Electrophoretic Study of Casein Breakdown during Ripening of Goat's Milk Cheese

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The study concentrated on the changes in caseins during the ripening of a semisoft goat's milk cheese [mixed coagulation (4% acid bacteria starter plus 0.05% calf rennet), 60 days ripened]. Samples of milk and cheeses at 1, 21, 42, and 63 days of ripening were compared for the densitometric profile of the electrophoretic analyses of the nonsoluble protein fraction at pH 4.6. Experiments were also carried out on α_{s1}- and β-caseins of goats in isolation, and the action of rennet on these proteins was detected. The appearance of the degradation peptides α_{s1}-I, β-I, β-II, β-III, and β-IV was observed in these experiments. These experiments allowed identification, in the tests undertaken on cheese, of the degradation products α_{s1}-I, β-I, and β-II, previously identified in the bibliography of cow's milk cheese. Vigorous activity of microbial enzymes on α_{s1}-caseins was also observed. The resistance of β-casein to hydrolysis was illustrated by the 50% of β-casein remaining unaltered at the end of ripening.

Keywords: *Goat's cheese, ripening, casein breakdown*

INTRODUCTION

Many studies on ripening in bovine milk cheeses have already been undertaken. However, even today there is relatively little information available concerning ripening in goat's milk cheese.

The increasing interest in goat's milk products and their possible improvement is of importance to the European Community due to the need to control excess cow's milk production.

Among products derived from milk, cheeses are, in the case of goat's milk, the most valued, especially in certain countries such as France, Greece, and Spain.

The study of casein breakdown, the main constituent in cheeses and curds, brings vital information to a knowledge of the processes involved in ripening, an obligatory step toward improvements in the industrialization of production, which until now has been small scale and without much added value.

Many agents are involved in the hydrolysis of caseins during cheese ripening (Fox, 1989): indigenous milk enzymes, mainly alkaline protease or plasmin (Farkye and Fox, 1990, 1991, 1992) and acid protease (Kaminogawa et al., 1980); rennet enzymes (Bringe and Kinsella, 1986; Grappin et al., 1985) or rennet-substitute enzymes (Creamer et al., 1988; Fedrick and Fuller, 1988); starter enzymes (Desmazeaud et al., 1976; Gripon et al., 1977; Rank et al., 1985); and nonstarter bacteria enzymes (Bhowmik and Marth, 1990; Peterson and Marshall, 1990).

The level and type of proteolysis have therefore been intensively studied, and its progress in most types of cow's milk cheeses is well documented.

This work develops the study of the extent and nature of casein breakdown in a goat's milk cheese.

MATERIALS AND METHODS

Cheese Samples. The samples came from a traditionally farmed herd (150 Murciano-Granadina breed goats, with an average daily yield of 250 L). The cheese was made according to traditional methods. The characteristics of cheese and cheesemaking can be found in Carretero et al. (1992a) and Mor-Mur et al. (1992).

The goat's milk cheese Cendrat del Montsec is a semisoft, mixed coagulation cheese, made from untreated milk, covered with ash, and then ripened. The mixed curd was obtained by natural acidification [4% (v/v) acid bacteria starter] of previously renneted milk (0.05% v/v calf rennet) during 20 h at 10–15 °C. This procedure allows the milk to acquire progressively the strong acid character of curd.

Seven samplings were made over a 2-year period between 1988 and 1990 covering the cheesemaking months December to June. Samples were taken at six stages of production: from milk in the curd vat, from two cheeses after molding and at 1 (unsalted), 21, 42, and 63 days during ripening. A final sample was taken from the finished product.

Electrophoresis. The separation of caseins was made by isoelectric point (pH 4.6) precipitation.

The electrophoretic separation of the caseins was undertaken using the Akroyd method (Akroyd, 1968).

A 0.7-mm-thick gel was used of 8.8% (w/v) polyacrylamide (neurotoxic reagent), with 5 M urea in 3 M tris(hydroxymethyl)-aminomethane/0.5 N HCl buffer at pH 8.9.

