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Influence of goat breeds and starter culture systems on gross composition and proteolysis in Gokceada goat cheese during ripening

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ABSTRACT

Milk from two different breeds (Gokceada and Turkish Saanen) and three different starter culture systems (starter-free, mesophilic and thermophilic cultures) were used in the manufacture of Gokceada goat cheeses. Milk from the two breeds differed in dry matter, protein, fat and ash contents. These differences were reflected on the yield and chemical composition of the cheese. The pH values and soluble nitrogen fractions (in water, 12% trichloroacetic acid, and 5% phosphotungstic acid) were significantly influenced by the starter culture systems, but not the types of milk. The degradation of α_s-caseins and its degradation products sharply increased after 60 days of ripening, especially in the cheese made using mesophilic starters. Greater changes were observed in RP-HPLC peptide profiles of the cheeses made using mesophilic starter cultures during ripening; however, the breed has minor effect on peptide profile. In conclusions, cheeses made using mesophilic starter culture exhibited different proteolysis patterns during ripening and the milk from Gokceada breed increased the gross composition parameters and cheese yield.

1. Introduction

According to the data published yearly by FAO (2011), world production of goat's milk increased in the recent years and Asia countries account for about 42% of the total goat milk production. Goat production is also important under extensive conditions in Mediterranean countries (Silanikove et al., 2010). In Turkey, goat's milk production increased from 272,811 tons in 2010 to 320,588 tons in 2011 (TUIK, 2012). A large amount of homemade goat milk products (mainly cheese) is produced and the production volume increases. Due to market demands, the state supported efforts to encourage dairy products production from goat milk (Hayaloglu and Karagul-Yuceer, 2011). Goat milk is an alternative to cow milk in the manufacture of products such as yogurt and cheese in Turkey. This milk has some special and unique aroma and flavor characteristics as well as nutritional and health values. Such properties can be influenced by many factors including breed, genetic, physiology, feed, environment, and production technology (Raynal-Ljutovac et al., 2008). Artisanal goat cheese called Gokceada is produced in Gokceada (Imbros) Island (near Canakkale province of Turkey) during about 5 months year (from April to August). Cheese milk was clotted with homemade kid rennet in the past; however, presently commercial calf rennet is used. Following the coagulation, curds are drained with a cotton cloth and pressed for about 6 h.

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After pressing, the cheese cut into blocks with weighing 400 g. This cheese is a semi-hard type with acid and salty taste. The ripening time varies between 2 and 8 months (Hayaloglu et al., 2013).

Proteolysis during cheese ripening has an important role in the flavor and texture development. Proteolysis is evaluated by measuring the nitrogenous compounds soluble in various solvent using classical methods, High pressure liquid chromatography (HPLC), and gel electrophoresis. The products generated by proteolysis reactions are non-volatile and contribute to cheese flavor. Many studies have been conducted to determine proteolysis indices in goat milk cheeses including both classical methods including nitrogen fractions, total free amino acids, etc. (Olerte et al., 2000; Serhan et al., 2010; Delgado et al., 2011) and modern techniques including casein electrophoresis, peptide or individual amino acid profiles, etc. (Mallatou et al., 2004; Pino et al., 2009; Bontinis et al., 2012). Due to the complexity of proteolysis, it is very difficult to determine its extent by only one index. Fractionation of nitrogenous compounds and their analysis by Kjeldahl, spectrometry, electrophoresis and chromatographic analysis of peptides and quantitative determination of free amino acids provide a more complete view of proteolysis (Mallatou et al., 2004).

Proteolysis in cheese is influenced by many factors including cheese environment (pH, salt-in-moisture, and ripening time and temperature), and enzymes originated from milk, starter, non-starter and secondary starter microorganisms, rennet and environmental microorganisms (Hayaloglu et al., 2004). Starter microorganisms have an essential role in proteolysis due to their proteinase/peptidase system which is capable of hydrolyzing casein-derived peptides to small peptides and amino acids. Effects of starter cultures on non-goat milk cheeses have been extensively studied by various researchers (Law et al., 1993; Pappa and Anifantakis, 2001; Michaelidou et al., 2003; Hayaloglu et al., 2004, 2005). However, limited information is found on goat milk cheeses (Tzanetakis et al., 1995; Olerte et al., 2000; Simsek and Sagdic, 2012). Recently, Gokceada goat cheese has been manufactured in small dairy plants by using pasteurized milk and the cheeses produced does not meet the consumers' demands due to lower milk yield of Gokceada goat breed. In the cheese production, either mesophilic or thermophilic cultures or both has been used; however, our hypothesis was these cultures result in different levels of proteolysis in cheese. The objective of the study is to determine the effects of milk from two different goat breeds and starter culture systems on gross composition, yield and proteolysis in Gokceada goat cheese during ripening.

