente debe pasar un tiempo mínimo de maduración. Las propiedades tales como el sabor y la textura toman cierto tiempo para desarrollarse (Walstra *et*

al., 1999). El almacenamiento y el mantenimiento de los quesos son caros debido a la inversión en edificios y maquinaria, así como los costes de energía y trabajo. Reducir el tiempo de maduración es por tanto atractivo desde el punto de vista económico. En los quesos normalmente están involucrados cambios microbiológicos, bioquímicos y físico-químicos que son responsables de desarrollo de la textura, sabor y aroma (McSweeney, 2004). El proceso de la aceleración de la maduración de los quesos normalmente tiene como objetivo acelerar la formación del sabor y aroma (por medio de una fuerte proteólisis y lipólisis), manteniendo al mismo tiempo una textura satisfactoria.

El uso de elevadas temperaturas para acelerar la proteólisis es uno de los métodos más prácticos para acelerar la maduración, pero con el riesgo de crecimiento de microorganismos no deseados. Azarnia *et al.* (2006) consiguieron disminuir el tiempo de maduración de queso Cheddar un 60-75% con buenos parámetros de sabor y textura a 12ºC.

El tratamiento con altas presiones es otra técnica que ha recibido especial atención en la última década. Se ha reportando que los quesos tras el tratamiento con altas presiones presentan una textura menos firme (Johnston y Darcy, 2000; O'Reilly *et al.*, 2000; Capellas *et al.*, 2001). Molina *et al.* (2000) trataron con alta presión leche parcialmente descremada (pasterizada y cruda) y elaboraron quesos reducidos en grasa, observando que estos quesos tenían un grado de proteólisis mayor que los quesos elaborados con leche pasteurizada sin tratar a alta presión, acelerando el desarrollo de la textura y el sabor, y disminuyendo la dureza tanto instrumental como sensorial.

La mayoría de los estudios sobre la aceleración de la maduración del queso, se han basado en la adición de varias enzimas lipasas o proteasas en la leche o la cuajada durante el procesado del queso (O'Keeffe *et al.*, 1978; Kwak *et al.*, 1989), para acelerar la lipólisis o proteólisis, respectivamente. El principal problema es que éstas se pierden fácilmente con el suero (hasta un 90%), a lo que se añade los posibles efectos sobre el suero que pueden imposibilitarlo para su reutilización, o bien estas enzimas no quedan homogéneamente

repartidas en la cuajada. Por tanto, se hace necesario encontrar un vehículo que mantenga las enzimas en la cuajada y que además las vaya liberando a lo largo del tiempo de la maduración (Kailasapathy y Lam, 2005; Madziva *et al.*, 2006). Azarnia *et al.* (2011) evaluaron quesos Cheddar a los cuales se les añadió aminopeptidasa recombinante de bacterias ácido lácticas encapsuladas, y encontraron que aumentaban la proteólisis considerablemente, al mismo tiempo que aquellos quesos con mayor concentración de proteasas obtuvieron las mayores puntuaciones positivas en los análisis sensoriales.

Las pastas de queso (cheese slurries) son papillas a base de productos lácteos a los cuales se les añade una mezcla de proteasas y lipasas, tras lo cual se incuban a 30-35ºC durante 4-5 días (Thakar y Upadhyay, 1992). Son utilizadas ampliamente en la industria alimentaria para dar el sabor a "queso" en muchos alimentos procesados. Estas pastas maduradas, altamente aromáticas, se pueden añadir en el proceso de la elaboración del queso para acelerar la maduración (Ammar El Tahra *et al.*, 1994). El principal inconveniente de esta técnica es el alto riesgo de contaminación microbiana.

El resultado de numerosos estudios sugiere que la plasmina puede ser una enzima de alto valor para acelerar la maduración y mejorar el desarrollo del aroma en quesos naturales (Bastian y Brown, 1996). Algunos investigadores han sugerido el uso de activadores del plasminógeno para aumentar la actividad de la plasmina, la cual se va perdiendo a lo largo de la maduración. De hecho, la aceleración de la maduración se ha conseguido en quesos Cheddar y tipo suizo al adicionarse activadores del plasminógeno exógeno (Bastian *et al.*, 1997; Barret *et al.*, 1999). Sin embargo, esta actividad tiene un gran coste y poca disponibilidad. Una posibilidad para seguir en esta línea de aceletación de la maduración podría ser activar el plasminógeno mediante activadores de plasminógeno endógenos, maximizando así la actividad de la plasmina, bajo condiciones controladas.

5. Los fluidos supercríticos

5.1. ¿Qué es un fluido supercrítico?

Un fluido supercrítico es definido como una sustancia que se encuentra por encima de su temperatura y presión crítica (punto crítico), tal como se indica en la Figura 5. En la región por encima del punto crítico, caracterizado por una sola fase, el fluido posee propiedades intermedias entre un gas y un líquido: líquido en cuanto a sus propiedades de densidad, y gas en cuanto a su viscosidad y difusividad. Con estas características, los fluidos supercríticos tienen al mismo tiempo un buen poder solvente a la par que buenas propiedades de transporte (Brunner, 1994).

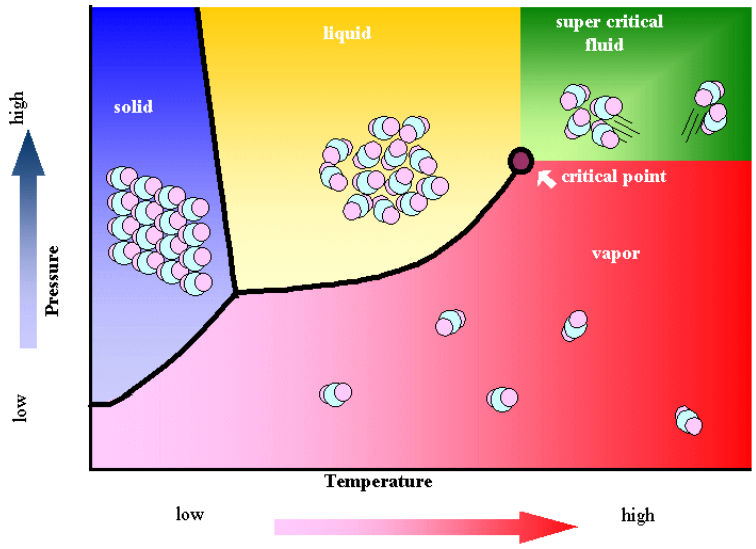

Figura 5. Diagrama de fases de una sustancia pura (www.che.tohoku.ac.jp).

5.2. El dióxido de carbono como fluído supercrítico

En las últimas décadas, se han propuesto diferentes sustancias como fluidos supercríticos. El CO_2 es el fluído supercrítico más usado debido a sus moderadas propiedades críticas: temperatura de 31ºC y 73 bares de presión, y porque es un gas que bajo condiciones ambientales permite una separación fácil del producto procesado (Chrisochoou y Schaber, 1996; Jackson y King, 1996). El CO_2 también es barato y se puede conseguir con una alta pureza, es una sustancia que no es tóxica, ni corrosiva ni inflamable, y es considerada como un

"solvente verde". El SC-CO_2 (dióxido de carbono supercrítico) es un solvente ideal para la eliminación de lípidos como triglicéridos y colesterol de matrices como el queso, debido a su comportamiento de solvente no polar (Arul *et al.*, 1994; Astaire *et al.*, 2003; Yee, 2006; Costa *et al.*, 2010).

La extracción con fluidos supercríticos (SFE, supercritical fluid extraction) es un proceso de separación en el cual el fluido supercrítico es el solvente. Este proceso consiste en dos pasos principales: la extracción, en la cual el fluido supercrítico disuelve el soluto desde la matriz sólida, y la separación entre el solvente y el soluto, lo cual normalmente se lleva a cabo al disminuir la solubilidad del fluido, que en caso del CO_2, consiste en la despresurización.

La capacidad de extracción del fluido supercrítico depende en gran medida de aquellas variables relacionadas con el diseño experimental, como es la matriz de la cual queremos extraer el componente, o de los parámetros operacionales (presión, temperatura, flujo, despresurización, etc).

5.3. Aplicaciones y uso en la industria

El uso de la tecnología de los fluidos supercríticos en la producción de componentes naturales ha adquirido un enorme interés en los últimos años, sobre todo cuando el producto final es de un alto valor para la industria alimentaria o farmacéutica.

El desarrollo de la SFE se ha incrementado notablemente en todo el mundo, y algunas plantas industriales extraen componentes naturales para su venta usando el SC-CO_2 como solvente. Hoy en día, muchos procesos están dirigidos hacia la extracción de componentes (ver Tabla 6, adaptada de Sahena *et al.*, 2009), aunque esta tecnología también se ha usado para el fraccionamiento de productos y cromatografía (Sandra *et al.*, 1988; Coleman *et al.*, 1999; Perretti *et al.*, 2004), mientras que otros procesos usan los fluidos supercríticos como medios para reacciones enzimáticas o formación de partículas (Knez, 2009). También se ha usado la extracción con fluidos supercríticos para eliminar compuestos no deseables de matrices que se

quieren purificar, como la desacidificación de aceites (Brunetti, 1989; Ayorinde y Hassan, 1995) y la descafeinización (Roselius *et al.*, 1974; Zosel, 1974). Los fluidos supercríticos también se han usado en los procesos de extrusión para la producción de nuevos alimentos, lo que permite el uso de ingredientes termolábiles (Berry, 2000).

Tabla 6. Resumen de algunos estudios sobre la extracción de componentes con fluidos supercrítico en la industria alimentaria (adaptado de Sahena *et al.*, 2011).

Producto	Analito	Presión (MPa)	Temp. (ºC)	Referencia
Huevo	Colesterol y lípidos	31	45	Froning *et al.* (1998)
Leche	Lípidos	10,0–35,0	50–70	Arul *et al.* (1987)
		6,9–24,1	40–80	Lim y Rizvi (1995)
Carne vacuno	Colesterol y lípidos	10,3–31,0	30–50	Chao *et al.* (1991)
		10,3–27,6	40	Merkle y Larick (1995)
Carne porcina	Lípidos	10,0–31,0	30–50	Chao *et al.* (1991)
Zanahoria	Carotenos	34,2–57,0	30–50	Vega *et al.* (1996)
Hojas de alfalfa	Caroteno y lutieno	10,0–70,0	40	Favati *et al.* (1998)
Batata	β-caroteno	13,8–41,4	38–48	Spanos *et al.* (1993)
Semillas de Bixa Orellana	Bixina	20,7–48,3	40–55	Degnan *et al.* (1991)
Pasta de tomate	β-caroteno y licopenos	20,0–30,0	35–65	Baysal *et al.* (2000)
Piel de tomate	Licopenos	40,5	60–110	Ollanketo *et al.* (2001)
Piel de uva roja	Antocianinos	7,0–15,0	80	Blasco *et al.* (1999)
Aceite de piel de naranja amarga	Terpenos	7,7–12,0	40	Chouchi *et al.* (1996)
Piel de naranja seca	Aceites esenciales	10,0–28,0	40–50	Blasco *et al.* (1999)
Jugo de frutas cítricas	Limonin	20,7–41,4	30–60	Kimball (1987)
Plantas aromáticas	Aceites esenciales	20	40	Blasco *et al.* (1999)
Setas	Oleo-resinas	11,5–14,9		del Valle y Aguilera (1989)

Semillas de Tamarindo	Antioxidantes	10,0–30,0	40–80	Tsuda *et al.* (1995)
Hojas de Eucalipto	Aceites con alta actividad antioxidante	20	50	Fadel *et al.* (1999)
Lúpulo	Humulona, lupulona and aceites esenciales	20	40	Langezaal *et al.* (1990)

Por otro lado, el SC-CO_2 tiene capacidad microbicida, lo que hace que tenga un gran potencial en el campo de la esterilización o reducción de carga microbiana (Perrut, 2011; Checinska *et al.*, 2011) y así garantizar la seguridad microbiológica de los alimentos. Spilimbergo *et al.* (2009) describió que, durante el tratamiento con SC-CO_2 a 36ºC y 100 bares de presión, existe una progresiva permeabilización de las células, y evidenció una correlación positiva entre la muerte célular y el CO_2 dentro de la células, el cual es efectivo tras 10 minutos de tratamiento. La cantidad de CO_2 acumulado en la fase lipídica podría entonces alterar la estructura y funcionalidad de la membrana celular al perderse la cadena lipídica al incrementar su fluidez (Isenschmid *et al.*, 1995).

En cuanto a su aplicación en productos lácteos, Astaire *et al.* (2003) y Costa *et al.* (2010), por ejemplo, usaron la tecnología de extracción con CO_2 supercrítico para obtener suero de mantequilla enriquecido aún más con fosfolípidos al extrae los lípidos no polares. Fatouh *et al.* (2007) ensayó la extracción con SC-CO_2 en un procedimiento por pasos para fraccionar la mantequilla de búfala, obteniendo 4 fracciones con propiedades químicas y físicas diferentes. En la primera fracción, obtuvieron colesterol, ácidos grasos de cadena corta y saturados a baja presión, mientras que los ácidos grasos de cadena larga y no saturados se concentraban en las siguientes fracciones a mayor presión. Spano *et al.* (2004) consiguieron fraccionar la grasa a partir de la leche de oveja a 40ºC y 250 bares de presión. La grasa fraccionada a partir de la grasa de la leche se puede comercializar con distintos propiedades

funcionales en la industria pastelera, confitería, del chocolate y la láctea (Gibon, 2006; Kontkanen *et al.*, 2011).

Yee *et al.* (2007) sugirieron que esta tecnología puede ser usada en la industria láctea para desarrollar quesos con menos grasa, reteniendo todo los componentes aromáticos de los quesos grasos, y que normalmente no se desarrollan en los típicos o más comunes procesos de producción de quesos reducidos o bajos en grasa. Yee *et al.* (2008) demostraron que la SFE puede ser aplicada a los quesos madurados, especialmente aquellos de baja humedad y duros. La aplicación de la tecnología de extracción con fluidos supercríticos a los quesos podría ser una alternativa ventajosa, sencilla y menos destructiva para la producción de quesos con menor contenido graso, que mantengan todas las características de aroma y textura de sus respectivos quesos originales, aumentando así su valor comercial.

Capítulo III:
Referencias Bibliográficas

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Capítulo IV:
Physicochemical analysis of full-fat, reduced-fat, and low-fat artisan-style goat cheese

1. Planteamiento y Metodología.
2. Trabajo experimental

Capítulo IV: Physicochemical analysis of full-fat, reduced-fat, and low-fat artisan-style goat cheese

1. Planteamiento y Metodología

1.1. Planteamiento

La demanda de leche de cabra y sus productos derivados, como el queso, se ha incrementado considerablemente en los últimos años como un reclamo a productos innovadores y como alternativa a la leche de cabra (Tziboula-Clarke, 2003). Por otro lado, existe un interés actual en preservar la producción de quesos tradicionales y artesanos, así como elaborados con leche cruda. En Canarias se producen 17.000 toneladas de queso cada año, consumido principalmente fresco (Fresno *et al.*, 2008), lo que apunta a una importante economía para el sector caprino.

Hay un creciente interés actual sobre los productos lácteos bajos en grasa (Koka y Metin, 2004), sin embargo, los quesos bajos en grasa normalmente están caracterizados por tener una textura gomosa y un sabor atípico comparado con su respectivo queso graso.

Existe una amplia información en la literatura sobre las características de textura de quesos de vaca (Rogers *et al.*, 2009) y de oveja (Lteif *et al.*, 2009), pero la información es escasa para quesos de cabra y no existe información sobre las características de quesos de cabra bajos en grasa elaborados con leche cruda y procedimientos no industriales.

1.2. Metodología

El material y los métodos usados en este experimento, así como los resultados han sido publicados con el título "Physicochemical analysis of full-fat, reduced-fat, and low-fat artisan-style goat cheese" (Análisis físico-químico de quesos artesanales de cabra enteros, reducidos y bajos en grasa) en Journal of Dairy Science (2010, 93:3950-3956).

En este trabajo se estudió la composición físico-química, recuento de células somáticas, densidad y pH de leche de cabra con 3 contenidos de materia grasa (5%, 1,5% y 0,4%), así como del suero resultante de la elaboración del queso, y la composición química básica y pH, así como el color instrumental y perfil de textura, también instrumental de los quesos madurados a 1, 7, 14 y 28 días.

De la leche y de los sueros resutantes de la elaboración del queso se midieron la composición química básica (grasa, proteína, lactosa y materia seca), el recuento de células somáticas, pH y densidad. En los quesos se midieron la composición química básica (grasa, proteína, humedad y grasa sobre materia seca), pH, color y textura instrumental.

Los datos se analizaron mediante el paquete estadístico SAS (versión 9.0; SAS Institute Inc., Cary, NC) mediante ANOVA de una vía (contenido graso de la leche) para los análisis de la leche y suero, y ANOVA medidas repetidas de dos vías (contenido graso de la leche de origen y tiempo de maduración) considerando un valor de $P<0,05$ como estadísticamente significativo. Para evaluar las diferencias entre grupos se usó el test de Tukey.

2. Trabajo experimental

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Physicochemical analysis of full-fat, reduced-fat, and low-fat artisan-style goat cheese[1]

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ABSTRACT

The objective of this study was to examine the physicochemical properties of cheese elaborated via traditional artisan methods using goat milk containing 5, 1.5, or 0.4% fat and ripened for 1, 7, 14, or 28 d. Seventy-two cheeses were produced (2 batches × 3 fat levels × 4 ripening times × triplicate). Proximal composition, pH, texture analysis, and color were recorded in each cheese. Protein and moisture were increased in cheese, and fat and fat in DM were decreased with decreasing fat in milk. Internal and external pH was higher in low-fat and reduced-fat cheese, and pH values decreased during the first 2 wk of ripening but increased slightly on d 28. Cheese fracturability, cohesiveness, masticability, and hardness increased with decreasing fat, whereas elasticity and adhesiveness decreased. Cheese lightness and red and yellow indexes decreased with decreasing fat content; during ripening, lightness decreased further but yellow index increased.
Key words: low-fat cheese, goat milk, raw milk, physicochemical property

INTRODUCTION

Over the past 15 yr, demand for goat milk and high-value goat milk products such as cheese has increased because of the increased demand for innovative products and for alternative products for consumers with cow milk intolerance (Tziboula-Clarke, 2003). Many varieties of goat milk cheese are produced around the world, and the quality of the products depends on the variety of the local animals, the milk composition, and the techniques used for manufacturing. The differences between different goat milk cheese types is mainly attributed to various physical and chemical changes that occur during the ripening process, which are highly influenced by the chemical composition of the milk and starter culture(s) and by the presence of additives (Park, 2001; Park et al., 2007).

Currently interest exists in preserving the production of traditional cheeses. In the Canary Islands of Spain, about 17,000 t of goat milk cheese is produced per year; most of these products are made with raw milk using traditional methods and are mainly consumed following short ripening periods (about 7 d; Fresno et al., 2008). It is generally agreed that pasteurization of milk causes changes that affect the flavor of cheese. The main agents involved in the flavoring of cheese are endogenous milk enzymes, rennet, and microbial enzymes from either local wild microflora or from commercial starters or adjunct cultures. Some endogenous milk enzymes, such as lipoprotein lipase, are inactivated by pasteurization; the autochthonous microflora are also partially eliminated, with a concomitant reduction in fermentation and degradation reactions (Grappin and Beuvier, 1997; Buchin et al., 1998).

Among the low-fat foods available worldwide, low-fat dairy products are in highest demand (Drake et al., 1996) and the demand for reduced-, low- and nonfat cheese has increased significantly since 1980 (Koca and Metin, 2004). Fat plays an important role in determining the cheese characteristics. Fat affects the body and texture of the cheese because it occupies the interstitial space in the mineral and protein structural networks and contributes substantially to the quality of taste (Jameson, 1987; McGregor and White, 1990). When fat is removed, caseins have a greater influence on the development of the texture. In low-fat cheeses, proteolysis of casein is inadequate, resulting in a relatively firm texture (Mistry et al. 1996; Mistry, 2001). Low-fat products are usually characterized as having a gummy body and an atypical flavor compared with their respective full-fat varieties.

Ample current information exists on the texture characteristics of low-fat cow (Rogers et al., 2009) and ovine milk cheeses (Lteif et al., 2009), but such information is scarce for low-fat goat milk cheese and no information is available regarding the characteristics

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of low-fat cheese generated from raw goat milk using nonindustrial procedures. The aim of this study was to analyze the chemical composition and the physical characteristics of cheese made using traditional methods from nonpasteurized goat milk containing various fat contents and ripened for different periods of time.

MATERIALS AND METHODS

Cheese Production

Animals and Cheese Formulations. Raw goat milk cheeses were elaborated according to the traditional handmade cheese practices common in the Canary Islands (Fresno and Álvarez, 2007) and were produced at the dairy farm of the Faculty of Veterinary, University of Las Palmas de Gran Canaria (Arucas, Spain). Chemical analysis and analysis of the physical and sensory cheese characteristics were performed at the Instituto Canario de Investigaciones Agrarias (La Laguna, Tenerife, Spain). Raw goat milk was obtained from an experimental herd of the Majorero goat dairy breed from the Animal Science Unit of the University of Las Palmas de Gran Canaria. Duplicate batches of experimental cheeses were produced and consisted of full-fat cheese (**FFC**), reduced-fat cheese (**RFC**), and low-fat cheese (**LFC**) ripened for 1, 7, 14, or 28 d in triplicate, resulting in a total of 72 cheese products (2 batches × 3 fat levels × 4 ripening times × triplicate).

Processing. An Elecrem skimmer (Elecrem, Fresnes, France) was used to obtain cream (35% fat content) and skim milk from 120 L of full-fat raw goat milk. Full-fat and skim milk were combined to obtain reduced-fat milk. Milk containing 3 different fat contents was therefore obtained: full-fat milk (**FFM**; 5% fat), reduced-fat milk (**RFM**; 1.5% fat), and low-fat milk (**LFM**; 0.4% fat). The same procedure was used to elaborate each type of cheese: raw milk containing 4 g/L of salt was heated in a cheese vat to 31°C. Animal rennet (Marshall rennet powder, Rhône-Poulenc Texel, Dangé-Saint-Romain, France) comprising 50% pepsin and 50% chymosin was added to obtain clotting within 35 min at 31°C. No starter cultures were added. Curd was cut with wire knives and allowed to stand an additional 5 min. More than 400 g of curd was placed in molds containing cheesecloth and pressed in a cheese press (Arroyo, Santander, Spain) at 2 kPa of pressure for 1 h. Cheese whey samples were removed for analysis. After pressing, the cheeses were 10 ± 0.1 cm in diameter and weighed 300 ± 15 g and were divided randomly into 4 groups of 6 cheeses and allowed to ripen for 1, 7, 14, or 28 d at 10 to 12°C and 80 to 85% relative humidity.

Analysis of the Physicochemical Properties of Milk, Cheese Whey, and Cheese

The proximal composition (fat, protein, lactose, DM, and nonfat solids) of each type of raw milk and cheese whey was evaluated using a DMA2001 Milk Analyzer (Miris Inc., Uppsala, Sweden). Somatic cell count was determined using a DeLaval somatic cell counter (DeLaval International AB, Tumba, Sweden), pH was determined using a GLP22 pH meter (Crison, Barcelona, Spain), and density was determined using a lactodensitometer (Alla France, Chemillé, France). Yield was calculated as the weight of cheese at 1 d of ripening divided by the initial weight of the milk. At 1, 7, 14, and 28 d of ripening, representative samples from each cheese were analyzed for proximal composition (fat, protein, moisture, and DM fat) using an Instalab 600 Product NIR Analyzer (Dickey-John Inc., Minneapolis, MN) according Adamopoulos et al. (2001). pH was measured at 3 internal (in the cheese center) and 3 external (1 cm from external cheese surface) locations for each cheese. Prior to texture analysis, a 0.5-cm layer was removed from the upper surface of each cheese to obtain a regular surface. Texture was analyzed using a cylindrical compression probe (samples were double compressed to 75% of their original height at a compression speed of 2 cm/min) in a TA-XT2i Texture Analyzer (Stable Micro Systems Ltd. Godalming, UK). Three cylindrical samples (2 cm in diameter and 5 cm in height) of each cheese were analyzed to determine the fracturability, hardness, cohesiveness, adhesiveness, and elasticity; masticability was indirectly determined (hardness × cohesiveness × elasticity). A Minolta colorimeter CR-400/410 (Illuminant D65, Konica Minolta, Osaka, Japan) was used to determine lightness (L*), yellow index (b*), and red index (a*) for each cheese. Measurements were taken at 4 external and 4 internal locations.

Statistical Analysis

Statistical analyses were performed using SAS (version 9.00, SAS Institute Inc., Cary, NC). The ANOVA procedure was used to compare physicochemical properties of milk and cheese whey. The MIXED procedure for repeated measurements was used to evaluate the effect of differing fat content and ripening time on the chemical composition, texture, and color of the raw goat milk cheeses. Tukey's test was used to evaluate the differences between groups.

RESULTS AND DISCUSSION

Milk and Cheese Whey

The physicochemical properties of the raw goat milk and cheese whey are shown in Table 1. Milk and cheese

Table 1. Proximal composition, pH, SCC, and density of full-fat milk (FFM), reduced-fat milk (RFM), low-fat milk (LFM), and cheese whey

	Raw goat milk				Whey			
Item	FFM	RFM	LFM	SEM	FFM	RFM	LFM	SEM
pH	6.77[a]	6.72[a]	6.64[b]	0.02	6.60	6.58	6.57	0.01
SCC, $\times 10^3$ cells/mL	1,753	1,811	1,795	63.53	173[a]	165[a]	99[b]	9.55
Fat, %	5.08[a]	1.57[b]	0.40[c]	0.48	0.54[a]	0.07[b]	ND[1]	0.06
Protein, %	3.47	3.35	3.33	0.04	1.07	0.96	1.07	0.04
Lactose, %	4.59[b]	4.84[a]	4.97[a]	0.05	5.45	5.48	5.45	0.03
DM, %	14.00[a]	10.51[b]	9.45[c]	0.47	7.49[a]	6.92[b]	6.87[b]	0.07
Density, g/cm^3	1.030	1.032	1.033	<0.01	1.023	1.026	1.026	<0.01

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]ND = not detectable.

whey pH decreased with decreasing milk fat content. In contrast, Rudan et al. (1999) observed an increase in pH when fat content in milk was reduced in Mozzarella cheese. Milk SCC ranged from 1,753 to 1,811 $\times 10^3$ cells/mL. The SCC in cheese whey after cheese manufacturing was 9.8, 9.1, and 5.5% of that in FFM, RFM, and LFM, respectively. Reduction of milk fat did not alter the milk SCC, but the SCC was lowest in cheese whey produced from LFM. Removal of fat may result in the formation of a stronger paracasein network (Banks, 2004), allowing for higher somatic cell retention in the LFM curd than in FFM and RFM curd. For FFM, milk fat percentages were in accordance with previous results for the same breed (Fresno and Álvarez, 2007). Milk and cheese whey fat percentages were decreased when fat was reduced by centrifugation in a milk skimmer. The fat percentage in cheese whey decreased according to the fat content of the milk from which it was produced; FFM cheese whey contained 0.54% fat, whereas RFM cheese whey contained 0.07% fat. No fat content was detectable in LFM cheese whey. The fat retention in the cheese products was higher as the fat content of the milk decreased. Rudan et al. (1999) suggested that when high-fat milk is used to make cheese, the curd matrix reaches the maximum fat-holding capacity. The optimum milk fat content should therefore be established to minimize fat loss in the cheese whey; however, it is also possible to reuse the cheese whey when cheeses are elaborated with high-fat milk.

The protein content in milk did not significantly decrease as fat was removed by centrifugation (Table 1). The amount of protein in whey was 3 times lower than in the milk from which it was produced, but no significant differences between the 3 types of cheese whey were observed. It has been reported that as the fat milk content decreases, protein increases and DM decreases (Rudan et al., 1999; Kahyaoglu and Kaya, 2003; Madadlou et al., 2005). Milk lactose ranged from 4.5 to 5%, increasing significantly as milk fat content decreased. However, the 3 types of cheese whey had similar lactose content. The removal of fat resulted in a slightly increased milk density.

Cheese Yield

As expected, cheese yield decreased by reducing fat in goat milk. Cheese yields for FFC, RFC, and LFC were 16.6 ± 0.54, 13.9 ± 0.26, and 12.4 ± 0.33%, respectively. An overall reduction in cheese yield is inevitable in the production of cheese from low-fat milk (Romeih et al., 2002) because the total amount of fat removed is not equal to the amount of moisture added (Mistry, 2001); therefore, the sum of the casein and fat content of milk, which are the principal components determining cheese yield, are reduced (Romeih et al., 2002).

Chemical Composition of Cheese

Table 2 shows the proximal composition and pH (external and internal) of cheeses during 28 d of ripening. As was expected, cheese fat content was higher in FFC than in RFC and LFC during the ripening period. The percentage fat in DM was higher in FFC than in RFC or LFC at all days tested during ripening. For all cheese (FFC, RFC, and LFC) the percentage fat in DM increased significantly during the ripening period because of moisture reduction. These values were within the ranges required by Spanish regulation for full-fat (60–45% fat in DM), half-fat (45–25% fat in DM), and low-fat (25–10% fat in DM) cheese designations.

On d 1 of ripening, the protein content was higher in LFC than in RFC and FFC. Throughout ripening, the difference between the protein content in LFC compared with RFC or FFC significantly increased. The moisture was higher in LFC than in RFC or FFC at 1 d of ripening and decreased in all 3 cheese types throughout the study because of surface water evaporation. The FFC protein content was close to the values reported

Table 2. Proximal composition and external and internal pH of full-fat cheese (FFC), reduced-fat cheese (RFC), and low-fat cheese (LFC) at 1, 7, 14, and 28 d of ripening

	Day of ripening				
Item	1	7	14	28	SEM
Fat, %					
FFC	$18.48^{d,x}$	$22.19^{c,x}$	$25.01^{b,x}$	$28.61^{a,x}$	0.89
RFC	$9.38^{b,y}$	$10.69^{b,y}$	$12.17^{ab,y}$	$13.61^{a,y}$	0.39
LFC	$1.12^{c,z}$	$2.60^{bc,z}$	$4.43^{b,z}$	$8.81^{a,z}$	0.64
Fat in DM, %					
FFC	$37.88^{d,x}$	$41.03^{c,x}$	$44.75^{b,x}$	$47.93^{a,x}$	0.87
RFC	$19.54^{c,y}$	$21.93^{b,y}$	$24.85^{a,y}$	$26.78^{a,y}$	0.68
LFC	$2.48^{d,z}$	$5.79^{c,z}$	$9.88^{b,z}$	$18.08^{a,z}$	1.28
Protein, %					
FFC	$18.94^{b,z}$	$20.51^{a,z}$	$20.47^{a,z}$	$19.96^{a,y}$	0.18
RFC	$20.97^{c,y}$	$23.01^{b,y}$	$24.38^{a,y}$	$24.65^{a,y}$	0.35
LFC	$22.84^{d,x}$	$25.90^{c,x}$	$29.37^{b,x}$	$32.66^{a,x}$	0.80
Moisture, %					
FFC	$46.95^{a,z}$	$45.92^{a,z}$	$44.12^{b,z}$	$40.32^{c,z}$	0.57
RFC	$52.00^{a,y}$	$51.61^{a,y}$	$50.98^{a,y}$	$49.20^{b,y}$	0.29
LFC	$55.69^{a,x}$	$55.28^{a,x}$	$55.15^{a,x}$	$51.18^{b,x}$	0.41
External pH					
FFC	6.58^{a}	5.03^{b}	4.92^{c}	$4.90^{c,x}$	0.15
RFC	6.57^{a}	5.10^{b}	4.89^{c}	$5.01^{b,y}$	0.14
LFC	6.59^{a}	5.10^{c}	4.99^{d}	$5.38^{b,z}$	0.13
Internal pH					
FFC	6.58^{a}	4.99^{b}	$4.74^{c,y}$	$4.81^{c,z}$	0.16
RFC	6.56^{a}	5.06^{b}	$4.82^{d,y}$	$4.94^{c,y}$	0.15
LFC	6.58^{a}	5.08^{c}	$4.91^{d,x}$	$5.32^{b,x}$	0.14

$^{a-d}$Means within a row with different superscripts differ ($P < 0.05$).
$^{x-z}$Means within a column with different superscripts differ ($P < 0.05$).

for Canarian goat cheeses by Fresno et al. (2005) for Palmero cheese and by Álvarez et al. (2007) for Majorero cheese. According to the literature (Bryant et al., 1995; Rudan et al., 1999; Lteif et al., 2009), low-fat cheeses contain significantly higher amounts of protein and moisture than their respective full-fat cheeses. Fat and moisture act as fillers in the casein matrix of the cheese. When the fat content is reduced, moisture does not replace the fat equivalents (Rudan et al., 1999), which results in higher percentages of protein as the fat content decreases, thereby increasing the ratio of protein to DM.

