

Full Length Research Paper

The effect of different packaging materials on proteolysis, sensory scores and gross composition of tulum cheese

Songul Cakmakci, Mustafa Gurses* and Engin Gundogdu

Department of Food Engineering, Agricultural Faculty, Ataturk University, 25240 Erzurum, Turkey.

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In this study, tulum cheese was manufactured using raw ewe's milk and was ripened in goat's skin and plastic bags. The effect of ripening materials (skin bag or plastic) on proteolysis was investigated during 120 days of ripening. In addition, sensory scores of the cheeses were assessed at the 90th and 120th days. The gross composition was also determined at the initial stage of ripening. The results showed that, some significant differences were noted between cheeses ripened in goat's skin and plastic bags in terms of gross composition due to the porous structure of skin bag, which causes moisture losses during ripening. Significant differences were observed in proteolysis indices including water, 12% trichloroacetic acid and 5% phosphotungstic acid-soluble nitrogen fractions among the cheese samples during ripening. Proteolysis levels were higher in tulum cheeses ripened in goat's skin.

Key words: Tulum cheese, packaging material, sensory analysis, ripening, proteolysis.

INTRODUCTION

Many cheese varieties which are known only in a restricted geographic area in the world are produced and consumed locally in certain quantities. There are more than 100 varieties of cheese in Turkey; however, three of them (beyaz, kasar and tulum cheeses) are the most popular cheeses. The annual production of Tulum cheese was 10,000 tonnes in 2004 (from the data of Turkish Statistical Institute, Ankara, Turkey). Its production underwent a large increase and this trend has continued over recent years in a number of cheese factories. Tulum cheese has a white or cream color, has high fat content and has a crumbly and semi-hard texture; it is dispersible in the mouth and has a buttery and pungent flavour. The name "tulum" means "goat's or sheep's skin bag", which is used for packaging and ripening (Cakmakci, 2010). The use of tulum for cheese ripening in the past was probably due to the absence of alternative materials for preserving and ripening the cheese. Nowadays, wooden, plastic or earthenware materials have been used for the

ripening of tulum cheese; plastic containers are the most popular for this purpose because it is available easily and cheaply. Also, plastic bags have pleasant appearance preferred by consumers compared with tulum (Cakmakci et al., 2008). The cheese is made in the mountains (plateaus) in Erzincan, Erzurum, Tunceli, Bingol and Elazig and East Anatolian region by the Savak tribe and was originally ripened in tulum for at least three months in caves.

Few studies are available for the effect of different packaging materials on the microbiological and chemical characteristics of tulum cheese. Microbiological qualities, physical and chemical properties of tulum cheeses ripened in goat's skin or polyethylene bags were compared by Guven and Konar (1994a, b). Similarly Sengul et al. (2001) studied the effect of packaging materials (wooden box, goat's skin or polyethylene bags) on the microbiology of tulum cheeses during ripening. Sengul and Cakmakci (1998) used polyethylene bags and wooden materials as alternatives to a tulum and the authors emphasized that the use of different packaging material has an effect on chemical and microbiological qualities of tulum cheese; however, they recommended further studies to determine the best material. Although cheese making from raw milk has continued, the use of

*Corresponding author. E-mail: mgurses@atauni.edu.tr, mustafa_gurses@hotmail.com. Tel: +90-442-2312488. Fax: +90-442-2360958.

pasteurized milk and starter culture for tulum cheese were recommended by Bostan and Ugur (1991) and Sengul et al. (2001).

The stated studies are related to only gross composition and microbiological status (hygienic aspects) of tulum cheese. Some authors (Güven and Konar, 1994a, 1995; Sengul et al., 2001; Hayaloglu et al., 2007) have pointed out that ripening in tulum or polyethylene bags significantly influenced the chemistry and microbiology of tulum cheese during ripening. According to the consumers' preference, the cheese ripened in tulum has a superior quality in comparison to the cheese ripened in polyethylene bags. In addition, Kocak et al. (1996) and Güler and Uraz (2003) studied on the physicochemical properties and proteolysis of tulum cheese samples collected from markets. Furthermore, due to increasing interests in traditional cheeses in Turkey and many other countries, new studies are needed to understand better the role of proteolysis and other profiles (volatile components, microbiological characteristics, etc.). To the authors' knowledge, no attempts were made by researchers to characterize the proteolysis of tulum cheese ripened in different packaging materials.

