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Investigation of Changes in Proteolysis and Lipolysis of Traditional Çanak Cheese Maturing Under the Soil

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Abstract: Çanak cheese which is matured in earthen were containers, is a common cheese type produced widely in Yozgat province of Turkey. Thirty Çanak cheese samples were investigated for routine chemical composition, lipolysis, proteolysis, and free fatty acids (FFAs). Dry matter, salt, salt in dry matter, fat, fat in dry matter, and acidity values in the analyzed samples were determined to be between 45.01 and 66.27 %; 2.11 and 8.89 %; 3.70 and 19.76 %; 13.00 and 40.00 %; 26.23 and 69.30 % and, 0.05 and 0.25%, respectively. WSN, TCA-SN, PTA-SN and lipolysis values were detected to be high in Çanak cheeses. Also, C14, C16, and C18:1 fatty acids were found to be higher than other fatty acids in all cheese samples.

Keywords: Çanak cheese, Fatty acids, Lipolysis, Proteolysis.

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1. INTRODUCTION

Çanak (Pan) cheese, which is matured in earthenware containers, is a common cheese type unique to the district of Sarıkaya, Sorgun, Boğazlayan, Çayıralan, Yerkoy and Şefaatli in Yozgat province in the Anatolia Region. Traditional Çanak cheese is commonly made by small-scale dairies using raw milk and traditional techniques. Fermentation relies on activities of indigenous milk microflora. The production methods of Çanak cheese and the ineffective control of temperature and relative humidity during storage cause differences in the quality of the final product. These variation changes in Çanak cheese show that there is no standardized method in the production of this cheese variety (Kamber, 2008a; Kirdar and Kursun, 2011).
Çanak cheese is white in color, slightly salty and has a homogeneous grain structure (Fig.1). This cheese variety is usually produced in June, July and August and is kept buried underground until September and November to fully mature (Kirdar and Kursun, 2011). It can be classified as a fat (fat content in dry matter <57 %) and hard cheese (Turkish Food Codex, 2015).

Traditional Çanak cheese is made from raw goat or sheep milk without the use of starter culture. Firstly, milk is coagulated using homemade calf rennet (for 30 kg of milk per quarter tea glass) in approximately 60-90 minutes (min.) at 32-35 °C. The curd is divided into four large pieces with a scoop, rested for 15-20 min. and transferred to cloth bags. After filtering, pressure is applied to the clot and left to rest for one hour. At the end of the straining process, the curd is cut into pea-sized pieces by hand on the cloth in the tray and salted. This cloth bag is placed on a rocky surface in a cool place (12–16°C) and pressure is applied with regularly shaped stones to create a weight of 10 kilograms for each kilogram of cheese, and the cheese is cut into potato wedges and crumbled. The pans are filled with a layer of potato slices and one layer of crumbling in alternating order until it is full. Finally, the lid of the pan is closed tightly as before and turned upside down; It sinks into the sand again, this time completely. Maturation is achieved in a cool and slightly moist environment, either by burial directly under the ground or by placing in a box filled with sand. The Çanak (Pan) is then buried underground for ripening for three months (Kirdar and Kursun, 2011).

Figure 1. Çanak cheese

Proteolysis is probably the main biochemical event during ripening for most cheese varieties. Nitrogen fractions are essential parameters for determining the extent of proteolysis (Fox, 1988). Proteolysis can vary greatly depending on the cheese type. The geographical region of the cheese, the season of production, the temperature and time of ripening, and the type of milk used in its production are factors that affect the proteolysis levels in cheese (Di Cagno et al., 2003).