The external pH was approximately 6.6 in FFC, RFC, and LFC at 1 d of ripening. These values were similar to the typical values reported for cheese from goat milk produced in the Canary Islands (pH 6.6–6.7; Fresno and Álvarez, 2007), were within the range reported by Martín-Hernández and Ramos (1984) and Juárez et al. (1991) for goat cheeses produced in Spain, and were also very similar to values obtained by Álvarez et al. (2007) for Majorero cheese. Similar results were observed for the internal pH at 1 d of ripening.

During the first and second weeks of ripening, external and internal pH values decreased in all cheese groups. At 28 d of ripening, the pH increased in RFC and LFC, likely because of the release of basic AA and because of NH_3 and lactate decomposition (Alais, 1985). In FFC at 28 d of ripening, the pH value was similar to that observed at 14 d of ripening. The pH increase observed on d 28 in RFC and LFC may be attributed to a concomitant decrease in the level of moisture in nonfat solids and, hence, in the lactate:protein ratio (Fenelon and Guinee, 2000). At 7 d of ripening no differences in external and internal pH were observed between cheese groups. At 14 d of ripening the external pH was the same between cheese groups but the LFC had a higher internal pH value. This difference was more pronounced at 28 d of ripening. Taken together, these data indicate that as the fat was reduced, the external and internal pH increased. Small increases in moisture in nonfat solids led to relatively large increases in available water, which in turn resulted in increases in the activity of microorganisms and enzyme and the degree of proteolysis in cheese (Ruegg and Blanc, 1981).

Instrumental Analysis of Cheese Texture

Texture, particularly in cheese, is one of the most important attributes that helps to determine the identity of a product. Casein gels in the milk from various livestock species (cow, goat, sheep) strongly influence the rheological properties and texture of the cheese and other dairy products that are produced from the milk (Tunick, 2000).

Table 3. Texture analysis or texture profiles of full-fat cheese (FFC), reduced-fat cheese (RFC), and low-fat cheese (LFC) at 1, 7, 14, and 28 d of ripening

	Day of ripening				
Item	1	7	14	28	SEM
Fracturability					
FFC	$20.34^{b,y}$	$17.35^{b,y}$	$13.72^{b,y}$	$37.53^{a,z}$	2.67
RFC	$35.10^{b,xy}$	$26.28^{b,xy}$	$35.30^{b,x}$	$83.48^{a,y}$	6.10
LFC	$39.62^{b,x}$	$36.38^{b,x}$	$45.12^{b,x}$	$190.04^{a,x}$	14.10
Hardness					
FFC	$24.91^{b,y}$	$21.37^{b,y}$	$21.77^{b,z}$	$51.32^{a,z}$	3.11
RFC	$44.13^{b,x}$	$38.00^{b,x}$	$40.33^{b,y}$	$106.47^{a,y}$	6.54
LFC	$57.14^{b,x}$	$46.02^{b,x}$	$59.32^{b,x}$	$192.60^{a,x}$	13.05
Cohesiveness					
FFC	$0.17^{ab,y}$	$0.18^{a,y}$	$0.14^{b,z}$	$0.13^{b,z}$	0.01
RFC	$0.18^{b,y}$	$0.18^{b,y}$	$0.22^{a,y}$	$0.24^{a,y}$	0.01
LFC	$0.21^{c,x}$	$0.22^{c,x}$	$0.32^{b,x}$	$0.42^{a,x}$	0.02
Adhesiveness					
FFC	$0.40^{c,x}$	$0.96^{b,x}$	$2.41^{a,x}$	$2.35^{a,x}$	0.19
RFC	$0.03^{c,y}$	$0.39^{b,y}$	$1.22^{a,y}$	$0.33^{bc,y}$	0.10
LFC	$0.01^{c,y}$	$0.14^{bc,y}$	$1.11^{a,y}$	$0.40^{b,y}$	0.10
Elasticity					
FFC	$71.02^{a,x}$	$64.69^{b,x}$	$66.20^{ab,x}$	$60.21^{b,x}$	1.07
RFC	$67.91^{a,xy}$	$57.30^{b,y}$	$58.91^{b,y}$	$51.56^{c,y}$	1.48
LFC	$63.76^{a,y}$	$51.54^{b,z}$	$53.18^{b,z}$	$38.21^{c,z}$	2.16
Masticability					
FFC	301.26^{y}	259.32^{y}	209.54^{z}	409.08^{z}	21.60
RFC	$541.28^{b,xy}$	$423.62^{b,xy}$	$522.88^{b,y}$	$1{,}288.03^{a,y}$	79.98
LFC	$772.58^{bc,x}$	$537.88^{c,x}$	$955.67^{b,x}$	$3{,}071.98^{a,x}$	224.06

$^{a-c}$Means within a row with different superscripts differ ($P < 0.05$).
$^{x-z}$Means within a column with different superscripts differ ($P < 0.05$).

Table 3 shows the texture analysis of FFC, RFC, and LFC during the ripening period. Fracturability was significantly higher in LFC than in RFC or FFC at all ripening times examined; statistical differences in hardness were observed only between LFC and FFC at 1 and 7 d of ripening. Removal of fat has been shown to result in the formation of a much tighter paracasein network and produce a more firm cheese (Banks, 2004). Similarly, Küçüköner and Haque (2006) and Rogers et al. (2009) concluded that the hardness of Cheddar cheese increased as the fat decreased. On d 28 of ripening, fracturability and hardening increased significantly for all cheeses. This can be related to decreased moisture (Buffa et al., 2001; Fresno et al., 2006).

Cohesiveness, which results from the force exerted by internal links in the food, was higher in LFC than in RFC or FFC. Similar results were reported by others (Bryant et al., 1995; Rudan et al., 1999; Sahan et al., 2008) in which reduction of fat significantly increased the cohesiveness values. Adhesiveness was lower in RFC and LFC than in FFC during the course of ripening, increased in the 3 types of cheeses until d 14, and decreased significantly on 28 d of maturation in RFC and LFC. Elasticity of FFC decreased slightly over the 28-d ripening period but decreased significantly in RFC and LFC. Differences between the 3 types of cheeses were observed; specifically, RFC and LFC were much less elastic than FFC, as described for Cheddar cheese (Küçüköner and Haque, 2006). In Kashar cheese (Sahan et al., 2008), elasticity was reported to decrease with age but low-fat cheese had the highest value throughout ripening. Masticability, defined as the energy required to chew the cheese samples, was more or less constant over the 28-d ripening period for FFC; it increased with ripening in RFC and LFC and was higher in LFC than FFC and RFC at all 4 ripening times examined.

Instrumental Analysis of Cheese Color

Lightness decreased during maturation, and external lightness values were always lower than internal values, except in LFC at 28 d of ripening (Table 4 and 5). The decrease in lightness during storage is probably associated with increased protein hydration, which reflects a decrease in the number of free moisture droplets and thus a reduced degree of light scattering (Sheehan et al., 2005). Khosrowshahi et al. (2006) also reported that whiteness decreased in Iranian white cheese during ripening. In a solid material such as cheese, light penetrates the superficial layers and is scattered by milk fat globules (Lemay et al., 1994) and whey pockets (Paulson et al., 1998). As ripening progresses, whey in

Table 4. External color analysis of full-fat cheese (FFC), reduced-fat cheese (RFC), and low-fat cheese (LFC) at 1, 7, 14, and 28 d of ripening

	Day of ripening				
Item[1]	1	7	14	28	SEM
L*					
FFC	91.41^{a}	85.98^{b,x}	80.16^{c,x}	77.88^{d,x}	1.12
RFC	91.13^{a}	80.81^{b,y}	76.26^{c,y}	75.94^{c,xy}	1.38
LFC	91.95^{a}	78.98^{b,y}	72.55^{c,z}	74.32^{c,y}	1.60
a*					
FFC	−1.31^{b,x}	−0.69^{a,x}	−0.92^{a,x}	−0.81^{a,x}	0.07
RFC	−1.90^{b,y}	−1.24^{a,y}	−2.10^{b,y}	−1.92^{b,y}	0.11
LFC	−2.47^{c,z}	−1.94^{a,z}	−2.97^{b,z}	−2.23^{a,y}	0.09
b*					
FFC	10.52^{c}	16.10^{b,x}	18.45^{a,x}	18.55^{a,x}	0.78
RFC	11.04^{b}	13.40^{a,y}	15.01^{a,y}	14.58^{a,y}	0.46
LFC	10.26^{b}	14.27^{a,xy}	12.87^{a,z}	12.88^{a,y}	0.39

$^{a-d}$Means within a row with different superscripts differ ($P < 0.05$).
$^{x-z}$Means within a column with different superscripts differ ($P < 0.05$).
[1]L* = lightness; a* = red index; b* = yellow index.

serum pockets diffuses from the cheese body out into the brine, accompanied by moisture loss. The surface area occupied by light-scattering centers therefore decreases.

As milk fat decreased, external and internal red index values varied during the ripening of each type of cheese but were higher in RFC and LFC than in FFC. On the other hand, yellow index on d 1 of ripening was similar in all cheeses and increased during maturation. According to Rohm and Jaron (1996) and Fresno et al. (2006), many biochemical modifications occur in the maturation process of cheeses. In addition, cheeses acquire aromas and flavors and reach darker shades and increased hue angles, which results in increased yellowing.

CONCLUSIONS

Fat reduction of handmade raw milk cheeses produced using traditional methods has important effects on the chemical composition, pH, texture, and color of the resulting cheese product. As fat was reduced in milk, cheese yield decreased and protein and moisture percentages increased in cheese. Ripened (28 d) low-fat cheese displays higher fracturability, hardness, cohesiveness, and masticability values than full-fat cheese; conversely, low-fat cheese was less elastic and yellow than full-fat cheeses. All these physicochemical characteristics resulting from reducing the fat in cheese must be evaluated under a sensorial prism in the future.

Table 5. Internal color analysis of full-fat cheese (FFC), reduced-fat cheese (RFC), and low-fat cheese (LFC) at 1, 7, 14, and 28 d of ripening

	Day of ripening				
Item[1]	1	7	14	28	SEM
L*					
FFC	92.79^{a}	89.75^{b}	87.77^{bc,x}	86.31^{c,x}	0.61
RFC	93.06^{a}	89.94^{b}	85.83^{c,x}	84.25^{c,x}	0.90
LFC	93.28^{a}	89.07^{b}	80.13^{c,y}	64.98^{d,y}	2.31
a*					
FFC	−1.25^{ab,x}	−1.11^{a,x}	−1.33^{b,x}	−1.24^{ab,x}	0.05
RFC	−1.85^{a,y}	−1.90^{b,y}	−2.47^{c,y}	−2.64^{c,y}	0.07
LFC	−2.32^{a,z}	−2.64^{b,z}	−3.35^{c,z}	−3.19^{c,z}	0.10
b*					
FFC	8.21^{c}	10.31^{b,xy}	11.43^{a,x}	11.51^{a}	0.29
RFC	8.24^{c}	9.98^{b,y}	11.17^{a,x}	11.63^{a}	0.29
LFC	7.89^{c}	10.57^{b,x}	10.41^{b,y}	11.94^{a}	0.32

$^{a-d}$Means within a row with different superscripts differ ($P < 0.05$).
$^{x-z}$Means within a column with different superscripts differ ($P < 0.05$).
[1]L* = lightness; a* = red index; b* = yellow index.

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Capítulo V:
Short communication: Lipolysis and proteolysis profiles of fresh artisanal goat cheese made with raw milk with three different fat contents

1. Planteamiento y Metodología.
2. Trabajo experimental

Capítulo V: Short communication: Lipolysis and proteolysis profiles of fresh artisanal goat cheese made with raw milk with three different fat contents

1. Planteamiento y Metodología

1.1. Planteamiento

Existen tres rutas primarias de actividad bioquímica en los quesos durante la maduración: proteólisis, lipólisis y el metabolismo del lactato, citrato y lactosa residual (McSweeney, 2004a). La proteólisis contribuye a la maduración del queso a través de la formación de componentes aromáticos y cambiando la textura del queso debido a la rotura de la red proteica (Sousa *et al.*, 2001). La lipólisis se debe principalmente a la presencia de enzimas lipolíticas que liberan ácidos grasos de los triglicéridos, los cuales contribuyen, junto con los ácidos grasos ramificados, a las peculiares características de la leche y queso de cabra (Salles *et al.*, 2002). Información acerca de la inadecuada proteólisis (Tunick *et al.*, 1995; Fenelon and Guinee, 2000) y lipólisis (Banks *et al.*, 1989; Aly, 1994; Kondyli *et al.*, 2002) de los quesos bajos en grasa es bastante extensa, pero escasa en quesos de cabra y no existiendo la misma sobre el perfil proteico y nivel de lipólisis de quesos frescos artesanos elaborados con leche de cabra cruda.

1.1. Metodología

El material y los métodos usados en este experimento y resultados han sido aceptados para publicación con el título "Short communication: Lipolysis and proteolysis profiles of fresh artisanal goat cheese made with raw milk with three different fat contents" (Comunicación corta: Perfiles de lipólisis y proteólisis de queso fresco de cabra artesano hecho con leche cruda con tres contenidos de grasa diferentes) en Journal of Dairy Science (doi:10.3168/jds.2011-4423, en prensa).

En este trabajo se estudió el efecto de tres contenidos de materia grasa en el queso de cabra madurados a 1, 7, 14 y 28 días, descrito en el capítulo IV, sobre el nivel de lipólisis medido como contenido de ácidos grasos libres usando el método de "copper soap" modificado para quesos, y sobre la proteólisis medida mediante el perfil de proteínas en SDS-PAGE.

Los datos se analizaron mediante el paquete estadístico SAS (versión 9.0; SAS Institute Inc., Cary, NC) mediante una ANOVA de medidas repetidas de dos vías (contenido graso de la leche de origen y tiempo de maduración) considerando un valor de $P<0,05$ como estadísticamente significativo. Para evaluar las diferencias entre grupos se usó el test de Tukey.

2. Trabajo experimental

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Short communication: Lipolysis and proteolysis profiles of fresh artisanal goat cheese made with raw milk with 3 different fat contents

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ABSTRACT

The objective of this study was to describe the proteolysis and lipolysis profiles in goat cheese made in the Canary Islands (Spain) using raw milk with 3 different fat contents (0.5, 1.5, and 5%) and ripened for 1, 7, 14, and 28 d. β-Casein was the most abundant protein in all cheeses and at all ripening times. Quantitative analysis showed a general decrease in caseins as ripening progressed, and degradation rates were higher for α_{S1}-casein than for β-casein and α_{S2}-casein. Furthermore, the degradation rate during the experimental time decreased with lower fat contents. The α_{S2}-casein and α_{S1}-casein levels that remained in full-fat and reduced-fat cheeses were less than those in low-fat cheese. In contrast, β-casein also showed degradation along with ripening, but differences in degradation among the 3 cheese types were not significant at 28 d. The degradation products increased with the ripening time in all cheeses, but they were higher in full-fat cheese than in reduced-fat and low-fat cheeses. The free fatty acid concentration per 100 g of cheese was higher in full-fat cheese than in reduced- and low-fat cheese; however, when the results were expressed as milligrams of free fatty acids per gram of fat in cheese, then lipolysis occurred more rapidly in low-fat cheese than in reduced- and full-fat cheeses. These results may explain the atypical texture and off-flavors found in low-fat goat cheeses, likely the main causes of non-acceptance.
Key words: low-fat cheese, goat milk, proteolysis, lipolysis

Short Communication

Goat milk production, and its transformation into value-added products such as cheese, has great importance because of the increasing demand for diverse and distinctive products and as an alternative for people who cannot tolerate cow milk (Tziboula-Clarke, 2003). Raw milk cheeses are an important part of the economy and traditions of the Canary Islands (Spain); about 17,000 tons**[AU1: Is this metric tonnes? If not, please convert into metric tonnes.]** of goat milk cheese are produced per year. Most of these products are made with raw milk using traditional methods and are mainly consumed following short ripening periods (about 7 d; Fresno et al., 2008). The Canary Islands are officially free of caprine and ovine brucellosis, and this exceptional sanitary situation has allowed to about 500 artisan producers to obtain license to sell raw milk cheese within 60 d of ripening (Fresno and Álvarez, 2007). In contrast, new trends for healthier food choices and consumer interest for low-fat products have increased the attention for low-fat cheese (Johansen et al., 2011). Lipids are vital components of our diet; they are sources of energy and essential fatty acids. However, consumption of saturated fat is highly correlated with an increased risk of obesity, atherosclerosis, coronary artery disease, and elevated blood pressure (Watts et al., 1996; Van Horn and Ernest, 2001). Although this affirmation is nowadays in controversy (Astrup et al., 2011), the consumption of highly processed foods with refined starches, sugars, fats, and oils is increasing considerably and these foods often fail to contain the essential nutrients that are found in nutrient-dense foods (Kant, 2000). At the same time, labor-saving devices and advances in transportation have contributed to the highly sedentary lifestyle over the last 30 yr (Hill et al., 2003). Largely influenced by increase of consumer concerns, pressure has increased on the food industry to decrease the amount of fat, sugar, cholesterol, salt, and other components in the diet.

Three primary routes of biochemical activity exist in cheese during ripening: proteolysis; lipolysis; and the metabolism of lactate, citrate, and residual lactose (McSweeney, 2004a). Proteolysis is the most complex and, from the point of view of most investigators, the most important (McSweeney, 2004b). Proteolysis contributes to cheese ripening through a direct contribution to flavor via the formation of peptides and AA and by changing the texture of cheese from the breakdown of the protein network (Sousa et al., 2001). The technique

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of PAGE in the presence of SDS under reducing conditions (SDS-PAGE) is a fast, reproducible, and low-cost method that is widely used for quantifying, comparing, and characterizing proteins in goat cheese (Park, 2001).

Lipolysis in cheese is due to the presence of lipolytic enzymes that cleave the ester linkage between a fatty acid and the glycerol moiety of a triacylglycerol. It produces free fatty acids (**FFA**) with chain lengths ≥C4, glycerol, and mono- and diacylglycerols. Hexanoic, octanoic, and decanoic fatty acids have long been considered responsible for the characteristic aroma of goat (caprine) cheeses, giving rise to the terms caproic, caprylic, and capric acids. Additionally, certain branched-chain FFA contribute by themselves to the goaty flavor of cheese (Salles et al., 2002). Furthermore, the peculiarities of the goat milk lipolytic system (Chilliard, 1982) and the content of medium-chain fatty acids (Ha and Lindsay, 1993) could greatly change the FFA content, playing a major role in the distinctive flavor of goat milk (Chilliard et al., 2003).

Drake (2008) evaluated the consumer acceptance of low-fat cheeses, and identified various drawbacks to its use, including lack of flavor, rubbery texture, stickiness, low melt ability, and consumer perception as unnatural and unappealing compared with full-fat cheeses. Especially for some cheese varieties (e.g., hard Italian and blue-type cheeses), FFA are the major contributors to the development of their characteristic flavors (Fenelon and Guinee, 2000). Sensory profile and consumer preference tests have been made for the cheeses of the present study (D. Sánchez-Macías et al.**[AU2: Please provide initials and surnames for all those involved with this unpublished data. Also, please provide affiliation and location information for any of those who are not authors on the current paper.]**, unpublished data), and both judges and consumers preferred the full-fat cheese (**FFC**) cheeses, mainly because of the higher intensity, and they rejected the counterpart low-fat cheeses due to the off-flavors and higher chewiness. It is not yet clear whether the lack of flavor in low-fat cheeses is due to the lack of flavor precursors derived from the fat, the lack of fat as a solvent for flavor compounds, or the different physical structures of reduced-fat cheese, which may decrease the rate of some enzymatic reactions that are essential to the formation of flavor compounds (Urbach, 1997).

The gross composition, texture profile, and color of artisanal raw goat cheeses from the Canary Islands that were produced with milk with different fat contents have been reported (Sánchez-Macías et al., 2010), but information about the protein profile and lipolysis level of these cheeses has not been determined. The aims of this study were to quantify and compare the protein profile using SDS-PAGE and the lipolysis level (as total FFA) of cheeses made from raw goat milk with 3 different fat contents and ripened for 28 d using an artisanal method.

Raw goat milk cheeses were made according to traditional hand-made cheese practices common in the Canary Islands (Fresno and Álvarez, 2007) and were produced at the dairy farm of the Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria (Arucas, Spain). Raw goat milk was obtained from an experimental dairy goat herd (Majorera breed) from the Animal Science Unit of the Universidad de Las Palmas de Gran Canaria. Duplicate batches of experimental cheeses were produced and consisted of FFC, reduced-fat cheese (**RFC**), and low-fat cheese (**LFC**) ripened for 1, 7, 14, or 28 d in triplicate, resulting in a total of 72 cheese products.

An Elecrem skimmer (Elecrem SA, Fresnes, France) was used to obtain cream (35% fat content) and skim milk from 120 L of full-fat raw goat milk. Full-fat and skim milk were combined to obtain reduced-fat milk. The results were 3 different milk fat contents: full-fat milk (5% fat), reduced-fat milk (1.5% fat), and low-fat milk (0.4% fat). The procedure to make each type of cheese with raw goat milk was exactly the same, using the method described by Sánchez-Macías et al. (2010), using animal rennet (Marshall rennet powder; Rhône-Poulenc Texel, Dangé-Saint-Romain, France) comprising 50% pepsin and 50% chymosin and no starter cultures were added. Curd was pressed in a cheese press (Arroyo Laboratories, Santander, Spain) at 2 kPa of pressure for a 1-h period. After pressing, the cheeses were 10 ± 0.1 cm in diameter and weighed 300 ± 15 g. The cheeses for a given fat content were divided randomly into 4 groups of 6 cheeses and allowed to ripen for 1, 7, 14, or 28 d at 10 to 12°C and 80 to 85% relative humidity.

At 1, 7, 14, and 28 d of ripening, representative samples from each cheese were homogenized and analyzed for proximal composition (fat, protein, moisture, and fat in DM) using an Instalab 600 NIR product analyzer (Dickey-John Inc., Minneapolis, MN), a reflection spectrophotometer that is based on near-infrared reflectance. The pH was measured at 3 internal and 3 external random locations for each cheese.

All chemical reagents were of analytical grade from Panreac Química S.A.U. (Barcelona, Spain), Merck KGaA (Darmstadt, Germany), and Bio-Rad Laboratories Inc. (Hercules, CA). Water-soluble proteins were extracted from FFC, RFC, and LFC tested at 1, 7, 14, and 28 d of ripening using the method described by Tunick et al. (1995) with modifications. Briefly, 5 mL of buffer (0.166 *M* Tris, 0.001 *M* EDTA, pH 8.0) was added to 2 g of cheese and vortexed for 15 min. To this mixture, 5 mL of 7% SDS was added, and the

sample was vortexed again for 5 min. Then 2 mL of 10 m*M* dithiothreitol buffer was added, and the mixture was vortexed and held in an ice bath for 20 to 30 min. Samples were then centrifuged at 39,000 × *g* for 1 h at 4°C (Beckman J2-CI Centrifuge; Beckman Coulter Inc., Miami, FL). The supernatant was filtered, lyophilized, and stored at −20°C until analysis.

Lyophilized samples were prepared for SDS-PAGE; a final concentration of 1 mg/mL of cheese protein was reached using sample buffer (12.5% of 0.5 *M* Tris-HCl, pH 6.8; 25% glycerol; 2% SDS; and 0.4% of bromophenol blue). Each sample in buffer was boiled for 5 min at 100°C with 16 μL of β-mercaptoethanol, and the proteins (40 μg) were separated by SDS-PAGE (Laemmli, 1970) on a 12.5% gel. Gels were run at 100 V for 6 h and, after electrophoresis, gels were stained for 90 min using 10% acetic acid, 40% methanol, and 0.05% (wt/vol) Coomassie Blue R-250 solution, and then destained for 15 h using 10% acetic acid and 40% methanol solution. Images of stained gels were captured with a Bio-Rad imaging device (Gel Doc EQ; Bio-Rad Laboratories Inc.), and bands were quantified using Quantity One Quantitation Software (Bio-Rad Laboratories Inc.). Densitometry analysis of the gels was performed using lane-based background subtraction followed by measurement of the bands by the area under the intensity profile curve, and the values were then used for statistical analysis. Each sample was analyzed on duplicate gels. Individual protein species from the cheese samples were identified by comparing their relative mobilities with those of standard proteins (Bio-Rad Laboratories Inc.) from a previous SDS-polyacrylamide gel.

The total FFA content was determined using the copper soap method (Shipe et al., 1980; Ma et al., 2003, Melilli et al., 2004) as modified for cheese analysis. Ground cheese (0.25 g) at 4°C was placed in a centrifuge tube, 0.1 mL of 0.7 *N* HCl was added, and the tube was vortexed to allow thorough mixing of the acid and cheese. Then, 0.1 mL of 1% (vol/vol) Triton X-100 solution was added, and the mixture was vortexed. The copper soap reagent (2 mL) was added, and the mixture was vortexed again. Chloroform/heptane/methanol (49:49:2 vol/vol/vol) solvent (6 mL) was added to each tube without vortexing. The mixture had 2 distinct layers: the deep blue aqueous layer on the bottom and the colorless chloroform/heptane/methanol solvent layer on the top. The centrifuge tubes containing the reagents plus cheese samples were shaken for 60 min in a Heidolph Rotamax orbital platform shaker (Heidolph Instruments GmbH & Co., Schwabach, Germany) at 300 rpm. During shaking, the deep blue aqueous copper soap layer broke into pea-sized beads that were in continuous contact with the colorless solvent. When shaking was stopped, 2 distinct layers quickly formed, and then the tubes were centrifuged (Hettich Zentrifugen GmbH & Co. KG, Tuttlingen, Germany) at 3,500 × *g* for 10 min. The top colorless solvent layer (3.5 mL) was transferred from the centrifuge tubes into an acid-washed test tube containing 0.1 mL of the color reagent solution. After mixing, absorbance was measured immediately at 440 nm in a cuvette using a SmartSpec Plus spectrophotometer (Bio-Rad Laboratories Inc.). Two blanks (0.5 mL of deionized water instead of 0.25 g of cheese) were also prepared and analyzed with the cheese samples.

A standard curve was constructed using palmitic acid crystal grade. Eleven concentrations of palmitic acid were prepared: 0, 15, 30, 60, 120, 180, 240, 280, 360, 420, and 600 μg of palmitic acid/g of hexane. Each standard (1 mL) was added to a centrifuge tube. The hexane solvent was evaporated under the hood. After the solvent was completely removed, 1 mL of deionized water was added to the tube, and the set of standards was carried through the full analysis with the cheese samples and 2 blanks.

Absorbance readings of the standards were corrected by subtracting the average of the 2 blank readings, and a regression line was constructed that correlated the corrected absorbance with micrograms of palmitic acid. The level of FFA in the cheese samples was calculated from the standard curve. The final results were expressed as milligrams of FFA per 100 g of cheese and were also converted to milligrams of FFA per gram of fat in the cheese.

Statistical analyses were performed using the SAS program package (Version 9.00; SAS Institute Inc., Cary, NC). A PROC MIXED procedure (ANOVA with repeated measures) was used to evaluate the effect of different fat contents and times of ripening and to evaluate proteolysis and lipolysis during the ripening process in all 3 types of cheeses. Significantly different means were identified using the Tukey test.

The gross chemical composition and pH of cheeses throughout the 28 d of ripening (Sánchez-Macías et al., 2010) are shown in Table 1. As was expected, the percentage fat in DM was higher in FFC than in RFC or LFC at all days tested during ripening. On d 1 of ripening, the protein and moisture content was higher in LFC than in RFC and FFC. The moisture decreased in all 3 cheese types throughout the study because of surface water evaporation.

The external and internal pH was approximately 6.6 in FFC, RFC, and LFC at 1 d of ripening. During the first and second weeks of ripening, external and internal pH values decreased in all cheese groups. At 28 d of ripening, the pH increased in RFC and LFC, likely due to the release of basic AA and the decomposition of NH_3 and lactate (Alais, 1985). In FFC at 28 d of ripen-

Table 1. Chemical composition, external and internal pH values (means and SEM) of full-fat cheese (FFC), reduced-fat cheese (RFC), and low-fat cheese (LFC) at 1, 7, 14, and 28 d of ripening (Sánchez-Macías et al., 2010)

Item	Cheese type	Ripening duration 1 d	7 d	14 d	28 d	SEM
Moisture, %	FFC	$46.95^{a,x}$	$45.92^{a,x}$	$44.12^{b,x}$	$40.32^{c,x}$	0.57
	RFC	$52.00^{a,y}$	$51.61^{a,y}$	$50.98^{a,y}$	$49.20^{b,y}$	0.29
	LFC	$55.69^{a,z}$	$55.28^{a,z}$	$55.15^{a,z}$	$51.18^{b,z}$	0.41
Fat, %	FFC	$18.48^{d,x}$	$22.19^{c,x}$	$25.01^{b,x}$	$28.61^{a,x}$	0.89
	RFC	$9.38^{b,y}$	$10.69^{b,y}$	$12.17^{ab,y}$	$13.61^{a,y}$	0.39
	LFC	$1.12^{c,z}$	$2.60^{bc,z}$	$4.43^{b,z}$	$8.81^{a,z}$	0.64
Protein, %	FFC	$18.94^{b,x}$	$20.51^{a,x}$	$20.47^{a,x}$	$19.96^{a,x}$	0.18
	RFC	$20.97^{c,y}$	$23.01^{b,y}$	$24.38^{a,y}$	$24.65^{a,y}$	0.35
	LFC	$22.84^{d,z}$	$25.90^{c,z}$	$29.37^{b,z}$	$32.66^{a,z}$	0.80
Fat in DM, %	FFC	$37.88^{d,x}$	$41.03^{c,x}$	$44.75^{b,x}$	$47.93^{a,x}$	0.87
	RFC	$19.54^{c,y}$	$21.93^{b,y}$	$24.85^{a,y}$	$26.78^{a,y}$	0.68
	LFC	$2.48^{d,z}$	$5.79^{c,z}$	$9.88^{b,z}$	$18.08^{a,z}$	1.28
External pH	FFC	$6.58^{a,x}$	$5.03^{b,x}$	$4.92^{c,x}$	$4.90^{c,x}$	0.15
	RFC	$6.57^{a,x}$	$5.10^{b,x}$	$4.89^{c,x}$	$5.01^{b,y}$	0.14
	LFC	$6.59^{a,x}$	$5.10^{c,x}$	$4.99^{d,x}$	$5.38^{b,z}$	0.13
Internal pH	FFC	$6.58^{a,x}$	$4.99^{b,x}$	$4.74^{c,y}$	$4.81^{c,x}$	0.16
	RFC	$6.56^{a,x}$	$5.06^{b,x}$	$4.82^{d,y}$	$4.94^{c,y}$	0.15
	LFC	$6.58^{a,x}$	$5.08^{c,x}$	$4.91^{d,x}$	$5.32^{b,x}$	0.14

$^{a-d}$Means within a row with different superscript letters differ significantly ($P < 0.05$).

$^{x-z}$Means within a column for a specific item with different superscript letters differ significantly ($P < 0.05$).

ing, the pH value was similar to that observed at 14 d of ripening. The pH increase observed at d 28 in RFC and LFC may be attributed to a concomitant decrease in the level of moisture in non-fat solids and, hence, in the lactate-to-protein ratio (Fenelon and Guinee, 2000). Taken together, these data indicate that as the fat was reduced, the external and internal pH increased. Small increases in moisture in nonfat solids led to relatively large increases in available water, which in turn resulted in increases in the activity of microorganisms and enzyme and the degree of proteolysis in cheese (Ruegg and Blanc, 1981).

The SDS-PAGE patterns of proteins extracted from cheese with different fat contents and ripened for 1, 7, 14, and 28 d showed clear bands for caseins α_{S1}-CN, α_{S2}-CN, β-CN, para-κ-CN, and fragments of protein degradation (Figure 1). A densitometer protein-peptide distribution analysis is summarized in Table 2. In all cheese types and at all ripening times, the β-CN bands showed the highest intensities, followed by α_{S2}-CN and then α_{S1}-CN. The presence of para-κ-CN, which corresponds to the κ-CN (f1–105) fragment, was stable throughout the 28 d of ripening, with slight degradation apparent at the end of the experimental period.

In general, caprine milk contains no or relatively little α_{S1}-CN, whereas β-CN is the major protein (Tziboula-Clarke, 2003). The Canarian dairy breeds (Majorera, Tinerfeña, and especially Palmera) represent a particular case where 60% of the alleles for the caprine α_{S1}-CN locus are of the high type (A and B; Jordana et al., 1996); therefore, α_{S1}-CN is relatively abundant in milk and cheeses made from these breeds. On average, α_{S1}-CN in caprine milk represents 10% of total caseins, varying from 0 to 25% (Boulanger et al., 1984; Ciafarone and Addeo, 1984), depending on the animal genotype. In the present study, α_{S1}-CN was about 20 to 22% of the total intact caseins in all cheese types at 1 d of ripening.

