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Characterization of naturally goat cheese enriched in conjugated linoleic acid and omega-3 fatty acids for human clinical trial in overweight and obese subjects

C. Santurino[a], M.V. Calvo[a], C. Gómez-Candela[b], J. Fontecha[a,*]

[a] Bioactivity and Food Analysis Department, Food Lipid Biomarkers and Health Group, Institute of Food Science Research (CIAL, CSIC-UAM), Madrid, Spain
[b] Dietetic and Clinical Nutrition Unit, Nutrition and Functional Food Research Group, Hospital La Paz Health Research Institute, IdiPAZ, Madrid, Spain

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ABSTRACT

The growing interest in the development of strategies for improving the fatty acid composition of dairy products with potential health benefits is a challenge for the industry. A goat cheese naturally enriched in conjugated linoleic acid (CLA) and omega-3 was elaborated using milk from goats fed a supplement based on extruded linseed. Diet induced a linear increase in unsaturated fatty acids percentages at the expense of saturated fatty acids. Thus, the content of the omega-3 was 5-fold higher in enriched cheese (EC) than in control cheese (CC). A sizeable enhancement was also found in the concentration of CLA as well as of its precursor, *trans*-vaccenic acid. As the level of omega-6 remained almost unaffected, the n-6/n-3 ratio was reduced 7 fold. Besides, a reduction in the cholesterol content and a higher level of sphingomyelin also occurred in the EC. In conclusion, goat diet supplementation seems to improve the nutritional value of the traditional cheese without significant variations in its texture and sensory quality during storage. Thus, this study demonstrates that naturally enriched goat cheese in CLA and omega-3 can meet quality standards with a consistent bioactive lipid pattern for a large human clinical trial focusing on overweight and obese subjects.

1. Introduction

Lately the concept of food has undergone a radical transformation and its benefits have extended beyond its nutritional value or their sensory properties. Thus, a key role in health maintenance and the prevention of certain diseases has also ascribed to the food, including milk [10,64]. The scientific evidence, which confirms the close link that exists between nutrition and health, has contributed decisively to the rapid development of the functional food market [1,5,6]. In line with the current healthy eating trends, goat milk possess positive inherent properties, such as low allergenic potential, high digestibility and nutritional value which provides an attractive alternative to develop dairy products with high added value, as cheese [49]. The lipid composition of goat milk determines its nutritional quality because lipid components, particularly fatty acids (FA), are involved in the production and quality of dairy products and directly affect the taste aspects of milk derivatives. In Europe, the development of food products with potential benefits to long-term human health and that may contribute to attenuate issues related to public health in a positive way is a challenge for the dairy industry. These considerations explain the growing interest in the development of sustainable strategies for improving the FA composition of ruminant milks. In this context there have been numerous studies concluding that modulation of the FA composition of goat's milk can be achieved through the feeding, particularly with oilseeds rich in polyunsaturated fatty acids (PUFA). Adding linseed to goat diets has proved to be a useful tool to decrease the presence of saturated fatty acids (SFA) and significantly multiply concentrations of bioactive compounds such as C18:3 n-3 (alpha-linolenic acid, ALA) or *cis*-9, *trans*-11-C18:2 (rumenic acid, RA) in goat's milk fat [19,47,48,52,59]. More specifically, feeding the Lodyn-Milk supplement, based on extruded linseed [7] has shown to be an effective way to obtain a milk naturally enriched in ALA and RA, both in goats and ewes [33,38,45]. On the other hand, due to the negative effects of salt on health, such as decrease in calcium absorption and increase in blood pressure, associated with an elevated risk of cardiovascular disease (CVD), the European

* Corresponding author at: Bioactivity and Food Analysis Department, Food lipid biomarkers and health group. Institute of Food Science Research (CIAL, CSIC-UAM), C/ Nicolás Cabrera, 9, 28049, Madrid, Spain.
E-mail address: j.fontecha@csic.es (J. Fontecha).

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Commission has considered the reduction of salt intake derived from food as a priority, focusing its efforts on in certain foods, such as cheeses [22].

Within this framework, the aim of this work was to develop a traditional goat cheese made from milk naturally enriched in omega-3 and CLA at industrial scale. Besides to characterize its fatty acid composition, a special focus was devoted to the potential changes both in neutral and polar lipid (PL) fractions in the cheese. The sensory quality of the end-product, as well as, the effect of temperature storage on its lipid composition, was also assessed.

Food delivery systems for a human clinical trial require consistency of retention the bioactive ingredients as well as the quality of the final product during storage. As such, the objective of the current study was to assess the chemical quality of CLA and omega-3 fatty acids enriched goat cheese for clinical trials and demonstrate their acceptability among overweight and obese subjects enrolled in cardiovascular risk prevention human clinical trials.