Major biochemical changes take place during cheese ripening such as fermentation of lactose, degradation of proteins, hydrolysis of lipids and production of volatile aroma compounds. Proteolysis is the most complex and important phenomenon during cheese ripening and it plays a major role in internal bacterially ripened cheeses (Kristoffersen, 1985; Fox et al., 1993; Lyne, 1995). During proteolysis, proteins are converted from insoluble to soluble forms (proteose-peptones, polypeptides, amino acids and ammonia) by the coagulant, indigenous milk proteinases (Ling, 1963) and the proteinases and peptidases from starter and non-starter bacteria (Fox, 1989; Steele and Unlu, 1992; Fox et al., 2000; Cinbas and Kilic, 2006).

In this study, nitrogen fractions were performed to determine the extent of primary proteolysis to compare tulum cheese ripened in goat's skin or polyethylene bags during ripening. In addition, the typical properties of the cheese, the manufacturing procedure, the gross composition and sensory characteristics of tulum cheese are described.

MATERIALS AND METHODS

Cheese making

Tulum cheese was made in Erzurum Province (Ilica-Toprakkale, Erzurum, Turkey). Fresh raw ewe's milk was used for the manufacture. The pH, dry matter, fat and acidity values of raw milk were 6.59, 18.62, 7.3 and 0.25%, respectively. The milk was renneted using home-made calf rennet, obtained from the farmers. The homemade calf rennet was added at a level of 1.0% (v/v) and coagulation took place at 35°C for 2 h. Following coagulation, the coagulum was cut into pieces and transferred into cotton bags for whey drainage. Drainage was carried out at approximately 20°C for 24 h and the curd was pressed by piling the cotton bags on top of each other with regular turning. Stacking of the curd at this tempera-

ture allows the development curd acidity and increases removal of whey. Following this step, the curd was broken into the pieces to the size of peas by hand and dry-salted (3.0% (w/w)), kneaded and transferred into the bags and were piled on top of each other and turned regularly for 10 days. Afterwards, the curd was tightly filled into plastic or goat's skin bags and packaged samples were ripened at 4 to 6°C for 120 days (and for sensory analysis 90 and 120 days). Tulum (goat's skin bags) with a capacity of 10 kg of cheese were obtained from the farmers. Plastic materials (hardened cylindrical barrels), also with a 10 kg capacity, were obtained from local markets. Afterward, the plastic barrels (cheeses A and B) or tulum (cheeses C and D) were tightly filled with curd at 2 different dairies. Cheese making was performed in duplicate and the cheese samples were analyzed every 30 days.

Chemical analysis

Cheeses were analyzed in duplicate during ripening for moisture by the oven drying method at 102°C (IDF, 1982), salt was analyzed by titration with AgNO₃ (Bradley et al., 1993), fat by the Van Gulik method (Ardo and Polychroniadou, 1999) and total nitrogen (TN) contents by the Kjeldahl method (IDF, 1993). The pH of the grated cheese (10 g) slurried by macerating with 20 ml of water was measured using a digital pH meter (Hanna 211, USA). Titratable acidity was determined as g 100 g⁻¹ lactic acid using the procedure described in AOAC (1990).

Water-soluble extracts of the cheese were prepared by the method of Kuchroo and Fox (1982). 20 g of grated cheese were mixed with 40 ml of deionized water and homogenized by an ultra Turrax blender (Ultra-Turrax, TP 18/10, Janke and Kunkel KG, Staufen) for 1 min. The homogenate was held at 40°C for 1 h with frequent stirring. The samples were centrifuged at 3000 × g for 30 min at 4°C. The upper fat layer was thoroughly removed and the supernatant was filtered through white ribbon filter paper No. 589/2 (SandS, Dassel, Germany). The content of water-soluble nitrogen (WSN) was determined by the Kjeldahl method (IDF, 1993) and expressed as % of TN.

The 12% (v/v) trichloroacetic acid soluble nitrogen (TCA-SN) fractions were prepared by using 25 ml of WSN fraction which was added 25 ml 24% (w/v) TCA solution. The suspension was held at room temperature for 2 h and then filtered through white ribbon filter paper no. 589/2 (SandS, Dassel, Germany). The nitrogen content was determined by Kjeldahl method (IDF, 1993) and the TCA-SN was expressed as % of TN.