Quantitative analysis showed a general decrease in casein fractions as ripening progressed for all 3 cheese types, and the degradation of intact caseins were higher for α_{S1}-CN (60–70%) than for β-CN (38–45%) and α_{S2}-CN (25–40%) over the 28 d of maturation. Trujillo et al. (1997b) found exactly the same order of susceptibility of the different casein components to rennet activity. Furthermore, the casein degradation rate was lower in cheeses with reduced fat contents. At 28 d, the percentage of total caseins remaining was 62, 66.5, and 70% in FFC, RFC, and LFC, respectively, as compared with the amount at 1 d of ripening. The remaining α_{S2}-CN and α_{S1}-CN levels in FFC and RFC were lower than in LFC. α_{S1}-Casein degraded faster; the cheeses contained 28 to 40%**[AU3: 28 to 40% of what? Of alphaS1-casein?]** at 28 d of ripening. α_{S2}-Casein degraded more slowly in LFC than in FFC and RFC. Other authors have also found that decreasing the fat content results in higher levels of intact α_{S1}-CN and β-CN in cheddar (Fenelon and Guinee, 2000) and mozzarella (Tunick et al., 1993; 1995) cheeses made with cow milk. Fenelon and Guinee (2000) showed that fat in the range of 6 to

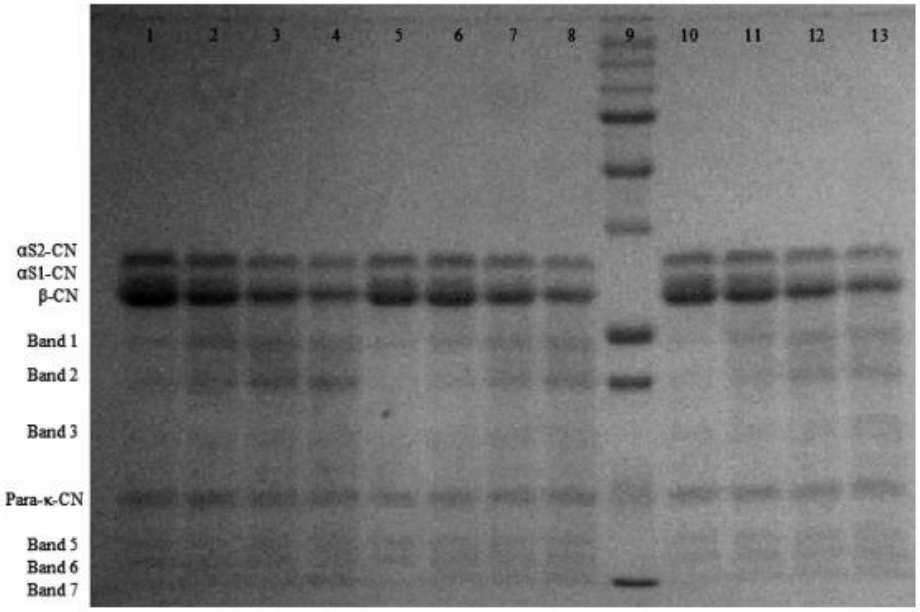

Figure 1. Sodium dodecyl sulfate PAGE pattern of caseins and other proteins (bands 1–3 and 5–7 are fragments of protein degradation) in goat milk cheese. Proteins were extracted from full-fat cheese (FFC), reduced-fat cheese (RFC), and low-fat cheese (LFC) and separated on a denaturing 12.5% polyacrylamide gel. Lanes 1 to 4 = degradation pattern of proteins from FFC aged for 1, 7, 14, and 28 d; lanes 5 to 8, degradation pattern of proteins from RFC aged for 1, 7, 14, and 28 d; lanes 10 to 13 = degradation pattern of proteins from LFC aged for 1, 7, 14, and 28 d; lane 9 = molecular weight protein standard (Precision Plus Protein Standards; Bio-Rad Laboratories Inc., Hercules, CA).

33% had a marked influence on the level of proteolysis in cheddar cheese manufactured using identical conditions but with a different level of moisture in nonfat solids. With a fixed concentration of casein, α_{S1}-CN degraded more slowly and β-CN degraded more rapidly as the fat content was decreased. In this study, the degradation slowed down only for α_{S1}-CN and α_{S2}-CN in LFC. This effect may be due to several associated factors: the decrease in the ratio of the residual rennet activity to the protein level (Fenelon and Guinee, 2000) and the high pH of low-fat cheeses, which is less favorable to the proteolytic activity of residual rennet (Tam and Whitaker, 1972; O'Keeffe et al., 1976). In low-fat cheeses, proteolysis of casein is inadequate, resulting in a relatively firm texture (Mistry et al., 1996; Mistry, 2001), and the results reported in the current paper are supported with those for higher instrumental hardness and masticability found by Sánchez-Macías et al. (2010) in the same cheeses. These authors found that fracturability, hardness, and cohesiveness were higher in low-fat cheese than full-fat cheese during 28 d of ripening. In the first 14 d of ripening, rubbery young cheese curd is rapidly changed to a smoother, more homogeneous texture, due to the casein network being considerably weakened through proteolysis (Creamer and Richardson, 1974; Lawrence et al., 1987).

Trujillo et al. (1995; 1997a,b) showed that the hydrolysis of goat casein by rennet and pepsin results in certain-sized peptides that can be identified by their migration rates in SDS-polyacrylamide gels. In this study, the breakdown products of casein in the experimental cheeses were quantitatively analyzed and generally increased over the ripening time for all cheeses. We have focused on the major peptides that appear, although other casein fragments may also have been present. The amount of these fragments was higher in FFC than in RFC and LFC. Thus, the degradation rate of caseins increased as the fat content in cheese increased, resulting in the appearance of more low-molecular weight bands in FFC. These low-molecular weight species are labeled bands 1 to 3, para-κ-CN, and bands 5 to 7 in Figure 1.

Band 1, just below β-CN, is related to γ-CN (Park, 2001) and did not vary significantly in cheese with different fat contents during 28 d of ripening. An increase in this fragment was observed in all cheese types at 7 d, but the level was stable between 7 and 28 d. A concomitant decrease in β-CN was observed with the increase in the band-1 protein, a finding that was also observed previously (Park, 2001). According to Trujillo et al. (1995, 1997a,b), the region where bands 2 and 3 appeared (with a molecular weight between those

Table 2. Comparison of densitometric values (intensity × millimeters) of SDS-PAGE protein bands in full-fat cheese (FFC), reduced-fat cheese (RFC), and low-fat cheese (LFC) at 1, 7, 14, and 28 d of ripening

	FFC				RFC				LFC				
Item[1]	1 d	7 d	14 d	28 d	1 d	7 d	14 d	28 d	1 d	7 d	14 d	28 d	SEM
α_{S2}-CN	69.16^{ab}	65.46^{b}	50.13^{c}	41.61^{e}	66.76^{ab}	69.40^{a}	50.25^{c}	44.53^{e}	65.68^{ab}	68.05^{ab}	53.07^{c}	49.05^{d}	0.98
α_{S1}-CN	50.93^{a}	39.58^{cd}	24.41^{ef}	17.03^{g}	55.69^{a}	44.48^{c}	28.07^{e}	15.83^{g}	56.10^{a}	47.96^{bc}	27.77^{e}	22.45^{f}	1.30
β-CN	125.69^{b}	103.07^{b}	77.71^{cd}	69.22^{e}	121.25^{b}	108.26^{b}	81.98^{c}	75.55^{d}	122.26^{a}	108.28^{b}	79.56^{cd}	72.95^{de}	1.86
Band 1	13.37^{c}	26.42^{a}	23.12^{ab}	24.50^{ab}	16.81^{c}	24.69^{ab}	21.07^{b}	24.42^{ab}	14.59^{c}	22.30^{ab}	22.25^{ab}	23.67^{ab}	0.56
Band 2	11.06^{f}	20.09^{de}	27.04^{cd}	44.67^{a}	10.97^{f}	18.56^{e}	24.27^{d}	37.28^{b}	8.32^{f}	16.39^{e}	23.84^{d}	30.80^{c}	1.03
Band 3	4.82^{e}	7.15^{bc}	9.77^{b}	13.03^{a}	5.27^{e}	6.57^{c}	5.99^{c}	9.66^{b}	5.22^{e}	6.07^{c}	6.94^{c}	8.43^{bc}	0.33
Para-κ-CN	40.80^{ab}	43.23^{a}	36.44^{b}	36.34^{b}	37.81^{b}	39.14^{b}	37.62^{b}	39.63^{ab}	41.02^{ab}	41.40^{ab}	35.74^{b}	39.49^{ab}	0.50
Band 5	13.57^{b}	15.56^{ab}	15.16^{ab}	16.93^{a}	14.54^{ab}	14.47^{ab}	13.68^{b}	15.29^{ab}	15.63^{ab}	15.32^{ab}	15.908^{ab}	15.45^{ab}	0.37
Band 6	13.25^{b}	15.92^{b}	15.68^{b}	16.38^{ab}	14.16^{b}	15.01^{b}	13.94^{b}	18.51^{ab}	15.20^{b}	14.15^{b}	16.02^{b}	19.05^{a}	0.41
Band 7	13.63^{bc}	15.66^{b}	15.00^{bc}	19.01^{a}	13.12^{bc}	11.45^{c}	17.60^{ab}	18.00^{ab}	12.35^{bc}	12.11^{c}	13.36^{bc}	17.35^{ab}	0.49

[a–g]Means within a row with different superscript letters differ significantly ($P < 0.05$).

[1]Bands 1 to 3 and bands 5 to 7 = fragments of protein degradation.

of β-CN and β-LG) is where the larger peptides from β-CN hydrolysis are expected to migrate, including the chymosin-generated series of β-CN (1–192, 1–163, and 1–139 fragments) and plasmin-generated β-CN (f29–207). For both bands 2 and 3, differences were found in the rate of appearance among cheese types; the bands emerged earlier in FFC than in RFC and LFC. Band 2 was more prevalent than band 3. Trujillo et al. (1997b) reported that the hydrolysis of α_{S2}-CN by rennet resulted in the appearance of low-molecular weight bands in the region between 10 and 14 kDa, where bands 5, 6, and 7 are presented on the gel. Bands 5 and 6 were present at a low level over time in the 3 types of cheese, except that band 5 and band 6 increased at 28 d in FFC and LFC, respectively. Band 7 clearly increased over the 28 d of maturation in all 3 cheese types to a similar extent.

Means of total FFA per 100 g of cheese and per gram of fat of cheeses throughout the 28 d of ripening are shown in Table 3. At all ripening times, FFC had the highest value of total FFA/100 g of cheese (Table 3a). Full-fat cheese exhibited a high increase in total FFA during the ripening time until 14 d; the value remained unchanged at 28 d of ripening. For RFC and LFC, the total values of FFA/100 g of cheese remained similar without significant differences, except for a significant increase in RFC on d 28 as compared with the first week of ripening. A decrease in the total FFA level occurs in other cheese varieties as the fat content is decreased (Banks et al., 1989; Aly, 1994; Kondyli et al., 2002).

The levels of total FFA per 100 g of cheese suggest that the lipolysis rate increases as fat content in cheese increases, but if the total FFA value is expressed per gram of fat in cheese (Table 3b), it is apparent that the fat was hydrolyzed faster in LFC than in FFC. After 1 d of ripening, total FFA in LFC per gram of fat was much higher than that in FFC and RFC. Thus, other parameters in the cheese appear to have an influence on lipolysis. Gunasekaran and Ding (1999) examined the 3-dimensional characteristics of fat globules in Cheddar cheese of varying fat content (4–34%) and found that at the lowest fat content, globule size was smallest but more globules were noted. Guinee et al. (2000) observed that, in Cheddar cheese (7–30% fat content), a decrease in the fat content resulted in progressive clumping and coalescence of the globules. Microstructural and physicochemical dynamics of fat globules also appear to influence the localization and retention of starter lactococci in cheese: compared with Cheddar with a 50% fat reduction, full-fat Cheddar cheese retains a higher bacterial population in the curds, and these bacteria are located on the periphery of the fat globules (Laloy et al. 1996). Thus, the smaller size (and consequent

Table 3. Total free fatty acids (FFA) in full-fat cheese (FFC), reduced-fat cheese (RFC), and low-fat cheese (LFC) at 1, 7, 14, and 28 d of ripening

	Ripening duration				
Item	1 d	7 d	14 d	28 d	SEM
Total FFA, mg/100 g of cheese					
FFC	$145.72^{c,z}$	$254.57^{b,z}$	$519.40^{a,z}$	$503.76^{a,z}$	35.37
RFC	$47.61^{ab,y}$	$32.68^{b,y}$	$57.41^{ab,y}$	$87.27^{a,y}$	6.94
LFC	$36.83^{a,y}$	$40.65^{a,y}$	$31.02^{a,y}$	$28.10^{a,x}$	2.66
Total FFA, mg/g of fat in cheese					
FFC	$7.58^{b,y}$	$11.60^{b,z}$	$20.78^{a,z}$	$17.62^{ab,z}$	1.24
RFC	$5.19^{a,y}$	$3.04^{a,y}$	$4.75^{a,y}$	$6.55^{a,y}$	0.57
LFC	$34.73^{a,z}$	$17.46^{b,z}$	$6.91^{c,y}$	$3.15^{c,y}$	3.09

[a–c]Means within a row with different superscript letters differ significantly ($P < 0.05$).

[x–z]Means within a column with different superscript letters differ significantly ($P < 0.05$).

higher total fat globule surface area and interface for lipase activity) and localization of lactic acid bacteria could explain the higher rate of lipolysis in LFC than in FFC.

Over the course of the 28-d ripening period, the total FFA per gram of fat in LFC decreased, possibly as a result of the catabolism of FFA. In RFC, the total FFA per gram of fat did not change over the ripening time, which may be due to equilibrium between FFA liberation and FFA degradation. The FFA per gram of fat in FFC increased during the first 14 d but decreased by d 28. The lack of fat and the faster FFA liberation in LFC and RFC may promote early FFA metabolism and, thus, modify the flavor that is characteristic of FFC.

Fat reduction in traditional hand-made cheese resulted in higher protein and moisture content and pH values. The degradation rate of caseins decreased with the lower fat content, resulting in fewer low-molecular weight bands in RFC and LFC. The FFA concentration per 100 g of cheese was always higher in FFC than in RFC and LFC, but lipolysis expressed as total FFA per gram of fat in cheese increased as the fat content decreased.

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Capítulo VI:
Sensory analysis of full-fat, reduce-fat, and low-fat cheese elaborated with raw gaot milk

1. Planteamiento y Metodología.
2. Trabajo experimental

Capítulo VI: Sensory analysis of full-fat, reduce-fat, and low-fat cheese elaborated with raw goat milk

1. Planteamiento y Metodología

1.1. Planteamiento

La tendencia actual de comer saludablemente, junto con la preferencia de los consumidores a comidas más bajas en grasa ha resultado en un creciente interés en los quesos bajos en grasa (Johansen *et al.*, 2011). Sin embargo, la percepción sobre los quesos reducidos y bajos en grasa no ha sido muy positiva, y su consumo aún es muy bajo debido a su inadecuado sabor y textura. Debido a la percepción integral de las características sensoriales de los productos lácteos, medir éstas es el paso final de muchos experimentos o aplicaciones. Un panel sensorial entrenado es una herramienta de gran valor para diferenciar y describir los quesos (Foegeding y Drake, 2007), así como las pruebas de consumidores nos darán una visión sobre el futuro comercial de un nuevo producto. El queso de cabra forma parte de la economía y tradición de las Islas Canarias, la mayoría producido con leche cruda y usando métodos tradicionales (Fresno *et al.*, 2008). No existen quesos canarios reducidos o bajos en grasa en el mercado. El presente estudio de análisis sensorial forma parte de un proyecto con el objetivo de demostrar los efectos de diferentes contenidos en materia grasa de la leche cruda de cabra sobre las características de queso elaborado artesanalmente y madurado 28 días.

1.2. Metodología

El material y los métodos usados en este experimento, así como los resultados obtenidos, están aceptados para publicación con el título "Sensory analysis of full-fat, reduce-fat, and low-fat cheese elaborated with raw gaot milk" (Análisis sensorial de quesos

enteros, reducidos y bajos en grasa elaborados con leche cruda de cabra) en Journal of Applied Animal Research.

El objetivo principal de este trabajo, siguiendo la línea de los dos capítulos anteriores, era profundizar en el entendimiento y proveer una línea base del efecto de la reducción de grasa en leche sobre las características sensoriales, diferencias y aceptabilidad de los quesos artesanos de leche cruda de cabra elaborados con tres contenidos de material grasa y madurados durante 1, 7, 14 y 28 días.

Para ello, se desarrollaron una variedad de métodos diferentes: test triangular para detectar pequeñas diferencia en el conjunto de atributos de los quesos, de acuerdo a la norma ISO 4120 (2004); test de preferencia por los consumidores, de acuerdo a la norma ISO 4121 (2003); y perfiles descriptivos por 7 expertos especializados en quesos de cabra, de acuerdo a la metodología descrita por Beròdier *et al.* (1996) y Lavanchy *et al.* (1999) y adaptados para quesos de cabra, según lo descrito por Fresno y Álvarez (2007).

Los datos de los perfiles sensoriales y resultados de las pruebas de aceptación se analizaron mediante el paquete estadístico SAS (versión 9.0; SAS Institute Inc., Cary, NC) mediante una ANOVA de medidas repetidas de dos vías (contenido graso de la leche de origen y tiempo de maduración) considerando un valor de $P<0,05$ como estadísticamente significativo. Para evaluar las diferencias entre grupos se usó el test de Tukey.

2. Trabajo experimental

Sensory analysis of full-, reduced- and low-fat cheese elaborated with raw goat milk

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Sensory analysis of full-, reduced- and low-fat cheese elaborated with raw goat milk

The market for goat milk cheese has grown due to the new tendencies to consume innovative products, and the fact that it has provided a profitable alternative to cow milk cheese due to its inherent health-promoting attributes. The trends toward healthier eating have increased the interest in low-fat cheese. The objective of this study is to enable an understanding and provides a baseline of the effect of fat reduction on sensory analyses and consumer acceptability of cheeses made from raw goat milk with three different fat contents and ripened for 28 d using an artisanal method. Odor and flavor intensity was lower as fat decreased in cheese, and LFC and RFC were firmer, friable, grainier, drier, acidic, and less adhesive and sweet than full-fat cheese. Both judges and consumers preferred the FFC cheeses, mainly because of the greater intensity and the combination of this with excessive hardness and high masticability was likely the main cause of non-acceptance.

Keywords: goat milk, low-fat cheese, sensory profile, consumer acceptance

Introduction

In affluent developed countries, more than 95% of dairy products consumed are derived from cow's milk. Exceptions to this rule include countries of the Mediterranean basin, where ewe and goat milk and their respective products form a fundamental part of their cultural heritage (Michaelidou, 2008). Goat milk comprises only 1.98% of the milk production in the world, with the Mediterranean area being the main producer (18%) after India (22%). According to recent literature reports, ewe and goat milk products can provide a profitable alternative to cow milk products due to their specific composition, sensory qualities, and inherent health-promoting attributes (Haenlein, 2004; Raynal-

Ljutovac et al., 2008). In addition, goat milk has been found to contain more easily digestible fat and protein than cow's milk, as well as an increased content of vitamin A, thiamine and niacin (Haenlein, 2001).

Currently, consumer demand for a diversification of cheese products requires the development of innovative products. Trends toward healthier eating and a consumer preference for lower fat foods have resulted in increased interest in low-fat cheeses (Johansen et al., 2011). However, consumer perception of low- and reduced-fat cheese has not necessarily been positive and consumption is still low because of inadequate taste and texture (Childs and Drake, 2009). To support this, the traditional characteristics of a cheese and its designation of origin have been found to be two of the most important factors influencing consumer preference in the market (Bertozzi and Panari, 1993), although flavor remains an important role for cheese purchase and consumption (Childs and Drake, 2009). Cheese is an important part of the economy and traditions of the Canary Islands (Spain): about 17,000 tons of goat milk cheese is produced per year, and most of these products are made with raw milk using traditional methods (Fresno et al., 2008). The Canary Islands have an important variety of cheeses, of which three of them are designated as being protected designation of origin (PDO): Palmero, Majorero, and de Guía and Flor de Guía cheeses. As well as the caprine and forage genetic richness, the islands have an exceptional sanitary situation due to being officially free of caprine and ovine brucellosis, which allows 500 artisan producers to sell raw milk cheese with shorter than 60 days of ripening (Fresno and Álvarez, 2007).

The sensory perception of dairy foods as flavorful and wholesome is one of the keys to their enjoyment by consumers. Due to the integral nature of sensory perception with dairy foods, measuring these aspects is the final step in many experiments or applications. A trained sensory panel is a valuable tool in understanding the aspects

differentiating cheeses (Foegeding and Drake 2007). Texture, color, taste, aroma and visual appearance can be used to define the sensory quality (Di Monaco et al., 2008).

No low-fat or reduced-fat Canary cheeses are available on the market. This study is part of a project aimed at demonstrating the effects of reducing fat in goat milk on the characteristics of fresh cheese elaborated using artisan procedures. The gross composition, texture profile, and color of artisanal raw goat cheeses from the Canary Islands that were produced with milk with different fat contents have been reported by Sánchez-Macías et al. (2010), and lipolysis and proteolysis profiles of the same cheeses have shown significant differences between FFC, RFC, and LFC (Sánchez-Macías et al. in press), but information about sensory characteristics or consumer acceptance has not been determined. This study enables an understanding and provides a baseline of the effect of fat reduction on the sensory profile by experts, differences and acceptability by consumers of cheeses made from raw goat milk with three different fat contents and ripened for 28 days using an artisanal method.

Materials and methods

Cheese Production

Animals and Cheese Formulations

Raw goat milk cheeses were prepared according to traditional hand-made cheese practices common in the Canary Islands (Fresno and Álvarez, 2007), and were produced at the dairy farm of the Faculty of Veterinary, Universidad de Las Palmas de Gran Canaria. Analysis of the chemical and sensory cheese characteristics was performed at the Instituto Canario de Investigaciones Agrarias. Raw goat milk was obtained from an experimental herd of the Majorera goat dairy breed from the Animal Science Unit of Universidad de Las Palmas de Gran Canaria. Duplicate batches of experimental cheeses

were produced and consisted of full-fat cheese (FFC), reduced-fat cheese (RFC) and low-fat cheese (LFC) ripened for 1, 7, 14 or 28 days in triplicate, resulting in a total of 72 cheese products.

Processing

The procedure to elaborate each type of cheese with raw goat milk was exactly the same, using the method described by Sánchez-Macías et al. (2010), using animal rennet (Marshall rennet powder, Rhône-Poulenc Texel, Dangé-Saint-Romain, France) comprising 50% pepsin and 50% chymosin and no starter cultures were added. Curd was pressed in a cheese press (Arroyo, Santander, Spain) at 2 kPa of pressure for 1 h. After pressing, the cheeses were 10 ± 0.1 cm in diameter and weighed 300 ± 15 g. The cheeses for a given fat content were divided randomly into four groups of six cheeses and allowed to ripen for 1, 7, 14, or 28 d at 10–12ºC and 80–85% relative humidity.

Analysis of the Physicochemical Properties of Cheese

At 1, 7, 14 and 28 d of ripening, representative samples from each cheese were analyzed for proximal composition (fat, protein, moisture and fat in dry matter) using an Instalab 600 Product NIR Analyzer (Dickey-John Inc. Minneapolis, MN). pH was measured at three internal and three external locations for each cheese.

Sensory Evaluation

Sensory analysis was performed using a variety of methods:

(1) Triangle tests to detect small differences that may affect the set of attributes or a particular attribute between FFC and RFC or LFC were performed at 1, 7, 14, and 28 d of ripening according to ISO 4120 (2004). Fifty untrained consumers who were unaware of the experimental conditions were provided with 3

samples, 2 of which were the same. Cheese samples were coded in a uniform manner, using three-digit numbers, chosen randomly. All the possible sequences of the three products were given to the consumers, and each consumer indicated which one of the samples was different from the other two. The consumers were also asked for the attributes that they found in the different sample. Normal distribution, as an approximation for binomial distribution, was used for data analysis. Statistics were performed at the 95% confidence level.

(2) A preference test, according to ISO 4121 (2003) at 1, 14 and 28 days of ripening was performed using 50 untrained consumers, with the goal of contributing to market research for introducing or evaluating the product. In this case a hedonic scale from 1 to 5 was used to quantify the degree of acceptance.

(3) A description profile was compiled using seven specialized expert assessors. The methodology for the description profile was in accordance with that described by Beródier et al. (1996) for odor and flavor attributes, and texture was determined following the guidelines published by Lavanchy et al. (1999); both tests were adapted for goat cheese sensory analysis as described by Fresno and Álvarez (2007). Sensory panelists evaluated randomly coded cheeses ripened for 28 d. The judges cleansed their palettes between samples using unsalted crackers, Granny Smith apples, and then water containing a very low level of minerals. Sensory texture terms can be separated into “texture with fingers” (roughness, surface moisture, and springiness) and “texture in mouth” (firmness, friability, adhesiveness, solubility, moisture in mouth, and granularity), as described in Table 1. The attributes sweetness, acidity, pungency, odor and flavor intensity comprised the odor and flavor analysis. In addition, each judge was allowed to describe the odor and flavor of each sample

by selecting descriptors from the following list of terms: milky, vegetable, fruity, toasted, animal, and floral. All evaluations were scored on a structured scale from 1 to 7 and cheeses were maintained at 20 ± 1ºC during the evaluation period.

The sensory analyses were performed in a special room following the instructions given by the norm ISO 8589 (2007), including absence of noises and odors, good lighting, white walls, adequate temperature and humidity conditions. The individual booths were arranged next to each other on both sides of a corridor through which the randomly coded samples were given to consumers and specialized expert assessors. Samples were allowed to equilibrate at room temperature (20ºC) for 1 h prior to evaluation in order to achieve optimum conditions for sensory evaluation and to obtain homogeneous cuts. The cheeses were cut into pieces of 2 x 2 x 1 cm, a size sufficient for the consumers to be able to taste the samples properly, and placed on an individual odor-free serving plate coded with three-digit random codes.

Statistical Analysis

Statistical analyses were performed using SAS, Version 9.00 (SAS Institute Inc. Cary, NC). The SAS PROC MIXED procedure for repeated measurements was used to evaluate the effect of differing fat content and ripening time on the chemical composition, sensory attributes of the sensory profile and acceptance test. Tukey's test was used to evaluate the differences between groups. The ANOVA procedure was used to compare sensory attributes.

Results and discussion

Chemical Composition of Cheese

Table 2 shows the gross chemical composition and pH of cheeses throughout the 28 d of ripening (Sánchez-Macías et al. 2010). As was expected, the percentage of fat in DM was higher in FFC than in RFC or LFC at all days tested during ripening, whereas protein and moisture content was higher in LFC than in RFC and FFC. The moisture decreased in all three-cheese types throughout the study because of surface water evaporation.

The external and internal pH was approximately 6.6 in FFC, RFC and LFC at day one of ripening. During the first and second weeks of ripening, external and internal pH values decreased in all cheese groups. At 28 d of ripening, the pH increased in RFC and LFC, likely due to the release of basic amino acids and the decomposition of NH_3 and lactate (Alais, 1985). In FFC at 28 d of ripening, the pH value was similar to that observed at 14 d of ripening. The pH increase observed at day 28 in RFC and LFC may be attributed to a concomitant decrease in the level of moisture in non-fat solids and, hence, in the lactate-to-protein ratio (Fenelon and Guinee, 2000). Taken together, these data indicate that as the fat was reduced, the external and internal pH increased.

Sensory Analysis of Cheese

Triangle test

The triangle test performed revealed significant differences between FFC, and RFC or LFC concerning organoleptic characteristics at the four evaluated ripening times. The sensory attributes differentiating the RFC and LFC from FFC were as follows: a) hardness and firmness, which were considered high; b) flavor, which was considered acidic, with poor cheese intensity; c) moisture content, which was considered high; and

d) color, which had low lightness and more yellow hue. These attributes were responsible for the consumers being able to distinguish the low and reduced-fat goat artisan cheeses from its counterpart full-fat cheese. These comments are accordance with those reported by Sánchez-Macías et al. (2010), who showed that fat reduction of handmade raw goat milk cheese resulted in higher fracturability, hardness, cohesiveness, and masticability values in cheese at 28 days of ripening, moreover the instrumental color analyses revealed that lightness decreases and yellow index increased as fat content was reduced in cheese.

Acceptance test

Figure 1 shows the results of the preference testing. As expected (Sipahioglu et al., 1999; Madadlou et al., 2005; Lteif et al., 2009), consumers preferred FFC, although RFC ripened for 14 and 28 d were scored slightly below FFC without significant differences. In general, cheese ripened for 14 d had better scores than cheese ripened for 1 and 28 d. Panelists listed some characteristics of each cheese sample and the results were as follows:

FFC: at 1, 14, and 28 d, the cheese was described as having good texture, flavor, and smoothness. At 14 and 28 d, it was slightly acidic, and at 28 d it was creamy.

RFC: at 1 d, the consumers rated the cheese slightly hard and dry, gummy, and an off-flavor; at 14 d, it retained the off-flavor, but had good texture and slight acidity; and at 28 d, the cheese was rated as dry, creamy, and slightly acidic, with an off-flavor. In general, the consumers described RFC as intermediate between FFC and LFC.

LFC: at 1 d, the cheese had an off-flavor and was gummy, very hard and dry; at 14 d, the cheese was described as dry, creamy, gummy, bitter, very acidic, and with off flavor; and at 28 d, the cheese was dry, gummy, bitter, and acidic, with an

off-flavor.

Drake (2008) has evaluated the consumer acceptance of low-fat cheeses, and has identified various drawbacks to its use, including lack of flavor, rubbery texture, stickiness, low melt ability, and consumer perception as unnatural and unappealing compared to full-fat cheeses. Childs and Drake (2009) found that most of consumers are not accepting of lower fat Cheddar or mozzarella cheese when their flavor and texture are different from the full-fat version of the cheese, even if those differences are small. Conceptually, consumers were willing to sacrifice flavor and texture of cheese slightly if they were interested in having a cheese that was reduced in fat. However, reality showed that consumer interest in this option is low if flavor and texture are sacrificed.

Description profile

Table 3 shows the results of the averaged scores obtained for the different descriptive sensory attributes of texture with fingers. The roughness increased during 14 d of ripening in all cheese types without significant differences between them, and only after 28 d was LFC considered to have lower rough surface than FFC and RFC. Surface moisture decreased during the ripening time in all cheeses. Although the sensory score of this attribute was higher in RFC and LFC than in FFC during the first 2 weeks of ripening, at 28 d FFC were considered to have more moisture than RFC and LFC. Springiness decreased along the experimental time, without significant differences between cheeses. Sánchez-Macías et al. (2010) reported that the instrumental springiness parameter decreased during the ripening time, but instead, they found significant differences between cheeses, specifically, RFC and LFC were much less elastic than FFC. Rogers et al. (2009) showed clear distinctions between full-, reduced-, and low-fat cheese too, low-fat cheese being more springy.

The means of the scores of the attributes of texture in mouth are summarized in Table 4.

Firmness scores decreased in cheeses over the 28 d of ripening. At d 1, the scores were higher in LFC than in RFC and FFC, but after 2 weeks, RFC and LFC had similar scores, indicating that the decreasing of firmness was faster in LFC. Rogers et al. (2009) reported that firmness at first-bite decreased during the first month in full-, reduced- and low-fat Cheddar cheese, which can be attributed to the higher rate of proteolysis during the first 14 d of aging (Lawrence et al., 1987). Quantitative analysis of proteolysis in the same cheeses (Sánchez-Macías et al. in press) showed a general reduction in caseins as ripening progressed and the degradation rate during the experimental time decreased with lower fat contents, which is correlated with the firmness profile found in the sensory analysis. FFC had lower values for friability, and these values increased during the ripening time for FFC and RFC, while they decreased slightly in LFC. Adhesiveness scores tended to increase over the experimental time in cheese; the values were similar in all cheeses at the beginning of the ripening, but at 14 and 28 d it increased as fat content increased in cheeses. These results are in accordance with those obtained from the instrumental texture analyses (Sánchez-Macías et al., 2010), although during the 28 d of ripening, RFC and LFC had similar values, which were lower than those for FFC. Solubility averaged scores increased during the experimental time and FFC received the higher scores, while granularity decreased as time passed and LFC was considered the grainiest during the experiment. Panouillé et al. (2011) working with dairy model gels found that those with higher protein content or lower fat content were firmer and stickier than control dairy models. Gwartney et al. (2002), Brown et al. (2003) and Yates and Drake (2007) reported that the majority of reduced-fat cheeses were characterized by chewiness, hardness, firmness, fracturability and springiness, and displayed lower adhesiveness and cohesiveness.