Lipolysis is a biochemical parameter of great importance that is frequently used in the analysis of short and medium chain (ie, C4-Cl2) free fatty acids (FFA) and in the chemical characterization of cheese ripening (Woo et al., 1984; De La Fuente et al., 1993). This type of FFA makes significant contributions to the flavor of different types of cheese, as it is reported that the characteristic flavor of cheese is provided by a well-balanced concentration of chemical compounds. The lipolysis values vary significantly between different cheese types, from Dutch low-grade cheeses (Walstra et al., 1993) to mold-ripened, surface-ripened and Italian hard cheeses (Battistotti and Corradini, 1993). Many studies have been conducted on the composition of fatty acids and sensory properties of various cheese types (Kondyli et al., 2002; Guler and Uraz, 2003; Mallatou et al., 2003; Perotti et al., 2005; Atasoy and Turkoglu, 2008; Atasoy et al., 2008; Atasoy and Turkoglu, 2009; Georgala et al., 2016; Fusaro et al., 2019; Serrapica et al., 2020). In recent years, different types of cheese have been studied in Turkey in order to increase the studies on the effect of proteolysis on the ripening of some cheeses (Cinbas and Kılıc, 2006; Hayaloglu and Karabulut, 2013; Tuncturk et al., 2014; Salum et al., 2018; Ozer and Kesenkas, 2019). The knowledge about Çanak cheese technology and chemical composition is inferior. In contrast, there has been no study on the proteolysis and lipolysis of Çanak cheese.

There have been few studies on the gross composition and microbiological characteristics of Çanak cheese (Akyuz and Gulumser, 1984; Coskun et al., 2002; Kirdar and Kursun, 2011).

The aim of this investigation was to obtain information about fat hydrolysis, proteolysis degree and fatty acids in Çanak cheese and to determine the correlation between chemical and biochemical properties of this cheese.

2. MATERIAL AND METHOD

2.1. Materials

Thirty ripened Çanak cheeses (~250g each) from different towns located near Yozgat in Turkey were purchased and quickly brought to the laboratory at 4 °C. The samples were storaged at 4±1 °C during the analysis.

2.2. Chemical analyses

The pH values of the cheeses were determined using a pH meter (HANNA). Acidity of samples was detected according to the AOAC method by titration (AOAC, 2000) and the fat content (%) was analyzed by the Gerber method (IDF, 1997). The total solids content was found by gravimetric (IDF, 1982) and the salt content was determined by the Mohr method (IDF, 1979). The amount of salt and fat in the dry matter was obtained by calculation.

2.3. Determination of proteolysis

The total nitrogen (TN) content was determined using the Kjeldahl method (IDF, 1993). The total protein, water-soluble nitrogen (WSN), 12% trichloroacetic acid soluble nitrogen (TCA-SN) and 5% phosphotungstic acid soluble nitrogen (PTA-SN) were detected using the Kjeldahl method (Bütikofer et al., 1993). The ripening index (RI) was also calculated by taking the percentage of WSN to TN ratio.

2.4. Determination of lipolysis

The lipolysis in cheese samples was evaluated by determining the acid degree value (ADV) and free fatty acid (FFA) content. Determination of ADV was done using the BDI method. In order to determine the ADV (mM/100 g fat) in cheese samples, finely ground 10 g samples were placed in special butyrometers. 20 mL reagent (obtained by dissolving 30 g Triton X-100 and 70 g sodium tetra phosphate in 1 L of distilled water and adjusting the pH to 6.6 with o-phosphoric acid) was added to it, and the oil was released by placing the butyrometers in boiling water. The mixture was then centrifuged for 1 min. and enough aqueous methanol (1 water: 1 methanol) was added to bring the oil column to the butyrometer neck. The samples were centrifuged again for 1 min. and incubated in a water bath. All of the upper oil was withdrawn with a syringe, 5 mL of oil solvent (4 petroleum ether: 1 n-propanol) containing 0.1 g/L thymol blue indicator was added and titrated with 0.01 N tetra n-butyl ammonium hydroxide. As a result, the ADV ratios were calculated and determined according to Case et al. (1985).

