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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4(7): 881-883, 2001

Proteolysis in Golot Cheese

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Abstract: The 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 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 the casein fractions (α_{s}-, β-, and γ_caseins) and some chemical properties (pH, soluble nitrogen, and ripening index).

Key words: Cheese, proteolysis, casein fractions, ripening

Introduction

Golot cheese, is one of the most important traditional cheese produced in the region of 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 is heated to 37°C and separated from the fat. An appropriate amount of rennet and yogurt whey are added to the non-fat milk and then heated until the precipitation (65-70°C). The curd is then transferred into the cheese cloth for whey drainage about 15 hours. The cheeses are placed in a 50 kilo polypropylene bags; granular cheeses are added between each Golot cheese and waited for one week. The Golot cheeses are pressed into wooden containers with cover and ripened between 6 months and 1 year based on the consumption period (Yazici and Dervisoglu, 2001).

All rennet coagulated cheeses have very similar properties at the end of the manufacturing step. Most fresh cheeses have a rubbery texture and a week flavor. To improve texture and flavor most cheeses are ripened. Cheese ripening may be accomplished by (1) rennet or rennet substitute; (2) indigenous milk enzymes; (3) starter proteinases and peptidases; (4) enzymes from secondary starters, and (5) non-starter bacteria (Fox *et al.*, 1993). Ripening conditions have also very important role in cheese ripening. These are ripening time, temperature, relative humidity, air, and packaging material. Proteolysis is probably the most important biochemical event during the ripening of most cheese varieties (Fox, 1988). Proteolysis includes the changes in α_{s}-, β, and γ- caseins and peptides. These changes can be detected by electrophoresis techniques. Electrophoretic patterns of cheese proteins during the 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 in contact (Schultz, 1977). The overall breakdown of β-casein in Cheddar cheese is generally small and affected by the salt concentration (Edwards and Sandine, 1981). Degradation of α_{s} and β-casein in Kulek cheese packed with wooden material at the end of 90 days of ripening is 19.54 and 41.72%, respectively. γ_caseins gradually increase during the ripening (Dervisoglu and Yazici, 2001). Hard (Parmesan) and semi-hard (Cheddar) cheeses show proportionally less α_{s} and β-casein and proportionally more γ_{1}- and γ_{3}- caseins than the semi-soft (Port salut) and soft (Brie) cheeses (Marshall *et al.*, 1988).

Some studies have been conducted by Caglar *et al.* (1998) on chemical composition and by Yazici and Dervisoglu (2001) on microbiological properties of Gobt cheese, but there is no data about proteolysis.

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

Materials and Methods

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

Chemical analyses: Total Nitrogen (TN) was determined according to the methods of Bradley *et al.* (1992). Water Soluble Nitrogen (SN) was determined by the method of Kuchroo and Fox (1982). The ripening index was calculated from the ratio SN to TN. pH was measured using a pH meter (inoLab, Weilheim, Germany) according to the Official Methods of AOAC (1992).

Preparation of cheese proteins and electrophoresis: One gram of cheese sample was defatted by diethyl ether extraction. After 1 hour, 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 (Mayer, 1996).

Cellulose acetate electrophoresis of casein fractions was performed 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, UK). A 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 de-stained three times in 5% acetic acid solution until the background was white. The cellulose acetate was fixed with an absolute methanol for 5 min. The final destaining was done in a mixture of methanol, glacial acetic acid, and clear aid (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 Sci. 2 densitometer (Helena Laboratories, UK). The positions of the casein fractions were tentatively identified according to Hassan and El-Deeb (1988). The ratios of individual casein fractions were calculated as the percentage of total scanning area.

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).

Results and Discussion

Casein fractions of Golot cheese are presented in Table 1. α_s-casein fraction of Golot cheese 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 in Provolone cheese as 40.1% and in Kariesh cheese as 55.3%. α_s-casein ratios of soft goat cheese (Martin-Hernandez *et al.*, 1988), Kulek cheese ripened ninty days Dervisoglu and Yazici, 2001),

Table 1: Average ratios of casein fractions (% of total scanning density), and 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

and for other cheese, the ratios of Kashkaval cheese (Albubai, 1979) were 13.1 – 55.3, 49.4, and 28.1 – 54.9%, respectively. Present results are higher than these values. Since there is no starter culture addition to the Golot cheese, α_s-casein in Golot cheese may not be broken down as much as the other cultured cheeses.

β-casein ratio of Golot cheese had a range of 12.2 - 26.0%, averaging 21.9%. β-casein ratios of cheeses were reported as 17.6 – 26.5% in Kashkaval cheese (Albubai, 1979), 11.5% in Roquefort 17.5% in Mish (Hassan and El-Deeb, 1988), 21.7-29.9% in soft goat cheese (Martin-Hernandez *et al.*, 1988), and 18.3 – 22.4% in Kulek cheese ripened 90 days (Dervisoglu and Yazici, 2001). Our results are closer to the finding of Martin-Hernandez *et al.* (1988). A 60 day-ripened Kulek cheese had a similar β-casein pattern to our Golot cheese (Dervisoglu and Yazici, 2001).

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 *et al.* (1988) determined the γ caseins plus polypeptide fraction of soft goat cheese between 31.0 and 36.1%. Among the nine cheese varieties, Kariesh cheese had the lowest of 27.1% and Roquefort the highest of 49.0% γ_caseins (Hassan and El-Deeb, 1988). While fresh Kulek cheese had 7.1% γ-caseins, 90 day-ripened cheeses showed a 32.3% γ_casein ratio (Dervisoglu and Yazici, 2001).

The pH and ripening index of Golot cheese ranged from 4.8 to 6.5 and 7.9 to 70%, respectively. A very broad range for pH and ripening index imply that some cheeses had a little ripening, while others much extensive ripening.

Based on the casein fraction and the chemical analysis, Golot cheeses collected from retail markets indicated a very broad range of β-casein, γ-casein, pH and ripening index, implying the lack of standardization of most manufacturing steps, especially the ripening conditions.

Table 2 shows the correlation coefficients among the casein fractions, pH, soluble N, and ripening index of Golot cheese. Since γ-caseins are degradation products of β-casein (Mayer, 1996, 1997), a highly negative correlation between β-casein and γ-caseins was observed in Golot cheese. There is a highly significant and negative correlation between pH and the fractions of α_s-, β-casein and positive correlation between pH and γ-caseins ($P< 0.01$). Similar changes in pH were observed by Hassan and El-Deeb (1988). During the ripening, the pH of cheeses increases due to extensive proteolysis with subsequent deamination of amino acids and the metabolism of organic acids (Madkor *et al.*, 1987). The correlation between the casein fractions and soluble nitrogen or ripening index was also highly significant ($P< 0.01$). This indicates that degradation of α_s-and β-casein or accumulation of γ-caseins

Table 2: The Correlation Coefficients between Casein Fractions and Some Chemical Properties[1]

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**
Ripening Index (%)					

[1] * Significant at 0.05% level of probability. ** Significant at 0.01% level of probability.

during the ripening process parallel the increase in soluble nitrogen or ripening index. Golot cheese samples collected from retail markets had a very broad range of proteolysis. There was a very high correlation among the casein fractions, pH, soluble nitrogen, and ripening index of Golot cheese. In order to categorize the proteolysis in Golot cheese, more attention should be given on standardization of manufacturing steps and ripening conditions.

Acknowledgments

This study was financially supported by Ondokuz Mayis University Research Fund.

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