In Table 5 are summarized the score means of the taste attributes. The saltiness attribute

increased, but only at 28 d of ripening was FFC considered saltier than RFC and LFC. The increase of salty perception in the presence of fat had already been reported in cheese (Wendin et al., 2000; Romeih et al., 2002) and is attributed to a relatively higher concentration of salt in the aqueous phase (Shamil et al., 1992). Panouillé et al. (2011) reported in dairy models that fat addition significantly increased salty perception, whereas protein addition decreased it, regardless of water content. Acid taste was detected in cheeses after two weeks of ripening, which significantly decreased at 28 d of ripening. At both times, FFC had lower scores than RFC and LFC. The acid taste scores' evolution during the ripening time is correlated with pH values shown in Table 2, although in this case, pH values at 14 and 28 d were higher in LFC than RFC and FFC. On the other hand, averaged scores obtained for sweetness evolved in the opposite way of acid taste scores.

The scores about odor and flavor are summarized in Table 6. External assessment was similar in the three cheese types at four ripening times (data not shown). Odor and flavor intensities increased during the experimental time and LFC always had the lowest scores. This perception was traduced in lower scores in odor and flavor assessments for LFC than RFC and FFC. Odor and flavor persistence scores increased with the maturation too, and FFC had higher persistence values than LFC at 14 and 28. With regard to taste and flavor, the lower intensity scores for LFC and RFC likely resulted from the lack of fat precursors. This is due to the role of fat in providing a medium for the dissolution of flavor compounds and the inhibition of certain enzymatic reactions essential for the formation of flavors (Banks, 2004). Lowered levels of fatty acids such as butanoic and hexanoic acids and methyl ketones, as well as increased levels of other compounds, have been attributed to the atypical flavor in low-fat Cheddar cheese (Carunchia Whetstine et al., 2006). They found that flavor profiles of full-fat cheese and

the same full-fat cheese with the fat removed after ripening were almost identical. Yee et al. (2007) suggested that supercritical fluid extraction technology can be used in the dairy industry to develop cheese products lower in fat, retaining flavor compounds that may not be typically fully developed with alternative methods of low-fat cheese processing.

Total assessment scores of the three cheese types reveled that FFC was considered the best, and LFC the worst. Anyway, the scores decreased for FFC at 28 d, while for LFC and RFC the total assessments increased significantly at 28 d compared to the first two weeks of ripening.

These results were consistent with claims that low-fat cheeses are considered less acceptable to consumers, and that defects in the texture include increases in strength, hardness, dryness and granularity (Olson and Jonhson, 1990). Increased elasticity of LFC and RFC products has also been reported (Olson and Jonhson, 1990; Madadlou et al., 2005), but was not reproduced in the present study. These results indicate that the properties of low-fat and non-fat cheese produced using traditional artisan methods need to be improved. Significant technological advances in low-fat cheese production are available to minimize sensory and functional deficiencies (Banks, 2004) and alternative methods that include variations in processing techniques or the inclusion of additives have been developed. Madadlou et al. (2005) reported that doubling the rennet concentration in low-fat cheese improved the rheological properties and sensory impression of texture, as well as increasing meltability in Iranian white cheese. Using natural rennet (Fresno et al., 2006) can be another alternative to improve the quality of low-fat goat cheese. As fat is removed from the cheese milk, cheese-making procedures that are generally used for manufacturing full-fat cheese should be modified to correct

the associated textural and flavor defects, including improving moisture retention and reducing acid accumulation. Fat replacers improve the sensory and functional properties of low-fat cheeses by the bulking effect associated with moisture retention and give a sense of lubricity and creaminess (Romeih et al., 2002), but Drake and Swanson (1995) have concluded that the manufacture of acceptable cheeses with a fat reduction of up to 33% and retention of good taste and texture is commercially feasible and could be achieved without the need for fat substitutes. Different methods have been employed to accelerate cheese ripening time: elevate ripening temperatures, attenuation of started bacteria, use of adjunct cultures, addition of curd slurries, addition of exogenous enzymes and microencapsulation of enzymes (Law, 2001), even the use of microencapsulated aminopeptidase from starters over expressed in E. coli (Azarnia et al., 2011). But in general, full-fat cheeses have always been more accepted than their counterparts that are lower in fat.

Conclusion

Fat reduction of raw goat milk to elaborate handmade cheeses using artisan methods has important effects on the sensory characteristics and consumer acceptance. Odor and flavor intensity was lower as fat decreased in cheese, and LFC and RFC were firmer, friable, grainier, drier, acidic, and less adhesive and sweet than full-fat cheese. Both judges and consumers preferred the FFC cheeses, mainly because of the higher intensity and the combination of this with excessive hardness and high masticability was likely the main cause of non-acceptance. To compete in local markets, the properties of low- and reduced-fat artisan cheeses must be improved; newly developed additives and cheese-making technologies must therefore be considered.

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Table 1. Standardized definitions of attributes used for the sensory profile.

	Attribute	Definition
Texture with fingers		
	Roughness	Perception of a granular surface
	Surface moisture	Perception of a surface water film
	Springiness	Ability of a cheese sample to rapidly regain its initial thickness after compression and deformation
Texture in mouth		
	Firmness	Resistance of the sample to a very slight opening and shutting of the jaws
	Friability	Capacity of a sample to break up into numerous pieces from the beginning of mastication
	Adhesiveness	The effort needed for the tongue to detach a product stuck to the palate and the teeth
	Solubility	A sensation which emerges when sample melts extremely fast in the saliva
	Moistness in mouth	Perception of the degree of humidity in the sample
	Granularity	Perception in the final stages of mastication of thin rounded grains in the chewed mass
Basic taste		
	Saltiness	The taste of sodium chloride
	Sweetness	The perception associated with the sweet taste of milk
	Acidic	The taste of lactic acid
	Pungent	Irritant sensation

Table 2. Means and standard error of the mean (SEM) of the proximal composition and external and internal pH of FFC, RFC and LFC[1] at 1, 7, 14 and 28 d of ripening.

		1 d	7 d	14 d	28 d	SEM
Fat, %						
	FFC	$18.48^{d,x}$	$22.19^{c,x}$	$25.01^{b,x}$	$28.61^{a,x}$	0.89
	RFC	$9.38^{b,y}$	$10.69^{b,y}$	$12.17^{ab,y}$	$13.61^{a,y}$	0.39
	LFC	$1.12^{c,z}$	$2.60^{bc,z}$	$4.43^{b,z}$	$8.81^{a,z}$	0.64
Fat in DM, %						
	FFC	$37.88^{d,x}$	$41.03^{c,x}$	$44.75^{b,x}$	$47.93^{a,x}$	0.87
	RFC	$19.54^{c,y}$	$21.93^{b,y}$	$24.85^{a,y}$	$26.78^{a,y}$	0.68
	LFC	$2.48^{d,z}$	$5.79^{c,z}$	$9.88^{b,z}$	$18.08^{a,z}$	1.28
Protein, %						
	FFC	$18.94^{b,z}$	$20.51^{a,z}$	$20.47^{a,z}$	$19.96^{a,y}$	0.18
	RFC	$20.97^{c,y}$	$23.01^{b,y}$	$24.38^{a,y}$	$24.65^{a,y}$	0.35
	LFC	$22.84^{d,x}$	$25.90^{c,x}$	$29.37^{b,x}$	$32.66^{a,x}$	0.80
Moisture, %						
	FFC	$46.95^{a,z}$	$45.92^{a,z}$	$44.12^{b,z}$	$40.32^{c,z}$	0.57
	RFC	$52.00^{a,y}$	$51.61^{a,y}$	$50.98^{a,y}$	$49.20^{b,y}$	0.29
	LFC	$55.69^{a,x}$	$55.28^{a,x}$	$55.15^{a,x}$	$51.18^{b,x}$	0.41
External pH						
	FFC	6.58^{a}	5.03^{b}	4.92^{c}	$4.90^{c,x}$	0.15
	RFC	6.57^{a}	5.10^{b}	4.89^{c}	$5.01^{b,y}$	0.14
	LFC	6.59^{a}	5.10^{c}	4.99^{d}	$5.38^{b,z}$	0.13
Internal pH						
	FFC	6.58^{a}	4.99^{b}	$4.74^{c,y}$	$4.81^{c,z}$	0.16
	RFC	6.56^{a}	5.06^{b}	$4.82^{d,y}$	$4.94^{c,y}$	0.15
	LFC	6.58^{a}	5.08^{c}	$4.91^{d,x}$	$5.32^{b,x}$	0.14

[a-d]Means within a row different superscripts differ ($P < 0.05$).

[x-z] Means within a column different superscripts differ ($P < 0.05$).

[1]FFC, full-fat cheese; RFC, reduced-fat cheese; LFC, low-fat cheese; DM, dry matter.

Table 3. Means and standard error of the mean (SEM) of sensory analysis of FFC, RFC and LFC[1] at 7 d of ripening: texture with fingers. Attributes were scored on a 7-point scale.

Texture with fingers						
		1	7	14	28	SEM
Roughness						
	FFC	1.00^{c}	1.00^{c}	2.43^{b}	$2.79^{a,z}$	0.16
	RFC	1.00^{c}	1.00^{c}	2.14^{b}	$2.71^{a,z}$	0.15
	LFC	1.00^{b}	1.00^{b}	2.29^{a}	$2.42^{a,y}$	0.14
	SEM	0.00	0.00	0.10	0.06	
Surface moisture						
	FFC	$5.79^{a,y}$	$5.85^{a,y}$	4.14^{b}	$4.07^{b,z}$	0.17
	RFC	$6.21^{a,z}$	$6.21^{a,z}$	4.14^{b}	$3.28^{c,y}$	0.25
	LFC	$6.21^{a,z}$	$6.21^{a,z}$	4.14^{b}	$3.07^{c,y}$	0.26
	SEM	0.07	0.06	0.05	0.10	
Springiness						
	FFC	3.50^{a}	3.26^{a}	2.42^{b}	1.64^{c}	0.15
	RFC	3.50^{a}	3.36^{a}	2.79^{b}	1.50^{c}	0.10
	LFC	3.50^{a}	3.07^{a}	2.57^{b}	1.64^{c}	0.12
	SEM	0.05	0.06	0.10	0.08	

$^{a-c}$Means within a row different superscripts differ ($P < 0.05$).

$^{x-z}$ Means within a column different superscripts differ ($P < 0.05$).

[1]FFC, full-fat cheese; RFC, reduced-fat cheese; LFC, low-fat cheese.

Table 4. Means and standard error means (SEM) of sensory analysis of FFC, RFC and LFC[1] at 7 d of ripening: texture in mouth. Attributes were scored on a 7-point scale.

Texture in mouth						
		1	7	14	28	SEM
Firmness						
	FFC	$2.00^{a,x}$	$2.00^{a,x}$	$1.50^{b,y}$	$1.64^{b,y}$	0.05
	RFC	$2.50^{ab,y}$	$2.36^{b,y}$	$2.71^{a,z}$	$2.50^{ab,z}$	0.06
	LFC	$4.07^{a,z}$	$4.07^{a,z}$	$2.64^{b,z}$	$2.64^{b,z}$	0.17
	SEM	0.20	0.21	0.13	0.12	
Friability						
	FFC	$1.21^{b,x}$	$1.21^{b,x}$	$1.00^{b,x}$	$1.64^{a,y}$	0.06
	RFC	$1.50^{c,y}$	$1.50^{c,y}$	$2.21^{b,y}$	$2.86^{a,z}$	0.11
	LFC	$3.21^{a,z}$	$3.14^{a,z}$	$2.71^{b,z}$	$2.79^{b,z}$	0.07
	SEM	0.20	0.20	0.17	0.13	
Adhesiveness						
	FFC	1.79^{c}	1.71^{c}	$2.86^{b,z}$	$4.85^{a,z}$	0.25
	RFC	1.78^{c}	1.86^{c}	$3.14^{b,z}$	$4.00^{a,y}$	0.18
	LFC	1.79^{c}	1.64^{c}	$2.21^{b,y}$	$2.86^{a,x}$	0.10
	SEM	0.05	0.08	0.10	0.19	
Solubility						
	FFC	$2.00^{c,z}$	$2.00^{c,z}$	$4.57^{b,z}$	$5.79^{a,z}$	0.32
	RFC	$1.00^{c,y}$	$1.00^{c,y}$	$2.64^{b,y}$	$4.78^{a,y}$	0.30
	LFC	$1.00^{c,y}$	$1.07^{c,y}$	$2.64^{b,y}$	$3.07^{a,x}$	0.18
	SEM	0.11	0.10	0.22	0.26	
Granularity						
	FFC	$4.00^{a,x}$	$4.00^{a,x}$	$1.86^{b,x}$	$1.36^{c,x}$	0.23
	RFC	$4.50^{a,y}$	$4.28^{a,y}$	$2.93^{b,y}$	$2.64^{c,y}$	0.16
	LFC	$5.21^{a,z}$	$5.21^{a,z}$	$3.50^{b,z}$	$3.29^{b,z}$	0.22
	SEM	0.12	0.12	0.11	0.19	
Moisture in mouth						
	FFC	$5.21^{a,z}$	$5.14^{a,z}$	$4.29^{b,z}$	$3.29^{c,z}$	0.17
	RFC	$3.00^{ab,y}$	$2.93^{ab,y}$	$3.07^{a,y}$	$2.71^{b,y}$	0.04
	LFC	2.00^{x}	2.07^{x}	2.14^{y}	2.14^{x}	0.09
	SEM	0.30	0.29	0.16	0.12	

[a-c]Means within a row different superscripts differ ($P < 0.05$).

[x-z] Means within a column different superscripts differ ($P < 0.05$).

[1]FFC, full-fat cheese; RFC, reduced-fat cheese; LFC, low-fat cheese.

Table 5. Means and standard error means (SEM) of sensory analysis of FFC, RFC and LFC[1] at 7 d of ripening: basic tastes. Attributes were scored on a 7-point scale.

		Basic taste				
		1	7	14	28	SEM
Saltiness						
	FFC	2.21^{b}	2.21^{b}	2.35^{b}	$3.89^{a,z}$	0.15
	RFC	2.21^{b}	2.14^{b}	2.21^{b}	$3.00^{a,y}$	0.08
	LFC	2.21^{b}	2.21^{b}	2.21^{b}	$3.21^{a,y}$	0.10
	SEM	0.06	0.05	0.08	0.10	
Acid						
	FFC	0.00^{c}	0.00^{c}	$3.21^{a,y}$	$2.14^{b,y}$	0.27
	RFC	0.00^{c}	0.00^{c}	$4.14^{a,z}$	$3.21^{b,z}$	0.36
	LFC	0.00^{c}	0.00^{c}	$3.96^{a,z}$	$3.07^{b,z}$	0.34
	SEM	0.00	0.00	0.11	0.11	
Sweetness						
	FFC	2.21^{a}	2.21^{a}	$1.07^{b,z}$	0.00^{c}	0.18
	RFC	2.21^{a}	2.14^{a}	$0.00^{b,y}$	0.00^{b}	0.21
	LFC	2.21^{a}	2.14^{a}	$0.00^{b,y}$	0.00^{b}	0.21
	SEM	0.06	0.05	0.11	0.00	
Pungent						
	FFC	ND[2]	ND	ND	ND	--
	RFC	ND	ND	ND	ND	--
	LFC	ND	ND	ND	ND	--
	SEM	--	--	--	--	

[a-c]Means within a row different superscripts differ ($P < 0.05$).

[x-z] Means within a column different superscripts differ ($P < 0.05$).

[1]FFC, full-fat cheese; RFC, reduced-fat cheese; LFC, low-fat cheese.

[2]ND, non detectable.

Table 6. Means and standard error means (SEM) of sensory analysis of FFC, RFC and LFC[1] at 7 d of ripening: odor and flavor assessments. Attributes were scored on a 7-point scale.

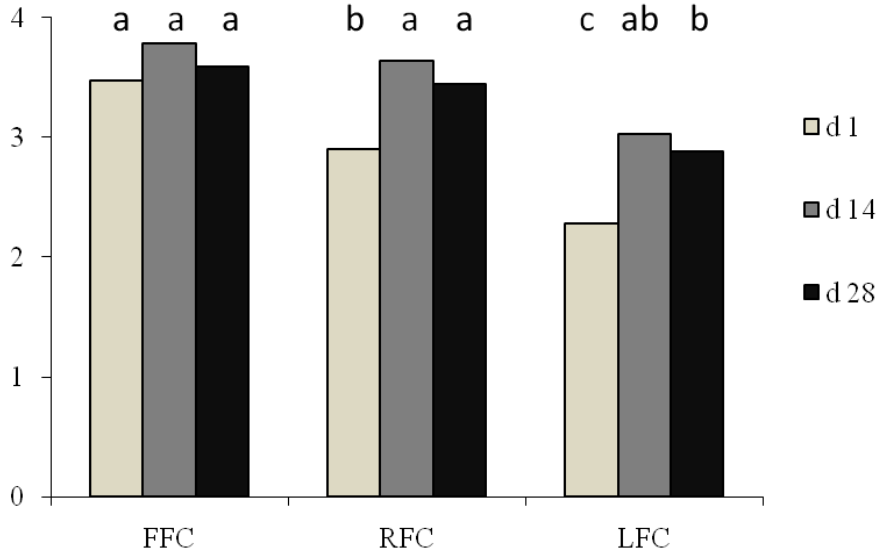
	Odor and Flavor				
	1	7	14	28	SEM
Odor Intensity					
FFC	$1.21^{c,z}$	$1.36^{c,z}$	$2.64^{b,z}$	$4.78^{a,z}$	0.28
RFC	$0.71^{c,y}$	$0.86^{c,y}$	$2.71^{b,z}$	$4.00^{a,y}$	0.27
LFC	$0.71^{c,y}$	$0.92^{c,y}$	$2.00^{b,y}$	$3.21^{a,x}$	0.20
SEM	0.07	0.07	0.14	0.15	
Odor Assessment					
FFC	$3.71^{c,z}$	$3.78^{c,z}$	$5.28^{a,z}$	$4.28^{b,z}$	0.13
RFC	$3.21^{b,y}$	$3.21^{b,y}$	$3.14^{b,y}$	$4.29^{a,z}$	0.10
LFC	3.21^{y}	3.21^{y}	3.14^{y}	3.14^{y}	0.05
SEM	0.08	0.08	0.23	0.14	
Flavor Intensity					
FFC	2.21^{c}	2.21^{c}	$3.21^{b,z}$	$4.14^{a,z}$	0.16
RFC	2.21^{c}	2.21^{c}	$3.28^{b,z}$	$4.21^{a,z}$	0.17
LFC	2.00^{b}	2.07^{b}	$2.00^{b,y}$	$3.43^{a,y}$	0.12
SEM	0.05	0.05	0.15	0.09	
Flavor Assessment					
FFC	$4.21^{b,z}$	$4.14^{b,z}$	$3.79^{c,z}$	$4.93^{a,z}$	0.09
RFC	$4.21^{a,z}$	$4.14^{a,z}$	$3.14^{c,y}$	$3.71^{b,y}$	0.09
LFC	3.21^{y}	3.14^{y}	3.21^{y}	3.21^{x}	0.09
SEM	0.11	0.12	0.15	0.18	
Odor and Flavor Persistence					
FFC	1.50^{c}	1.43^{c}	$3.29^{b,z}$	$4.57^{a,z}$	0.25
RFC	1.50^{c}	1.29^{c}	$2.71^{b,y}$	$4.36^{a,z}$	0.25
LFC	1.50^{c}	1.29^{c}	$2.21^{a,x}$	$2.14^{a,y}$	0.12
SEM	0.06	0.11	0.10	0.26	
Total Assessment					
FFC	$5.21^{a,z}$	$5.29^{a,z}$	$5.21^{a,z}$	$4.86^{b,z}$	0.06
RFC	$3.21^{b,y}$	$3.21^{b,y}$	$3.07^{b,y}$	$3.79^{a,y}$	0.07
LFC	$2.21^{b,x}$	$2.14^{b,x}$	$3.07^{a,y}$	$3.07^{a,x}$	0.09
SEM	0.28	0.30	0.23	0.17	

[a-c]Means within a row different superscripts differ ($P < 0.05$).

[x-z] Means within a column different superscripts differ ($P < 0.05$).

[1]FFC, full-fat cheese; RFC, reduced-fat cheese; LFC, low-fat cheese.

Figure 1. Least square means of the acceptability scores (rated on a 5-point category hedonic scale) of FFC, RFC and LFC[1] at 1, 14 and 28 days of ripening.



[1]FFC, full-fat cheese; RFC, reduced-fat cheese; LFC, low-fat cheese.

Capítulo VII:
Objetive characterization and in isolation effects of somatic cells on finished goat milk cheese

1. Planteamiento y Metodología.
2. Trabajo experimental

Capítulo VII: Objetive characterization and in isolation effects of somatic cells on finished goat milk cheese

1. Planteamiento y Metodología

1.1. Planteamiento

De acuerdo a la FAO (Food and Agriculture Organization) de las Naciones Unidad, la población mundial de cabras de aptitud lechera ha alcanzado los 179 millones en el 2008, y la producción de leche sobrepasa las 15,2 millones de toneladas (FAOSTAT, 2010). Dubeuf y Boyazoglu (2009) han sugerido que este incremento durante los últimos 25 años significa que las cabras ya no sólo son "las vacas de los pobres", y este creciente interés debe ser consolidado para explotar el comportamiento de los consumidores y las potenciales oportunidades del mercado (Dubeuf, 2005).

En muchos países se han establecido ciertos criterios de acuerdo a los requerimientos higiénicos, tecnológicos y sensoriales. En Estados Unidos se ha impuesto un límite de 1.000.000 de células somáticas por mL en la leche de cabra, mientras que en Europa no existe un límite legal. Sin embargo, algunos países usan este criterio como parte del sistema de pago con penalizaciones. Paape *et al.* (2007) y Raynal-Ljutovac *et al.* (2007) han reportado que los ganaderos de Norte América tienen dificultades para mantener el recuento de células somáticas de la leche de cabra recogida por debajo del límite. Esta situación se debe principalmente a los factores no-infecciosos, los cuales tienen un mayor impacto en los recuentos de la leche de cabra (Haenlein y Hinckey, 1995; Paape *et al.*, 2001; Fernández *et al.*, 2009). El resultado es que muchas granjas eliminan la leche de cabra que excede del límite para el consumo humano o para hacer queso, lo que incrementa las pérdidas económicas.

Raynal-Ljotovac *et al.* (2007) ha revisado intensamente los aspectos analíticos, sanitarios, productivos y tecnológicos del efecto del recuento de células somáticas sobre la leche y queso de

cabra y oveja. La mayoría de los estudios compararon las características de la leche y queso a partir de agrupaciones de animales en función de su recuento de células somáticas, o animales con mamitis y sanos. Los trabajos revisados fueron muy diferentes unos de otros: distintos procedimientos de elaboración, leche cruda o pasteurizada, coagulación enzimática o ácida, etc. Debido a esto, no está muy claro el efecto de las células somáticas sobre la leche, el queso, la lipólisis o proteólisis. En el experimento presentado en este capítulo se aislaron células somáticas de leche de alto recuento procedente de cabras sanas y se añadieron a leche de cabra de bajo recuento pasteurizada y sin pasteurizar, de manera que el único factor de variación fue el nivel de células somáticas.

Se ha propuesto los quesos en miniatura como la mejor alternativa a los experimentos en plantas piloto, ya que pueden ser preparados bajo condiciones controladas y porque se obtienen más económica, reproducible y fácilmente (Shakeel-Ur-Rehman *et al.*, 2001).

1.2. Metodología

El material y los métodos usados en este experimento, así como los resultados obtenidos han sido enviados y están bajo revisión en formato con el título "Objetive characterization and in isolation effects of somatic cells on finished goat milk cheese" (Caracterización objetiva y aislada de los efectos de las células somáticas en quesos de leche de cabra) a la revista International Dairy Journal.

Mediante el proceso de centrifugación (1500 rpm x 20 min a 4ºC) se obtuvo un pool de células somáticas procedente de 2 cabras sanas con alto recuento. La leche procedente de 4 cabras sanas de bajo recuento se separó en gupos: una parte se dejó como leche cruda y la otra sufrió un proceso de pasteurización (63ºC durante 30 min). Luego, ambos grupos de leche se subdividió a su vez en 3, los cuales presentaron distintos niveles de células somáticas: leche de bajo recuento (sin adición de células del pool), leche de recuento medio (1.000.000 de células por mL) y leche de alto recuento (aproximadamente 2.500.000 células por mL). Se elaboraron

miniquesos de la siguiente manera: a 40mL de leche se le añadió cuajo animal en tubos de centrífuga de 50mL a 32ºC. La cuajada se cortó y se centrifugó a 4500 rpm durante 20 min. Se recogieron muestras de leche y suero, para medir composición química, recuento de células somáticas, densidad, pH, acidez titulable, estabilidad en etanol y tiempo de coagulación. Los quesos resultantes se dejaron madurar 1 y 7 días a 10-12ºC y 75-80% de humedad relativa. De los quesos se midieron composición química básica, color, pH, proteólisis mediante perfil proteico en SDS-PAGE y lipólisis como nivel de ácidos grasos libres.

Los datos se analizaron mediante el paquete estadístico SAS (versión 9.0; SAS Institute Inc., Cary, NC) mediante un ANOVA de una vía para las muestras de leche y suero, y un ANOVA de medidas repetidas de dos vías (pretratamiento de la leche y tiempo de maduración) considerando un valor de $P<0,05$ como estadísticamente significativo. Para evaluar las diferencias entre grupos se usó el test de Tukey.

2. Trabajo experimental

Objective characterization and in isolation effects of somatic cells on finished goat milk cheese

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ABSTRACT

The interest in goat milk and products has increased potential marketing opportunities. However, the milk quality criteria and limits for SCC established in many countries makes very difficult maintaining bulk tank goat milk SCC below the threshold due to non-infection factors linked to goat physiology. The aim of this study was to objectively verify the effects of somatic cells (200,000, 1,000,000, and 2,500,000 cells mL-1) on fresh goat cheese. Somatic cells were recovered from pooled healthy goat milk, which were added to low SCC raw or pasteurized goat milk. Miniature cheeses were made and evaluated after 1 and 7 d. Somatic cells intensively affected lipolysis in cheese, increasing FFA regardless if milk was raw or pasteurized. Two important conclusions about proteolysis were found in this work: somatic cell effect on proteolysis is specific for caseins, and, in addition, the proteolytic effects of somatic cells on main caseins in cheese are different if cheeses are elaborated with raw or pasteurized milk. The somatic cells addition affected the incremental color: lightness decreased in 7 d in all cheeses, while a* and b* values increased only in pasteurized milk cheeses. We concluded that somatic cells themselves in goat milk affect some cheese parameters, which could results in a different product.

Keywords: goat milk cheese, somatic cell count, proteolysis, lipolysis.

1. Introduction

Currently, goats and goat milk have showed an increased demand to be used in diverse products and as alternative products for consumers with cow milk intolerance (Tziboula-

Clarke, 2003). According to Food and Agriculture Organization of the United Nations (FAOSTAT, 2010), the worldwide dairy goat population reached 179 million in 2008 and goat milk production surpassed 15.2 million tons, representing significant increases of 15 and 17%, respectively, as compared with a decade ago. The population of dairy goats in The United States of America and European Union in 2008 was 305,000 and 8,093,429, respectively.

According to Haenlein (2004) and Park and Haenlein (2007) there are three reasons for the demand of goat milk: increased home consumption due to a growing human population, interest in goat milk products, and medical purposes. Ribeiro and Ribeiro (2010) and Silakinove, Leitner, Merin, & Prosser (2010) reinforce the healthy properties of milk and products from goats in reviews justifying its high quality and benefits. Mowlen (2005) reported that people are not overly concerned too much about the cost of both health and specialty foods markets if they can see the benefits of consuming goat products.

Milk quality criteria have been established in many countries according to hygienic, technological and sensorial requirements. These milk quality criteria are part of the payment system of milk, which will, in return, ensure a better quality (hygienic and sensorial) of the final products (Raynal-Ljutovac, Laborit & Lauret, 2005). In the United States the legal limit established for SCC in milk by the Food and Drug Administration for cows is 750,000 mL^{-1} and for goats 1,000,000 mL^{-1}. In the European Union (Directive 92/46ECC Council, 1992) the legal limit for cows is 400,000 mL^{-1} and there is no legal limit for goats and sheep. Some authors (Paape et al., 2007; Raynal-Ljutovac, Pirisi, Crémoux & Gonzalo, 2007) have reported that North American goat dairymen have difficulty maintaining bulk tank goat milk SCC below the threshold of the United States limit. This is due to non-infectious factors (Fernández, Rodríguez-Alcala, Abilleira, de la Puente & Fontecha, 2009; Haenlein and Hinckley, 1995; Paape, Poutrel, Contreras, Marco & Capuco, 2001) that have major impact

on counts in goat milk, and this should be taken into account when the European Union establishes limits on SCC in goat milk.

Numerous studies have shown that increased SCC in cow milk produces a decrease in milk production and affect the milk composition, which causes a reduction in the ability to produce cheese and decreases the shelf-life of consumer milk (Erden, Atasever & Kul, 2010; Ma et al., 2000; Politis and Ng-Kwai-Hang, 1988;). The main component of the economic loss associated with SCC is the reducing milk production in cows with mastitis (Huijps, Lam & Hogeveen, 2008). Raynal-Ljutovac et al. (2005) concluded that somatic cells, although they are an indicator of the hygienic quality of goat milk, do not seem to necessarily impair cheese making. However, Raynal-Ljutovac et al. (2007) intensively reviewed analytical, sanitary, productive and technological aspects of somatic cells on goat, and controversies among the conclusions of the studies were found, probably due to the different variables, as the variety of cheese-making or using different animals in each experimental group. Despite these differences, it is not clear the effects of somatic cells on milk properties, cheese quality, lipolysis and proteolysis. Marino, Considine, Sevi, McSweeney & Kelly (2005) recovered somatic cells from mastitic cow milk and added them to good quality milk, then evaluated their contribution to proteolysis during Cheddar cheese ripening. Caroprese et al. (2007) studied the effect of macrophages to proteolysis and plasmin (**PLe**) activity in ewe bulk milk. Somatic cells own a range of lysosomal enzymes, like B, cathepsin D, cathepsin G and elastase (Le Roux, Laurent & Moussaoui, 2003), many of which pass to the milk where they cause proteolysis and lypolysis more intense in the milk (Albenzio et al., 2005; Le Roux, Colin & Laurent, 1995). Many of the enzymes that originate from somatic cells have been found or measured in fractions of milk other than the cells themselves (e.g., in skimmed milk or whey), suggesting that leakage or secretion of enzymes occurs, or that the cells lyse and release their intracellular enzymes (Kelly and Fox, 2006). Moreover, the increase of somatic

cells or its cause usually is accompanied by imbalances in other parameters in milk, so it is very difficult to hold somatic cells responsible for changes in milk and cheese.

Milk pasteurization is commonly practiced before making cheese. This heat-treatment functions in reduction of microorganisms, activation–inactivation of indigenous milk pro-enzymes and enzymes, denaturation of serum proteins, and modification of starter bacteria activity. It also concomitantly reduces fermentation and degradation reactions (Grappin and Beuvier, 1997; Buchin et al., 1998). Marino et al. (2005) reported that proteolysis during ripening of cheese increased with somatic cell addition, although this effect was reduced by pasteurization after cell addition. A relationship between milk proteolysis and SCC has been described by Hachana and Kraiem (2009).

In the Canary Islands, Spain, 91 tons of goat milk is produced per year for use in cheese-making, and most of these are made with raw milk using traditional methods and are mainly consumed following short ripening periods (Fresno et al., 2008). The agricultural economy has benefitted in recent years by the higher interest in goat cheese, and it can be feasible for many small-scale farmers to use traditional practices in making high quality goat cheese. Currently, there is interest in preserving the traditional cheese production methods. Sánchez-Macías et al. (2010) examined the possibility of using hand-made practices to amply the goat cheese market and to compete with industrial low-fat cheese.

Collectively, few studies about the direct effect of somatic cells from healthy animals on cheese have been reported in the literature, and no objective study has been reported about the contribution of somatic cells on goat cheese elaborated by a simple rennet cheese-making process using raw milk. The aim of this study was to objectively verify the effects of somatic cells on chemical composition, proteolysis, lipolysis and color in fresh goat cheese, and at day 1 and 7 of ripening.

