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Effects of packaging materials on some ripening characteristics of Tulum cheese

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Abstract

In this study the effects of different packaging materials on Tulum cheese made with cloth, stomach, animal skin, pot and plastic materials were investigated. Dry matter, ash, titratable acidity, salt, salt in dry matter, fat, total protein, water soluble nitrogen (WSN), trichloroacetic acid soluble nitrogen (TCA-SN), phosphotungstic acid soluble nitrogen (PTA-SN), electrophoretic casein fraction analyses, L^*, a^* and b^* values in colour analyses and sensory characteristics were analysed. Dry matter 54.74-66.37 %, ash 3.93-4.79 %, titratable acidity 1.22-1.80 % as lactic acid, salt 2.48-3.93 %, salt in the dry matter 3.78-6.20 %, fat 21-43 %, total protein 17.20-23.16 %, WSN ratio 5.58-16.81 %, TCA-SN ratio 4.33-13.59 %, PTA-SN ratio 2.06-5.12 %, β-CN ratio 31.01-52.16 %, α_{s1}-CN ratio 33.52-47.84 %, and in colour measurements L^*, a^* and b^* values ranged from 82.18-84.39, 0.93-1.72 and 8.41-16.51, respectively. Accordingly it was clarified that the use of different packaging materials affected the composition of Tulum cheese and was related to from the cheese origin as well.

Key words: Tulum cheese, packaging material, ripening characteristics

Introduction

Tulum cheese can be produced from whole, semi skimmed or non-fat sheep's, goat's, cow's or buffalo's milk or their mixture. Traditionally, Tulum cheese is made from non-fat milk remaining after butter production and sometimes yoghurt can be added to give flavour (Erdoğan et al., 2003; Gürses and Erdoğan, 2006). Tulum cheese is characterized as white or cream coloured, containing a high dry matter and protein ratio, not easily crashed, medium-hard, homogenous textured cheese with a characteristic taste which melts in the mouth revealing an easily felt original butter taste (Kurt et al., 1991). Tulum cheese is often produced from raw milk by different processing methods depending on the local dairies, farmers and production areas (Kirdar et al., 2015).

Generally, proteolysis of cheese is influenced by several factors including chymosin, pH, moisture levels in curds, salt content, storing temperature and time. Proteolysis is probably the most important biochemical event, having a major impact on flavor and texture of most cheese varieties. Proteolysis contributes to the softening of cheese texture during ripening due to hydrolysis of the casein matrix of the cure and through a decrease in the water activity of the cure due to changes in water binding by the new carboxylic acid and amino groups formed by hydrolysis (McSweeney, 2004). The proteinases from starter and nonstarter microorganisms can also be active in degradation of cheese proteins and peptides. Lactic acid bacteria are weakly proteolytic, but possess a very comprehensive range of proteinases/peptidases capable of hydrolysing casein-derived peptides to small peptides and amino acids (Hayaloğlu et al.,

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2004). Proteolysis and lipolysis are two primary processes in cheese ripening with a variety of chemical, physical, and microbiological changes taking place usually under controlled environmental conditions. Proteolysis in cheese involves a complex and dynamic series of events. In order to better understand the development of proteolysis in cheeses it is necessary to investigate the nitrogen fractions formed during ripening (McSweeney and Fox, 1993). Using that traditional method it is very difficult to produce a high quality product due to a lack of standardization of most manufacturing steps, especially the ripening conditions.