2. Materials and methods

2.1. Cheese-making

Experimental cheeses were manufactured in a private dairy plant (Imroz Co. Inc., Gokceada, Canakkale, Turkey) using 90 kg of milk from two goat breeds, Gokceada and Turkish Saanen. Cheeses were manufactured with or without starter cultures. Three trials of cheese making were carried out with 20-day intervals. The mixture of *Lactococcus lactis* subsp. *lactis* + *L. lactis* subsp. *cremoris*, code FD-DVS R-704 (Peyma-Hansen, Istanbul Turkey) was used as a mesophile starter while *Streptococcus thermophilus* code FD-DVSST-B01 (Peyma-Hansen, Istanbul, Turkey) represented the thermophile culture. Calf rennet (REN-NA® Ozel (Mayasan, Istanbul, Turkey)) at 1/18,000 MCU/mL (means that 1 mL rennet per 18 L of milk) was used to coagulate milk. The manufacturing protocol for goat milk cheese is shown in Fig. 1. Gross chemical composition was assayed after 1 day of ripening; however, pH, titratable acidity and proteolysis indices were determined after 1, 30, 60 and 90 days of ripening.

2.2. Cheese yield and chemical analysis of cheese milk and cheese samples

Cheese yield were calculated by the method given by Soryal et al. (2005). Cheese samples were analyzed in duplicate for moisture, fat, total nitrogen, titratable acidity (% lactic acid) and ash by the methods described in Hayaloglu et al. (2011). For pH measurement, 10 g of cheese were macerated in 10 mL of distilled water and the pH of the resultant slurry was measured using a digital pH meter (pH 211, Microprocessor pH Meter, Hanna Instruments, Italy).

2.3. Proteolysis

The water-soluble nitrogen (WSN), 12% trichloroacetic acid soluble nitrogen-soluble (TCA-SN) and 5% phosphotungstic acid-soluble (PTA-SN) fractions as % of total nitrogen of cheese were determined by the methods described by Hayaloglu et al. (2011). For urea-polyacrylamide gel electrophoresis (urea-PAGE) experiment, 20 g of grated cheese was homogenized with 40 mL of distilled water for 5 min using a stomacher (Stomacher 400, Seward Medical, London, UK). The homogenate was kept at 40 °C for 1 h and separated by centrifugation at 3000 × *g* for 30 min at 4 °C. The pellet was then freeze-dried. A Bio-Rad gel electrophoresis unit (Protean II Xi vertical slab gel) equipped with a power supply (Power Pac™ Universal Power Supply) was used (Bio-Rad Laboratories Ltd., Herts, UK). The pH 4.6-insoluble samples (10 mg of freeze dried sample) were dissolved in buffer containing 49% (v/v) urea. A 7 μL of the sample was loaded onto the gel. Electrophoresis was carried out through the stacking and separating gels at 280 and 300 V, respectively. The gels were stained overnight with Coomassie Brilliant Blue G-250. After destaining, gel slabs were scanned using a scanner HP Scanjet G4010 (Hewlett Packard Co., Palo Alto, CA, USA). The WSN fractions of the cheeses were also freeze-dried and analyzed by reverse-phase high performance liquid chromatography (RP-HPLC) as described by Hayaloglu et al. (2011) using a Shimadzu LC 20 AD Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan). A Phenomenex Jupiter C18 column 250 mm × 4.6 mm × 5 μm, 300 Å pore size (Phenomenex Co, Torrance, CA, USA) was used. The solvents were as follows: (A) 0.1% (v/v) trifloroacetic acid (TFA, sequencing grade; Sigma–Aldrich Laborchemikalien GmbH, Seelze, Germany) in deionized HPLC-grade water (Milli-Q system, Waters Corp., Molsheim, France) and (B) 0.1% (v/v) TFA in acetonitrile (HPLC grade, Merck KGaA, Darmstadt, Germany) at a flow rate of 0.75 mL/min. A 10 mg WSN fraction was dissolved in solvent A (10 mg/mL), filtered through a 0.45 μm cellulose acetate filter (Sartorius GmbH, Gottingen, Germany) and an aliquot (40 μL) of filtrate was injected into the column. The samples were eluted initially with 100% solvent A for 5 min, then with a gradient from 0% to 50% solvent B over 55 min, maintained at 50% solvent B for 6 min, followed by a linear gradient from 50% to 60% solvent B over 4 min and finally with 60% solvent B for 3 min. The elute was monitored at 214 nm.

2.4. Statistical analysis

An experimental design which incorporated 6 goat milk cheeses (2 breeds × 3 starter systems) and 4 ripening points × 3 trials was used to analyze the data from the variables. The analysis of variance was performed using the GLM procedure of SAS (SAS, 1999) according to following model:

$$Y_{ijkl} = \mu_{ijkl} + B_i + S_j + R_k + BS_{ij} + BR_{ik} + SR_{jk} + BSR_{ijk} + e_{ijkl}$$

where Y_{ijkl} = observed mean of the traits; μ_{ijkl} = overall mean; B_i = fixed effect of goat breed (Gokceada, Turkish Saanen); S_j = fixed starter cultures (starter-free, thermophilic and mesophilic cultures); R_k = fixed effect of ripening days (1, 30, 60, 90); BS_{ij} = interactions of goat breeds and starter cultures; BR_{ik} = interactions of goat breeds and ripening days;