2. Material and methods

2. 1. Recovery of Somatic Cells

Somatic cells were recovered by centrifugation (1500 rpm x 20 min at 4˚C) (Beckman, J2-CI Centrifuge, Beckman Coulter Inc., Miami, FL) from high SCC raw milk (approximately 1.78 x 10^6 cells/mL) from 2 healthy Majorera breed goats. The resulting pellet was resuspended in phosphate buffered saline at pH 6.8, as described by Verdi and Barbano (1991) until a pool with approximately 21 x 10^6 SCC/mL was obtained. The SCC of this stock preparation, before and after dilution in PBS, was determined using a DCC Device counter (DCC**,** DeLaval International AB, Tumba, Sweden). The type of somatic cell in the pool was not specifically determinate, but is likely that the somatic cell population in goat milk is predominated by polymorphonuclear neutrophils (45-74%), followed by macrophages (15-41%) and lymphocytes (9-20%) (Dulin, Paape, Schultze & Weinland, 1983).

2. 2. Cheese Production

2. 2. 1. Cheese Formulation

Miniature cheese systems have been proposed as the best alternative to pilot plant experiments, as closers to cheese than any other model proposed to date. Moreover, they can be prepared under controlled conditions and are more economical, reproducible and easier to obtain (Shakeel-Ur-Rehman, Fox, McSweeney, Madkor & Farkye, 2001).

Raw goat milk with low SCC (approximately 2 x 10^5 SCC/mL) was obtained from four goats from a healthy experimental herd of Majorera dairy goat breed from the Animal Science Unit of Universidad de Las Palmas de Gran Canaria, and was used to elaborate the cheeses. Four batches of experimental cheeses were produced consecutively over four days using six different experimental treatments (three levels of SCC and pasteurization or non-pasteurization). Each day the raw goat milk was separated into two groups: raw milk or

pasteurized milk (63ºC for 30 min); and both groups were then divided into three subgroups with different levels of the somatic cells: low SCC milk (without somatic cells added), medium SCC milk (mean value approximately 1 x 10^6 SCC/mL), and high SCC milk (mean value approximately 2.5 x 10^6 SCC/mL). The finished cheeses were ripened for 1 and 7 d in duplicate, resulting in a total of 96 cheese products (4 batches x raw or pasteurized milk x 3 level of SCC x 2 ripening times x in duplicate).

2. 2. 2. Manufacture of Miniature Cheese.

Cheeses were elaborated with six different milk treatments: raw milk low SCC (**RL**) cheese, raw milk medium SCC milk (**RM**) cheese, raw milk high SCC (**RH**) cheese, pasteurized milk low SCC (**PL**) cheese, pasteurized milk medium SCC (**PM**) cheese, and pasteurized milk high SCC (**PH**) cheese. Milk was salted with NaCl at 4%. The same procedure was used to elaborate each type of miniature cheese: 40 mL of milk were poured in four 50 mL-centrifuge tubes, which were then placed in a water bath at 32°C. Animal rennet (commercial rennin powder, Marshall rennet power), comprised of 50% pepsin and 50% chymosin was added**.** No starter cultures were added. Curd was cut after 45 min of clotting time with a stainless-steel spatula and allowed to stand for additional 5 min. The curds were centrifuged at 4500 x rpm for 20 min and the whey was discarded. Cheeses were divided randomly into two groups and allowed to ripen 1 or 7 d at 10-12ºC and 75-80% relative humidity.

2. 3. Gross composition, pH and intrumental color of cheese

A Minolta colorimeter CR-400/410 (Illuminant D65, Konica Minolta, Osaka, Japan) was used to determine external and internal lightness (L), yellow index (b*), and red index (a*), as well the pH at central location, for each cheese in triplicate. After that, cheese samples

were cut into small pieces, weighed and freeze-dried. The weight difference before and after the freeze-drying was used to calculate the percentage of moisture content. The rest of the analysis was performed with the resultant dried matter. Soxhlet extraction was performed to quantify the fat content in dry basis using a Büchi 810 Soxhlet fat extraction apparatus (Büchi Labortechnik, Flawil, Switzerland). Protein content was determinate in duplicate as % N x 6.38 using a LECO CNS-2000 nitrogen analyzer (Leco Corporation, St. Joseph, MI).

2. 4. Extraction of Proteins and Electrophoretic Analysis

All chemical reagents were of analytical grade from Panreac (Barcelona, Spain), Merck (Darmstadt, Germany), and Bio-Rad (Hercules, CA). Water-soluble proteins were extracted from the cheeses tested at 1 and 7 d of ripening using the method described by Tunick, Malin, Smith & Hilsinger (1995) with modifications. Briefly, 2.5 mL of buffer (0.166 *M* Tris, 0.001 *M* EDTA, pH 8.0) was added to 0.5 g of freeze-dried cheese and vortexed for 15 min. To this mixture, 2.5 mL of 7% SDS was added, and the sample was vortexed again for 5 min. Then 1 mL of 10 m*M* dithiothreitol buffer was added, and the mixture was vortexed and held in an ice bath for 20–30 min. Samples were then centrifuged for 1 h at 4ºC at 18,000 rpm (Beckman, J2-CI Centrifuge; Beckman Coulter Inc., Miami, FL). The supernatant was filtered, lyophilized, and stored at –20ºC until analysis.

Lyophilized samples were prepared, separated by SDS-PAGE (Laemli, 1970), and stained according to Sánchez-Macías et al. (2011). Images of stained gels were captured with a Bio-Rad imaging device (Gel Doc EQ; Bio-Rad Laboratories, Hercules, CA), and bands were quantified using Quantity One Quantitation Software (Bio-Rad Laboratories). Densitometry analysis of the gels was performed using lane-based background subtraction followed by measurement of the bands by the area under the intensity profile curve, and the values were then used for statistical analysis. Each sample was analyzed on duplicate gels.

Individual protein species from cheese samples were identified by comparing their relative mobilities with those of standard proteins (Bio-Rad Laboratories) from a previous SDS-polyacrylamide gel.

2. 5. Total FFA (Copper Soap Method)

The total FFA content was determined using the copper soap method (Melilli et al., 2004; Shipe, Senyk, & Fountain, 1980) as modified for cheese analysis according to Sánchez-Macías et al. (In press). Absorbance was measured at 440 nm in a cuvette using a SmartSpec Plus Spectrophotometer (Bio-Rad Laboratories). A standard curve was constructed using palmitic acid crystal grade at increasing concentration in hexane. Each standar (1mL) was added to a centrifuge tube, and let to evaporate the solvent under the hood. After the solvent was completely removed, 1 mL of deionized water was added to the tube, and the set of standards was carried through the full analysis with the cheese samples and two blanks.

Absorbance readings of the standards were corrected by subtracting the average of the two blanks readings, and a regression line was constructed that correlated the corrected absorbance with micrograms of palmitic acid. The level of FFA in the cheese samples was calculated from the standard curve. The final results were expressed as milligrams of FFA per 100 g of dry matter of cheese and were also converted to milligrams of FFA per g of fat in cheese.

2. 6. Statistical Analysis.

Statistical analyses were performed using the SAS program package (Version 9.00, SAS Institute Inc., Cary, NC). A PROC MIXED procedure (analysis of variance with repeated measures) was used to evaluate the effect of different SCC level and times of ripening on the chemical composition, pH, lipolysis level, primary proteolysis and lightness

of the raw or pasteurized goat milk cheeses. Significantly different means were identified using the Tukey's test.

3. Results and discussion

3. 1. Gross Chemical Cheese Analysis and pH values

The gross chemical composition and pH of cheeses at 1 and 7 d of ripening are shown in Table 1. At day 1 of ripening, the moisture was higher in pasteurized milk cheeses than raw milk cheeses, though no differences were found among RH and PH cheeses. This data concurs with Pearse Linklater, Hall, & MacKinlay (1985), who reported an adverse effect of heat treatment on syneresis of rennet-induced milk gels using model systems. This effect on syneresis is probably due to the presence of denatured whey protein on the surface of the micelles, which retard aggregation and fusion of the rennet-treated micelles (Rynne, Beresford, Kelly, & Guinee, 2004). Henry, Mollé, Morgan, Fauquant, & Bouhallab (2002) reported that more water is bounded in cheese when β lactoglobulin-κ casein complex is formed after milk pasteurization. In this study, slightly increase and decrease of moisture content in RH and PH, respectively, were found compared with their counterparts low in SCC, but similar among them. Marino et al. (2005) suggested in a similar study that somatic cells themselves may contribute to the increase moisture content in cheese, perhaps the inclusion of cells physically interferes with the process of curd syneresis. Moisture content decreased after 7 d of ripening in all cheeses because of surface water evaporation, and no differences were found in cheeses. Fat percentage in DM cheeses during the ripening period were within the ranges required by Spanish regulation for full-fat (60-45% fat in DM) cheese designation, and very similar to those found by Sánchez-Macías et al. (2010) in full-fat cheese elaborated with milk from the same breed.

Protein content in DM was similar among cheeses from the different treatments at both ripening times; however, an increase of the content was found in cheeses after 7 d of ripening. Van Vliet and Walstra (1994) reported that about 90% of the water present in milk gels is mechanically enclosed between the casein strands forming the network. It is the structure of the casein aggregates, which determines the ease of removal of water from the gel. The increased found in protein content in dry basis in this experiment can be explained due to the more possible water that is available at 7 d of ripening and can be extracted from cheeses after casein network is broken due to proteolysis of caseins. pH in cheeses was approximately 6.6 at 1 d of ripening. These values were similar to the typical values reported for Majorera goat milk cheese (Álvarez et al., 2007; Sánchez-Macías et al., 2010). During the first week of ripening, pH values decreased in all cheese groups, though PL cheese presented the significant highest value. Pirisi et al. (2000) and Vianna, Mazal, Santos, Bolini, & Gigante (2008) reported that higher somatic cell levels in milk result in increase pH in cheese, while other authors (Jaeggi, Govindasamy-Lucey, Berger, & Johnson, 2003) did not observe significant variations, possibly due to other changes produced in milk accompanying the SCC increasing. Marino et al. (2005), adding somatic cells from mastitic milk to milk, did not find significant differences on cheeses. In this study, we used somatic cells from healthy goats, and we found that pH decrease in cheese as somatic cells added increase in milk.

3. 2. Lipolysis of Cheese

FFA mean values are summarized in Table 2. The greater proportion of small fat globules naturally produced in goat milk may have different lipolytic characteristics compared with cow milk, and the higher levels of moisture and short- or medium-chain fatty acids in goat cheeses are likely to have greater lipolytic effects than their cow counterparts (Park, 2001). The indigenous lipoprotein lipase (**LPL**) hydrolyses the 1,3-position in mono-,

di-, and triglycerides to give free fatty acids. Large proportions of goat milk LPL are bound to cream (46%), and not to caseins (8%), unlike in cow milk (78%), which could explain that milk lipolysis is well correlated to milk LPL activity in this specie (Chilliard, Selselet-Attou, Bas, & Morand-Ferh, 1984). FFA levels did not differ significantly among the cheeses after 1 d of ripening. However, slight FFA content increases were found as somatic cells was increased in cheese milk.

Quantitative analysis of the total FFA content showed a general increase in all cheeses after 7 d of ripening, and the levels were higher as somatic cells count was increased in milk. Moreover, RL cheese resulted in higher FFA level than PL cheese. Other authors have found lower levels of FFA comparing pasteurized and raw milk cheese: 38% in Manchego Cheese (Gaya, Medina, Rodriguez.Marin, & Nuñez, 1990) and 50% in Cheddar cheese (McSweeney, Fox, Lucey, Jordan, & Cogan, 1993a). Morgan and Gaborit (2001) reported that the transformation of the goat milk, physical treatment, and cold storage increase the lipolysis level in the milk, whereas pasteurization decreases the lipolysis level. Driessen (1989) reported that LPL is nearly completely inactivated by pasteurization at 78ºC during 10s, but latter author (Jandal, 1995) observed than pasteurization, and even boiling, do not completely inactivate lipase in goat milk. Medium and high SCC cheeses had similar FFA content in both raw and pasteurized milk. Chen, Wang, Van Kessel, Ren, & Zeng (2010) reported that SCC levels in pasteurized goat milk cheese did not have significant effects on FFA content, and similar results were obtained by Jaeggi et al. (2003) in hard ewe milk cheese. In both cases, cheese milk was collected from grouped animals according to SCC, and thus, not only somatic cells themselves were involved on cheese lipolysis, but other changes in milk. Moreover, in this work somatic cells were added to the milk after pasteurization, which means that somatic cells enzymes were not affected by heating. In this study, lipolysis clearly depended on somatic cells. This process only occurs when the membrane of the fat globules is

broken, and the fat is accessible to the enzyme. Azzara and Dimick (1985) found that macrophages from mastitic cow milk secreted lipolytic enzymes which may bind to the fat globule membrane and damage it exposing the fat to degradation by lipoprotein lipase.

In cheese, lipid hydrolysis results in the formation of FFA, especially those short- and intermediate-chain FFA that may contribute to cheese flavor and also serve as substrates for further reactions producing highly flavored catabolic end products (Collins, McSweeney, &Wilkinson, 2003). Jaubert, Bodin, & Jaubert (1996) observed that milk having a more intense goat flavor, presented higher lipolysis and higher SCC. Sensory studies are needed to evaluate if somatic cells themselves are able to affect the sensory characteristics of goat cheese.

3. 4. Protein Profile and Proteolysis of Cheese

A densitometer-derived protein distribution analysis is summarized in Table 3. Primary proteolysis measures essentially the extent of main casein breakdown. This occurs mainly as result of the action of residual coagulant, PLe, and other somatic cell proteinases. However, microorganisms are also active in the degradation of caseins (Fox, 1993; Fox and McSweeney, 1996). Harmon (1994) and Moslehishad and Ezzatpanah (2010) have described that the elevation of somatic cell count of milk affect casein micelle microstructure and properties due to a hydrolysis of casein particles by increasing proteolytic activity of enzymes such as PLe, neutral, and acidic proteases, therefore, casein could be more susceptible to the degradation.

In this experiment, in all cheese groups at both ripening times, β-CN bands had the highest values, followed by α_{S2}-CN and α_{S1}-CN. Caprine milk contains relatively little α_{S1}-CN, whereas β-CN is the major protein (Tziboula-Clarke, 2003). The Canarian dairy breeds (Majorera, Tinerfeña, and especially Palmera) represent a particular case where 60% of the

alleles for the caprine α_{S1}-CN locus are of the high type (A and B; Jordana et al., 1996); therefore, this casein is relatively abundant in milk and cheese made from these breeds. In the present study, α_{S1}-CN was about 17-20% of the total caseins in all cheese types at 1 d of ripening.

Quantitative analyses showed a general reduction in casein fractions as ripening progressed, and the degradation was higher for β-CN (26-43%), than for para-κ-CN (23-37%), α_{S2}-CN (20-30%), and α_{S1}-CN (10-25%). Different patterns of degradation were found as SCC was increased in goat milk for raw or pasteurized milk cheeses.

Regard to α_{S2}-CN, RL cheese presented significantly lower content than PL cheese. Somatic cell addition to raw goat milk resulted lower α_{S2}-CN degradation rate in RM and RH cheeses compared to RL cheese; however, the degradation of this casein was accelerated in pasteurized goat milk cheese as more somatic cells were added. The α_{S1}-CN in cheese was not affected by the heat treatment or somatic cells addition during the experimental time; only a general reduction was found after 7 d of ripening.

In this study, β-CN content was similar in all cheese groups at the beginning of the maduration; and a significant reduction of this casein was found at 7 d of ripening. β-CN degradation rate was similar in RL and PL. However, the breakdown in cheese followed a similar pattern than α_{S1}-CN when somatic cells were added to raw or pasteurized goat milk. In raw milk cheeses, there were no differences in β-CN degradation, but it slightly decreased as SCC level was increased. On the contrary, β-CN degradation rate increased as SCC level was higher in pasteurized milk cheese.

Hachana, Kraiem, & Paape (2010) reported that the addition of somatic cell extracts to UHT bovine milk have significant effects on casein fraction: increasing the concentration of somatic cell proteinase caused a reduction of β-CN, followed by α_S-CN, and lastly κ-CN. The same findings were reported by Kahina, Moussaoui, Hebia, Laurent, & Le Roux (2005), Le

Roux et al. (2003), and Saeman, Verdi, Galton, & Barbano (1988). Leroux et al. (1995) observed a decrease of β-CN and α_S-CN when SCC exceeded 250.000 in bovine raw milk, but κ-CN did not vary significantly. According to Verdi and Barbano (1991), κ-CN were the most resistant to proteolysis by somatic cells proteinase. In this study, para-k-CN, derived from the cleavage of k-CN, was breakdown during the first week of ripening, and no differences were found among the cheese groups at the end of the experiment.

Marino et al. (2005) found that adding somatic cells from mastitic milk to cow milk resulted in a higher α_{S1}-CN degradation in cheese at 15 d of ripening than in control cheese, indicating accelerated proteolysis of this casein. This difference could be due to using somatic cells from healthy goats in this study, which have predominantly polymorphonuclear cells, as in mastitic cow milk, but it is possible that somatic cell activities differ between species or between healthy and unhealthy animals. Anyway, in the present study, cheeses were ripened until 7 d, and no data were recovered in cheeses after this time. When isolated and lysed macrophages from ewe bulk milk were incubated on Na-caseinate, changes in percentage composition of intact casein and casein degradation products (γ-CN and α_S-CN products) after 24 h of incubation were observed, and β-CN did not undergo appreciable degradation, whereas α-CN underwent hydrolysis (about 20%) (Caroprese et al., 2007).

PLe is the main indigenous proteolytic enzyme in milk, associated to the casein micelles, and contains the entire PLe system: PLe, plasminogen (**PG**), PLe inhibitors, PG activators, and inhibitors of PG activators, and PG activator have been associated with somatic cells fraction in goat milk (Politis et al., 1994). PLe acts mainly on β-casein, which constitutes approximately 60% of the total casein in goat milk. Trujillo, Buffa, Casal, Fernández, & Guamis (2002) found lower levels of β-CN in pasteurized goat milk cheese compared with raw goat milk cheese, which was explained by the activation of PG or

inactivation of inhibitors of PG activators, resulting in a net increase in PLe activity in pasteurized milk (Prado, Sombers, Ismail, & Hayes, 2006).

Increased PLe activity in pasteurized milk, which is concomitant with a decrease in PG concentration, is due to the denaturation of the inhibitor(s) of the PG activator(s) (Bastian and Brown, 1996) and the inactivation of PLe inhibitors (Farkye and Imafidon, 1995). In this study, pasteurization combined with a high somatic cell level in the milk increased the proteolytic activity in the finished cheese after 7 d of ripening. Heegaard et al. (1994) reported that polymorphonuclear cells possess PG activators, which may explain the accelerated hydrolysis of β-CN in pasteurized milk cheese versus raw milk cheese with greater SCC. The general consensus is that proteolysis in low SCC milk is dominated by PLe, with a minor contribution from cathepsin D; however, as the SCC increases, the relative significance of PLe activity decreases, while the relative activity of other enzymes increases (Kelly, O'Flaherty, & Fox, 2006). However, in raw milk cheese, adding somatic cells to the goat milk resulted in an inhibition of the proteolytic activity on β-CN. This could be due to high SCC inhibiting enzyme activity and slowing β-CN hydrolysis or due to inhibitors of somatic cell proteases in raw milk.

Cathepsin D is ostensibly a lysosomal enzyme (Larsen, Benfeldt, Rasmussen, & Petersen, 1996), and its level in correlated significantly with SCC (O'Driscoll, Rattray, McSweeney, & Kelly, 1999). Kaminogawa, Yamauchi, Miyazawa, & Koga (1980) and McSweeney et al. (1995) showed that cathepsin D partially purifed from milk degraded α_{S1}-CN to a peptide with the same molecular mass or electrophoretic mobility as α_{S1}-CN (f24–199), which is one of the primary peptides produced by chymosin. These reports could explain the higher degradation rate of α_{S2}-CN in pasteurized milk cheeses as somatic cells were added.

Somatic cells also contain several other proteinases, including cathepsins B, L and G, and elastase, which have received limited attention to date (Kelly and McSweeney, 2003). Cathepsin B showed some similarity to chymosin in its cleavage sites on both α_{S1}-CN (McSweeney, Oloson, Fox, Heulym & Hϕjrup, 1993b) and β-CN (Visser and Slangen,1977), suggesting that cathepsin B, like cathepsin D, may contribute to chymosin-like activity in dairy products.

From an enzymatic viewpoint, a significant question is whether different cell types have a different complement of enzymes, and hence whether milks of different differential SCC have quantitatively or qualitatively different enzyme activities (Kelly and Fox, 2006). Moreover, the different behaviors of somatic cells in raw or pasteurized goat milk cheese need to be deeply studied.

3. 5. Instrumental Analysis of Cheese Color

The internal and external lightness values of cheeses ripened at 1 and 7 d are shown in Table 4. Lightness values were always higher in pasteurized milk cheese than raw milk cheeses at both ripening time. At 1 d of ripening, external values were similar to internal values in pasteurized goat milk cheeses, but in raw milk cheeses were lower. Lightness decreased during maduration; it has also reported that in Iranian white cheese (Khosrowshahi, Madadlou, Mousavi, & Emam-Djomeh, 2006) and fresh goat cheese (Sánchez-Macías et al., 2010) whiteness decreased during ripening. In Figure 1, relations between red and yellow indexes are shown for external and internal measurements at 1 and 7 d of ripening times. In all cases, raw milk and pasteurized milk cheese groups were clearly separated each other. Internal and external red index (a*) values decreased as time passed, and external values were generally higher than internal values. At 7 d of ripening, red index data in pasteurized milk

cheeses were significantly higher than raw milk cheeses, and no differences were found as SCC level was increased in cheese (statistical data no shown).

Regarding to yellow index (b*), the internal values did not vary significantly as ripening time passed, but pasteurized milk cheeses had lower values than raw milk cheeses, and no differences were found at different SCC levels. The external b* values were in all cheeses higher than their respective internal values. At d 1 of ripening yellow index was higher in RL and RM cheeses than in PL and PM cheeses, but there were no differences between RH and PH cheeses. In raw milk cheeses, b* values slightly decreased as SCC level increased but, on the contrary, this value increased in pasteurized milk cheese when somatic cells were added. At d 7 of ripening, there were not significant differences as SCC was increased in cheeses, and slight higher values were found in raw milk cheeses than in pasteurized milk cheeses.

4. Conclusions

It is necessary that there be technical and effective solutions for the breeders that own economically viable production systems. Lipolysis clearly depends of somatic cell counts, which may have important consequences on cheese flavor. Two important conclusions about proteolysis were found in this work: somatic cell effect on proteolysis is specific for caseins, and, in addition, the proteolytic effects of somatic cells on main caseins in cheese are different if cheeses are elaborated with raw or pasteurized milk. Lightness decreased in a short time as 7 d in all cheese, always higher in pasteurized goat milk cheeses than their counterparts elaborated with raw goat milk, while a* and b* values increased only in pasteurized milk cheeses, being more homogeneous as SCC increases and time passes in raw milk cheeses. This study has shown that the behavior and objective effects of somatic cells on cheese

depend on the pretreatment of milk. The use of milk with high SCC will result in a different cheese than those elaborated with low SCC.

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Table 1. Chemical composition and pH of raw or pasteurized goat milk cheese with three levels of SCC at 1 and 7 d of ripening.

		Treatment[1]						
Item	Time	RL	RM	RH	PL	PM	PH	SEM[2]
Moisture, %	1	48.86^{b}	49.65^{b,z}	51.98^{ab,z}	55.27^{a,z}	55.02^{a,z}	53.37^{ab,z}	0.57
	7	43.75	43.72^{y}	44.18^{y}	43.43^{y}	43.83^{y}	44.32^{y}	0.61
	SEM	2.61	2.70	2.90	3.41	3.33	3.07	
Fat in DM, %	1	44.19	45.82	46.58	45.59	47.05	46.47	0.42
	7	45.44	46.46	44.60	46.89	47.57	48.74	0.35
	SEM	0.21	0.17	0.37	0.40	0.12	0.39	
Protein in DM, %	1	35.53^{y}	36.58^{y}	36.30^{y}	35.50^{y}	35.34^{y}	36.72^{y}	0.36
	7	38.81^{z}	40.13^{z}	39.61^{z}	38.05^{z}	38.21^{z}	39.48^{z}	0.43
	SEM	0.32	0.21	0.47	0.11	0.19	0.28	
pH	1	6.56^{z}	6.61^{z}	6.62^{z}	6.60^{z}	6.61^{z}	6.60^{z}	0.01
	7	5.67^{ab,y}	5.68^{ab,y}	5.53^{a,y}	6.23^{c,y}	5.83^{b,y}	5.72^{b,y}	0.08
	SEM	0.12	0.12	0.15	0.06	0.12	0.13	

[a-c]Means within a row with different superscripts differ ($P < 0.05$).

[z-y]Means within a column with different superscripts differ ($P < 0.05$).

[1]Treatments: RL = raw goat milk with low SCC; RM = raw goat milk with medium SCC; RH = raw goat milk with high SCC; PL = pasteurized goat milk with low SCC; PM = pasteurized goat milk with medium SCC; PH = pasteurized goat milk with high SCC.

[2]SEM = Standard error of the mean.

Table 2. Total FFA in cheese elaborated with goat milk at three levels of SCC and raw or pasteurized at 1 and 7 d of ripening.

	Treatment[1]						
Time	RL	RM	RH	PL	PM	PH	SEM[2]
	a. Total FFA (mg) per 100gr of DM						
1	52.92^{y}	54.21^{y}	60.96^{y}	48.59^{y}	56.26^{y}	63.75^{y}	3.14
7	243.5^{c,z}	441.01^{b,z}	641.58^{a,z}	113.94^{d,z}	468.10^{b,z}	581.34^{a,z}	35.10
SEM	33.09	55.19	86.11	11.60	59.52	72.49	
	b. Total FFA (mg) per g of fat in cheese						
1	1.15^{y}	1.17^{y}	1.31^{y}	1.04^{y}	1.20^{y}	1.36^{y}	0.07
7	5.22^{c,z}	9.41^{b,z}	13.81^{a,z}	2.47^{d,z}	10.14^{b,z}	12.49^{a,z}	0.64
SEM	0.70	1.17	1.83	0.24	1.21	1.47	

$^{a-c}$Means within a row with different superscripts differ ($P < 0.05$).

$^{z-y}$Means within a column with different superscripts differ ($P < 0.05$).

[1]Treatments: RL = raw goat milk with low SCC; RM = raw goat milk with medium SCC; RH = raw goat milk with high SCC; PL = pasteurized goat milk with low SCC; PM = pasteurized goat milk with medium SCC; PH = pasteurized goat milk with high SCC.

[2]SEM = Standard error of the mean.

Table 3. Comparison of densitometric values (intensity x mm) of SDS-PAGE protein bands in cheese elaborated with goat milk at three levels of SCC and raw or pasteurized at 1 and 7 d of ripening.

		Treatment[1]						
Item	Time	RL	RM	RH	PL	PM	PH	SEM[2]
α_{S2}-CN	1	846.28^{c,z}	1021.21^{b,z}	1032.35^{ab,z}	1100.04^{a,z}	970.83^{b,z}	978.84^{b,z}	15.59
	7	671.25^{bc,y}	713.26^{b,y}	765.31^{ab,y}	805.98^{a,y}	707.89^{b,y}	619.35^{c,y}	11.99
	SEM	27.05	45.64	38.25	41.40	37.00	48.68	
α_{S1}-CN	1	566.74^{z}	634.59^{z}	677.69^{z}	722.22^{z}	657.42^{z}	638.01^{z}	25.71
	7	421.99^{y}	543.86^{y}	520.9^{y}	583.18^{y}	520.58^{y}	483.11^{y}	23.98
	SEM	41.90	38.60	44.79	63.39	39.05	35.97	
β-CN	1	1587.84^{z}	1624.15^{z}	1661.73^{z}	1694.79^{z}	1654.67^{z}	1516.92^{z}	31.05
	7	1089.08^{ab,y}	1131.18^{ab,y}	1219.80^{a,y}	1114.22^{ab,y}	961.97^{b,y}	864.50^{b,y}	32.30
	SEM	55.95	76.76	73.09	102.34	101.59	90.66	
para-κ-CN	1	495.04^{b,z}	607.29^{ab,z}	696.29^{a,z}	726.21^{a,z}	554.96^{b,z}	599.74^{b,z}	21.12
	7	368.33^{y}	464.81^{y}	491.48^{y}	465.10^{y}	382.29^{y}	372.57^{y}	18.43
	SEM	26.03	33.04	43.87	51.08	37.96	41.09	

[a-c]Means within a row with different superscripts differ ($P < 0.05$)

[z-y]Means within a column with different superscripts differ ($P < 0.05$)

[1]Treatments: RL = raw goat milk with low SCC; RM = raw goat milk with medium SCC; RH = raw goat milk with high SCC; PL = pasteurized goat milk with low SCC; PM = pasteurized goat milk with medium SCC; PH = pasteurized goat milk with high SCC.

[2]SEM = Standard error of the mean.

Table 4. Internal and external lightness analysis in cheese elaborated with goat milk at three levels of SCC and raw or pasteurized at 1 and 7 d of ripening.

		Treatment[1]						
	Time	RL	RM	RH	PL	PM	PH	SEM[2]
Internal	1	$97.47^{b,z}$	$97.21^{b,z}$	$97.45^{b,z}$	$100.01^{a,z}$	$99.84^{a,z}$	$99.52^{a,z}$	0.20
	7	$76.21^{b,x}$	$75.75^{b,x}$	$74.43^{c,x}$	$78.13^{a,y}$	$78.68^{a,y}$	$78.79^{a,y}$	0.42
External	1	$94.79^{c,y}$	$95.26^{c,y}$	$96.05^{c,y}$	$99.5^{a,z}$	$99.16^{ab,z}$	$98.00^{b,z}$	0.32
	7	$76.66^{b,x}$	$75.74^{b,x}$	$75.46^{b,x}$	$78.49^{a,y}$	$79.49^{a,y}$	$78.80^{a,y}$	0.26
	SEM	4.06	4.05	4.06	4.25	4.30	4.27	

[a-c]Means within a row with different superscripts differ (P<0.05).

[z-x]Means within a column with different superscripts differ (P<0.05).

[1]Treatments: RL = raw goat milk with low SCC; RM = raw goat milk with medium SCC; RH = raw goat milk with high SCC; PL = pasteurized goat milk with low SCC; PM = pasteurized goat milk with medium SCC; PH = pasteurized goat milk with high SCC.

[2]SEM = Standard error of the mean.

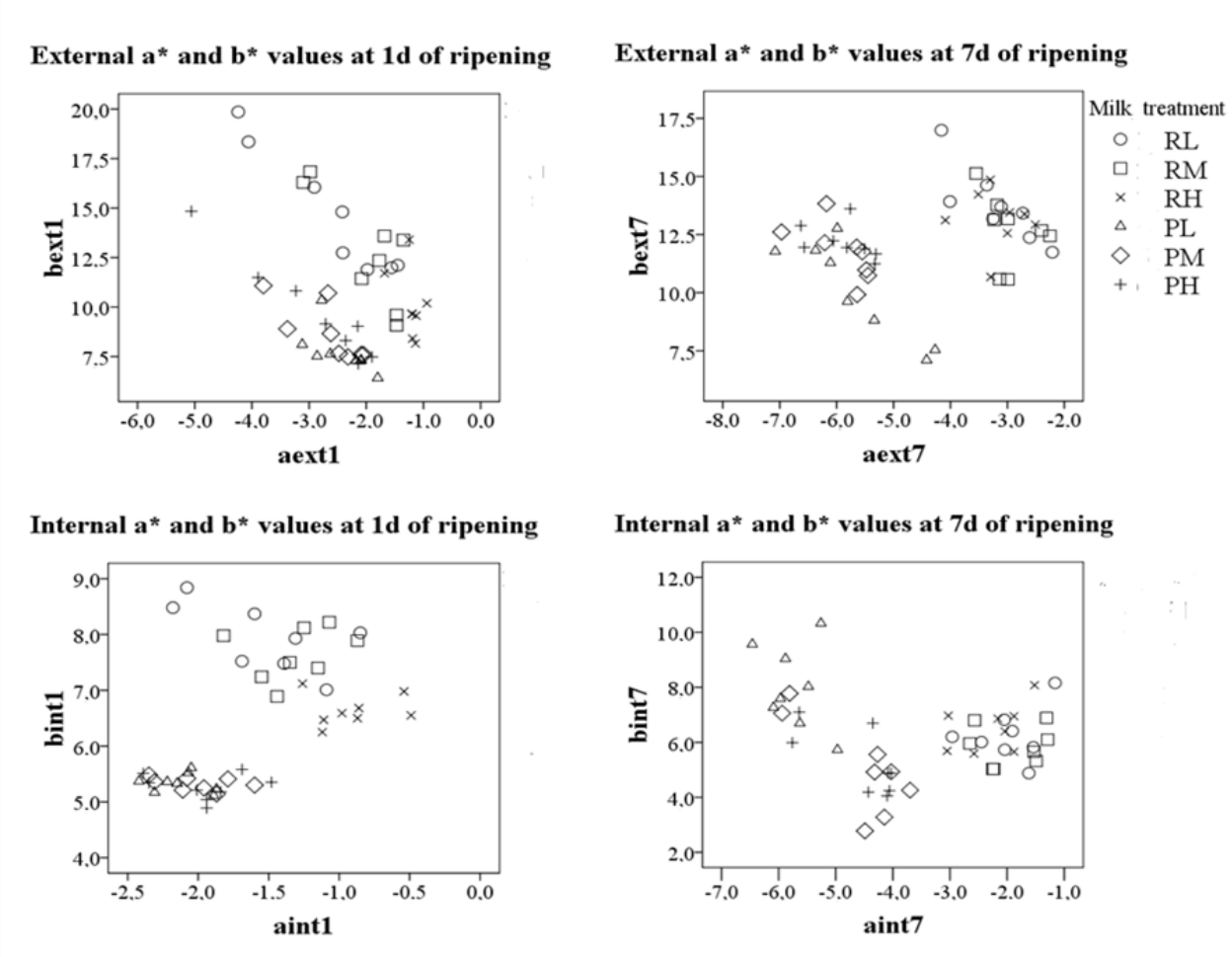

Fig. 1. Scatterplots of internal and external a* and b* values (aint, aext, bint, and bext, at 1 and 7 d of ripening, respectively) in cheeses elaborated with raw or pasteurized goat milk at three levels of SCC.