Studies on Tulum cheese are limited, although some research was focused on this variety in the past decade. These studies concentrated on the chemical and microbiological properties of Tulum cheeses sold on Turkish markets. Only few studies dealing with the effect of different packaging materials on the microbiological and chemical characteristics of Tulum cheese are available. The microbiological quality of Tulum cheeses ripened in goat's skin and polyethylene packaging materials were compared by Güven and Konar (1994). Similarly, Şengül et al. (2001) studied the effect of packaging materials (wooden box, goat's skin, stomach, class or polyethylene packaging materials) on the properties of Tulum cheese during ripening. The use of different types of milk (cow's, ewe's or goat's milk) in the manufacture of Tulum cheese was compared by Güven et al. (1995), who reported that the type of milk significantly influenced the microbiology of Tulum cheese during 210 days of ripening. Şengül and Çakmakçı (1998) used polyethylene packaging materials and wooden materials as alternatives and emphasized that the use of different packaging materials affected the chemical and microbiological quality of Tulum cheese. However, the authors recommended further studies to determine the optimal packaging material. Although raw milk continued to be used for Tulum cheese production, Bostan and Uğur (1991) and Şengül et al. (2001) recommended the use of pasteurized milk and a starter culture. Under the term "Tulum cheese" in Turkey, Erzincan Savak Tulum cheese is implied. Thus, the term "Tulum cheese" is used throughout the text to refer to Erzincan Savak Tulum cheese. Goat's skin packaging materials are stronger than sheep's skin packaging materials and Tulum cheeses are permeable to water and air because of their porous structure. In the past, the tulum was probably used for cheese packaging because of the absence of alternative packaging materials for preserving and ripening the cheese. Nowadays, hardened plastic barrels are usually used for ripening the Tulum cheese. Since there is no standard production method, the chemical structure of the cheese is quite variable. In the past years mainly animal skin was used for packaging but it since it is expensive and due to some difficulties related to storage, easy access bins and cloth materials were also often used. The aim of this study was to investigate the physical and chemical composition of Tulum cheeses ripened in different packaging materials and determine the extent of primary and secondary proteolysis by electrophoreses.

Materials and methods

Tulum cheeses ripened in cloth, skin, pot, stomach and plastic were obtained from grocery stores and markets. In total, 20 samples were examined among which 9 were made with cloth, 2 in stomach, 4 in animal skin, 3 in pot and 2 were packaged into plastic packaging materials.

Physicochemical analyses

The cheese samples were analysed for acidity (lactic acid %) with 0.1 N NaOH and pH with a pH meter (Ohaus, Starter 3100), total nitrogen content using micro Kjeldahl digestion and distillation units (Velp Scientifica, Italy), salt content using the Mohr method and ash content using a muffle furnace at 550 °C for 24 h. Fat content of cheeses was measured by Gerber method; dry matter content by weight difference using a drying oven (Nüve, Ankara, Turkey).

Nitrogen fractions of Tulum cheeses were determined using the method described by Kuchroo and Fox (1982). For preparation of water soluble extracts, 20 g of grated cheese was mixed with 40 mL of deionized water and homogenized with a homogenizer (WiseTis_HG-15D, Daihan, Korea) for 1 min. Then it was centrifuged at 3000x*g* for 30 min at 4 °C. The fatty layer was removed and the supernatant was filtered through Whatman 42 paper. To determine the content of water-soluble

nitrogen (WSN), 10 mL of filtrate was taken and the Kjeldahl method was performed (IDF, 1993). The 12 % trichloroacetic acid-soluble nitrogen (TCA-SN) fractions were prepared by mixing 25 mL of the WSN fraction with 25 mL of 24 % (w/v) TCA solution. The mixture was held at room temperature for 2 h and then filtered through Whatman 42 paper. The 5 % phosphotungstic acid-soluble nitrogen (PTA-SN) fractions of the cheeses were prepared as follows: 3.0 mL of a 33 % (w/v) PTA solution and 7.0 mL of a 3.95 M H_2SO_4 solution were added to 10 mL of the WSN fraction. The mixture was kept at 4 °C overnight and filtered through Whatman 42 paper. The nitrogen contents were determined using the Kjeldahl method (IDF, 1993) and WSN, 12 % TCA and 5 % PTA values were expressed as the percentage of the total nitrogen content of the cheese (Jarrett et al., 1982).