The fatty acids extracted from cheese were determined according to the Deeth et al. (1983) method (Table 1) by gas chromatography (Agilent 6890 Series GC system). The cheese samples (1.5-2.0 g) were weighed into the test tube. Then 2.5 g of sodium sulfate, 5 mL of heptanoic acid and 300 µL of 4 N H2SO4 were added. The mixture was stirred in the vortex for 30 sec. 5 mL of hexane was added over and stirred again. After the covers of the samples are wrapped with parafilm, they were kept on hold for 2 hours in a dark environment. At the end of the period, in the top layer of the sample in the test tubes the hexane / diethyl ether layer containing the lipids is carefully transferred to each biorad column (100 mm height and 10 mm diameter) which with 1 g of deactivated alumina and glass wool is washed once with 5 mL of diethyl ether / hexane (1/1 v / v) mixture. The column is washed twice with 5mL of 1: 1 diethyl ether /

hexane solution, the wash solution is poured into the waste tray. After the diethyl ether / hexane solution was completely filtered, the air was given to the upper part of the columns by a fan and allowed to dry for 60 min. The dried alumina was transferred into 13 mm closure glass tubes. 2 mL formic acid (6%) prepared in diethyl ether was added to the test tube, the tubes were closed for 15 seconds after mixing and centrifuged for 10 min. at 2000 rpm. The clear part collected on the upper surface was transferred to the colored vials using a Pasteur pipette and closed with a unique sealing device. Samples were stored at -18ºC until GC analysis. The samples were allowed to dissolve free fatty acids for 30 min at room temperature before being injected into the GC, and 5 µL of the vials were injected from the vials under appropriate GC conditions.

Table 1. The working conditions of Gas Chromatography

Detector	Flame ionization detector (FID-Flame Ionization Detector)
Column	Agilent –FFAP Capillary 300x250 µm x 0.25 µm
Detector temperature	230ºC
Injector temperature	220ºC
Split ratio	1:20
Injection volume	1 µL
Mobile phase	Nitrogen (N2) flow rate: 2 mL / min Hydrogen (H2) flow rate: 33 mL / min
Air flow rate:	370 mL / min

2.5. Statistical analysis

The mean, minimum, maximum and standard deviation values for all parameters were obtained using SPSS v.19 (IBM Corp., Armonk, NY, USA). Significance assessment was done by analyzes of variance followed by Spearman correlation (Draper and Smith, 1998).

3. RESULTS AND DISCUSSIONS

3.1. Gross composition

The chemical content of the Çanak cheese samples is shown in Table 2. The mean value of fat, salt, pH, titratable acidity, and total dry matter was determined 22.92%, 4.22%, 5.34, 0.14 and 52.10%, respectively.

Dry matter of samples contents vary according to the type and duration of syneresis, salting type, curd size, pre-ripening (Ucuncu, 2004). The dry matter content of Çanak cheese showed to be similar to Sıkma cheese, Tulum cheese, and Küp cheese. For the rates of fat in dry matter in the samples of Çanak cheese, 30% and 50% were more concentrated. Five samples fat rate in dry matter have been detected to contain more than 50%.

Fat is the main nutrient of milk, also has important functions in cheese which is the and affects the hardness, stickiness, mouthfeel and flavor of the cheese (Fox et al., 1993; Fox et al., 1999). It was change that fat content of the Çanak cheese samples varied in a wide range.
The fat ratio of Çanak samples showed significant variations. The differences in fat content may be due to the fact that this product does not have a standard production technique. The fat content of Çanak cheese was similar found to that of Sıkma cheese and higher than the other similar cheeses (Kamber, 2008b; Guven and Karaca, 2004).

Table 2 shows that the minimum, maximum and average pH values of Çanak cheese are 4.54, 6.22 and 5.34, respectively. Kinik et al. (1999) determined that the values of 5.22 and 5.46 pH in Sepet cheese were found to be similar and higher than the value of 4.68 pH found by Polat and Yetismeyen (2004) in Civil cheese. In addition, TS591 (2006) stated that the minimum pH value should be 4.5 in white cheeses and it was determined that the results obtained in the study were higher than this value.

