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PROTEOLYSIS IN GOLOT CHEESE

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In this research, the degree of proteolysis and correlations among the casein fractions, pH, soluble nitrogen, and ripening index in Golot cheese were investigated. The range and mean values of α_s-, β-, γ-caseins, pH, and ripening index were found to be 56.3–66.4, 61.9%; 12.2–26.0, 21.9%; 6.5–30.6, 16.2%; 4.8–6.5, 5.6, and 7.9–70.0, 27.9%, respectively. There was a highly significant correlation between casein fractions (α_s-, β-, and γ-caseins) and chemical properties (pH, soluble nitrogen, and ripening index). A very broad range of β-casein, γ-casein, pH, and ripening index values indicated the lack of standardization of manufacturing steps and the ripening conditions of Golot cheese.

Keywords: cheese, proteolysis, casein fractions, ripening

Golot cheese is one of the most important traditional cheese varieties produced in the region of the East Black Sea, Turkey. The average composition of Golot cheese is 43.51% total solids, 5.31% fat, 33.64% protein and 3.12% salt (CAGLAR et al., 1998). The mixture of morning and night milk supplies is heated to 37 °C and skimmed. An appropriate amount of rennet and yogurt whey are added to the non-fat milk and heated until coagulation (65–70 °C). The curd is then transferred into the cheese cloth for whey drainage, which takes about 15 h. The cheeses are put in 50 kg polypropylene bags with granular cheeses being placed between Golot cheeses and held for 1 week. The Golot cheeses are pressed into wooden containers with cover and ripened for 6 months to 1 year. The duration of ripening determines the shelf life of the product (YAZICI & DERVISOGLU, 2001).

All rennet coagulated cheeses have very similar properties at the end of the manufacturing process. Most fresh cheeses have a rubbery texture and a mild flavour. To improve texture and flavour most cheeses are ripened. Cheese ripening may be accomplished by (1) rennet or rennet substitute; (2) indigeneous milk enzymes; (3) starter proteinases and peptidases; (4) enzymes from secondary starters and (5) non-starter bacteria (FOX et al., 1993). Ripening conditions also have a very important role in cheese ripening. These include ripening time, temperature, relative humidity, air and packaging material. Proteolysis is probably the most important biochemical process during the ripening of most cheese varieties (FOX, 1988). Proteolysis results in changes in the quantity of α_s-, β, and γ-caseins and peptides. These changes can be detected by

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electrophoresis techniques. Electrophoretic patterns of cheese proteins during ripening may vary with the cheese variety. In Cheddar cheese and other cheeses, α_{s1}-casein is always the first to be hydrolyzed. Complete hydrolysis of α_{s1}-casein in Cheddar cheese occurs within 35 days, whereas β-casein remains intact (SCHULTZ, 1977). The overall breakdown of β-casein in Cheddar cheese is rather limited and is affected by the salt concentration (EDWARDS & SANDINE, 1981). At the end of 90 days of ripening, degradation of α_s and β-caseins in Kulek cheese packed in wooden material is 19.54% and 41.72%, respectively. The quantity of γ-caseins gradually increases during ripening (DERVISOGLU & YAZICI, 2001). Hard (Permesan) and semi-hard (Cheddar) cheeses show proportionally less α_s- and β-caseins and proportionally more γ_1- and γ_3-caseins than the semi-soft (Port Salut) and soft (Brie) cheeses (MARSHALL et al., 1988).

Studies have been conducted by CAGLAR and co-workers (1998) on chemical composition and by YAZICI and DERVISOGLU (2001) on microbiological properties of Golot cheese, but there are no data on proteolysis.

The objectives of this study were to determine the degree of proteolysis in Golot cheese and correlate these results with some relevant chemical parameters.

1. Materials and methods

Thirty Golot cheese samples were purchased from randomly selected retail markets and public bazaars in the East Black Sea Region of Turkey.

1.1. Chemical analyses

Total nitrogen (TN) was determined according to the methods described by BRADLEY and co-workers (1992). Water soluble nitrogen (SN) was determined by the method of KUCHROO and FOX (1982). The ripening index was calculated from the ratio of SN to TN. pH was measured using a pH meter (inoLab, Weilheim, Germany) according to BRADLEY and co-workers (1992).

1.2. Preparation of cheese proteins and electrophoresis

One gram of cheese sample was defatted by diethylene ether extraction. After 1 h, the resulting cheese was dissolved in a solvent containing 1 ml EDTA (1%), 1 ml sodium deoxycholate (1%), and 5 ml urea (50%, w/v). The pH of the solution was adjusted to 7.0 and stored at 4 °C for 24 h (KURDAL, 1982; MAYER, 1996).