Preparation of cheese proteins and electrophoresis

The electrophoretic analysis of protein patterns was conducted with the method given by Creamer (1991) with some modifications (Tarakçi et al., 2004). Sample buffers (pH=8.4) consisted of EDTA 0.0925 g, Tris 1.08 g, boric acid 0.55 g and urea 36.0 g were dissolved in 100 mL. A cheese sample (0.5 g) was homogenized in 25 mL sample buffer, and then centrifuged at 3000x*g* for 30 min; 2 mL of central portion was transferred into a small tube and stored at -20 °C. Casein standard was prepared using sodium caseinate obtained from cow milk by dissolving in urea. The resolving gel buffer was prepared with Tris 9.2 g, Urea 54 g and solved in 100 mL of distilled water, filled up to 200 mL (pH 8.8); 15 mL of 30 % acrylamide/bis-acrylamide (37.5:1) solution, 35 mL of separating gel buffer and 15 μL TEMED were used for resolving gel solution. After degassing, 70 μL of ammonium persulphate (APS) solution (0.1 mg/L) was added and immediately poured into gel apparatus. A volume of 0.5 mL of distilled water was placed on gel solution. After polymerization of the resolving gel, water was removed and the comb was inserted. Stacking gel solution was prepared with Tris 1.08 g, urea 36.0 g, boric acid 0.55 g, EDTA 0.092 g and 5 g of acrylamide/bis-acrylamide (37.5:1), and dissolved in 100 mL (pH=8.4); 15 mL of this solution was taken, and 15 μL of TEMED was added. After degassing, 50 μL of APS was added. This solution was poured on the previous gel, and after polymerization, the comb was removed. Finally the gel was placed in the electrophoresis unit (Wealtec Bioscience Co., Ltd, Taiwan). 3 % mercaptoethanol and 2 % bromophenol blue (0.1 %) were added to frozen cheese samples; 40 μL solution from cheese samples were taken and placed into the slots. Stock chamber buffer was prepared with EDTA 3.7 g, Tris 43.2 g and boric acid 22 g and dissolved in 1 L (pH=8.4), and the buffer was diluted with distilled water (1:4) before use. The conditions for performing electrophoresis were (25±1 °C) maximum 280 V, 70 mA and 20 W. Protein bands were stained with Coomassie BBR-250 solution (1 g of Coomassie brilliant blue R-250, 500 mL of isopropanol and 200 mL of glacial acetic acid, and dissolved in 2 L). Then, bands were destained with destaining solution (200 mL of isopropanol and 200 mL acetic acid dissolved in 2 L).

The gels were scanned with a scanner and pictures were transferred to the computer. Electrophoretograms by a scanner (EPSON III) were used to quantify bands using TotalLab Quant densitometry software (TotalLab Limited, Newcastle, UK), α_{s1}-CN and β-CN were quantitatively determined by integration of peak volumes.

Colour characteristics analyses

Colour measurements were performed using a colorimeter (Minolta Chroma Meter, CR-400, Osaka, Japan). The L^*, a^* and b^* colour measurements were determined according to the CIELab colour space system, where L* corresponded to light/dark chromaticity (changing from 0 % dark to 100 % light), a^* to green/red chromaticity (changing from -60 % green to 60 % red) and b^* to blue/yellow chromaticity (changing from -60 % blue to 60 % yellow). Three readings were taken for each sample and arithmetic means were calculated.

Sensory analysis

Sensory assessment of Tulum cheeses was carried out by a six-member panel of the University staff selected on the basis of interest and experience in sensory evaluation of Tulum cheeses. Prior

to assessment, each cheese was cut into 10 g cubes, equilibrated to room temperature (20 °C) after laying for 4 h at room temperature and served to the panelists randomly. The overall sensory quality was assessed using a scaling method (1-10 points), where 1 reflected a very bad and 10 a very good score for appearance and colour, body and texture, flavour and acceptability. Panel members were also instructed to report any defects in colour and appearance (wet, dry, cracks), texture (hard, soft, pasty, crumbly, grainy), odour, flavour (rancid, salty, bitter, sour) and thw overall acceptability as well. Water was provided for mouth rinsing between evaluations of the samples (Tarakçı et al., 2011).

Statistical analysis

Statistical analysis was performed using the Minitab software program (Release 16.1; Minitab, 2010). Data was analysed using the one-way analysis of variance (ANOVA) and Fisher's multiple range tests. The significance levels of $p<0.05$ were used for statistical differences.