The pH values of Çanak cheeses have a significant negative correlation with the acidity values (Table 3). Accoding to the TS 591 standard (2006) recommends that titratable acidity as lactic acid in cheeses should not exceed 3%. As a matter of fact, when the acidity values obtained in the study were examined, it was seen that the titratable acidity values of cheeses did not exceed 3%.

Salt is an important ingredient that contributes to flavor and taste development. When Table 2 is examined, it is seen that the average salt content of Çanak cheeses is 4.22%, the maximum salt content is 8.89% and the minimum salt content is 2.11%. There are significant differences in salt content between both producers and production periods. Since Çanak cheese is salted by hand, inhomogeneously after cheese making, salt distribution is observed immediately, causing limitation of rate of proteolysis in cheeses during ripening (Thomas and Pearce, 1981). In addition, the high salt in moisture (S/M) contact of the cheeses may have reduced the acidity of lactic acid bacteria (Prasad and Alvorez, 1999). Similar results were obtained by Cinbas and Kiliç (2006).

Table 2. Biochemical properties of Çanak Cheese

Parameters	Mean±SD	Minimum	Maximum
pH	5.34±0.44	4.54	6.22
Titratable asidity(LA %)	0.14±0.07	0.05	0.25
Fat[b]	22.92±5.92	13.00	40.00
Dry matter[b]	52.10±4.68	45.01	66.27
Salt[b]	4.22±1.99	2.11	8.89
Fat in dry Matter[b]	43.72±8.30	26.23	69.30
Salt in Dry matter[b]	8.25±4.05	3.70	19.76
Total nitrogen[b]	1.93±0.11	0.49	4.42
Total protein[b]	12.11±6.22	3.13	28.21
WSN[c]	0.33±0.13	0.09	0.68
RI[c]	24.49±16.16	7,38	60,42
TCA	0.26±0.12	0.08	0.64
TCA-SN[c]	17.95±1.71	2.64	59.71
PTA	0.06±0.00	0.01	0.16
PTA-SN[c]	3.85±0.34	0.54	18.86
ADV value	10.34±11.01	1.04	46.24

[*a] [*]Mean data of Çanak cheese analysed in triplicate
[a] All data are expressed as means± standard error
[b] Values for moisture, protein, total nitrogen, total protein, fat and sodium chloride, are g 100/gof cheese.
[c]WSN: water-soluble nitrogen; TCA-SN: 12% trichloroacetic acid-soluble nitrogen; RI: ripening index; PTA-SN: 5% phosphotungstic acid-soluble nitrogen.

Table 3. The correlation between chemical and ciochemical properties of Çanak cheese

	pH	Titratable acidity	Fat	Dry matter	Salt	Fat in dry matter	Salt in dry matter	Total nitrogen	protein	WSN%	TCA%	PTA%	ADV value
pH	1.000												
Titratable acidity	-0.727**	1.000											
Fat	-0.096	0.216	1.000										
Dry matter	-0.315**	0.420**	0.667**	1.000									
Salt	0.442**	-0.370**	-0.034	-0.30	1.000								
Fat in dry matter	-0.028	0.055	0.917**	0.397**	0.08	1.000							
Salt in dry matter	0.442**	-0.403	-0.131	-0.140	0.980**	-0.063	1.000						
Total nitrogen	-0.485**	0.497**	0.507**	0.556**	0.068	0.375**	0.031	1.000					
Total protein	-0.457**	0.460**	0.514**	0.572**	0.085	0.390**	0.043	0.979**	1.000				
WSN%	0.354**	-0.318**	-0.495**	-0.601**	-0.246**	-0.413**	-0.116	-0.789**	-0.800**	1.000			
TCA%	0.175	-0.115	-0.420**	-0.470**	-0.255**	-0.365**	-0.219*	-0.668**	-0.703**	0.854**	1.000		
PTA%	0.341**	-0.258*	-0.282**	-0.280**	-0.025	-0.241*	0.082	-0.524**	-0.554**	0.701**	0.670**	1.000	
ADV value	-0.044	0.078	-0.241*	-0.121	-0.076	0.299**	-0.028	-0.129	-0.162	-0.440**	0.470**	0.399**	1.000