The electrode buffer was 0.4 M glycine, 0.05 M tris(hydroxymethyl)aminomethane, pH 8.4, diluted 10% (v/v).

The caprine casein standards were separated and purified in the Laboratoire de Génétique Biochimique del INRA de Jouy-en-Josas (France) (Mercier et al., 1968).

Preparation of Samples. Purified casein (10 mg) was dissolved in 2 mL of 7 M urea, 40 μL of this solution was mixed with 5 μL of 0.05% aqueous solution bromophenol blue, and then 15 μL was used for the electrophoresis.

Electrophoretic Conditions. Electrophoresis was carried out in a vertical vat (LKB 2001), using an Atom 502 power supply, at a constant intensity of 40 mA.

Gel Staining. This was done according to the method of Conejero and Semancik (1977), with Coomassie Blue R-250 solution (Sigma).

Densitometer. Band scanning was carried out with a laser densitometer (LKB 2202 Ultroscan), connected to a Hewlett-Packard 3390A integrator: absorbance range, 0.5–1 unit, 600 nm; scanning velocity, 30 mm/min; integration factor, 1.

Quantitative determination of caseins was made by peak area integration of the densitometer traces.

Rennet Activity on α_{s1}- and β-Casein and Whole Casein. Whole casein was separated by acid precipitation from skimmed goat's milk. The α_{s1}- and β-caseins were prepared from whole casein according to precipitation methods (Brignon et al., 1976; Fox and Guiney, 1972).

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Table 1. Mean Compositional Changes during Cheesemaking and Ripening

	total solids, g/kg of edible part	ash, g/kg of edible part	total N, g/kg of edible part	soluble N,[a] g/kg of edible part	non-protein N,[a] g/kg of edible part	fat, g/kg of edible part	pH
milk	135.7	7.8	5.6	29.46	10.53	49	
1-day cheese	456.3	9.9	24.1	7.88	4.52	273	4.02
21-day cheese	505.1	20.4	27.7	12.81	9.60	290	4.12
42-day cheese	531.3	19.3	30.3	19.44	14.98	319	4.35
63-day cheese	526.6	22.8	30.5	28.59	20.29	321	4.40

[a] Percent of total nitrogen.

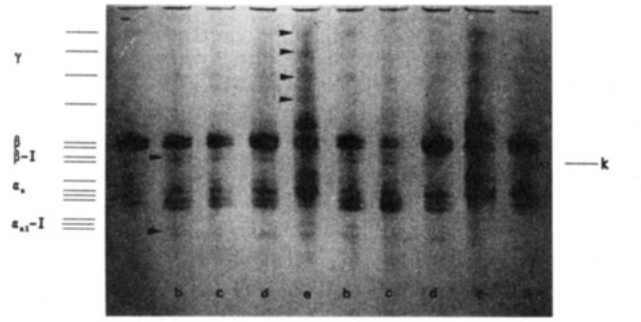

Figure 1. Electrophoregram of the pH 4.6 insoluble proteins of milk (a) and cheeses of 1 (b), 21 (c), 42 (d), and 63 (e) days of ripening.

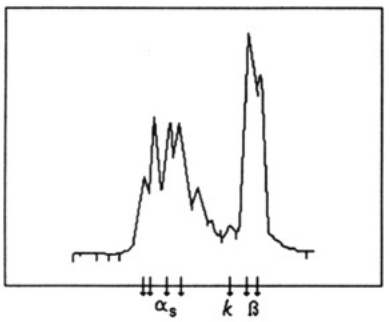

Figure 2. Densitometric profile of milk caseins.

Solutions of whole casein, α_{s1}-casein, and β-casein (25 g/L) in 0.05 M sodium acetate buffer (pH 5.4), containing 0.02% thimerosal to prevent microbiological activity, were heated at 80 °C for 30 min to prevent milk protease activity during incubation (Mulvihill and Fox, 1978).

