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GOAT MILK: A CHALLENGE FOR RESEARCH AND DEVELOPMENT SECTOR FROM ROMANIA

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In the past 20 years the study of goat caseins genetic polymorphisms received a considerable research interest. In particular the researches were focused on CSN1S1 locus which is the most polymorphic, 17 alleles being known so far. These alleles are associated with four different levels of protein synthesis, affecting significantly goat milk quality and its technological properties. In this context the present review is describing recent literature data at international and national level, which allow the understanding of goat milk quality variation in relation with its physical-chemical characteristics, milk protein genetic polymorphisms and physiological factors. We also describe the current stage of researches concerning the use of milk protein polymorphisms in identifying possible adulterations from dairy industry and also studies on milk allergenic potency.

Key words: milk, goat caseins, polymorphisms, cheese, authenticity, allergies

Introduction

1. Nutritional and physico-chemical properties of goat milk

Among all foods, milk is the most complete and balanced in nutritional contents. Goat milk has a significant important role in human nutrition because of its nutritional and dietetic characteristics. In many countries goats milk production represents 55% of total milk production, like in Bangladesh, and 26% in Northern Mediterranean countries France, Italy etc. (Haenlein, 2004).

Physico-chemical characteristics of goat milk are currently well known. Many studies were carried out in different countries some of these describe milk biochemical composition and nutritional characteristics in detail (Jaubert, 1997, Park et al., 2007). These studies concluded that goat milk is a good source of healthy fats (short and medium chain fatty acids), important minerals (calcium, phosphorus, magnesium, potassium), as well as numerous vitamins such as riboflavin (vitamin B₂). Because of their unique role of providing energy instead of lipids, medium chain fatty acids (especially caprylic - C₈ and capric - C₁₀), are used in the treatment of patients with malabsorption symptoms, metabolic

disorders, cholesterol problems, anemia, bone demineralization and infant malnutrition (Haenlein, 2004).

Among the characteristics of goat milk, flavor is one quality component of a particular importance to cheese production. The formation of goat specific flavor of goat dairy products is closely linked to lactation period, nutritional factors, genotype in α S1-casein locus, nature of various milk constituents, biochemical and enzymatic factors (lipase activity) (Jaubert, 1997).

Materials and Methods

2. Milk protein polymorphisms in goat

In goat milk there are six major proteins, codified by 6 genes (α S1-casein, β -casein, α S2-casein, k-casein located on chromosome 6, α -lactalbumin located on chromosome 5 and β -lactoglobulin located on chromosome 11), which are specific expressed in mammary gland epithelial cells during lactation, being divided in two groups depending on their behavior at pH 4.6:

*a) The insoluble fraction, named **whole casein***, is composed of four caseins: α S1-casein (CSN1S1) - 199 aa, β -casein (CSN2) - 207 aa, α S2-casein (CSN1S2) - 208 aa (which are hydrophobic) and k-casein (CSN3) - 171 aa (which has a hydrophobic part: para-k-casein and a hydrophilic part: glycomacropeptide). Because of its amphoteric properties, k-casein plays a colloid-protector role of the other three caseins and constitutes with them micelles, which are dispersed in milk. It has a significant influence on milk technological properties and cheese making efficiency (Martin et al., 2002). Casein fraction represents in goats milk 77% from whole protein content.

*b) The soluble fraction, named **whey proteins***, is composed of several different proteins, the most important being α -lactalbumin (LALBA) - 123 aa and β -lactoglobulin (LGB)-162aa, representing in goat milk 23% from whole protein content.

The mutations which appeared over the years in these 6 genes structure induced the appearance of new alleles, with a positive or negative effect on different new genetic variants expression levels (Vlaic et al., 2008).

CSN1S1 locus was the most intensively studied because it's high degree of polymorphism (Boulanger et al., 1984; Grosclaude et al., 1987). Until now 17 alleles, associated with four different expression levels, were identified in this locus: **strong alleles** (A, B1, B2, B3, B4, C, H, L and M) producing 3,6 g CSN1S1/kg milk/allele; **intermediate alleles** (E and I) with 1,6g/kg; **weak alleles** (D, F and G) with 0,6g/kg and **null alleles** (O₁, O₂ and N) with no CSN1S1 in milk of homozygous animals (Ramunno et al., 2005).