Capítulo VIII:
Somatic cells: a potential tool to accelerate the ripening of low-fat goat cheese

1. Planteamiento y Metodología.
2. Trabajo experimental

Capítulo VIII. Somatic cells: a potential tool to accelerate the ripening of low-fat goat cheese

1. Planteamiento y Metodología

1.1. Planteamiento

Las nuevas tendencias más saludables de los consumidores y su mirada hacia los quesos bajos en grasa (Johansen *et al.*, 2011) chocan drásticamente con la mala percepción sensorial que se tiene de este producto debido a sus inusuales e inapetecibles características (Drake, 2008). La red proteica es más compacta y existe una inadecuada proteólisis (Fenelon y Guinee, 2000; Aryana and Haque, 2001; Rahimi *et al.*, 2007). Se han empleado muchos métodos para mejorar los quesos bajos en grasa, entre los cuales destaca el uso de enzimas exógenas que normalmente no participan en el proceso de elaboración del queso.

Las células somáticas son una fuente de enzimas lisosomales, como las catepsinas B, D y G, y elastasa (Le Roux *et al.*, 2003), muchas de las cuales pasan a la leche y causan proteólisis y lipólisis más intensa (Le Roux *et al.*, 1995; Albenzio *et al.*, 2005). Muchas de estas enzimas han sido encontradas o medidas en fracciones de la leche más que en las propias células, lo que sugiere que existe una secreción o liberación desde las células, o que las células se lisan y liberan las enzimas intracelulares (Kelly y Fox, 2006). Harmon (1994) y Moslehishad y Ezzatpanah (2010) describieron que una elevación del recuento de células somáticas en la leche afecta a la microestructura y propiedades de las micelas de caseína, debido a la hidrólisis de las partículas de caseína por un incremento de la actividad proteolítica de enzimas como la plasmina, proteasas neutras y ácidas, lo que las hace más susceptibles a la degradación. Marino *et al.* (2005) encontró una mayor degradación de α_{S1}-caseína en los quesos a los 15 días de maduración, indicando una acelerada proteólisis de esta caseína. Delandes (1998) reveló la existencia de actividad de lipasa derivada de células somáticas de la leche de cabra. También otros autores (Azzara y Dimick, 1985) encontraron que los macrófagos derivados de leche mamítica de vaca segregan enzimas

lipolíticas. Jaubert *et al.* (1996) ha observado que la leche de cabra que presentó un flavor caprino más intenso contenían mayores recuentos de células somáticas.

Con estos datos, nuestra hipótesis principal es que las células somáticas, proveniente de leche de cabra saludable, podrían usarse como potenciales aceleradores de la maduración de los quesos desnatados a través de la mejora de la proteólisis.

1.2. Metodología

El material y los métodos usados en este experimento, así como los resultados obtenidos han sido enviados en formato y están bajo revisión con el título "Somatic cells: a potyencial tool to accelerate the ripening of low-fat goat cheese" (Células somáticas como una herramienta para acelerar la maduración de los quesos de cabra bajo en grasa) a Journal of Dairy Science.

La obtención de células somáticas se realizó del mismo modo que el descrito en el capítulo VII. La leche procedente de 4 cabras sanas de bajo recuento se separó en dos grupos: una parte se dejó como leche entera y la otra sufrió un proceso de desnatación por medio de la centrifugación. Estos grupos se subdividieron en dos: una parte se dejó como leche cruda y la otra sufrió un proceso de pasteurización (63ºC 30 min). A su vez, ambos grupos de leche desnatada se subdividieron en 2 partes, a una de las cuales se le añadió células somáticas del pool hasta un nivel de 2 millones por mL. Se elaboraron miniquesos de la siguiente manera: a 40mL de leche se le añadió cuajo animal en tubos de centrífuga de 50mL a 32ºC. La cuajada se cortó y se centrifugó a 4500 rpm 20 min. Los quesos resultantes se dejaron madurar 1 y 7 días a 10-12ºC y 75-80% de humedad relativa. De los quesos se midió la composición química básica, color, pH, proteólisis mediante perfil proteico en SDS-PAGE y lipólisis como nivel de ácidos grasos libres.

Los datos se analizaron mediante el paquete estadístico SAS (versión 9.0; SAS Institute Inc., Cary, NC) mediante un ANOVA de una vía para las muestras de leche y suero, y un ANOVA de medidas repetidas de dos vías (pretratamiento de la leche y tiempo de maduración)

considerando un valor de P<0,05 como estadísticamente significativo. Para evaluar las diferencias entre grupos se usó el test de Tukey.

2. Trabajo experimental

Interpretive summary

Somatic cell contribution in low-fat goat milk cheese. By: Sánchez-Macías.

The atypical characteristics associated with low-fat cheese can be avoided using a number of methods. Although in cow milk is associated with inflammation or infection, particularly mastitis, a high somatic cell count in goat milk is highly associated with non-infection factors. This study examines the potential of goat milk somatic cells to increase the casein breakdown in the excessively hard matrix of low-fat cheese. The work demonstrated differences between pasteurized and raw milk used in goat milk cheese, showing it is possible to increase the degradation of casein using somatic cells in low-fat raw goat milk cheese whereas in pasteurized goat milk cheese degradation was reduced. Moreover, the addition of these cells increased the luminosity and decreased the high chrome associated with low-fat goat cheese.

Running head: SOMATIC CELL IN LOW-FAT GOAT MILK CHEESE

TITLE

Somatic cells: a potential tool to accelerate the ripening of low-fat goat cheese

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ABSTRACT

The current demand for healthy products and for a diversification of dairy products has increased the interest in both low-fat and goat's cheeses. When these two characteristics are combined, the result is a flawed cheese, with similar shortcomings to other low-fat cheeses. As a result, it is necessary to seek alternatives. This research proposes the use of somatic cells as a potential tool to improve low-fat goat cheeses. The aim of this study was to evaluate the effects of somatic cells taken from healthy goats on fresh low-fat cheese made with raw or pasteurized goat milk. Miniature cheeses were made and ripened for 1 and 7 days. Protein and moisture were increased as fat decreased in milk, but there were no effects found on the chemical composition of low-fat cheeses when somatic cells were added. Somatic cells increased the proteolysis of α_{S1}-CN, α_{S2}-CN, and para-κ-CN in low-fat cheese from raw milk; although the degradation of all the protein was reduced when low-fat cheese was made from pasteurized milk. The addition of somatic cells to low-fat milk resulted in whiter cheeses, reduced the negative red index, and did not change the yellow index very much. In conclusion, somatic cells may be used to increase the softness low-fat cheese texture, thereby improving it. Further results concerning instrumental texture and sensory analysis are needed to confirm the indirect results found in this study.

INTRODUCTION

Current health and nutrition trends have changed consumer preferences, and there is now a greater focus on dairy products, for example low-fat cheese (Johansen et al., 2011).

However, when the product is made with low-fat milk,consumers detect unusual and unappealing properties such as poor sensory characteristics. (Drake, 2008). Cheese made with low-fat milk results in a more compact casein network than full-fat cheese (Aryana and Haque, 2001; Rahimi et al., 2007), it is harder (Küçüköner y Haque, 2006; Rogers et al., 2009) and more cohesive (Sahan et al., 2008).

Demand for a diversification of cheese products has led to increased interest in goat milk products as a profitable alternative to cow's milk due to their sensory qualities and inherent health-promoting attributes (Haenlein, 2004; Raynal-Ljutovac et al., 2008; Ribeiro and Ribeiro, 2010). Sánchez-Macías et al. (2010) recently have reported significant differences in physicochemical composition, instrumental texture profile, in addition to instrumental color in full-fat goat cheese, low- and reduced-fat goat cheese when raw milk is used. These authors concluded that low-fat cheese displayed higher fracturability, hardness, cohesiveness, and masticability values, as well as less instrumental color intensity than full-fat goat's cheese. An inadequate proteolysis of casein has been reported in the same cheeses (Sánchez-Macías et al., In press), in accordance with other studies made with low-fat cow's milk cheese (Fenelon and Guinee, 2000; Tunick et al., 1995).

Several methods have been employed to improve low fat cheeses. Examples include: modifications inf cheese-making conditions (Mistry, 2001; Guinee and McSweeney, 2006, Johnson et al., 2009); inclusion of additives and fat replacers (amply reviewed by Larsen, 2009); using starter strains (Awad et al., 2005; Dabour et al., 2006; Jimenez-Guzman et al., 2009); and increasing the rennet concentration (Madadlou et al, 2005). Moreover, technologies such as ultrafiltration (Drake and Swanson, 1995) and homogenization (Madadlou et al., 2007) have also been used.

Acceleration of the ripening process with the objective of increasing the flavor (through proteolysis and lipolysis), whilst maintaining a satisfactory texture. This reduces

time and costs associated with cheese ripening, and could, therefore, be of interest to commercial cheese producers. Some techniques used involve temperature (Azarnia et al., 2006), high pressure (Molina et al., 2000; Capellas et al., 2001; Trujillo et al., 2002), and enzyme addition (Kailasapathy and Lam, 2005; Azarnia et al., 2011). The results of many studies have suggested that plasmin could be a high value enzyme to accelerate maturing and improve the flavor development in natural cheeses (Bastian and Brown, 1996). Exogenous activators of plasminogen have been proposed and used to increase the plasmin activity, which decrease during the ripening (Bastian et al., 1997; Barret et al, 1999). However, this process is expensive and not easily available. Another possibility is to activate the plasminogen through endogenous plasminogen activators.

Many countries use the somatic cell count (**SCC**) as a sanitary criterion, establishing a limit to ensure the quality of dairy products (Raynal-Ljutovac et al., 2005). However, it is important to note that non-infectious factors also have a major impact on goat milk SCCs (Haenlein and Hinckley, 1995; Paape et al., 2001; Fernández et al., 2009). The effects of somatic cells on milk properties, cheese quality, lipolysis and proteolysis (Raynal-Ljutovac et al., 2007) are not clear. A relationship between milk proteolysis and SCC has been described by Hachana and Kraiem (2009). Marino et al. (2005) reported that proteolysis during cheese ripening (as measured by levels of pH 4.6-soluble nitrogen) increased when somatic cells were added from mastitic cow's milk, although this effect was reduced by pasteurization after cell addition. Caroprese et al. (2007) studied the contribution of macrophages to proteolysis and plasmin activity in bulk ewe milk, observing an increase of proteolysis linked to macrophage concentration. Somatic cells present lysosomal enzymes, such as cathepsin B, cathepsin D, cathepsin G and elastase (Le Roux et al., 2003), many of which pass to the milk where they cause more intense proteolysis and lypolysis (Le Roux et al., 1995; Albenzio et al., 2005). Many of the enzymes that originate from somatic cells have been found or

measured in fractions of milk other than the cells themselves (e.g. in skimmed milk or whey), suggesting that leakage or secretion of enzymes occurs, or that the cells lyse and release their intracellular enzymes (Kelly and Fox, 2006). Politis et al. (2004) reported that somatic cells from goat milk have a plasminogen activator; and Leitner et al. (2004) reported that the plasmin and plasminogen activator activities are higher in goat milk than in cow and sheep milk, increasing as somatic cells count increase in milk.

To date, no studies concerning the effects of somatic cells on low-fat cheese have been published in academic literature. Somatic cells from healthy goat milk are proposed in this work as a potential tool to accelerate the ripening of low-fat cheese and improve it. There is a possibility that this method could also be extended to full-fat cheese industry.

The aim of this study was to evaluate the effects of somatic cells on chemical composition, proteolysis, lipolysis and color of fresh goat cheese elaborated with full-fat and low-fat goat milk, pasteurized and non-pasteurized milk, and ripened at 1 and 7 days.

MATERIAL AND METHODS

Recovery of Somatic Cells

Somatic cells were recovered by centrifugation (1500 rpm x 20 min at 4˚C) (Beckman, J2-CI Centrifuge, Beckman Coulter Inc., Miami, FL) from high SCC raw milk (approximately 2 x 10^6 cells/mL) from two healthy Majorera breed goats. The resulting pellet was re-suspended in phosphate buffered saline at pH 6.8, as described by Verdi and Barbano (1991), until a pool with approximately 21 x 10^6 SCC/mL was obtained. The SCC of this stock preparation, before and after dilution in PBS, was determined using a DCC Device counter (DCC**,** DeLaval International AB, Tumba, Sweden). The type of somatic cell in the pool was not specifically determined, but is likely that the somatic cell population in goat milk is

predominated by polymorphonuclear neutrophils (45-74%), followed by macrophages (15-41%) and lymphocytes (9-20%) (Dulin et al., 1983).

Cheese Production

Cheese Formulation. Raw goat milk with a low SCC (approximately 3 x 10^5 SCC/mL) was obtained from four goats from a healthy experimental herd of Majorera dairy goat breed located at the Animal Science Unit of Universidad de Las Palmas de Gran Canaria, and was used to elaborate the cheeses. Four batches of experimental cheeses were produced consecutively over four days using six different experimental treatments, as shown in the Figure 1. Each day, a proportion of the raw goat milk was separated to obtain skimmed goat milk (approximately 0.5% of fat content) using an Elecrem skimmer (Fresnes, France). Full-fat and low-fat milk were subdivided into two groups; raw milk and pasteurized milk (63ºC for 30 min). Subsequently, low-fat milk was subdivided again into two subgroups with two levels of somatic cells: low SCC milk (without somatic cells added), and high SCC milk (approximately 2 x 10^6 SCC/mL). Miniature cheeses were made and ripened for 1 and 7 d in duplicate, resulting in a total of 96 cheese products.

Manufacture of Miniature Cheese. Miniature cheese systems have been proposed as the best alternative to pilot plant experiments, as they are closer to cheese than any other model proposed to date. Moreover, they can be prepared under controlled conditions and are more economical, reproducible and easier to obtain (Shakeel-Ur-Rehman et al., 2001). Cheeses were made using the six different milk treatments: full-fat raw milk low SCC (**FR**) cheese, full-fat pasteurized milk low SCC (**FP**) cheese, low-fat raw milk low SCC (**LRL**) cheese, low-fat raw milk high SCC (**LRH**) cheese, low-fat pasteurized milk low SCC (**LPL**) cheese, and low-fat pasteurized milk high SCC (**LPH**) cheese. The same procedure was used to make each type of miniature cheese: 40 mL of milk were poured in four 50 mL-centrifuge tubes,

which were then placed in a water bath at 32°C. Animal rennet (commercial rennin powder, Marshall rennet power), comprising 50% pepsin and 50% chymosin was added**.** No starter cultures were added. Curd was cut after 45 min of clotting time with a stainless-steel spatula and allowed to stand for additional 5 min. The curds were centrifuged at 4500 x *g* for 20 min. Cheese whey was recovered for analysis and cheeses were divided randomly into two groups and allowed to ripen 1 or 7 d at 10-12ºC and 75-80% relative humidity.

Analysis of the Physicochemical Properties of Cheese

A Minolta colorimeter CR-400/410 (Illuminant D65, Konica Minolta, Osaka, Japan) was used to determine external and internal lightness (L), yellow index (b*), and red index (a*) for each cheese in triplicate. Cheese samples were cut into small pieces, weighed and freeze-dried. The weight difference before and after the freeze-drying was used to calculate the percentage of moisture content, and the rest of the analyses were performed with the resultant dry matter. Soxhlet extractions were performed to quantify the fat content in dry basis in cheeses with a Büchi 810 Soxhlet fat extraction apparatus (Büchi Labortechnik, Flawil, Switzerland). The protein content was determined in duplicate as % N x 6.38 using a LECO CNS-2000 nitrogen analyzer (Leco Corporation, St. Joseph, MI).

Extraction of Proteins and Electrophoretic Analysis

Water-soluble proteins were extracted from the cheeses tested at days 1 and 7 of ripening using a modified version of the method described by Tunick et al. (1995). Briefly, 2.5 mL of buffer (0.166 *M* Tris, 0.001 *M* EDTA, pH 8.0) was added to 0.5 g of freeze-dried cheese and mixed for 15 min in vortex. 2.5 mL of 7% SDS was added to this mixture, and the sample mixed again for 5 min. 1 mL of 10 m*M* dithiothreitol buffer was then added, and the mixture was held in an ice bath for 20-30 min. Samples were then centrifuged for 1 h at 4ºC at 39,000

x *g* (Beckman, J2-CI Centrifuge; Beckman Coulter Inc., Miami, FL). The supernatant was filtered, lyophilized, and stored at -20ºC until analysis.

Lyophilized samples were prepared for SDS-PAGE; a final concentration of 1mg/mL of cheese protein was achieved using sample buffer for SDS-PAGE. Each sample in buffer was boiled for 5 min at 100ºC with 16 µL of β-mercaptoethanol, and the proteins (30 µg) were separated by SDS-PAGE (Laemmli, 1970) on a 12.5% gel, including a molecular weight patron (Precision Plus Protein Standards, Bio-Rad Laboratories). Gels were stained with Coomassie Blue R-250, and destained. Images of gels were captured with a Bio-Rad imaging device (Gel Doc EQ; Bio-Rad Laboratories, Hercules, CA), and bands were quantified using Quantity One Quantitation Software (Bio-Rad Laboratories). Densitometry analysis of the gels was performed using lane-based background subtraction followed by measurement of the bands by the area under the intensity profile curve, and the values were then used for statistical analysis. Each sample was analyzed on duplicate gels. Individual protein species from cheese samples were identified by comparing their relative mobility with those of standard proteins from a previous SDS-polyacrylamide gel.

Total FFA (Copper Soap Method)

The total FFA content was determined using the copper soap method (Shipe et al., 1980; Melilli et al., 2004) modified for cheese analysis. Lyophilized cheese (0.12 and 0.06 g of 1 and 7 d of ripening time, respectively) and 1 mL of deionized water were placed in a centrifuge tube. Subsequently, 0.1 mL of 0.7 *N* HCl, 0.1 mL of 1% (vol/vol) Triton-X 100 solution, and the copper soap reagent (2 mL) were added and the mix was vortexed after each addition. Chloroform/heptanes/methanol (49:49:2 vol/vol/vol) solvent (6mL) was added to each tube without vortexing. The centrifuge tubes containing the reagents plus cheese samples were shaken for 60 min in a Heidolph Rotamax orbital platform shaker (Heidolph Instruments

GmbH & Co, Schwabach, Germany) at 300 rpm. After shaking, two distinct layers quickly formed, and the tubes were then centrifuged (Hettich Zentrifugen, Tuttlingen, Germany) at 4,500 x *g* for 10 min. The top colorless solvent layer (3.5 mL) was transferred from the centrifuge tubes into an acid-washed test tube containing 0.1 mL of the color reagent solution. After mixing, absorbance was measured immediately at 440 nm using a SmartSpec Plus Spectrophotometer (Bio-Rad Laboratories). Two blanks (1 mL of deionized water) were also prepared and analyzed with the cheese samples.

A standard curve was constructed using palmitic acid crystal grade diluted in hexane. After the solvent was completely removed under a hood, 1 mL of deionized water was added to the tube, and the set of standards was carried through the full analysis with the cheese samples and two blanks. Absorbance readings of standards were corrected by subtracting the average of the two blanks readings, and a regression line was constructed that correlated the corrected absorbance with micrograms of palmitic acid. The level of FFA in the cheese samples was calculated from the standard curve. The final results were expressed as milligrams of FFA per 100 g of dry matter of cheese and per 100 g of fat in cheese.

Statistical Analysis

Statistical analyses were performed using the SAS program package (Version 9.00, SAS Institute Inc., Cary, NC). A PROC MIXED procedure (analysis of variance with repeated measures) was used to evaluate the effect of fat content, somatic cell level, and time of ripening on the chemical composition, pH, lipolysis level, primary proteolysis, and color parameters of goat milk cheeses. Significantly different means were identified using the Tukey's test.

RESULTS AND DISCUSION

Gross chemical composition of cheese

Table 1 shows the proximal composition and pH of cheeses. As expected, cheese fat in DM content was higher in FRL and FPL than in the rest of low-fat cheeses during the experimental period. These values were within the ranges required by Spanish regulation for full-fat (60–45% fat in DM), and for low-fat (less than 10% fat in DM).

However, protein in DM content was higher in lower in fat cheeses compared to full-fat cheese. The FRL and FPL protein contents were close to the values reported for Canarian goat cheeses (Fresno et al., 2005; Álvarez et al., 2007; Sánchez-Macías et al., 2010). Moisture content was significantly higher in LRL and LRH than FRL, and in LPL and LPH than FPL, respectively. According to the literature (Lteif et al. 2009; Sánchez-Macías et al., 2010), low fat cheese contains significantly higher amounts of protein and moisture than their respective full-fat cheeses. Moreover, pasteurized goat milk cheeses were found to have higher moisture content than those elaborated with raw goat milk. These results concur with Pearse et al. (1985), who reported an adverse effect of heat treatment on syneresis of rennet-induced milk gels using model systems. This effect on syneresis is probably due to the presence of denatured whey protein on the surface of the micelles, which slow aggregation and fusion of the rennet-treated micelles (Rynne et al., 2004). Henry et al. (2002) reported that more water is bound in cheese when β lactoglobulin-κ casein complex is formed after milk pasteurization. After 7 d of ripening, there were fewer differences in moisture content in cheese due to the milk pasteurization. Those differences between full-fat and low-fat cheese, however, were still present at the end of the experimental time. The addition of somatic cells to low-fat goat milk did not affect the moisture content in cheese.

Marino et al. (2005) suggested that somatic cells in may contribute to the increase moisture content in cheese, as there is a possibility that the inclusion of cells physically interferes with the process of curd syneresis. In this study no higher moisture content in

cheese when somatic cells were added to the low-fat goat milk was found. It is well known that low-fat cheese has a tighter protein network than full-fat cheese (Gunasekaran y Ding, 1999; Guinee et al. 2000; Rogers et al., 2010), and this may hinder moisture retention in the curd when somatic cells are added to the milk.

Proteolysis

A densitometer protein-peptide distribution analysis is summarized in Table 2. The β-CN bands showed the highest intensities, followed by α_{S2}-CN and α_{S1}-CN in all cheeses and at all ripening times. In general, goat milk contains no or relatively little α_{S1}-CN, whereas β-CN is the major protein (Tziboula-Clarke, 2003). The Canarian dairy breeds (Majorera, Tinerfeña, and especially Palmera) represent a particular case where 60% of the alleles for the caprine α_{S1}-CN locus are of the high type (A and B; Jordana et al., 1996). In the present study, α_{S1}-CN was about 20-22% of the total intact casein in all cheese types at d of ripening, similar to data found by Sánchez-Macías et al. (2011) in artisan cheeses made from the same goat breed.

Quantitative analysis showed a general reduction in casein fractions at 7 d compared with 1 d of ripening for all cheeses, and the degradation of intact casein was higher for α_{S1}-CN than for β-CN and α_{S2}-CN over the 7 d of maturation. Trujillo et al. (1997, 2002) and Sánchez-Macías et al. (2011) found exactly the same order of susceptibility of the casein to rennet activity in goat cheese.

Regarding α_{S2}-CN after 1 d of ripening, there were no differences among cheeses made with raw milk that had higher values than pasteurized milk cheeses. In pasteurized goat milk cheeses, reducing fat content in milk resulted in high content of α_{S2}-CN compared with full-fat cheese. However, when somatic cells added into the milk a lower level of this casein was shown. It appears that the pasteurization, combined with high levels of somatic cells, resulted in faster α_{S2}-CN degradation at only 1 d of ripening. After 7 d of ripening, both FR

and FP cheeses had similar values. All cheeses made with raw milk had similar α_{S2}-CN content, although somatic cells contributed to slightly increasing the degradation of this casein. On the contrary, milk pasteurization resulted in lower α_{S2}-CN content in cheeses with lower fat content, but no effect was found when somatic cells were added. The intensities of both low-fat high somatic cell level made with raw or pasteurized milk were not significantly different at the end of the experimental time compared with LRL and LPL. Sánchez-Macías et al. (2011) also reported that α_{S2}-CN degradation was similar in raw goat milk cheeses with different fat content during the first 14 d of ripening, but at 28 d the degradation rate in low-fat cheese was lower than its counterpart higher in fat.

Full-fat cheeses both from raw and pasteurized milk had similar α_{S1}-CN content at d 1 of ripening, slightly lower in the latter. LRL and LPL cheeses showed higher content of this casein than FR and FL, respectively. However, somatic cells addition to low-fat milk resulted in higher degradation rate of lower content of α_{S1}-CN. At 7 d there was a general reduction of α_{S1}-CN in all cheeses except in FP, which had higher value than FR cheese. This result differs from that found by Trujillo et al. (2002), who reported that pasteurized goat milk cheese presented lower values of intact α_{S1}-CN than raw goat milk cheese after 15 d of ripening. At the end of the experimental time, LRL cheese had more α_{S1}-CN than FR, but on the contrary, LPL presented lower content than its counterpart full-fat and even than LRL, as was found for α_{S2}-CN. Somatic cell addition only slightly increased the degradation rate in LRH, but not in LPH. The para-κ-CN profile had exactly the same degradation patron as α_{S1}-CN.

On the first day of ripening, β-CN content did not vary significantly as fat content was decreased or the somatic cell level was increased in the goat milk, nevertheless, it was higher in raw-milk cheeses than pasteurized ones. This result is in accordance with data observed by Trujillo et al. (2002) concerning the amount of β-CN found in pasteurized and raw goat milk cheese, and similar to that reported by Sánchez-Macías et al. (2011) in raw goat milk cheeses

with different fat content. After 7 d, both FR and FP cheeses presented similar intensity values for β-CN. Only in pasteurized milk group were low-fat cheeses found to have lower amounts of this casein, and no differences were found among raw milk cheeses. The addition of somatic cell to pasteurized low-fat goat milk resulted in a lower degradation rate.

Plasmin acts mainly on β-CN, which constitutes approximately 60% of the total casein in goat milk. Pasteurization involves the activation of plasminogen or inactivation of inhibitors of plasminogen activators, resulting in a net increase in plasmin activity in pasteurized milk (Prado et al., 2006). These facts explain the lower β-CN content in pasteurized low-fat milk cheeses in this experiment at 1 d of ripening. However, the somatic cell addition to the low-fat milk did not increase this casein degradation; on the contrary, it decreased it. Some authors have reported that during the first two weeks of ripening, the residual rennet activity is more intense than the plasmin activity, (Fox, 1993; Grappin et al., 1985; McSweeney and Sousa, 2000). Longer experimental time is needed to confirm if somatic cells increase the β-CN degradation rate in low-fat cheese. Caroprese et al. (2007) showed that isolated macrophages from ewes' milk contributed to proteolysis, but not to the plasmin activity. In this work, the comparison between LRL and LPL, which are very rich in protein, demonstrated that plasmin activity increases after pasteurization. The decreased degradation rate found in LPH suggests that somatic cells, not affected by pasteurization, may have possibly inhibited plasminogen activation.

The second proteinase identified in milk is cathepsin D (Kaminogawa and Yamauchi, 1972), which is ostensibly a lysosomal enzyme (Larsen et al, 1996). As with plasmin, cathepsin D is part of a complex system, including inactive precursors of this enzyme (Hurley et al., 2000). The level of cathepsin D in milk is correlated significantly with SCC (O'Driscoll et al., 1999), although it is not clear whether this reflects increased production of cathepsin D, increased activation of precursors or both (Hurley et al., 2000). Kaminogawa et al. (1980) and

McSweeney et al. (1995) showed that cathepsin D, partially purifed from milk, degraded α_{S1}-CN to a peptide with the same molecular mass or electrophoretic mobility as α_{S1}-CN (f24–199), which is one of the primary peptides produced by chymosin. These reports could explain the higher degradation rate of α_{S1}-CN and α_{S2}-CN in LRH cheeses compared to FR and LRL cheeses, but not in pasteurized milk cheeses.

Somatic cells also contain several other proteinases, including cathepsins B, L and G, and elastase, which have received limited attention to date (Kelly and McSweeney, 2003). Cathepsin B showed some similarity to chymosin in its cleavage sites on both α_{S1}-CN (McSweeney et al.,1993a) and β-CN (Visser and Slangen,1977), suggesting that cathepsin B, like cathepsin D, may contribute to chymosin-like activity in dairy products. Cathepsin B, if retained in the curd, may be influential during cheese ripening. As a result, both enzymes may be used to increase the softness of low-fat cheese texture by using high somatic cell count goat milk or its addition if these enzymes are purified. Also, another alternative could be to produce these enzymes as potential tools to improve the low-fat cheese, or even to accelerate the maturation of full-fat goat cheese.

Lipolysis

Means of total FFA per 100 g of dry matter are shown in Table 3 (a). Quantitative analysis of the total FFA content showed that full-fat cheese at both 1 and 7 d of ripening had the highest values than those from low-fat cheese. However, LPH cheese at 1 d of ripening had higher values than the other low-fat cheeses. A reduction in the total of FFA content occurs as the fat content is reduce have been reported in other cheese varieties (Banks et al., 1989; Aly, 1994; Kondyli et al., 2002) and in raw goat milk cheeses made with artisan methods (Sánchez-Macías et al., 2011). The higher value found in LPH compared with LRH suggests that somatic cells or their enzymes have a higher lipolysis activity on fat from pasteurized milk

that has been skimmed using an aggressive centrifugation, as in this experiment. Transformation of goat milk, physical treatment and posterior cold storage were reported to increase the lipolysis level in milk, whereas pasteurization decreased the lipolysis level (Morgan and Gaborit, 2001). The two pre-treatments of the milk in this experiment, centrifugation and posterior pasteurization, may have caused greater damage to the fat globule, exposing the fat to lipolytic enzymes from somatic cells not affected by heating. RL cheese resulted in higher FFA level than PL cheese. Other authors have found lower levels of FFA content in comparing pasteurized and raw milk cheese: 38% in Manchego Cheese (Gaya et al., 1990) and 50% in Cheddar cheese (McSweeney et al., 1993).

The total FFA content increased after 7 d of ripening compared to 1 d in all the cheese, although for LPH it remained unchanged. When comparisons in full-fat cheeses were made at 7 d the pasteurization treatment resulted in a slightly decreased FFA level. Gaya el al. (1990) and McSweeney et al., (1993) reported lower levels of FFA comparing pasteurized and raw milk in Manchego cheese and Cheddar cheese, respectively. In low-fat cheeses, the pasteurization or somatic cell level did not show significant effects on the FFA content.

Theses results suggest that the lipolysis rate increases as fat content in cheese increases, or it is inhibited in low-fat cheeses, as was suggested by Dimos et al. (1996). In low-fat cheeses, when somatic cells are added, total the FFA level remained constant, with values similar to this from LPH at d 1 of ripening. However, when total FFA content was expressed on the basis of fat (Table 3,b), it was possible to obtain results from another point of view. After 1 d of ripening, low-fat cheeses had the highest FFA levels compared with full-fat cheeses, according to the results found in low fat goat cheese (Sánchez-Macías et al., 2011), suggesting that other parameters in low-fat appear to have an influence on lipolysis, as the small globule size (Gunasekaran and Ding, 1999) found as fat content decrease in cheese, more susceptible to lipase action.