After cooling, 10 μL of 0.5% (v/v) commercial calf rennet containing 520 mg of chymosin/L (International Dairy Federation, 1987) solution was added and then incubated at 20 °C for different times (30 min and 1 and 2 h for α_{s1}-casein and 15 and 30 min and 1, 2, 3, 6, 24, and 48 h for β-casein). The enzymatic reaction was stopped by addition of urea solution to a fine concentration of 7 M, and this solution was immediately frozed in liquid nitrogen and stored at −25 °C. Fifteen microliters of 0.5% protein content samples were taken for electrophoretic separation.

Composition Analysis. Standard methods were used to determine the milk and cheese compositions (Richardson, 1985).

The cheese nitrogen was fractionated according to the procedures of Kuchroo and Fox (1982). The pH 4.6 soluble proteins were determined as described by Carretero et al. (1992b).

RESULTS AND DISCUSSION

Table 1 shows the mean composition data of milk and mean composition and pH values of the cheese samples at different stages of ripening. Extensive proteolysis with high values of non-protein nitrogen fraction in the last samples was observed.

Figure 1 shows the electrophoretic pattern of casein evolution from milk to ripened cheese.

Figure 2 is a representative densitogram of a milk sample.

Including purified standards in the gel, we identified the main caseins of goat's milk; the results were consistent with previous observations (Addeo et al., 1988; Ono and Creamer, 1986). The first group of bands are α_s-caseins; several peaks, caused by the genetic polymorphism described for this type of protein in goat's milk, can be seen (Boulanger et al., 1984). This group of peptides, whose number varies between three and five depending on each sample, contains the α_{s1}-caseins. These are the fastest peptides according to the standards. Next, α_{s2}-casein is observed in more abundance (Assenat, 1985; Remeuf and Lenoir, 1985; Grosclaude et al., 1987) in all of the samples studied. Quantitatively, the α_s fraction constitutes a mean of 35% of the total amount of casein, with a standard deviation of 3.9. The variability observed may be explained by the individual and seasonal differences goat's milk shows (Grappin et al., 1981; Grosclaude et al., 1987; Quiles et al., 1991).

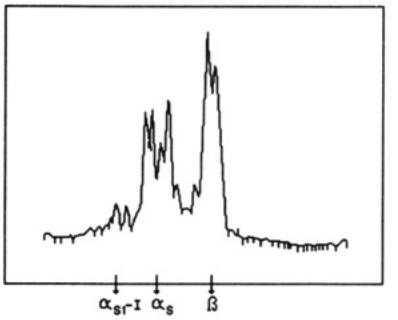

Figure 3. Densitometric profile of pH 4.6 insoluble proteins of 1-day-ripened cheese.

β-Casein is divided into its two varieties, β_1 and β_2, which differ in their level of phosphorylation (6/5) (Richardson and Creamer, 1974); there is no genetic polymorphism in this case. β- plus κ-caseins correspond quantitatively to 62.4% of the total casein as a mean value, and the standard deviation (4.8) is due to the differing degradation level between samples.

κ-Casein has an electrophoretic mobility very close to that of the fastest β-casein, which makes it impossible to quantify using this method. In the cheese samples, κ-casein disappeared due to the specific cleavage action of the chymosin, producing a highly hydrophobic peptide 1–105 (para-κ-casein) and an acidic soluble peptide 106–171 (caseinomacropeptide) (Mercier et al., 1976). In this electrophoretic system para-κ-casein is not visible because it runs off the gel in the electrophoretic conditions used.

Figure 3 shows the densitogram of the insoluble protein fraction (pH 4.6) of 1-day-old cheese before salting.

Only a slight difference in the densitometric profile of insoluble protein of the milk can be observed. However, in all cases two or three peptides of greater electrophoretic mobility than α_s-caseins are evident. These degradation products are identical in number to those occurring when pure α_{s1}-casein is incubated with rennet as shown in Figure 4. In all of the tests, three peptides were observed from the hydrolysis of α_{s1}-casein at 30 min of incubation. These peptides correspond to α_{s1}-I, previously identified for other cheeses in the literature (Addeo et al., 1988; Fox, 1989).