*= Significant at ($p< 0.05$) ** = Significant at ($p< 0.01$)

Total nitrogen was used to determine the evolution of the total protein content and the specify of the maturation index. Total nitrogen was negatively correlated with pH, and positively correlated with dry matter, total acidity, fat and fat in dry matter (Table 3) ($p < 0.01$).

One of the parameters used to determine the level of proteolysis in cheese is the water-soluble nitrogen content. The water-soluble nitrogen ratio, which is considered as an indicator of ripening, is a value that mainly explains the environment of ripening, and therefore the level of large and medium molecular weight nitrogen fractions formed by the hydrolysis of casein (Koçak et al., 1997). WSN/TN, TCA-SN and PTA-SN levels were detected to be high in Çanak cheese.

TCA-SN and PTA-SN values also differed among the Çanak cheese samples. Only small peptides and amino acids are soluble in 12% TCA used in the extraction of nitrogen dissolved in 12% trichloroacetic acid. Therefore, this fraction is also called the non-protein nitrogen (NPN) fraction and can be evaluated as an indicator of proteinase activity in cheese (Tuncturk, 1996). The average TCA-SN values obtained in our study (17.95%) are higher than the results (7.08%) determined by Tarakci et al. (2004) for Van herby cheese and lower than (19.35%) determined by Durmaz et al. (2004) for Surk cheese.

The average of PTA-SN values were found in this study was 4.46%, and the lowest and highest values ranged between 0.54% and 28.81%. These results were higher than the values obtained by Tarakci et al. (2004) in Van herby cheese (3.85%). This can be elucidated by the different compositions of raw milk or the lack of current standard production methods (composition and quality of milk) and production stages (packaging and ripening conditions).

A significant negative correlation was observed between salt in dry matter and TCA-SN values at $p<0.05$ level. Similar results were found in the study by Ercan et al (2012). The cheese ripening coefficient is between 33-66% for fully mature , from 33% in younger cheeses described as less mature. According to this analysis, twenty Çanak cheese was less mature. Using the maturity level of proteolysis to determine the coefficients of the Çanak cheese samples by considering the classification criteria, it was found that 6 of them were less than 10% of the maturation factor, 3 of them 10-20%, 3 of them between 20-30%, and 8 of them between 33-66%.

Table 4. Fatty acid values of Çana cheese samples

Fatty acids										
Saturated fatty acid[1]										
	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0
Min	0.00	0.12	0.28	0.95	2.55	0.58	0.31	35.62	0.37	0.28
Max	1.90	1.65	1.17	4.60	6.10	14.72	2.35	46.06	11.69	10.84
Mean	0.94±0.25	1.03±0.21	0.86±0.13	2.81±0.50	3.65±0.51	7.78±2.14	1.13±0.32	41.24±1.64	2.93±1.80	6.09±1.76

Unsaturated fatty acids [2]							
	C14:1	C15:1	C16:1	C17:1	C18:1	C18:2	C18:3
Min	0.10	0.12	0.22	0.05	4.80	0.5	0.16
Max	12.99	2.68	2.47	1.27	23.56	8.27	13.93
Mean	2.63±2.08	0.73±0.40	1.06±0.38	0.36±0.28	17.51±2.99	4.48±1.65	2.65±2.56

[1]C4:0 = Butiric, C6:0 = Caproic, C8:0 = Caprilic, C10:0 = Capric, C12:0 = Lauric, C14:0 = Myristic, C15:0 = Pentadecanoic, C16:0 = Palmitic, C17:0 = Margaric, C18:0 = Stearic acids.
[2]C14:1 = Myristoleic, C16:1 = Palmitoleic, C17:1 = 10-heptadesenoic, C18:1 = Oleic, C18:2 = Linoleic, C18:3 = Linolenic acids.