The 5% (v/v) phosphotungstic acid-soluble nitrogen (PTA-SN) fractions of the cheeses were prepared as follows: A 3.0 ml of a 33% (w/v) PTA solution and 7.0 ml of a 3.95 M H₂SO₄ solution were added to 10 ml of the WSN fraction. The mixture was allowed to stand overnight at 4°C and filtered through white ribbon filter paper no. 589/2 (SandS, Dassel, Germany). The nitrogen content was determined by the Kjeldahl method (IDF, 1993) and the PTA-SN was expressed as % of TN (Jarrett et al., 1982).

Sensory analysis

The sensory properties of the cheeses were assessed at 90 and 120 days of ripening by eight panelists who were familiar with tulum cheese. A scoring test was used by the panel. Coded cheese samples were removed from the refrigerator about 1 h prior to evaluation and kept at room temperature. The cheese samples (approximately 30 g) which were wrapped with aluminum foil were placed on white plates. The cheeses were evaluated according to the method of Bodyfelt et al. (1988) and the sensory criteria were modified considering the characteristics of tulum cheese. Each panel member assessed the cheeses for 10 sensory attributes including appearance, odour, texture, flavour, off-flavour/or unclean,

Table 1. Chemical composition and pH of tulum cheeses ripened in plastic (A and B) and goat's skin (C and D) bags at 30th day of ripening¹.

Cheese	pH	Titrateable acidity ²	Dry matter (%)	Fat (%)	Salt in dry matter (%)
A	4.72 ^c	1.40 ^a	48.04 ^d	28.25 ^a	2.91 ^c
B	4.79 ^b	1.40 ^a	49.28 ^c	28.50 ^a	2.84 ^c
C	4.82 ^b	1.22 ^b	54.00 ^a	28.50 ^a	3.57 ^b
D	4.88 ^a	1.35 ^a	50.72 ^b	28.75 ^a	4.49 ^a
SED ³	0.02	0.04	0.09	0.45	0.05

^{a,b,c,d} Means within a row with no common superscript differed at $p < 0.05$. ¹Presented values are the means of two replicates ²expressed as g lactic acid per 100 g of cheese; ³standard error of difference.

bitter taste, oxidized taste, salty, creamy and overall acceptability. All sensory attributes were recorded on a 1 (poor) to 9 (excellent) point scales. Water and normal bread were also provided to the panel members to cleanse their palates between the samples were assessed.

Statistical analysis

A randomized complete block design which incorporated four treatments (cheeses A, B, C and D), four ripening periods (30, 60, 90 or 120 days) and two blocks (trials) were used to analyze the response variables relating to proteolysis data. ANOVA was performed using a general linear model procedure (SAS, 1995), where the effect of treatment and replicates were estimated for response variables. Duncan's multiple comparison test was used as a guide for pair comparisons of treatment means. The level of significance of differences between treatments was determined at $p < 0.05$.

RESULTS

Gross composition

The gross composition of tulum cheeses ripened in plastic and goat's skin bags are shown in Table 1. Significant differences were observed between the samples in terms of pH, titrateable acidity, dry matter and salt in the dry matter contents ($P < 0.05$); however, fat content in the cheeses did not differ.

Nitrogen fractions

The concentrations of nitrogen fractions are shown in Table 2. The levels of total nitrogen (TN) increased during ripening because the moisture contents of the cheese decreased. The highest levels were close to 2.6% for A and B cheeses and 3.1 to 3.3% for C and D cheeses, respectively, at 120 days of ripening.

Sensory characteristics

Sensory properties of the cheeses are shown in Table 3. The trained panelists evaluated the cheeses after 90 and

120 days of ripening and the cheeses ripened in tulum were awarded the highest scores for appearance, odour, texture, saltiness and overall acceptability, whereas the highest scores for flavour, off-flavour, bitterness, oxidized taste and creaminess were given to cheeses ripened in plastic after 90 days of ripening. After 120 days of ripening, similar trends were observed for all samples; however, the highest flavour scores were obtained in the cheese ripened in a tulum.