Peptides from β-casein degradation are also evident. These are products of rennet hydrolysis, identified in cow's

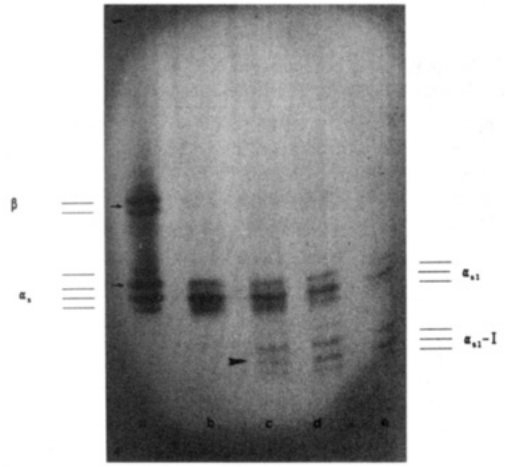

Figure 4. Electrophoregram of α_{s1}-casein treated with rennet: whole casein (a); untreated α_{s1}-casein (b); incubated with rennet α_{s1}-casein for 30 min (c), 1 h (d), and 2 h (e).

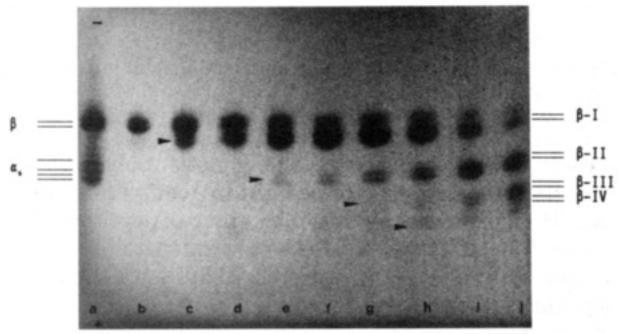

Figure 5. Electrophoregram of β-casein treated with rennet: whole casein (a); untreated β-casein (b); incubated with rennet β-casein for 15 min (c), 30 min (d), 1 h (e), 2 h (f), 3 h (g), 6 h (h), 24 h (i), and 48 h (j).

milk cheeses as β-I (Gripon et al., 1975; Noomen, 1978). The origin of these peptides was verified in the laboratory by incubating the purified goat's milk β-casein in solution; the results are shown in Figure 5. The two β-I peptides were seen after 15 min of incubation with rennet. They had greater electrophoretic mobility than β-casein. The two peptides β-II were observed after an hour when pure β-casein was incubated with rennet but are not present in any samples of 1-day-old cheese. After more than 2 h of degradation, peptides β-III are observed, and finally, when the hydrolysis is prolonged (more than 6 h), β-IV peptides appear (slightly observed at 6 h and more evident at 48 h). These peptides are not evident in 1-day-old cheese samples either (Figure 3).

In all cases, more hydrolysis peptides are visible in the incubation tests than in the cheese due to the inhibitory action of NaCl and the low pH of the cheese. This agrees with the observations of Mulvihill and Fox (1977, 1979) in cow's milk casein.

Figure 6 shows the densitogram of a cheese sample after 21 days of ripening. Important changes in the group of bands corresponding to α_s-casein can be seen in all of the cases studied, and the first bands increase. In solution (Figure 7) β-II bands show electrophoretic mobilities very close to that of the α_s-casein bands. The densitometrical increase on the α_s-caseins zone during the latter stages of ripening could be explained by the β-II peptides formation.

The three peptides with the highest mobility (α_{s1}-I), already seen in the previous sample, did not change. This indicates that they were produced only in the first moments of ripening, which agrees with the affirmations of Desmazeaud and Gripon (1977).