Lipolysis is the separation of milk fat into mono- and diglycerides and fatty acids as a result of hydrolytic decomposition with natural lipase of milk or lipases secreted by bacteria contaminated with milk, or with the combined effect of both (Coskun, 2005). All factors that have a positive/negative effect on fungal growth (water activity, NaCl, fat ratio, salting pattern, salt gradient in the mass, pH and pH gradient in the mass, O_2 and CO_2 concentration of the cheese environment, temperature, presence and concentration of antimicrobial substances produced by other microorganisms), antifungal agent(s) produced during cheese production and its concentration, cheese variety, and other conditions during cheese production and ripening) affect lipolysis in cheese (Cantor et al., 2004). Table 2 shows that the ADV values of Çanak cheese vary in a wide range between 1.04 and 46.24 and this is thought to be due to the reasons mentioned above.

Table 4 demonstrate the fatty acid composition of the cheeses. There is no information about fatty acid (C4:0 to C18:1) concentrations in Çanak cheese, which has high lipolysis values. In general, the FFA content of Çanak cheese was lower than other cheese varieties. These results can be attributed to the type and composition of milk used in cheese production, heat treatment applied to milk, production methods, ripening and storage temperatures. Long chain fatty acids found in cheeses are mainly myristic (C14:0), palmitic (C16:0), stearic (C18:0) and oleic (C 18:1) acids. The major fatty acids were palmitic (C16:0) and oleic (C18:1) acids and other predominant fatty acids were myristic (C14:0) and stearic (C18:0) acids. A similar situation was reported by Perotti et al. (2005) for "Reggianito and Parmigiano Reggiano" cheese, Cakır et al. (2009) for Keş cheese and Yılmaz et al. (2005) and Guler and Uraz (2003) for Tulum cheese. Palmitic and oleic acid were found

to be the major FFAs in other hard cheese types (Kondyli and Katsiari, 2001).

One of the important features of the quality of dairy products is the determination of the fatty acid profile (Ivanova et al., 2009). The myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids were found to have the highest values among saturated fatty acids, and oleic acid (C18:1) among unsaturated fatty acids. The oleic acid

(C18:1) and palmitic (C16:0) acid values were found to be the highest in fresh and ripened Örgü cheese samples determined by Turkoglu (2011), similar to the data obtained in our study. In addition to Örgü cheeses, it is stated that the main FFAs in Turkish white cheese are palmitic, oleic, myristic, and stearic acids (Hayaloglu et al., 2008; Ozer et al., 2011).

4. CONCLUSIONS

In this investigation, the chemical and biochemical properties were determined of Çanak cheese produced by the traditional method. The dry matter, protein and fat levels of the cheeses varied in a wide range. These differences are thought to be due to the type of raw milk and composition production techniques and storage conditions. When the nitrogen fraction values of cheese samples were examined, it was concluded that this cheese was exposed to high but low to medium depth proteolysis. Therefore, Çanak cheese take part in class of low quality and less mature cheeses. Since Çanak cheese is usually produced from raw milk, lipase in milk remains in milk and cheese, so it was causing high lipolytic activity. In addition to the factors listed above, the geographical region where the cheese is made and the production season are other factors that affect the lipolysis and proteolysis levels in cheese. In addition, it was determined that stearic and myristic fatty acids were dominant and follow by palmitic and oleic acids in cheese samples.

Author Contributions

Conceptualization:S.S.K.; Investigation:S.S.K., S.K; Material and Methodology: S.S.K., S.K.; Supervision: S.S.K.; Visualization: S.S.K.; Writing-Original Draft: S.S.K., S.K; Writing-review & Editing: S.S.K., S.K; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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