Results and discussion

Physicochemical properties in Tulum cheeses

Dry matter, acidity, salt, salt in dry matter, total protein, WSN, TCA-SN and PTA-SN values for the analysed cheese samples are given in Table 1. Dry matter contents of cloth cheeses were significantly ($p<0.05$) higher than those of pot cheese and were followed by stomach, skin and plastic cheeses, respectively. The samples in cloth packaging had higher amounts of dry matter (66.37 %) but the lowest value of the total protein content (17.20 %) and acidity (1.22 %). Such findings could be explained by the fact that cloth packages probably transferred water through the cloth which lead to protein losses by water. Bayar and Özrenk (2011) indicated that dry matter content was high in the Tulum cheeses packaged in skin (57.87 %) followed by cloth (54.92 %) and plastic (53.83 %) packaged Tulum cheeses. Çakmakçı et al. (2011) determined that goat's skin packaged Tulum cheese had 54.00 % dry matter while plastic packaged Tulum cheeses had a dry matter of 48.04 %. Fat content of the cheese

Table 1. Chemical composition of Tulum cheeses ripened in cloth, stomach, animal skin, pot and plastic packaging materials

Properties	Packaging material types				
	Cloth	Stomach	Skin	Pot	Plastic
Dry matter (%)	66.37±2.06^{a}	61.36±7.49^{a}	61.96±1.31^{a}	65.18±9.42^{a}	54.74±3.47^{a}
Ash (%)	3.93±0.10^{a}	4.01±0.14^{a}	4.63±0.11^{a}	4.32±0.49^{a}	4.79±1.16^{a}
Titratable acidity[1]	1.22±0.11^{a}	1.26±0.18^{a}	1.62±0.07^{a}	1.38±0.22^{a}	1.80±0.54^{a}
pH	5.50±0.07^{a}	5.23±0.30^{ab}	5.09±0.03^{b}	5.12±0.11^{b}	4.73±0.35^{b}
Salt (%)	2.48±0.10^{c}	2.98±0.18^{b}	3.83±0.13^{a}	3.93±0.14^{ab}	3.25±0.25^{b}
Salt in dry matter (%)	3.78±0.21^{c}	4.90±0.31^{b}	6.20±0.32^{a}	5.35±0.52^{ab}	5.05±0.17^{ab}
Fat (%)	40.33±1.60^{a}	33.50±0.50^{ab}	33.25±0.63^{ab}	43.83±8.61^{a}	21.00±3.00^{b}
Total protein (%)	17.20±1.15^{b}	21.02±0.42^{ab}	23.16±2.15^{a}	18.59±1.68^{ab}	21.24±3.49^{ab}
WSN/TN(%)	9.34±1.01^{b}	5.58±0.15^{b}	16.57±1.63^{a}	11.56±2.97^{ab}	16.81±2.71^{a}
TCA-SN/TN (%)	5.98±0.71^{b}	4.33±0.21^{b}	11.87±1.19^{a}	8.32±3.07^{ab}	13.59±2.67^{a}
PTA-SN/TN (%)	4.47±1.28^{a}	2.06±0.32^{a}	3.83±0.09^{a}	3.55±1.11^{a}	5.12±0.45^{a}

Values with different letters within the same column represent significant differences ($p<0.05$)
[1]Titratable acidity expressed as grams of lactic acid/100 grams of cheese
WSN: Water Soluble Nitrogen, TCA-SN/TN: Trichloroacetic Acid Soluble Nitrogen,
PTA-SN/TN: Phosphotungstic Acid Soluble Nitrogen

samples changed similarly to the dry matter contents. The observed fat contents were in the range of 21.00 % and 43.83 % while the highest fat content was detected in samples of pot cheeses.

The brine phenomenon and the effect of increasing the ionic strength on the structure of the casein matrix could be explained by chemical reactions occurring during brining. More precisely, when a cheese mould is placed into the brine, there is a net movement of Na^+ and Cl^- ions from the brine into the cheese due to osmotic pressure difference between the cheese structure and the brine. Moisture in cheese diffuses out through the cheese matrix until the osmotic pressure becomes equal. The migration of salt or water is impeded by the casein matrix due to its narrow pores. The loss of water is about twice higher than the rate of NaCl entering, which is in proportion to their molecular sizes (Guinee and Fox, 1993; Guinee, 2004; Güven et al., 2006). In the present study it was observed that salt content of pot cheeses was higher than in all other analysed types of Tulum cheese. Çakmakçı et al. (2011) concluded that skin packaged Tulum cheeses (4.49 % in dry matter) had more salt content than plastic packaging materials (2.91 % in dry matter) Tulum cheeses. Similarly Bayar and Özrenk (2011) demonstrated the salt content of skin, plastic and cloth packaged cheeses were in descending order 3.99 %, 3.83 % and 3.79 %, respectively. Analysis showed that cloth packaged Tulum cheeses had the lowest ash content while the plastic packaged Tulum cheeses had the highest ash content (Table 1). Ceylan et al. (2007) indicated that the ash contents of skin, ceramic pot and plastic packaged Tulum cheeses were 7.23 %, 6.75 % and 6.04 %, respectively.