After 7 d of ripening time, both raw and pasteurized low-fat milk cheeses, regardless the somatic cell count, showed the highest FFA content. Azzara and Dimick (1985) found that macrophages from mastitic cow milk secreted lipolytic enzymes which may bind to the fat globule membrane and damage it, exposing the fat to degradation by lipoprotein lipase. As suggested by Sánchez-Macías et al. (2011), the lack of fat and the faster FFA liberation in low-fat cheeses may promote early FFA catabolism that occurs in cheese (Collins et al., 2003), which could not reflect higher FFA content in that cheeses with high somatic cell level compared to those with low SCC, but a equilibrium.

Instrumental color

The mean values of external and internal L* (lightness), a* and b* parameters of the cheeses ripened at 1 and 7 d are shown in Table 4. At d 1, both external and internal lightness showed similar values among the cheeses made with the different milk treatments. This parameter decreased significantly after 7 d of ripening. External values were always lower than those from internal measures. Similar results have been found by other authors (Khosrowshahi et al., 2006; Sánchez-Macías et al., 2010). The decreased lightness was explained by a reduced degree of light scattering due to the decrease in the number of free moisture droplets during ripening (Sheehan et al., 2005). The lower values of the external lightness could be a result of faster evaporation of the surface water in cheese, compared to the internal matrix. Low-fat cheeses obtained lower values than full-fat cheeses. These results concur with those found by Sánchez-Macías et al. (2010) in raw goat milk cheeses elaborated with three different fat contents. This can be explained as being a result of decreasing the milk fat in cheese, thereby scattering the light that penetrates the superficial layers (Lemay et al., 1994). Somatic cell addition to low-fat goat milk resulted in an increase of the lightness in cheese at the end of the experimental time.

The internal and external red indexes (a*) were similar in each cheese group at 1 d of ripening and this increased during the experimental time. After 7 d of ripening, pasteurized milk cheeses obtained higher values than raw milk cheeses. Fat content decrease in raw milk resulted in higher values of this parameter in cheese, compared with full-fat cheese, at both 1 d and 7 d of ripening. However, in pasteurized milk cheeses, low-fat cheese showed lower means at the end of the experiment, compared with their respective full-fat counterparts. In all cases, the addition of somatic cells to low-fat milk decreased the red index in cheese when comparing with the low-fat cheese control. Cheese made with low fat content milk appears to have a higher negative red index, which have been attributed to a reduced percentage of fat (Merril et al., 1994; Rudan et al., 1998a; Tunick et al., 1993). Rudan et al. (1998b) observed that when a fat substitute was added, cheese became whiter than respective low-fat counterparts.

At d 1 of ripening pasteurized milk cheeses had lower yellow index (b*) values than raw milk cheeses. Reducing fat in cheeses did not change the mean with respect to the full-fat cheeses. However, adding somatic cells to low-fat milk resulted in a slightly less yellowish color, compared with the control low-fat cheese. The internal values did not vary significantly as ripening time passed. On the contrary, the external b* values increased in all cheeses, being higher than their respective internal values. After 7 d of ripening, LPL had a lower mean for internal b* compared to FP, and the somatic cell addition did not change the values significantly. However the reduced fat content in raw milk showed cheeses with lower yellowness levels (external b*), in accordance with results found by Sánchez-Macías et al. (2010), and no effects of somatic cell were found. On the other hand, no effect of reducing fat in pasteurized milk cheese was revealed compared with full-fat cheeses, although a higher value was achieved when somatic cells were added to the milk. In summary, somatic cell addition to low-fat milk resulted in whiter cheeses, reduced the negative red index, and did

not change considerably the yellow index, with an exception in the external part of pasteurized milk cheese.

CONCLUSION

Somatic cells addition increased the proteolysis of both α_{S1}-caseins and α_{S2}-caseins, and para-κ-casein in low-fat cheeses elaborated with raw goat milk after 7 d of ripening; however, it was reduced for all the caseins when pasteurized goat milk was used in cheese-making. The FFA concentration in dry matter basis was always higher in full-fat cheeses than low-fat cheeses, but lipolysis expressed in fat basis in cheese increased as fat content decreased. Somatic cell addition to low-fat milk resulted in whiter cheeses, reduced the negative red index, and did not significantly change the yellow index. This study proposes the use of somatic cells as a tool to increase the softness low-fat cheese texture, thereby improving it. Furthermore, its use could be extended to full-fat cheese to accelerate the maturing process.

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Table 1. Chemical composition and pH means of cheeses at 1 and 7 d of ripening.

		Treatment[1]						
Item	Time	FRL	LRL	LRH	FPL	LPL	LPH	SEM[2]
Moisture, %	1	47.27^{c,z}	51.00^{b}	52.86^{b}	53.88^{b,z}	58.05^{a}	58.86^{a}	1.02
	7	42.93^{b,y}	50.76^{a}	48.74^{a}	42.89^{b,y}	49.66^{a}	49.65^{a}	0.97
	SEM	1.84	1.57	2.41	2.33	2.40	2.78	
Fat in DM, %	1	46.47^{a}	5.35^{b}	6.06^{b}	46.27^{a}	5.83^{b}	6.27^{b}	3.87
	7	46.59^{a}	5.85^{b}	6.23^{b}	45.61^{a}	5.99^{b}	6.50^{b}	3.60
	SEM	0.86	0.18	0.10	0.59	0.15	0.22	
Protein in DM, %	1	34.87^{b}	67.66^{a}	66.67^{a}	34.01^{b}	66.86^{a}	64.88^{a}	5.00
	7	35.22^{b}	65.43^{a}	65.97^{a}	35.70^{b}	65.97^{a}	64.76^{a}	4.77
	SEM	0.51	0.78	0.94	0.30	0.41	0.28	
pH	1	6.52^{z}	6.52^{z}	6.49^{z}	6.48^{z}	6.45^{z}	6.49^{z}	<0.01
	7	5.62^{b,y}	5.65^{b,y}	5.58^{b,y}	6.20^{a,y}	5.64^{b,y}	5.67^{b,y}	0.05
	SEM	0.14	0.13	0.16	0.05	0.14	0.12	

[a-c]Means within a row with different superscripts differ ($P < 0.05$)

[z-y]Means within a column with different superscripts differ ($P < 0.05$)

[1]Treatments: FR = raw full-fat goat milk; LRL = raw low-fat goat milk with low SCC; LRH = raw low-fat goat milk with high SCC; FP = pasteurized full-fat goat milk; LPL = pasteurized low-fat goat milk with low SCC; LPH = pasteurized low-fat goat milk with high SCC.

[2]SEM = Standard error of the mean.

Table 2. Comparison of densitometric values (intensity x mm) of SDS-PAGE protein bands in the cheeses at 1 and 7 d of ripening.

		Treatment[1]						
Item	Time	FFRL	LFRL	LFRH	FFPL	LFPL	LFPH	SEM[2]
α_{S2}-CN	1	$469.00^{a,z}$	$467.98^{a,z}$	$455.99^{a,z}$	$395.15^{c,z}$	$433.48^{b,z}$	$403.35^{bc,z}$	6.12
	7	$365.66^{a,y}$	$369.94^{a,y}$	$337.00^{ab,y}$	$368.07^{a,y}$	$297.67^{b,y}$	$315.43^{b,y}$	6.34
	SEM	13.18	14.16	15.74	9.79	18.99	13.74	
α_{S1}-CN	1	$407.36^{bc,z}$	$481.42^{a,z}$	$428.4^{b,z}$	372.55^{c}	$392.21^{bc,z}$	$347.84^{c,z}$	8.90
	7	$300.63^{b,y}$	$329.38^{ab,y}$	$267.37^{bc,y}$	355.27^{a}	$202.58^{c,y}$	$240.23^{c,y}$	9.90
	SEM	19.67	19.68	23.27	13.43	23.97	17.06	
β-CN	1	$1146.54^{a,z}$	$1181.76^{a,z}$	$1145.80^{a,z}$	$1054.41^{b,z}$	$1052.89^{b,z}$	$1068.30^{b,z}$	11.77
	7	$888.93^{a,y}$	$831.69^{ab,y}$	$876.81^{a,y}$	$903.58^{a,y}$	$610.83^{c,y}$	$763.50^{b,y}$	17.73
	SEM	38.79	44.19	39.50	20.98	53.10	37.85	
Para-κ-CN	1	$423.69^{bc,z}$	$539.75^{a,z}$	$440.31^{b,z}$	$345.35^{c,z}$	$391.66^{bc,z}$	$360.92^{c,z}$	13.07
	7	$363.70^{a,z}$	$388.27^{a,y}$	$324.15^{ab,y}$	$280.65^{b,y}$	$203.52^{c,y}$	$229.40^{bc,y}$	13.32
	SEM	19.34	25.08	20.32	19.04	30.21	21.65	

[a-c]Means within a row with different superscripts differ ($P < 0.05$)

[z-y]Means within a column with different superscripts differ ($P < 0.05$)

[1]Treatments: FR = raw full-fat goat milk; LRL = raw low-fat goat milk with low SCC; LRH = raw low-fat goat milk with high SCC; FP = pasteurized full-fat goat milk; LPL = pasteurized low-fat goat milk with low SCC; LPH = pasteurized low-fat goat milk with high SCC.

[2]SEM = Standard error of the mean.

Table 3. Free fatty acid content (mg) per 100 gr of cheese dry matter and per 100gr of fat in cheese.

	Treatment[1]						
Time	FRL	LRL	LRH	FPL	LPL	LPH	SEM[2]
	a. FFA (mg) per 100 g of DM of cheese						
1	$49.12^{a,y}$	$20.69^{c,y}$	$22.49^{c,y}$	$47.02^{a,y}$	$19.76^{c,y}$	39.63^{b}	1.90
7	$166.31^{a,z}$	$35.83^{c,z}$	$38.81^{c,z}$	$103.24^{b,z}$	$36.56^{c,y}$	37.85^{c}	8.78
SEM	20.69	3.45	4.02	10.3	2.61	2.1	
	b. FFA (mg) per 100 g of fat in cheese						
1	$107.58^{c,y}$	$352.87^{b,y}$	$377.16^{b,y}$	$102.97^{c,y}$	$336.65^{b,y}$	665.54^{a}	27.71
7	$364.24^{b,z}$	$595.17^{a,z}$	$630.14^{a,z}$	$223.87^{c,z}$	$608.78^{a,z}$	614.63^{a}	40.74
SEM	45.27	38.33	40.32	22.14	44.24	19.74	

[a-c]Means within a row with different superscripts differ ($P < 0.05$)

[z-y]Means within a column with different superscripts differ ($P < 0.05$)

[1]Treatments: FR = raw full-fat goat milk; LRL = raw low-fat goat milk with low SCC; LRH = raw low-fat goat milk with high SCC; FP = pasteurized full-fat goat milk; LPL = pasteurized low-fat goat milk with low SCC; LPH = pasteurized low-fat goat milk with high SCC.

[2]SEM = Standard error of the mean.

Table 4. Internal and external color analysis of cheeses at 1 and 7 d of ripening.

		Treatment[1]						
Item	Time	FR	LRL	LRH	FP	LPL	LPH	SEM[2]
		Lightness						
Internal	1	99.06^{z}	99.28^{z}	99.43^{z}	100.16^{z}	98.97^{z}	99.02^{z}	0.09
	7	84.61^{a,y}	80.91^{b,y}	87.07^{a,y}	85.51^{a,y}	79.16^{b,y}	88.6^{a,y}	1.42
External	1	98.14^{z}	98.26^{z}	98.84^{z}	99.69^{z}	98.59^{z}	98.81^{z}	0.11
	7	80.96^{a,y}	73.19^{b,x}	81.98^{a,y}	81.77^{a,y}	71.92^{b,x}	77.60^{a,x}	1.33
	SEM	1.78	2.45	2.64	1.85	2.96	2.04	
		a* index						
Internal	1	-1.85^{a,z}	-3.34^{b,z}	-3.10^{b,zy}	-2.18^{a,z}	-2.38^{a,z}	-2.09^{a,z}	0.10
	7	-2.26^{a,z}	-4.12^{b,y}	-3.66^{b,y}	-5.82^{c,y}	-5.14^{c,y}	-4.28^{b,y}	0.23
External	1	-1.97^{a,z}	-3.33^{b,z}	-2.65^{ab,z}	-2.35^{ab,z}	-2.96^{b,z}	-2.46^{ab,z}	0.11
	7	-4.91^{a,y}	-6.03^{b,x}	-5.13^{a,x}	-8.13^{d,x}	-6.95^{c,x}	-6.76^{bc,x}	0.23
	SEM	0.27	0.25	0.26	0.52	0.42	0.41	
		b* index						
Internal	1	6.05^{a,x}	5.85^{a,x}	5.68^{a,x}	4.14^{b,w}	4.32^{b,x}	4.24^{b,x}	0.16
	7	5.02^{b,x}	4.91^{b,x}	4.22^{b,x}	6.58^{a,y}	3.84^{c,x}	4.00^{bc,x}	0.32
External	1	8.63^{a,y}	8.84^{a,y}	7.00^{b,y}	6.47^{b,y}	7.25^{ab,y}	6.73^{b,y}	0.23
	7	19.14^{a,z}	13.37^{c,z}	13.91^{c,z}	16.44^{b,z}	15.62^{b,z}	18.57^{a,z}	0.41
	SEM	1.18	0.98	0.80	0.99	1.01	1.26	

$^{a-d}$Means within a row with different superscripts differ ($P < 0.05$)

$^{z-x}$Means within a column with different superscripts differ ($P < 0.05$)

[1]Treatments: FR = raw full-fat goat milk; LRL = raw low-fat goat milk with low SCC; LRH = raw low-fat goat milk with high SCC; FP = pasteurized full-fat goat milk; LPL = pasteurized low-fat goat milk with low SCC; LPH = pasteurized low-fat goat milk with high SCC.

[2]SEM = Standard error of the mean.

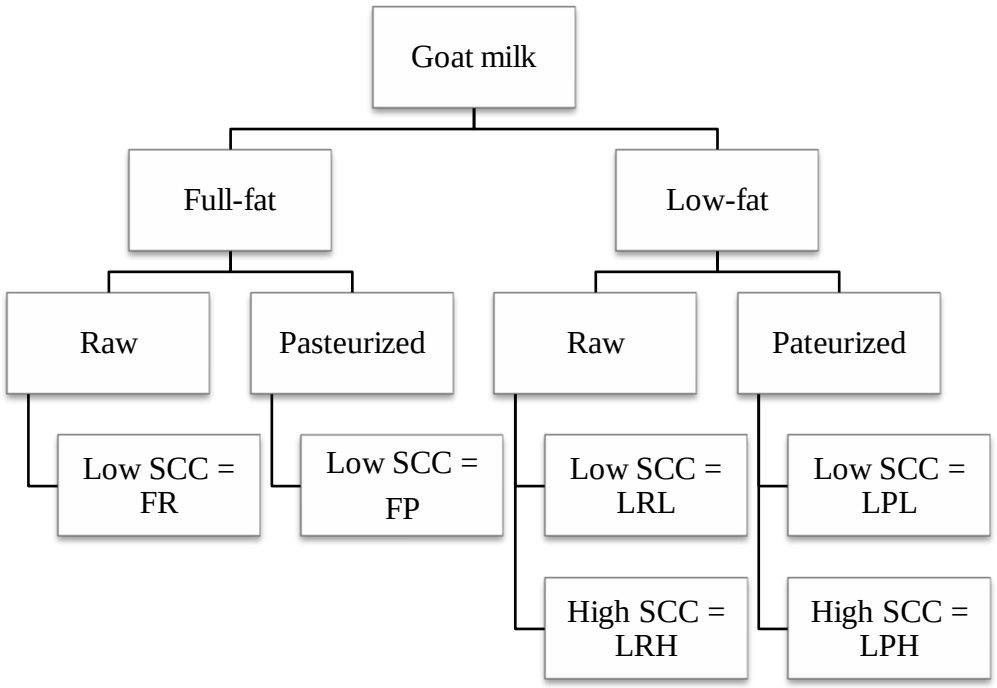

Figure 1. Subdivision of goat milk in six different milk treatments before cheese-making.

Capítulo IX:
Effects of supercritical fluid extraction pressure on chemical composition, microbial quality, polar lipids profile and microstructure of goat cheese

1. Planteamiento y Metodología.
2. Trabajo experimental

Capítulo IX: Effects of supercritical fluid extraction pressure on chemical composition, microbial quality, polar lipids profile and microstructure of goat cheese

1. Planteamiento y Metodología

1.1. Planteamiento

Los cambios de conciencia de los consumidores hacia comidas más saludables y que aporten algún beneficio extra, ha derivado en que las industrias se vean presionadas a reducir grasa, colesterol, sal, azúcares y otros componentes en sus productos. Pero el consumidor prefiere un alimento que al mismo tiempo le resulte delicioso, lo cual no ocurre con los quesos bajos en grasa (Drake *et al.*, 2008).

La grasa en los quesos forma cavidades en la matriz proteica, lo que la hace más porosa y abierta, mejorando la masticabilidad de los quesos (Johnson *et al.*, 2009), lo cual no ocurre en quesos desnatados (Rahimi *et al.*, 2007, Yates y Drake, 2007). Por otro lado, durante la maduración, la proteólisis en los quesos desnatados es inadecuada (Fenelon y Guinee, 2000), lo cual disminuye los cambios de textura (Sousa *et al.*, 2001); y la falta de grasa y ácidos grasos libres (Kondyli *et al.*, 2002), los cuales son considerados responsables de las características del aroma de los quesos de cabra, hace que estos quesos no sean aceptados.

Se han propuesto muchos métodos para mejorar las características de los quesos elaborados con leche desnatada, pero se ha explorado muy poco en la producción de quesos reducidos o bajos en grasa después del proceso de maduración (Nelson y Barbano, 2004; Yee et al, 2007). La tecnología de fluidos supercríticos usando el CO_2 como solvente se presenta como una interesante alternativa debido a sus moderadas propiedades de temperatura y presión, y por su selectividad al eliminar lípidos de la matriz del queso y al retener todos los

componentes del sabor (Yee *et al.*, 2008) que típicamente no se producen en los quesos desnatados tradicionales.

1.2. Metodología

El material y los métodos usados en este experimento, así como los resultados obtenidos han sido enviados en formato y están bajo revisión con el título "Effects of supercritical fluid extraction pressure on chemical composition, microbial quality, polar lipids profile and microstructure of goat cheese" (Effecto de la extracción con CO_2 supercrítico sobre la compositión química, calidad microbiológica, perfil de lípidos polares y microestructura del queso de cabra) a Journal of Dairy Science.

Se obtuvieron del mercado dos variedades de queso de cabra, queso Majorero y queso Gouda de cabra, madurados durante 5 meses, con similares características de contenido en humedad, proteína, dureza y firmeza sensorial. Ambos quesos fueron divididos en seis tratamientos diferentes: control (4ºC), control en la estufa (35ºC), P100, P200, P300 y P400, (quesos tratados a 100, 200, 300 y 400 bares de presión en la extracción con CO_2 supercrítico, respectivamente).

Los quesos fueron manejados de la forma más aséptica posible. Se rallaron a mano y aproximadamente 100gr de queso se distribuyeron en bolsas de filtro. Los quesos control se mantuvieron a 4ºC, los control en la estufa se mantuvieron a 35ºC durante 50 minutos, y el resto se trataron con CO2 supercrítico a 35ºC, con un flujo de 20g/min, durante 50 minutos, y variando la presión en función del tratamiento al que fueron asignados (100, 200, 300 ó 400 bares).

Se analizó la composición química de los quesos (grasa, proteína, humedad y materia seca), la calidad microbiológica (aerobios totales, lactococcos y lactobacilus), el perfil de lípidos polares, y la microestructura.

Los datos se analizaron mediante el paquete estadístico SAS (versión 9.0; SAS Institute Inc., Cary, NC) mediante una ANOVA de una vía para la composición química básica y los recuentos microbianos usando los 6 tratamientos del queso como variable y considerando un valor de $P<0,05$ como estadísticamente significativo. Para evaluar las diferencias entre grupos se usó el test de Tukey.

2. Trabajo experimental

Running head: SUPERCRITICAL FLUID EXTRACTION ON GOAT MILK CHEESE

TITLE

Effects of supercritical fluid extraction pressure on chemical composition, microbial quality, polar lipids profile and microstructure of goat cheese

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ABSTRACT

New trends to healthier food choices and consumer preference for low-fat products have increased the interest in low-fat cheese and nutraceutical dairy products. However, consumers desire to purchase delicious food, and low- and reduce-fat cheeses are not completely accepted due to their unnatural and unappealing properties compared to full-fat cheeses. Using CO_2 as supercritical fluid offers an alternative to reduce fat in cheese after ripening, maintaining the initial characteristics and flavor. The aim of this experiment was to evaluate the effect of pressure (100, 200, 300 and 400 bar) of the supercritical CO_2 on the amount of fat extracted, microbial quality, polar lipids profile, and microstructure of two varieties of goat cheese: Majorero, a PDO cheese from Spain, and Gouda-type cheese. The amount of fat was reduced 50-57% and 48-55%, for Majorero and goat Gouda-type cheeses, respectively. Higher content of sphingomyelin and phosphatidilcholine were found in Majorero cheese compared to the control and goat Gouda-type cheese; and the micrographs from confocal laser scanning microscopy showed a more open matrix and whey pockets which could explain the easiness to extract fat and reduce considerably the microbial content in the cheese after the treatment with SC-CO_2. The microbial population was reduced after the supercritical fluid extraction in both cheeses, and the lethality was higher as pressure increased in Majorero cheese, most noticeably on lactococcus and lactobacillus bacteria. Gouda-type cheese did not contain any lactobacillus. The results of this study demonstrated that the supercritical fluid extraction with CO_2 process has a great potential in the dairy industry and commercial applications. We can obtain not only cheese lower in fat, but due to lower microbial load, have a longer self-live in the market avoiding economic losses due to this perishable product. In addition the resulting cheeses are enriched in phospholipids due to a selective reduction in triglycerides. Majorero cheese

obtained after the SFE treatment is an excellent candidate to be considered as phospholipid enriched lower in fat goat cheese, with all the healthy benefits of goat milk, phospholipids and a lower content of triacylglicerides and cholesterol.

Keyword: goat cheese, supercritical fluid extraction, microstructure, phospholipids

INTRODUCTION

Lipids are vital components of our diet; however, consumption of saturated fat is highly correlated with an increased risk of obesity, atherosclerosis, coronary artery disease, and elevated blood pressure (Watts et al., 1996; Van Horn and Ernest, 2002). The consumption of highly processed foods with refined starches, sugars, fats, and oils is increasing considerably and often fail to contain the essential nutrients that are found in nutrient-dense foods (Kant, 2000). Largely influenced by increase of consumer concerns, there has been pressure on the food industry to reduce the amount of fat, sugar, cholesterol, salt and other components in the diet. New trends for healthier food choices and consumer preference for low-fat products have increased the interest in low-fat cheese (Johansen et al., 2011). Food industry is aware of the consumer's desire to purchase delicious, convenient and nutritious foods, and cheese has been proposed as a potential nutrient solid matrix which can be use as vector of bioactive components to improve nutrition and health of the consumers (Turgeon and Rioux, 2011).

Fat forms cavities in the cheese and hence give it an open structure. In full fat or reduced fat cheese the protein matrix is more open and the spaces are occupied by fat globules. Weak spots in the discontinuous protein matrix are created which improves the chewability of the cheese (Johnson et al., 2009). In low fat cheese the protein matrix is

more compact (Aryana and Haque, 2001; McMahon et al., 1996; Rahimi et al., 2007). Some authors (Gwartney et al., 2002; Brown et al., 2003; Yates and Drake, 2007) have reported that the majority of reduced-fat cheeses were characterized by chewiness, hardness, firmness, fracturability and springiness, and displayed lower cohesiveness. More recently, Sánchez-Macías et al. (2010) have reported significant differences in physicochemical composition, instrumental texture profile, as well instrumental color, between full-fat goat cheese and their counterpart low- and reduced-fat goat cheese.

Proteolysis contributes to cheese ripening through a direct contribution to flavor and texture (Sousa et al., 2001). Lipolysis in cheese produces FFA which have long been considered responsible for the characteristic aroma of goat cheeses and certain branched-chain FFA contribute by themselves to the "goaty" flavor of cheese (Salles et al., 2002). In low-fat goat cheeses, proteolysis of casein is inadequate (Sánchez-Macías et al., 2011a), resulting in a relatively firm texture and higher instrumental hardness and masticability (Sánchez-Macías et al., 2010). The lack of fat and the faster FFA liberation of the remaining fat in low- and reduced-fat cheese (Sánchez-Macías et al., 2011a) may promote early FFA metabolism and thus modify the flavor that is characteristic of full-fat goat cheese. Drake (2008), Childs and Drake (2009), and Sánchez-Macías et al. (2011b) have evaluated the consumer acceptance of low-fat cow and goat cheeses, and have identified various drawbacks to its use, including lack of flavor, rubbery texture, stickiness, low meltability, and consumer perception as unnatural and unappealing compared to full-fat cheeses.

Several methods have been employed to modify and improve low fat cheese texture. Alteration of cheese-milk processing conditions, modification of make procedure (Mistry, 2001; Guinee and McSweeney, 2006), inclusion of additives and fat replacer

(Larsen, 2009), and using of starter strains which produce exopolysaccharides have been proposed as mechanisms to improve the quality of reduced-fat cheeses (Awad et al., 2005; Dabour et al., 2006; Jimenez-Guzman et al., 2009). But in general, full-fat cheeses have always been more accepted than their counterparts lower in fat.

There has been little exploration of manufacturing a full-fat, flavorful, matured cheese, and then removing the fat from the product. Nelson and Barbano (2004) using centrifugal fat removal process produced 55% reduced-fat Cheddar cheese. Supercritical fluid extraction (**SFE**) technology offers a less destructive alternative to fat extraction. Fat removal in sample matrices is based on lipid solubility in the solvent, carbon dioxide in this case, without any harmful chemical residues (Nielsen, 2003). Carbon dioxide is the most commonly used supercritical fluid due to its mild critical properties, its critical temperature 31ºC, and because it is a gas under ambient conditions that allows an easy separation of the processed products. It is also cheap and achievable with high purity; it is non-toxic, non-corrosive, non-flammable and considered a "green solvent". However, supercritical carbon dioxide (**SC-CO_2**) is an ideal solvent for removing lipids such as triglycerides and cholesterol from cheese matrices due to its behavior as a nonpolar solvent (Arul et al., 1994; Yee, 2006). The application of SFE on cheese may be an advantageous alternative for reduced-fat cheese-making process. Yee et al. (2007) suggested that SFE technology can be used in the dairy industry to develop cheese products lower in fat, retaining flavor compounds that may not be typically fully developed with alternative methods of low-fat cheese processing.

Currently, consumer demand for a diversification of cheese products has focused on small ruminant cheeses. Ewe and goat milk products can provide a profitable alternative to cow milk products due to their specific composition, sensory qualities, and inherent health-

promoting attributes (Haenlein, 2004; Raynal-Ljutovac et al., 2008; Ribeiro and Ribeiro, 2010). Cheese is an important part of the economy and traditions of the Canary Islands (Spain): about 17,000 tons of goat milk cheese is produced per year (Fresno et al., 2008). The Canary Islands have an important variety of cheeses, of which 3 of them are designated as Protected Designation of Origin and 2 elaborated with pure goat milk: Palmero and Majorero cheese. Goat milk Gouda cheese is available in the market as an alternative to the cow milk counterpart, and was chosen for this experiment for its similitude of gross composition (protein and moisture content), sensorial hardness and firmness, and type of rennet used in its cheese-making, with the Majorero cheese.

The objective of this study is to evaluate the influence of 4 different pressures in a dynamic flow of 1000gr supercritical CO_2 at 35ºC and on the chemical composition, especially the amount of removed fat, microbial inactivation, lipid profile and microstructure of Goat Gouda-type and Majorero cheese aged over 5 months.

MATERIALS AND METHODS

Experimental design

Samples at 4ºC temperature and at 35ºC for 50 min in incubator were taken as control and incubator control (**CI**), respectively. Variable parameters of the process were treatment pressure (100, 200, 300 and 400bar) and cheese type (Gouda-type and Majorero goat cheeses). Each treatment was carried out in triplicate. The designation of cheese was as follow: control (control in fridge), CI (control held in an incubator set at 35ºC for 50 min), **P100** (treatment at 100 bar) **P200** (treatment at 200 bar), **P300** (treatment at 300 bar), and **P400** (treatment at 400 bar). A total of 36 samples were collected: 6 treatments x 2 cheese varieties x triplicated.

Sample preparation

Comercial goat milk Gouda-type from Holland and Majorero cheese from Canary Island (Spain) aged over 5 months was purchased for experiments. The goat Gouda-type cheese was chosen for being similar to Majorero cheese in chemical composition (moisture and protein content), and sensorial hardness and firmness parameters, as well the animal rennet used in its cheese-making. Approximately 100 g of hand grated cheese into a plastic bag were weighed in hygienic conditions on an analytical balance separately and evenly distributed into filter bags (Filter Fabrics Incorporated, Goshen, IN), sealing both ends of the bag. After processing, the goat cheese samples were removed from the filter bag and reweighed to determine the yield of finished processed cheese.

Supercritical fluid extraction

The SFE unit used was a laboratory-scale SFE system (model SFE 500; Thar Technologies, Inc., Pittsburgh, PA) with an extraction volume of up to 500 mL, maximum flow rate of 50 g/min, maximum operating pressure of 600 bar, maximum temperature of 150ºC, cyclone separator capacity of 500mL, including an automated back pressure regulator (model ABPR-200, Thar Technologies). The carbon dioxide tanks used for extraction were supplied by Air Gas (50-lb., San Luis Obispo, CA). The process unit was operated at the dynamic mode, which allowed the sample to be continuously supplied with fresh supercritical CO_2. The flow rate was kept constant at 20 g/mL. A total of 1000 g of CO_2 was allowed to run when the desirable pressure was reached, equivalent to 50 min of treatment.

Cheese Compositional analysis

Fat content of cheese samples was determinate by the Babcock method for cheese as per Standard Methods for the Examination of Dairy Products (Bradley et al., 1992) in duplicate, and the average was taken as the sample value. Protein content in cheese samples was determined by the Kjeldahl method in the AOAC International methods for cheese (920.123 Nitrogen in Cheese, 1995). The moisture and solids contents in samples were measured using the AOAC International methods for cheese (977.11 microwave oven, Nielsen, 2003) utilizing a LabWave 9000 Microwave Moisture/Solids Analyzer (CEM Corporation, Matthews, North Carolina), programmed for the appropriate method parameters. Approximately three g of sample was needed for each analysis conducted in duplicate.

Lipid characterization

Thin layer chromatography (TLC) was used to chemically analyze the lipid profile of the cheese and lipid extracted from SFE processing. Polar plates were run to characterize the polar lipid fractions, such as phospholipids remaining in the cheese. Fat obtained by the Mojonnier test was used to obtain the lipid samples as per Standard Methods for Examination of Dairy Products (Bradley et al., 1992). Lipid samples were diluted to a 10 mg/mL concentration with choloform:methanol (1:2, vol:vol) solvent mixture. Polar standards phosphatidylethanolamine (**PE**), phosphatidylcholine (**PC**) and sphingomyelin (**SM**) (Sigma Chemicals Co., St. Louis, MO) were prepared into a 2 mg/mL concentration to detect specific phospholipids in cheeses. Cheese lipid samples and extracted fat from control, P100, P300 and P400 treatments, and polar standards were applied in 25 µL amounts for all plates using a syringe. Precoated silica gel plates 10 × 20 cm in size (EMD

Chemicals Inc., Gibbstown, NJ) were used for separation. The plates were placed in a polar solvent tank consisting of chloroform:methanol:water (65:25:4, vol:vol) and then were developed with iodine (Sigma Chemicals Co., St. Louis, MO) to detect the polar lipids present in the samples.

Microbiological analysis

To assess the microbial quality of cheeses after the SFE treatment, 10 g of cheese samples were homogenized in a sterile plastic bag and mixed with 90 mL of NaCl solution (0.9%) using a stomacher lab-blender. The samples were serially diluted in saline solution and then plated in 3M Petrifilm Aerobic Count Plate (3M Microbiology, St. Paul, MN, USA) for aerobic bacteria, and onto M17 agar and Man-Rogosa-Sharpe agar, for lactococcus and lactobacillus, respectively, using standard pour-plate techniques. All plates were incubated at 30ºC and the colonies were enumerated after 48-72 h. The obtained results were expressed as log CFU/g of cheese.