In the other three casein loci were also identified many alleles with different expression levels: CSN1S2 locus - 8 alleles (A, B, C, D, E, F, G and O) with 0-2g/kg milk/allele; CSN2 locus: 7 alleles (A, A₁, B, C, D, O₁ and O₂) with 0-5g/kg milk/allele, CSN3 locus: 13 alleles with 4-6 g/kg milk/allele. Because of the complex genetic polymorphisms in caseins loci (not found in other species) and

their additive effects, there are high variability limits concerning milk content in coagulable casein, according to the alleles inherited from parents (Balteanu V.A., et al. 2007).

Results and Discussions

3. The impact of goat α 1-casein polymorphism on milk processing

The effects of CSN1S1 polymorphisms on goat milk yield, composition, renneting properties, cheese yield and their flavor have been intensively studied in French breeds (Remeuf et al., 1993; Vassal et al., 1994; Manfredi et al., 2000). The obtained results were: **A.** No differences were observed between CSN1S1 genotypes and milk yield; **B.** A significant effect of CSN1S1 A allele was found on fat content; **C.** CSN1S1 strong alleles (A, B) have a significant effect (positive), in comparison with intermediate and weak alleles (E, F), on: **1.** Milk quality: whole protein, casein and fat contents; **2.** Milk manufacturing properties: rate of coagulum formation, coagulation time and curd firmness; **3.** Cheese making efficiency: higher cheese quantities obtained from AA genotypes in comparison with FF with up to + 14,8%; **4.** Cheese obtained from AA genotypes had a weaker specific goat flavor in comparison with FF genotypes which had a more pronounced goat flavor. The influence of CSN1S1 genotypes on goat cheeses flavor is explained by the fact that milk from goats with FF genotype in CSN1S1 locus has a higher lipase activity and as consequence a higher spontaneous lipolysis, in comparison with milk from AA genotypes (Delacroix et al., 2000). Furthermore, it has been shown that caprine CSN1S1 is required for efficient transport of the other caseins from endoplasmic reticulum to Golgi apparatus. In FF genotypes this transport is not made in an efficient way, so there is an accumulation of other caseins in mammary gland which raise health problems (Chanat et al., 1999).

Similar results were obtained in Spanish, Italian, Norwegian and USA goat breeds (Sanchez et al., 2005). In France the selection for high expression alleles in CSN1S1 was introduced since 1996 as selection criteria in goat breeding programs.

4. Milk protein polymorphism in Romanian goat breeds

In Romania there are two local goat breeds, Carpathian Goat and White Banat Goat. About 80% of the population is represented by Carpathian breed, a rustic breed but with low production and high heterogeneity of milk quality (fat 3.2-6.9% and protein 2.5-5%), representing a high breeding potential. The rest of 20% is represented by White Banat breed, an improved breed with higher milk production.

At the national level studies concerning goat milk polymorphism are few. Important results concerning the biodiversity study of Romanian and European goat populations (using genetic markers), were obtained by Vlaic et al. (2006) in the European project ECONOGENE. Recent results concerning the study of major milk protein genetic polymorphisms of some Romanian native goat populations from Transylvania, were obtained in Farm Animal Genotyping Laboratory

belonging to USAMV Cluj-Napoca (Balteanu V.A. et al., 2007; Pop F.D., et al., 2007) (Fig. 1).

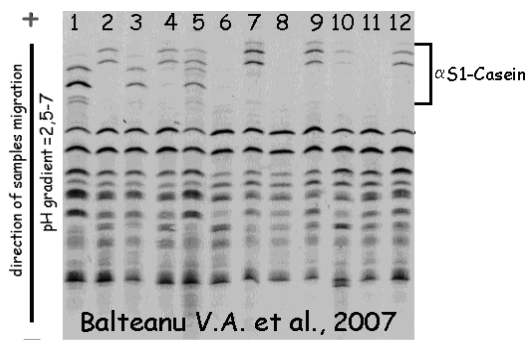


Figure 1. IEF profile belonging to some Carpathian goat individuals.