DISCUSSION

Use of plastic or goat's skin bag as packaging material in the manufacture of tulum cheese has a significant role in the chemical composition of the cheese. Significant differences were noted between cheeses during ripening ($p < 0.05$). The concentrations of water-soluble nitrogen (WSN) as a percentage of TN increased significantly ($P < 0.05$) during ripening (Table 2). The initial concentrations of WSN in the cheeses ripened in plastic material were higher than those of the cheeses ripened in tulum and the WSN reached 26.82% at 120 days of ripening in C cheese. The levels of WSN were essentially similar in all cheeses. The similar levels of WSN in the cheese can be attributed to the same enzymes produced by NSLAB, fungal flora or home-made rennet extract; they make a considerable contribution to the formation of the WSN. Ripening in different material had no significant effect on the WSN contents of the cheeses. Similarly, Guven and Konar (1994b) and Sengul and Cakmakci (1998) reported that, the ripening in tulum or plastic material did not influence the WSN content of tulum cheeses. The concentrations of 12% trichloroacetic acid-soluble nitrogen (TCA-SN) increased significantly during maturation and the initial levels of TCA-SN were lower in A cheese than in the other cheeses ($P < 0.05$). The greatest increases in the TCA-SN, which corresponded to a final value 10.99% of TN, occurred in C cheese. Differences in the levels of TCA-SN between the cheeses were significant during ripening ($P < 0.05$). The TCA-SN contains only small peptides with a chain length of between 2 and 20 amino acid residues and free amino acids and the amount of TCA-SN increase during ripening due to the action of

Table 2. Nitrogen fractions in tulum cheeses ripened in plastic (A and B) or goat's skin (C and D) bags during ripening¹.

Variable	Day	Cheese			
		A	B	C	D
Total N (%)	30	2.72 ± 0.04 ^{ab,A}	2.75 ± 0.04 ^{a,A}	2.64 ± 0.00 ^{b,C}	2.74 ± 0.02 ^{a,C}
	60	2.70 ± 0.03 ^{a,A}	2.72 ± 0.08 ^{a,A}	2.83 ± 0.03 ^{a,B}	3.05 ± 0.04 ^{b,B}
	90	2.69 ± 0.05 ^{b,A}	2.68 ± 0.03 ^{b,A}	3.05 ± 0.04 ^{a,A}	3.13 ± 0.01 ^{a,B}
	120	2.61 ± 0.04 ^{c,A}	2.62 ± 0.01 ^{c,A}	3.10 ± 0.08 ^{b,A}	3.34 ± 0.02 ^{a,A}
Water-soluble N (% of total N)	30	15.16 ± 0.11 ^{a,D}	14.21 ± 0.49 ^{ab,C}	14.35 ± 0.47 ^{ab,C}	13.75 ± 0.29 ^{b,D}
	60	18.44 ± 0.02 ^{b,C}	19.02 ± 1.28 ^{b,B}	21.20 ± 1.70 ^{a,B}	18.35 ± 0.51 ^{b,C}
	90	22.62 ± 0.44 ^{b,B}	24.21 ± 0.28 ^{a,A}	24.45 ± 0.07 ^{a,A}	22.47 ± 0.50 ^{b,B}
	120	24.80 ± 0.70 ^{b,A}	25.36 ± 0.13 ^{ab,A}	26.82 ± 0.01 ^{a,A}	25.71 ± 0.24 ^{ab,A}
12%TCA-soluble N (% of total N)	30	6.18 ± 0.66 ^{b,B}	6.68 ± 0.13 ^{a,C}	7.16 ± 0.10 ^{a,C}	6.76 ± 0.28 ^{a,C}
	60	7.19 ± 0.35 ^{b,B}	7.88 ± 0.03 ^{b,B}	9.51 ± 0.45 ^{a,B}	7.46 ± 0.47 ^{b,C}
	90	8.49 ± 0.05 ^{c,A}	9.76 ± 0.24 ^{b,A}	10.26 ± 0.26 ^{a,AB}	9.05 ± 0.34 ^{b,B}
	120	9.65 ± 0.09 ^{c,A}	10.26 ± 0.25 ^{b,A}	10.99 ± 0.13 ^{a,A}	10.90 ± 0.11 ^{a,A}
5% phosphotungstic acid-soluble N (% of total N)	30	2.90 ± 0.18 ^{ab,B}	2.40 ± 0.41 ^{b,C}	3.26 ± 0.16 ^{a,B}	2.94 ± 0.02 ^{ab,C}
	60	3.53 ± 0.03 ^{a,B}	3.13 ± 0.04 ^{b,B}	3.41 ± 0.18 ^{a,B}	3.48 ± 0.04 ^{a,B}
	90	3.92 ± 0.23 ^{b,A}	4.03 ± 0.13 ^{b,A}	4.53 ± 0.19 ^{a,A}	4.14 ± 0.01 ^{ab,A}
	120	4.13 ± 0.17 ^{b,A}	4.21 ± 0.06 ^{b,A}	4.64 ± 0.01 ^{a,A}	4.37 ± 0.04 ^{ab,A}