Microstructure of cheese samples

Cheese samples were prepared and stained according to Lopez et al. (2007). Acridine Orange fluorescent dye (1:500, vol:vol) for protein network, and lipid-soluble Nile Red fluorescent dye (20:500, vol:vol) to label fat were dissolve in acetone and mixed. About 0.5mL of the mix staining solution was put on slices of cheese (approximately 5 mm x 5 mm x 3 mm) in a concave glass slide. The solvents were evaporated in the dark, then 20 microliters of agar 5% was added and a cover slip was put on the slide. The microstructural analysis was performed using an Olympus FV1000 inverted confocal laser scanning microscope (Olympus America Inc., Center Valley, PA) was used allowing confocal laser

scanning microcopy (**CLSM**) in dual-beam fluorescent mode, with excitation wavelengths of 568 and 488 nm for fat and protein, respectively. Each cheese sample was subjected to CLSM, and at least five images from each sample were taken at x 400 level of magnification. The micrographs presented in this work were considered by the authors to be representative of the different cheese samples analyzed.

Statistical analysis

Statistical analyses were performed using the SAS program package (Version 9.00, SAS Institute Inc., Cary, NC). The ANOVA procedure was used to compare control cheeses and SC-CO_2 treated cheese to evaluate the effect of the pressure level on yield, chemical composition, and microbiological counts. Significantly different means were identified using the Tukey's test.

RESULTS AND DISCUSSION

Yield and Chemical Composition of Cheese

In Table 1 is summarized the yield of cheese sample and the chemical composition of control cheeses and treated cheeses with SC-CO_2. Fifty min in the incubator resulted in a slightly lost weight, due to a moisture evaporation during the experimental time, but not statistical differences were found for both Majorero and goat Gouda-type cheeses. However, as expected, the weights of samples were reduced after the SFE treatment as pressure was increased.

Fat percentage was reduced in cheese samples after the treatment. In Majorero goat cheese, the reduction was over 40% for P100, P200 and P300, and 45% for P400; and for goat Gouda-type cheese the reduction was between 36 and 40% for all the treated samples,

without significant differences. Protein and moisture percentages increased in both type of cheeses after the SFE treatment due to effect of fat reduction in the matrix.

In Table 2 are shown the results of chemical composition in absolute values compared to the initial content, which are easy to calculate with the loss of weight in samples after the treatment. In this way, we can establish the real fat, protein and moisture losses. The fat content was reduced between 50-57% (15.79-17.95gr) and 48-55% (17.60-19.46gr), for Majorero and goat Gouda-type cheeses, respectively. In general, the reduction was higher as pressure was increased, but no significant differences were found between the experimental pressures used in Gouda cheese. Using similar parameters for SFE (350 bar of pressure, 35ºC temperature, and 1000 g of CO_2), Yee et al. (2008) reported in Cheddar (35% of fat) and Parmesan (28% fat) cheeses fat reductions of 24-25 g and 18-21 g of fat, respectively. The protein content did not suffer changes in cheeses after supercritical fluid extraction compared to control cheese, which means that not proteins are lost in the treatment, as Yee et al. (2008) reported for the other cheese varieties. Respect to moisture content, it was reduced after the CO_2-SFE over the 8-12% (3.86 g) and 10-12% (3.84 g) the moisture in Majorero and goat Gouda-type cheeses, respectively. Yee et al. (2008) found similar losses of moisture in the treated cheeses, which suggests that the moisture losses are independent of pressure treatment, and possibly dependent of the time exposure to the SC-CO_2. These authors suggested that the moisture content in cheese may influence in the supercritical fluid extraction of lipids, but they could not explain if the differences in fat removed from cheese samples were due to differences in moisture or differences in cheese types. Because the initial moisture content in both cheeses of the present work were similar, we suggest that exists a clear dependence of cheese variety on

the CO_2-SFE, although that initial fat content in cheese may have a role in the extraction of lipids with SC-CO_2: as higher fat content in cheese, higher amount of fat is extracted.

Selectivity of lipid extraction

The polar lipid profiles of cheeses are represented in Fig. 1. To detect lipid retention in the cheesematrix, polar lipids standards consisting of the three main bovine milk fat globule membrane phospholipids were used; phosphatidyl ethanolamine, phosphatidyl choline and sphingomyelin (Spitsberg, 2005). It is quite remarkable the higher content of the main phospholipids found in Majorero goat cheese compared to goat Gouda-type cheese.

The individual phospholipids, sphingomyelin and phosphatidilcholine were detected in Majorero cheese. In Gouda cheese, a little sphingomyelin was detected in the treated cheeses, but not phosphtidilcholine. No phosphatidiletanolamine was detected in both type of cheeses at any control or treatment. Yee et al. (2007) observed that Cheddar and Parmesan cow milk cheese treated with SC-CO_2 contained all three of phospholipids in the polar standard used in the present experiment. The different phospholipids proportions in cow and goat milk have been reviewed by MacGibbon and Taylor (2006), and they reported that phosphatidylcholine, phosphatidylethanolamine and sphingomyelin are found in similar proportions in various animal species, including cow and goat milk.

Analyzing the phospholipids content in different dairy products, Rombaut et al. (2007) affirmed that the ruptured membrane parts will preferentially migrate to the serum phases, resulting in an alteration of the phospholipid/lipid ratio in cheese. This can be noticed when examining the data of cheese, whey, cream, butter and buttermilk by these authors. For example, during cheese making, the fat is concentrated in the curd, the MFGM is damaged and these fragments are migrating to the cheese whey. As such, the

phopholipids content expressed on a fat basis of raw or full fat milk (0.6–0.7%) will be slightly higher than the value of full fat cheeses (0.4–0.6%), but much lower than the whey (5.3–6.2%) or derived whey products like Ricotta (2.7%). Milk homogenization is a nonthermal technology currently used by the dairy industry to prevent creaming in fluid milk by reducing the fat globule radius. One of the effects of homogenization is the disruption of fat globules and some MFGM components are displaced to the skim milk phase (Keenan et al., 1983). If we suppose that milk used to elaborate goat Gouda-type cheese was homogenized, then, it would explain the results of phospholipids profile found in this experiment, but we cannot confirm this affirmation because we do not know the possible milk pretreatment of this cheese. However, for Majorero goat milk, it is sure that the homogenization is not a common practice in the elaboration of Majorero cheese.

Yee et al. (2008) observed that nonpolar lipids were reduced and the polar phospholipids were concentrated in cow milk cheese after the supercritical fluid extraction with CO_2, and only nonpolar lipids and cholesterol were extracted during the process. Similar conclusion have been reported by Astaire et al. (2003) and Costa et al. (2010) in buttermilk powder samples treated with SC-CO_2, which selectively removed only nonpolar lipids material.

There is immense interest to develop low-cholesterol foods as reflected the large amount of low-cholesterol available in the market, and new trends are focusing in nutraceutical products (Bernal et al., 2011) in natural matrices, especially in dairy products (Turgeon and Rioux, 2011). Majorero cheese obtained after the SFE treatment in this experiment is an excellent candidate to be considered as a phospholipids enriched lower in fat goat cheese (because of PL/protein ratio or total solids is increased after the treatment,

due to the selectivity of lipid extraction of SC-CO_2), with all the healthy benefits of goat milk, phospholipids and a lower content of triacylglicerides and cholesterol.

Cheese Microstructure analysis with Confocal Laser Scanning Microscopy

Lopez et al. (2007) identified 4 forms of fat present in Emmental cheese: 1) intact milk fat globules, which are small, spherical globules dispersed throughout the cheese; 2) aggregates of milk fat globules that appear as clumps of circular globules; 3) coalesced milk fat globules are spherical but larger than the typical milk fat globules, and 4) nonglobular fat or free fat. These forms were identified in the Fig. 2 of the present work. The small intact milk fat globules are especially visible in Majorero cheeses treated at 100 bar of pressure, compared to the image of the control cheese, where the fat seems to have a coalesced or nonglobular appearance into the whey pockets. At higher pressure, the fat globules appear as aggregated (P200), then they became coalesced (P300), to finally coexist very small and spherical globules with coalesced ones (P400). This evolution of the fat globules can be produced by increasing the pressure applied, which favors its aggregation and coalescence of the fat, and the final state of fat in P400 Majorero cheeses can be produced by the disruption of fat in smaller globules due to the supercritical fluid extraction of fat, which is quite more reduced at in this cheeses. In Gouda-type control cheese, fat appears as nonglobular fat, as well in P100 cheeses. In P200 and P400 cheeses, the fat coexist in small globules with coalesced globules, while in the images of P300 cheeses the fat appears as coalesced. Rogers et al. (2010), working with low fat cheddar cheese, reported that CLSM images of cheese microstructure showed increasing fat globule size with increasing cheese fat content; low-fat cheeses (3-8% fat) had more spherical fat globules dispersed throughout the protein matrix and, in contrast, cheeses with 28-33% fat

content had clumped and coalesced fat globules into nonspherical shapes. Gunasekaran and Ding (1999) examined the fat globules in one month old Cheddar of varying fat contents (4-30%) and detected the smallest ones at lowest fat content, but at lowest fat content more globules were noted. The average globule size appeared to be inversely related to the total fat content of the cheese. These workers noted that the nature of protein matrix in low fat cheese may influence fat globules by preventing changes in their size and shape. Guinee et al. (2000) also examined the microstructure of Cheddar cheeses of fat contents, in the range 7-30% using CLSM. Reduction of fat content of cheese was accompanied by dispersion of discrete globules without clumping, while increasing fat content of cheese resulted in progressive clumping and coalescence of the globules. Lopez et al. (2007) observed that heating the curd grains induced the formation of fat globules aggregates, and pressing of the curd grains resulted in the greatest disruption of milk fat globules, their coalescence and the formation of nonglobular fat (free fat). Because heating the curd grains is part of the Gouda making-cheese, but not in Majorero, it could explain the large fat globules found in the images of control goat Gouda-type cheese, compared to control Majorero cheese.

Lopez et al. (2007) also suggested that the increase in the density of the protein matrix during the grain stirring and heating decreased the size of the pores left in the cheese matrix by whey; hence the fat may become too large and act as breakers in the protein matrix. In this case, in the Fig. 2, the images of control cheeses clearly differ, where Majorero cheese presented higher number and size of whey pockets. Because in full-fat cheese fat acts as breaker in the protein matrix and improves the chewability (Johnson et al., 2009), compared to low- and reduced-fat cheeses, which present a more compact matrix, fat is an important component in the cheese-making to obtain a high

quality cheese with a good texture. The more open matrix and whey pockets found in the control Majorero cheese could explain the more fat extracted, in relation to the initial content, than in Gouda-type cheese, as well the continuously fat decreasing as pressure of SC-CO_2 was increased.

Guinee et al. (2000) attributed the clumping and coalescence of globules to the destruction of the MFGM during processing and also to heating of curds during Cheddar cheese-making. The shape of fat in control Gouda-type cheese images obtained with CLSM in this study and the lower phospholipids content found in the TLC analysis are results that concord with the possible homogenization pretreatment of the milk and the normal heating curd grains.

Microbial quality

Results of microbiological counts obtained from Majorero and goat Gouda-type cheeses are summarized in Table 3. The initial microbial counts in goat Gouda-type cheese were 6.15 and 5.53-log for total aerobic bacteria and lactococcus, respectively. No lactobacillus was found in this cheese. In goat Majorero cheese the initial counts were 5.38, 9.28 and 8.70-log, for total aerobic bacteria, lactococcus and lactobacillus, respectively. An interesting observation was that in Majorero cheese, at all treatments, lactococcus and lactobacillus counts were always higher than aerobic bacteria. On the contrary, in goat Gouda-type cheese, aerobic bacteria counts were higher than lactococcus counts.

Spilimbergo et al. (2009) reported a progressive permeabilization of the cell during the treatment at 100 bar, 36ºC. The data evidenced a correlation between the cellular death and the CO_2 permeabilization inside the cell. They reported that the initial damage cellular envelope is not lethal for the cell. Just after 10 min, the treatment induces the irreversible

damage that causes its death, and about 4 min of treatment the fraction of survival cells is still nearly 100%. The amount of CO_2 accumulated in the lipid phase may then structurally and functionally disrupt the cell membrane due to an order loss of the lipid chain, which may increase the fluidity (Isenshchmid et al., 1995).

Lethality on microorganism in Gouda cheese decreased at all pressure treatment in 2.5-3 logs, and the highest reduction was at 100 bar of pressure. On the contrary, in Majorero cheese, lethality was increasing continuously as pressure was increased, and for lactobacillus and lactococcus the highest reduction was at 400 bar of pressure, while for total plate aerobic bacteria was at 300 bar. This results are quite correlated with the microstructure imaged discussed for the cheeses. The lack of whey pockets and closer matrix in Gouda-type cheese possibly blocked the SC-CO_2 getting through, which limited the potential microbial inactivation, unlike to Majorero cheese, where microbial population decreased with the increasing pressure.

We must not discard the option of the limit or not sterilized capacity of the supercritical CO_2. The main advantage of SC-CO_2 compared to thermal treatment is the extending shelf-life of the product while retaining all the original nutritional and organoleptic properties of the untreated one, which avoids the economic losses due to this perishable product.

Yee et al. (2007) found that removal of fat with SC-CO_2 resulted in partition of flavors between the cheese matrix and the lipids extracted, and a higher number of flavors detected in the treated cheese matrix. The analysis of the water soluble compounds in Cheddar and Parmesan cheeses treated with the SC-CO_2 did not differ from the control cheeses; however more compounds were detected after the treatment. Triangle test performed by these authors indicated that panelists could identify the difference between

the full-fat and SC-CO_2 treated Cheddar cheese, but could not differentiate among the Parmesan samples. Preference tests similarly indicated that panelists actually preferred the SFE treated Parmesan sample to the full-fat and commercial reduced-fat counterparts. Carunchia Whetstine et al. (2006) demonstrated that when fat was removed from aged full-fat Cheddar cheese, most of the flavor and flavor compounds remained in the cheese are were not removed with the fat. An informal sensory analysis was made to the goat cheeses treated in the present experiment. In the case of Majorero cheese, it was found that SFE treated cheeses were relatively tastier, with a stronger flavor and odor intensity, and drier texture, which reminded to a more time ripened cheeses. But more sensory and instrumental analyses are needed to explore the texture, flavor, odor and color of the resulting cheeses subjected to the supercritical fluid extraction.

CONCLUSION

These results demonstrate that the supercritical fluid extraction with CO_2 process has a great potential in the dairy industry and commercial applications. We can obtain not only cheese lower in fat, but the resultant cheeses are enriched in phospholipids. At the same time, the microbial population is reduced considerably after the cheese finished, which permits a longer self-life in the market, avoiding economic losses due to this perishable product. Majorero cheese obtained after the SFE treatment in this experiment is an excellent candidate to be considered as phospholipids enriched lower in fat goat cheese, with all the healthy benefits of goat milk, phospholipids and a lower content of triacylglicerides and cholesterol.

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Table 1. Yield and chemical composition in percentage of control and CO_2 supercritical fluid extraction treated Majorero and goat Gouda-type cheese at 4 different pressures.

	Treatment[1]						
	Control	CI	P100	P200	P300	P400	SEM[2]
Item	Majorero Cheese						
Weight before, gr	100.03	100.06	100.10	100.09	100.02	100.03	0.07
Weight after, gr	99.76^{a}	98.64^{a}	80.30^{c}	77.72^{d}	78.41^{cd}	77.79^{d}	0.72
Fat, %	31.17^{a}	31.33^{a}	19.17^{c}	19.50^{c}	18.75^{cd}	17.00^{d}	0.69
Protein, %	25.77^{c}	26.83^{c}	30.67^{b}	31.39^{ab}	31.47^{ab}	32.93^{a}	0.51
Moisture, %	36.03^{b}	34.72^{b}	41.19^{a}	40.55^{a}	41.36^{a}	40.84^{a}	0.97
Solids, %	64.30^{a}	65.28^{a}	58.81^{b}	59.45^{b}	58.64^{b}	59.16^{b}	0.88
Fat in DM, %	48.48^{a}	48.00^{a}	32.60^{b}	32.80^{b}	31.98^{b}	28.76^{b}	1.44
	Goat Gouda Cheese						
Weight before, gr	100.05	100.03	100.03	100.01	100.02	100.03	0.09
Weight after, gr	99.82^{a}	97.72^{a}	79.46^{c}	79.11^{c}	78.33^{cd}	76.72^{d}	0.82
Fat, %	36.33^{a}	37.33^{a}	23.33^{c}	23.67^{c}	22.00^{c}	22.00^{c}	0.69
Protein, %	24.72^{b}	25.00^{b}	29.20^{a}	30.05^{a}	29.64^{a}	30.97^{a}	0.53
Moisture, %	35.64^{c}	34.67^{c}	40.13^{ab}	40.65^{a}	40.85^{a}	40.65^{a}	0.70
Solids, %	64.36^{a}	65.33^{a}	59.87^{bc}	59.35^{c}	59.16^{c}	59.35^{c}	0.70
Fat in DM, %	56.46^{a}	57.15^{a}	38.97^{b}	39.88^{b}	37.20^{b}	37.07^{b}	1.23

[a-d]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: Control = cheese at 4ºC; CI = cheese in incubator at 35ºC for 50 min; P100, P200, P300 and P400 = cheese treated with 1000g of SC-CO2 at 35ºC for 50 min at 100, 200, 300 and 400 bar of pressure, respectively. [2]SEM = Standard error of the mean.

Table 2. Chemical composition in 100gr control base of control and CO_2 supercritical fluid extraction treated Majorero and goat Gouda-type cheese at 4 different pressures.

	Treatment[1]						
	Control	CI	P100	P200	P300	P400	SEM[2]
Item	Majorero Cheese						
Fat, gr	31.17[a]	30.89[a]	15.38[c]	15.14[cd]	14.70[cd]	13.22[d]	0.66
Protein, gr	25.77[ab]	26.45[a]	24.60[b]	24.33[b]	24.67[b]	25.61[b]	0.34
Moisture, gr	36.04[a]	34.23[ab]	33.04[b]	31.49[b]	32.43[b]	31.76[b]	0.95
Solids, gr	64.30[a]	64.36[a]	47.17[b]	46.16[b]	45.97[b]	46.00[b]	0.79
	Goat Gouda Cheese						
Fat, gr	36.33[a]	36.47[a]	18.53[c]	18.73[c]	17.23[c]	16.87[c]	0.67
Protein, gr	24.73	24.42	23.19	23.78	23.21	23.75	0.07
Moisture, gr	35.64[a]	34.87[a]	31.87[b]	32.16[b]	31.99[b]	31.18[b]	0.67
Solids, gr	64.36[a]	63.82[a]	47.56[b]	46.95[b]	46.32[b]	45.52[b]	0.87

[a-d]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: Control = cheese at 4ºC; CI = cheese in incubator at 35ºC for 50 min; P100, P200, P300 and P400 = cheese treated with 1000g of SC-CO2 at 35ºC for 50 min at 100, 200, 300 and 400 bar of pressure, respectively.

[2]SEM = Standard error of the mean.

Table 3. Effect of the different pressures of SC-CO2 extraction on the total aerobic, lactococci and lactobacilli counts.

	Treatment[1]						
	Control	CI	P100	P200	P300	P400	SEM[2]
Item	Majorero Cheese						
Aerobic bacteria	5.38^{a}	5.06^{ab}	3.93^{b}	3.24^{b}	2.66^{b}	3.28^{b}	0.48
Lactoccoci	9.28^{a}	8.78^{a}	7.43^{b}	7.22^{b}	6.97^{b}	4.27^{c}	0.55
Lactobacilli	8.70^{a}	8.53^{a}	6.19^{c}	5.65^{cd}	5.19^{d}	4.39^{e}	0.22
	Goat Gouda Cheese						
Aerobic bacteria	6.15^{a}	6.09^{a}	2.96^{d}	3.47^{cd}	3.60^{c}	3.55^{c}	0.19
Lactoccoci	5.53^{a}	5.46^{a}	2.73^{c}	3.17^{bc}	3.27^{b}	3.52^{b}	0.18
Lactobacilli	--	--	--	--	--	--	--

[a-e]Means within a row with different superscripts differ (P < 0.05).

[1]Treatments: Control = cheese at 4ºC; CI = cheese in incubator at 35ºC for 50 min; P100, P200, P300 and P400 = cheese treated with 1000g of SC-CO2 at 35ºC for 50 min at 100, 200, 300 and 400 bar of pressure, respectively.

[2]SEM = Standard error of the mean.

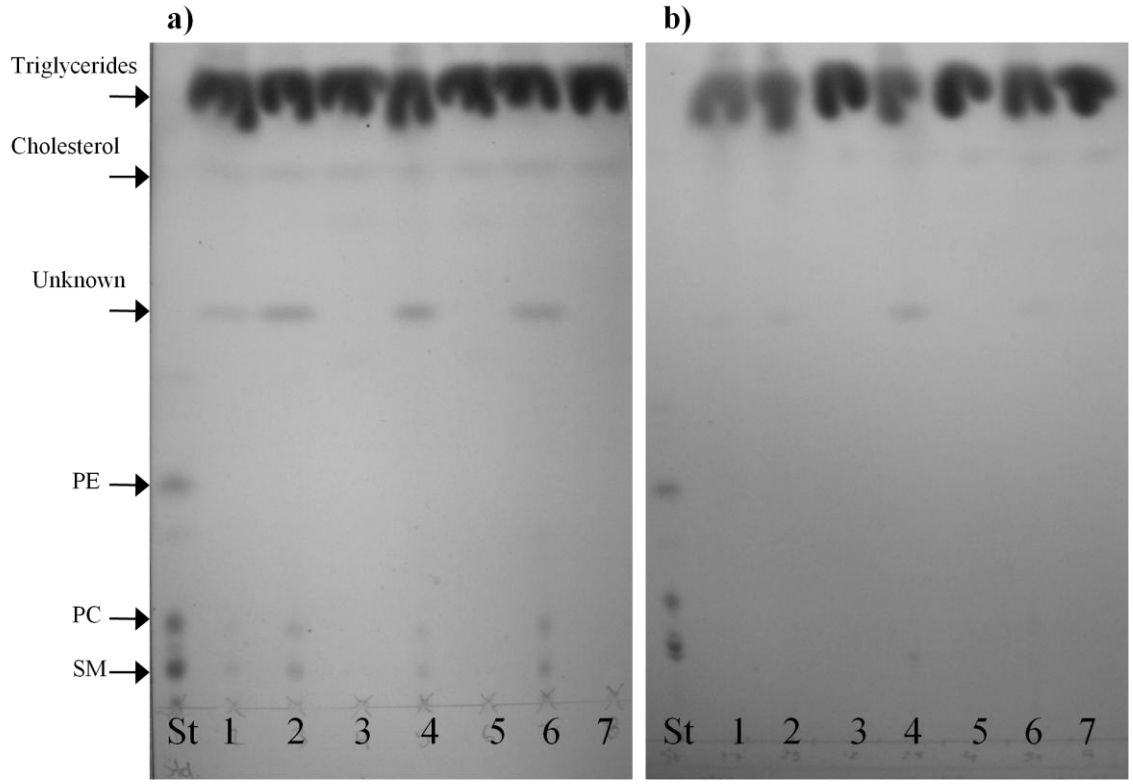

Fig 1. Thin.layer chromatography plate showing the polar lipid profile of the control (1) and SC-CO_2 P100 (2), P300 (4) and P400 (6) treated Majorero (a) and Gouda-type (b) cheeses, and their respective fat extracted at P100 (3), P300 (5) and P400 (7). St, standard; PE, phosphatidyk ethanolamine; PC, phosphatidilcholine; SM, sphingomyelin.

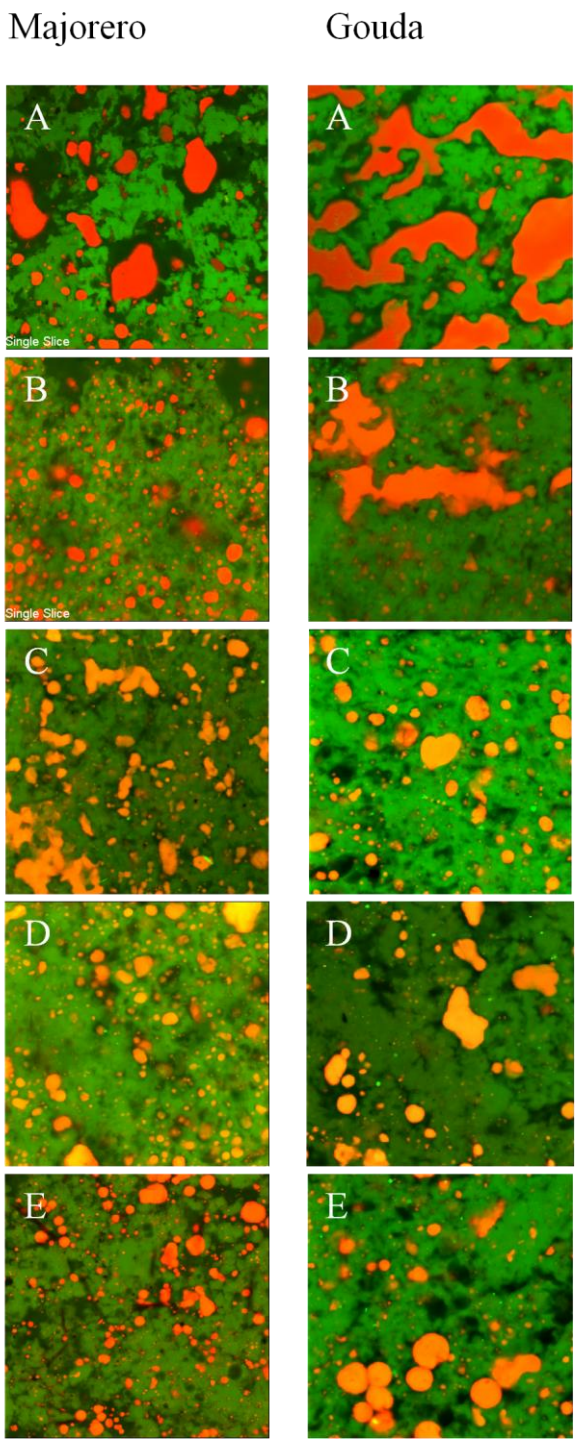

Fig 2. Confocal Laser Scanning micrographs of Majorero and goat Gouda-type control cheese (A), and cheese treated with SC-CO_2 at 100 (B), 200 (C), 300 (D), and 400 (E) bar of pressure. Fat is coded in orange and protein in green. The serum phase appears in black.

Capítulo X: Conclusiones

1. Conclusiones
2. Conclusions

Capítulo X: Conclusiones

1. Conclusiones

1.1. Capítulo IV

- Los quesos elaborados con leche cruda de cabra desnatada resultan en un incremento del porcentaje de proteína y humedad, así como del pH tras una la maduración durante 28 días, mientras que disminuye el porcentaje de grasa sobre materia seca y humedad sobre sólidos no grasos.
- Estos quesos además muestran una mayor fracturabilidad, cohesividad, dureza y masticabilidad, mientras que la adhesividad y elasticidad disminuyen.
- La luminosidad, y los índices de color rojo y amarillo disminuyen a medida que lo hace el contenido graso de los quesos.

1.2. Capítulo V

- La disminución de grasa en la leche de cabra y posterior elaboración de quesos con leche cruda revela una disminución del grado de proteólisis primaria durante los 28 días de maduración.
- El grado de degradación de α_{S2}-caseina y α_{S1}-caseina disminuyen a medida que lo hace el contenido graso en los quesos de cabra, mientras que el nivel de β-caseina no se ve afectado por este factor.
- La concentración de ácidos grasos libres es mayor durante toda la maduración en quesos de cabra con mayor contenido graso cuando se expresa en base a queso total; sin embargo, cuando el total de ácidos grasos libre se expresa sobre materia grasa, el grado de lipólisis durante la maduración en mayor a medida que el contenido graso disminuye en el queso.

1.3. Capítulo VI

- Los quesos de cabra elaborados con leche desnatada madurados durante 28 días, sensorialmente, presentan una menor intensidad de olor y aroma, así como que tienen mayor firmeza, friabilidad, granulosidad, sequedad y acidez, mientras que fueron valorados como menos adhesivos y dulces.
- Los jueces y consumidores prefieren los quesos de cabra elaborados con leche grasa, debido a su menor dureza y mayor intensidad de aroma y olor.

1.4. Capítulo VII

- La adición de células somáticas a la leche de cabra, y posterior elaboración de quesos, incrementa intensamente el grado de lipólisis, independientemente de si la leche es cruda o pasteurizada.
- El efecto de las células somáticas sobre la proteólisis del queso es específica según el tipo de caseína y depende en gran medida del pretratamiento térmico de la leche de cabra.
- Los quesos elaborados con leche de cabra pasteurizados resultan más luminosos y con mayor índice de color rojo y amarillo que aquellos elaborados con leche cruda.

1.5. Capítulo VIII

- Las células somáticas incrementan la proteólisis de las α-caseínas y para-κ-caseína en los quesos desnatados elaborados con leche de cabra cruda; mientras que la proteólisis se ralentiza cuando se usa leche de cabra pasteurizada.
- La combinación de leche desnatada pasteurizada y células somáticas incrementan la lipólisis de la grasa del queso rápidamente.
- La adición de células somáticas resulta en quesos más claros y reduce el índice de color rojo.
- Las células somáticas potencialmente podrían ser una herramienta efectiva para mejorar la textura de los quesos desnatados, así como podrían usarse para acelerar la maduración de los quesos en la industria láctea.

1.6. Capítulo IX

- La extracción con CO_2 supercrítico es una alternativa viable para reducir más del un 50% el contenido de grasa del queso de cabra curado, siendo específica para lípidos no polares.
- El queso Majorero tratado con CO_2 supercrítico muestra mayores niveles de fosfolípidos expresado en materia grasa que el queso control y que el queso de cabra tipo Gouda, obteniendo un queso enriquecido en estos lípidos.
- La matriz del queso Majorero sin tratar, observada en imágenes obtenidas con microscopía confocal de barrido con laser, es mucho más abierta que la del queso Gouda, lo que favorece la penetración del CO_2 supercrítico.
- El tratamiento con CO_2 supercrítico reduce la población microbiana; y esta reducción es mayor a medida que se incrementa la presión en el tratamiento de quesos Majoreros.

Chapter X: Conclusions

2. Conclusions

2.1. Chapter IV

- Low-fat raw goat cheese results in an increased protein and moisture content, as well the pH, after 28 days of ripening; while, fat on dry matter and moisture on non fat solids decrease.
- These cheeses show a higher fracturability, cohesiveness, hardness and chewiness, and lower adhesiveness and springiness.
- Lightness, and the red and yellow indexes decreases as fat content decreases in cheese.

2.2. Chapter V

- Using low-fat raw goat milk results in a lower primary proteolysis in cheese during 28 days of ripening.
- Degradation rate of both α_{S2}-casein and α_{S1}-casein decreases as fat content decreases in goat cheese, while β-casein content does not vary because of this parameter.
- Free fatty acid content is higher during the ripening of goat cheese in full-fat cheese when it is expresed on cheese basis; however, when this parameter is expresed on fat basis, the lipolysis rate is higher in low-fat cheeses during early states of maturation.

2.3. Chapter VI

- Low-fat raw goat cheeses during 28 days of ripening have a lower odor and flavor intensities. They have a higher firmness, friability, granulosity, dryness and acidity, while they are less adhesive and sweet.
- Judges and consumers prefer full-fat goat cheese more than reduced- and low-fat cheese, mainly because of the lower hardness and higher intensity of flavor and odor.

2.4. Chapter VII

- Somatic cells addition to goat milk increases the lipolysis rate in cheese, regardless of raw or pasteurized milk treatment.
- Somatic cells effect on cheese proteolysis is specific of casein type and it is highly dependent of the heat treatment of goat milk.
- Goat cheese elaborated with pasteurized milk results in a higher luminosity and red and yellow indexes than those made with raw goat milk.

2.5. Chapter VIII

- Somatic cells addition to low-fat goat milk increases the proteolysis rate of both α_{S1}-caseínas and α_{S2}-caseínas, and para-κ-caseín when raw milk is used. However, proteolysis rate decrease when pasteurized goat milk is used.
- Pasteurized low-fat goat milk combined with somatic cells addition increases the lipolysis rate in cheese during the first days of ripening.
- Somatic cells addition results in a higher luminosity, and reduces the negative red index in low-fat cheese.
- Somatic cells could be potentially used as a tool to improve the texture of low-fat cheese, as well to accelerate the ripening in cheese industry.

2.6. Chapter IX

- Supercritical CO_2 extraction is an alternative to reduce the fat content more than 50% in ripened goat cheese, being specific for non polar lipids.
- Majorero goat cheese treated with supercritical CO_2 shows higher levels of phospholipids expressed in fat basis, than control cheese, obtaining reduced-fat cheese enriched in these polar lipids.
- The microstructure of control Majorero goat cheese matrix is more open than Gouda goat cheese, which permits the supercritical CO_2 going into the cheese. This favors the microbial reduction as pressure parameter increase in the treatment.