The genotypes at the α S1-casein locus were: 1-AA; 2-EE; 3-AF; 4-EE; 5-AE; 6-FF; 7-BB; 8-FF; 9-BB; 10-EF; 11-FF; 12-BE;

Medium frequency of high expression allele was observed, which could explain the variation in protein and fat content of Carpathian goat milk (Balteanu V.A. et al., 2007). Due to the heterogeneity of each heard, studies in Carpathian and White Banat goat populations are in progress in order to characterize them on six major milk protein loci and to establish CSN1S1 allele's frequencies at national level. We will also study the influence of different CSN1S1 genotypes on goat milk quality, cheese making efficiency and goat flavor in an experimental Carpathian goat population, in order to evaluate the possibility of using this marker in national breeding programs.

5. Goat milk proteins and their allergenic potency

Genetic polymorphism of milk protein in different farm species plays an important role in understanding allergic reactions of some patients to milk consumption. The allergies caused by cattle milk proteins consumption are affecting up to 20% of human population, especially children. Researches carried out in the world, established that principal allergen from milk is CSN1S1 and in a smaller way CSN2. The hypersensitivity reactions are usually seen on skin, gastrointestinal and/or respiratory tracts. Goat milk could be an alternative for some patients, because there are individuals who are carrying these 2 loci null allele and therefore these two proteins are missing from milk (Ramunno et al., 2001b).

Anyway the overall judgments on goat milk as a substitute for cow milk are still conflicting. Many clinical trials reports concluded that a high percentage of patients with cow milk protein allergy (CMPA) tolerate goat milk. Others studies reported cross-reactions between cow and goat milk allergy or even rare cases of goat milk allergy, sometimes in absence of cow milk sensitivity (Marletta et al., 2004). Further studies are necessary to elucidate if different goat milk formula can be used as cow milk alternative for persons with allergic reactions.

Conclusions

6. Potential use of milk protein polymorphisms for authenticity identification of goat milk and other dairy products

Authenticity identification of dairy-products received in the last years an important attention in many countries. Because of its lower cost undeclared cow milk (produced at a lower price and in higher quantities), can be added into buffalo, goat or ewe milk, normally used to produce authentic dairy products, especially in the case of industrial processed milk (Vlaic et al., 2008).

To avoid this, many methodologies have been developed at the international level to identify possible adulterations from dairy products industry like: isoelectric focusing (IEF), capillary electrophoresis (CE), reverse phase high-performance liquid chromatography (RP-HPLC), immunochemical methods (ELISA), mass spectrometry (MS) and PCR techniques (Vlaic et al., 2008). These techniques allow the detection of unwanted cow milk addition in different goat, buffalo or ewe dairy products starting with 0,1% (Vlaic et al., 2008). Except DNA based techniques, all these methods are mainly based on major milk proteins genetic variants analysis or their digestion products (γ_2 and γ_3 -caseins or para- κ -casein) (Addeo et al., 1990). From all this methodologies, IEF technique was chosen by US and European Community as reference method to identify adulterations from milk industry (Vlaic et al., 2008).

At the national level data concerning the use of milk proteins for milk and cheeses authenticity identification are almost absent. If in others EU countries products adulteration cases are quite rare, in Romania undeclared cow milk is often added into buffalo, goat or ewe milk used for cheese making.

Recent results concerning the study of major milk proteins genetic polymorphisms from native farm species and possibility to use them as genetic markers for authenticity/origin identification of milk and other dairy products were obtained in Farm Animal Genotyping Laboratory, belonging to USAMV Cluj, using IEF technique (Balteanu V.A et al., 2005, 2007; Pop F.D., et al., 2007; Vlaic et al., 2008).

The results confirm the applicability of this high sensitivity method to identify possible adulteration of goat dairy products. Therefore the application at the national level can be done only after a complete characterization of the two native goat breeds in the six major milk protein loci.

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