Cellulose acetate electrophoresis of casein fractions was performed by using a Titan Plus Electrophoresis unit (Helena Laboratories, Beaumont, USA). Electra HR buffer (18.0 g Tris-barbital-Sodium Barbital buffer, pH = 8.6–9.0) and acid blue stain (100 ml of acid blue stain in 50% acetic acid) were obtained from Helena Laboratories (Helena Laboratories, Gateshead, UK). The procedure followed was explained in the

instruction manual. Sample application was done by filling each well in the sample plate with 5 µl of sample. After loading the applicator, the samples were applied to the cellulose acetate plate for 10 sec. Electrophoresis was carried out at voltage of 185 V for 15 min. The cellulose acetate plate was immersed in the acid blue stain solution for 25 min. The plate was destained three times in 5% acetic acid solution until the background was white. The cellulose acetate was fixed with absolute methanol for 5 min. The final destaining was done in a mixture of methanol, glacial acetic acid and polyethylene glycol (70:30:4, v/v/v). The cellulose acetate plate was dried at 70 °C for 8–10 min (MAYER, 1996; 1997).

Densitometric evaluation of electrophoretograms was performed at 595 nm using a Clinic Scien 2 densitometer (Helena Laboratories, Gateshead, UK). The positions of the casein fractions were tentatively identified according to MARCOS and co-workers (1979) and HASSAN and EL-DEEB (1988). The ratios of individual casein fractions were calculated as the percentage of total scanning area.

1.3. Statistical analysis

Linear regression analysis was done to correlate the casein fractions with each other and with pH, soluble nitrogen, and ripening index of Golot cheese using Minitab statistical software (MINITAB, 1996).

2. Results and discussion

The results of casein fraction of Golot cheese are presented in Table 1. Densitogram of sample No. 15 is given as an example of the separation of casein fractions by cellulose acetate electrophoresis (Fig. 1). The α_s-casein fraction ranged from 56.3% to 66.4% and had an average of 61.9%. HASSAN and EL-DEEB (1988) determined the casein fractions of nine commercial cheeses and found the α_s-casein fraction to be 40.1% in Provolone cheese and 55.3% in Kariesh cheese. α_s-Casein ratios of soft goat cheese (MARTIN-HERNANDEZ et al., 1988), Kulek cheese ripened for 90 days (DERVISOGLU & YAZICI, 2001) and Kashkaval cheese (ALRUBAI, 1979) were 13.1–55.3%, 49.4%, and 28.1–54.9%, respectively. Our results are higher than these values. As there is no starter culture addition to Golot cheese, α_s-casein may not be broken down as much as in the other cultured cheeses.

The β-casein ratio of Golot cheese had a range of 12.2 to 26.0%, averaging 21.9%. β-Casein ratios of cheeses were reported to be 17.6–26.5% in Kashkaval cheese (ALRUBAI, 1979), 11.5% in Roquefort, 17.5% in Mish (HASSAN & EL-DEEB, 1988), 21.7–29.9% in soft goat cheese (MARTIN-HERNANDEZ et al., 1988) and 18.3–22.4% in Kulek chese ripened for 90 days (DERVISOGLU & YAZICI, 2001). Our results are closer to the finding of MARTIN-HERNANDEZ and co-workers (1988). Kulek cheeses ripened for 60 days had a similar β-casein pattern to our Golot cheese (DERVISOGLU & YAZICI, 2001).

Table 1. Average ratios of casein fractions, pH and ripening index of Golot cheese

Sample No	Casein fractions			pH	Ripening index (%)
	α_s-Casein	β-Casein	γ-Caseins		
1	60.9	23.8	15.3	5.56	19.24
2	63.3	22.2	14.6	5.36	19.72
3	62.2	22.2	15.6	5.38	25.67
4	64.8	20.6	14.7	5.20	21.29
5	64.8	19.7	15.4	5.58	12.08
6	61.2	23.3	15.6	5.33	15.72
7	62.7	23.3	14.0	5.43	18.05
8	62.8	22.0	15.3	5.40	11.13
9	58.4	25.4	16.2	5.48	15.62
10	64.4	21.2	14.4	5.32	12.27
11	58.6	25.1	16.2	5.60	14.37
12	61.5	23.6	15.0	5.30	7.94
13	64.4	21.8	13.9	5.41	26.53
14	66.4	20.3	13.3	5.90	17.23
15	66.1	25.4	8.4	4.82	8.62
16	63.9	25.3	10.7	4.95	17.83
17	65.8	25.2	9.0	5.04	16.05
18	58.1	16.2	25.7	6.39	69.96
19	59.2	18.9	22.0	5.95	41.24
20	63.6	17.3	19.1	6.21	49.48
21	58.0	24.6	17.3	5.72	28.31
22	58.3	20.1	21.6	6.40	58.95
23	61.1	22.4	16.5	5.62	33.91
24	62.2	18.9	19.0	6.15	53.13
25	66.2	27.3	6.5	5.33	18.67
26	57.2	12.2	30.6	6.14	69.31
27	61.8	26.7	11.6	5.10	14.49
28	61.1	24.2	14.7	5.59	24.17
29	60.4	23.0	16.7	5.70	30.12
30	56.3	15.9	27.8	6.45	65.47
Minimum	56.3	12.2	6.5	4.8	7.9
Maximum	66.4	26.0	30.6	6.5	70.0
Average	61.9	21.9	16.2	5.6	27.9