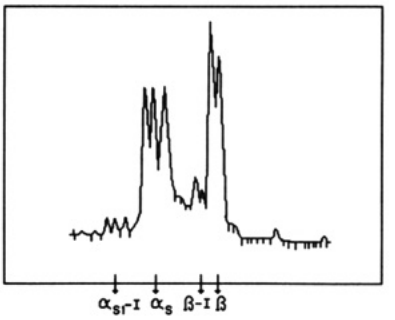

Figure 6. Densitogram of pH 4.6 insoluble proteins of 21-day-ripened cheese.

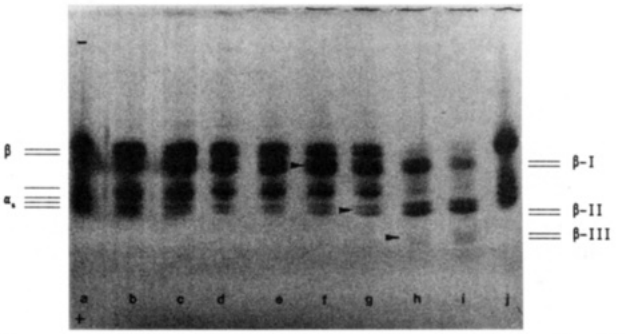

Figure 7. Electrophoregram of whole casein treated with rennet: untreated whole casein (a, j); whole casein incubated with rennet for 15 min (b), 30 min (c), 1 h (d), 2 h (e), 3 h (f), 6 h (g), 24 h (h), and 48 h (i).

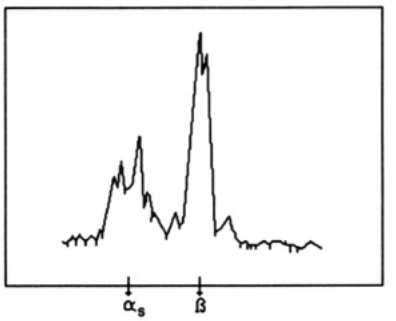

Figure 8. Densitogram of pH 4.6 insoluble proteins of 42-day-ripened cheese.

With respect to β-casein, degradation peptides β-I and β-II can be seen in all cases, although proteolysis affects this protein less. This has been observed in other cow's and sheep's milk cheeses (Buruiana and Seham-Farag, 1982).

Figure 8 represents the cheese at 42 days of ripening. The appearance of α_s-casein bands is very different from those seen previously and indicates an important degradation of all the caseins. The action of microbial enzymes is now obvious. The hydrolysis products do not appear in the protein fraction of the study because they are small-size peptides and free amino acids (Gripon et al., 1977). These raise the levels of soluble and non-protein fractions (Table 1).

Some bands with lesser mobility than β-casein appear in the electrophoregrams. These components are γ-caseins (Jaubert and Martin, 1992), probably produced from β-casein by goat plasmin.

Figure 9 represents the densitogram of the pH 4.6 insoluble protein fraction at 63 days of ripening, when the cheese is ready for consumption. The α_s-caseins are almost completely degraded, due to the action of lactic acid culture, surface molds, and other microorganisms, which mainly act on these proteins (Nath and Ledford, 1973).

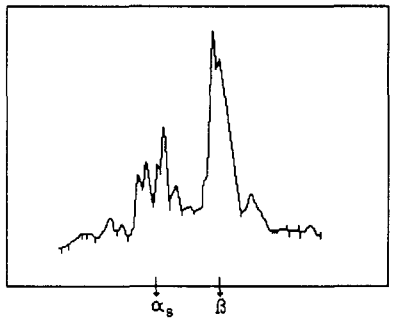

Figure 9. Densitogram of pH 4.6 insoluble proteins of 63-day-ripened cheese.

The coincidence with other degradation peptides makes a quantitative determination of this type of α_s-casein impossible.

The β-casein is not so affected by the proteolysis, a fact that is already known in cow's milk cheeses, and 50% of the initial amount of this protein remains unaltered in the fully ripened cheese.

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