^{a,b,c} Means within a row with no common superscript differed at $p < 0.05$. ^{A,B,C,D} Means within a column with no common superscript differed at $p < 0.05$; ¹presented values are the means of two replicate trials.

Table 3. Sensory scores of tulum cheeses ripened in plastic (A and B) or goat's skin (C and D) bags during ripening¹.

Attribute ²	Day	Cheese			
		A	B	C	D
Appearance	90	8.3	8.5	9.0	9.0
	120	8.0	7.8	7.5	9.0
Odour	90	7.5	7.3	8.0	7.0
	120	8.0	8.3	8.0	8.5
Texture	90	7.3	7.5	9.0	9.0
	120	7.3	7.5	9.0	9.0
Flavour	90	7.5	8.0	7.5	7.3
	120	7.0	8.0	8.5	9
Off-flavour	90	8.0	8.0	8.0	7.0
	120	8.0	7.5	8.0	8.5
Bitterness	90	7.0	7.0	5.0	7.5
	120	8.0	8.0	5.0	6.0
Oxidized taste	90	8.0	9.0	7.5	8.0
	120	8.0	8.0	6.0	5.0

Table 3. Contd.

Saltiness	90	7.8	7.8	8.0	8.3
	120	8.0	7.5	8.0	8.0
Creaminess	90	8.0	7.5	7.5	7.0
	120	8.0	7.5	7.5	7.5
Overall acceptability	90	7.8	8.0	9.0	9.0
	120	7.5	7.8	8.5	8.3

¹ Presented values are the means of two replicate trials; ² all sensory attributes were recorded on a 1 (poor) to 9 (excellent) point scales.

starter or non-starter lactic acid bacteria. The action of *lactococci* and *lactobacilli* in the cheeses was identically reflected in the concentrations of the TCA-SN. The levels of 5% phosphotungstic acid-soluble nitrogen (PTA-SN) ratio increased significantly ($P < 0.05$) and were higher (but not significant) in C cheese than in the other cheeses throughout ripening. PTA-SN contains small peptides with molecular weights below-600 Da and frees amino acids (McSweeney and Fox, 1997). The C cheese had the highest levels of PTA-SN which reached 4.64% as percentage of TN at 120 days of ripening. The ripening period had an effect on the formation of PTA-SN in tulum cheeses. Likely, the lactic acid bacteria which possess nutritional requirements for amino acids are responsible for the formation of PTA-SN in cheese (Macedo and Malcata, 1997).

Sensory scores in the cheeses ripened in tulum or plastic material were not apparently influenced by the use of different ripening material although, the cheeses ripened in tulum received the lowest scores for bitterness and oxidized taste which may be originated from methyl ketones and/or fatty acids. This could be related to the structure of tulum, that is, permeability to oxygen and water. The gross composition was also determined at the initial stage of ripening (30th day).

The results obtained showed that, the ripening of tulum cheese in tulum or plastic material influenced the gross chemical composition determined at the initial stage of ripening. Nitrogen fractions increased during ripening in the cheeses. The proteolysis of the cheeses ripened in tulum was differentiated from the cheeses ripened in plastic. The principal proteolytic agent responsible for primary proteolysis is the coagulant. The gross chemical composition of the cheeses ripened in tulum was clearly differentiated from the cheeses ripened in plastic due to the properties of water and air permeability of tulum. The highest flavour scores were received in the cheese ripened in a tulum. Proteolysis was higher in tulum cheeses ripened in goat's skin.

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