γ-Caseins including γ_1-, γ_2-, and γ_3-casein in Golot cheese ranged from 6.5 to 30.6%, averaging 16.2%. MARTIN-HERNANDEZ and co-workers (1988) determined that the γ-caseins plus polypeptide fraction of soft goat cheese were between 31.0 and 36.1%. Among the nine cheese varieties examined, Kariesh cheese had the lowest level of γ-caseins (27.1%) and Roquefort had the highest (49.0%) (HASSAN & EL-DEEB, 1988). While fresh Kulek cheese contained 7.1% γ-caseins, 90 day-ripened cheeses showed a γ-casein ratio of 32.3% (DERVISOGLU & YAZICI, 2001).

The pH and ripening index of Golot cheese ranged from 4.8 to 6.5 and 7.9% to 70%, respectively, showing that some cheeses were ripened for a very short period,

whereas others underwent extensive ripening. Ripening indexes of cheeses were reported to be 7.29 in fresh and 29.9 in 90-day-ripened Kulek cheeses (DERVISOGLU & YAZICI, 2001), 14.8 in Gouda and 31.7 in Ras cheeses (HASSAN & EL-DEEB, 1988).

As evidenced by the results of casein fractionation and the chemical analyses, Golot cheeses collected from retail markets had a very broad range of values for β-casein and γ-casein fractions, pH and ripening index, indicating a lack of standardization of most manufacturing steps, especially the ripening conditions.

Table 2 shows the correlation coefficients among the casein fractions, pH, SN, and ripening index of Golot cheese. Since γ-caseins are degradation products of β-casein (HASSAN & EL-DEEB, 1988; MARSHALL et al., 1988; MAYER, 1996; 1997), a highly negative correlation between β-casein and γ-caseins was observed in Golot cheese.

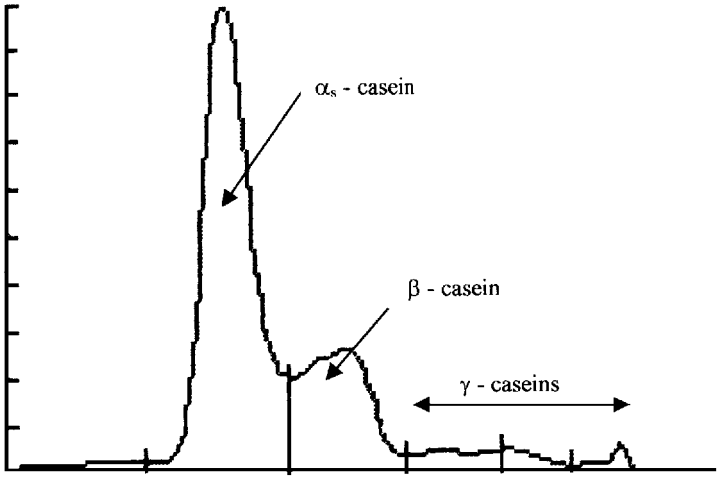

Fig. 1. Densitogram of casein fractions of Golot chese

Table 2. Correlation coefficients between casein fractions and some chemical properties

Variable	β-Casein	γ-Caseins	pH	Soluble N (%)	Ripening index (%)
α-Casein	0.362*	–0.788**	–0.614**	–0.574**	–0.598**
β-Casein		–0.859**	–0.766**	–0.750**	–0.796**
γ-Caseins			0.842**	0.810**	0.853**
pH				0.864**	0.869**
Soluble N (%)					0.984**

*: Significant at the P = 0.05 level
**: Significant at the P = 0.01 level

There is a highly significant and negative correlation between pH and the fractions of α_s-and β-caseins and a positive correlation between pH and γ-caseins ($P<0.01$). Similar changes in pH were observed by HASSAN and EL-DEEB (1988). During ripening, the pH of cheeses increases due to extensive proteolysis with subsequent deamination of amino acids and the metabolism of organic acids (GODINHO & FOX, 1982; MADKOR et al., 1987). The correlation between the casein fractions and SN or ripening index was also highly significant ($P<0.01$). This indicates that degradation of α_s- and β-casein and accumulation of γ-caseins during the ripening process result in increasing SN and the ripening index.

3. Conclusion

Golot cheese samples collected from retail markets were characterized by a very broad range of proteolysis. There was a very high correlation between the casein fractions, pH, SN, and ripening index of Golot cheese. In order to categorize the proteolysis in Golot cheese, more attention should be given to standardization of manufacturing steps and ripening conditions.

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