2. Materials and methods

2.1. Animals and dietary treatments

The study was performed under real field conditions and production in a farm located in the Region of Murcia (Spain). Briefly, the trial was conducted as follows. The experimental period lasted 12 weeks from November to January. Six hundred multiparous lactating dairy Murciano-Granadina goats were divided randomly on the basis of their age and milk yield into two homogeneous groups: control (C) and experimental (E). C group received a reference diet without the supplement meanwhile E group was initially fed a reference diet and subsequently the animals were gradually switched to a similar ration but including the supplement (Lodyn-Milk®, Ciudad Real, Spain) which is a commercial product under patent protection [7] based on extruded linseed. Once this intake was stabilized, animals were kept under experimental conditions for the following 4 weeks. Bulk milk samples of both groups were collected weekly until the level of the FAs of interest were stabilized. Previous studies have demonstrated that the use of this supplement allows achieving an improvement in the nutritional quality of dairy lipids from goats [45] and ewes [32] without detrimental effects on animal performance.

2.2. Cheese making

For each group (C and E), two batches of soft pressed goat's cheese were made in a local cheese factory (Murcia, Spain), according to the process of the Protected Denomination Origin (PDO) 'Queso de Murcia' regulatory board [9]. Cheesemaking procedures were carried out at industrial scale, employing 1200 L of pasteurized milk in order to obtain about 180 kg of cheese (500 g/piece).

According to EC Regulation 1924/2006 [56], to obtain a reduced-sodium product, the level of sodium chloride (NaCl) added to the enriched cheese (EC) was 25% lower than in the control cheese (CC). After a 21 d-ripening period at 10 °C, all cheeses were vacuum-packaged with an estimated shelf-life period of six months.

2.2.1. Refrigerated and frozen storage

The above-mentioned cheeses were regarded as the starting product to analyze the combined effect of vacuum packaging and storage temperature. Each batch of cheese was divided and assigned to one of the two-storage regimen: refrigerated storage, which remained in a walk-in-cooler at 4 °C; and frozen storage, which remaining in a −20 °C freezer. The storage period was 12 months.

2.3. Milk and cheese composition

Fat, total protein, lactose and total solids (TS) in milk were measured with an infrared spectrophotometer (Milko Scan FT-6000; Foss Electric, Barcelona, Spain). Chemical composition analysis of cheeses was determined in the Food Assays Laboratory (ALIA SAT No. 2439, Murcia, Spain).

2.4. Fat extraction

Milk and cheese fats were extracted as described by Castro-Gómez et al. [14]. All lipid extracts were collected in amber vials, flushed with nitrogen and stored at −35 °C until their chromatographic analysis.

2.4.1. Fatty acid methyl esters analysis

Fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis of the extracted FA fraction using 2N KOH in methanol as described by the ISO method [39,40]. FAME determination was done using a CPSil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2-μm film thickness; Agilent Technologies Inc., Palo Alto, CA) in an Agilent chromatograph (model 6890N; Agilent Technologies Inc.) equipped with a mass spectrometry detector. The column was temperature programmed as in Castro-Gómez et al. [14]. Briefly, the column was held at 100 °C for 1 min after injection and temperature-programmed at 7 °C/min to 170 °C, held there for 55 min, and then at 10 °C/min to 230 °C and held for 33 min. The injector temperature was set at 250 °C. Helium was the carrier gas with a column inlet pressure of 206.9 kPa. The mass spectrometry detector conditions were as follows: transfer line temperature: 250 °C, source temperature: 230 °C, quad temperature: 150 °C, electron impact ionization: 70 eV, and the range from 50 to 500 *m/z* was scanned. For identification of the peaks, the National Institute of Standards and Technology (NIST, Gaithersburg, MD) library and mass spectra of the standards used in our laboratory were used. The injection volume was 1 μL and the split ratio used was 1:25. Response factors were calculated using an anhydrous milk fat (reference butterfat BCR-164) and tritridecanoin as internal standard (200 μL; 1.3 mg/mL) was used. FAMEs were expressed as percentage of total methyl ester content. Each sample was analyzed in triplicate.

2.4.2. Triacylglycerides and cholesterol determination

Triacylglycerides (TAG) and cholesterol (CHOL) analysis in milk and cheese fat was performed following Fontecha et al. [29], on a Clarus 400 GC (PerkinElmer Ltd., Beaconsfield, UK) equipped with an automatic split/splitless injector and a flame ionization detector. An Rtx-65TAG fused-silica capillary column (30 m × 0.25

Table 1
Effect of diet supplementation on chemical composition of the control and enriched goat milk. Data correspond to the analysis of bulk milk samples within each treatment collected weekly over 12-w period.

Composition (g/100 g)	Goat milk	
	Control	Enriched
Fat	5.95 ± 0.20[b]	6.28 ± 0.22[a]
Protein	3.72 ± 0.15	3.76 ± 0.13
DM[c]	15.32 ± 0.23	15.65 ± 0.43
Lactose	4.82 ± 0.07	4.65 ± 0.14
β−Casein	3.04 ± 0.17	3.09 ± 0.32

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[c] DM = dry matter.