Titratable acidity of plastic cheeses was higher than skin cheeses followed by pot, stomach and cloth cheese. All of the samples had a titratable acidity expressed as lactic acid below 3.0 %, as was specified in the regulation for Tulum cheese in Turkish standards (Anonymous, 1995). There was no statistically significant difference in the acidity values within the analysed cheese samples ($p>0.05$). Samples in plastic containers had higher amounts of titratable acidity (1.80 %) but lowest amount of dry matter (54.74 %). Hayaloğlu et al. (2007) indicated that after 150 days of ripening plastic packaged Tulum cheeses had a titratable acidity of 0.63 % and skin packaged Tulum cheeses had 0.59 % and also Çakmakçı et al. (2011) represented that plastic packaged Tulum cheeses had 1.40 % and skin packaged Tulum cheeses had 1.22 % titratable acidity. In another study it was expressed that Tulum cheeses obtained from grocery stores and markets had a titratable acidity of 1.07 % (Morul and İşleyici, 2012). Acid development is a very important criterion in the manufacture of cheeses ripened under brine due to the inhibitory effect of lactic acid on undesirable microorganisms, and curd stability during brining (Bintsis and Papademas, 2002; Hayaloğlu et al., 2005). Titratable acidity increases and pH decreases due to the production of organic acids (primarily lactic acid), whereat lactic acid bacteria are mainly responsible for most of the sugar fermentation (Göncü and Alpkent, 2005). It was observed that skin packaged Tulum cheeses had the highest pH value while the Tulum cheeses ripened in plastic packaging materials had the lowest pH value (Table 1). These findings are in good agreement with the study carried out by Hayaloğlu et al., (2007). Çakmakçı et al. (2011) pointed out that pH of skin packaged Tulum cheeses (pH 4.88) were higher than plastic packaged Tulum cheeses (pH 4.72). Bayar and Özrenk (2011) presented that cloth packaged Tulum cheeses (pH 5.12) had higher pH value than skin packaged Tulum cheeses (pH 5.12).

Protein content of stomach ripened cheeses was 21.02 %, water-soluble protein and maturation index 5.58 %, salt 2.98 % and salt in dry matter value was 4.90 %. According to the examination of cheese samples it can be said that there was no relationship between protein content and dry matter content. Although the highest protein content was determined in the cheese made with skin, lowest protein content was in cloth cheese samples and there was significant difference in terms of protein content of cheeses ($p<0.05$). Bayar and Özrenk (2011) found that protein content of cloth, skin and plastic packaged Tulum cheeses were 24.31 %, 22.74 % and 21.67 % respectively. Also Hayaloğlu et al. (2007) demonstrated that skin packaged Tulum cheeses (21.31 %) had more protein content than plastic packaged Tulum cheeses (16.71%) and Çakmakçı et al. (2011) found these values as 21.31 % and 16.65 %, respectively.

Nitrogen fractions in Tulum cheeses

The water-soluble nitrogen (WSN): Proteolysis in the cheeses throughout ripening was evaluated by analysing the proteolytic indices, including water-soluble nitrogen (WSN), trichloroacetic acid-soluble nitrogen (TCA-SN) and phosphotungstic acid-soluble nitrogen (PTA-SN). WSN resulting from the decomposition of proteins, which is an indicator of ripeness, contains high, medium and low peptides, free amino acids and nitrogen compounds produced mainly from residual rennet or proteinases present in the curd such as plasmin or proteases from the cheese microflora (McSweeney and Fox, 1997; Sousa et al., 2001; Şengül et al., 2014). Although the highest WSN values were determined in the cheese made with plastic material, lowest WSN values in stomach cheese samples and there was significant difference in terms of WSN values of cheeses ($p<0.05$). The WSN fraction contains whey proteins, protease-peptone (soluble proteins, peptides, amino acids, amines, urea, ammonia), low molecular weight peptides (<15000 Dalton molecular mass) derived from casein hydrolysis (Güven et al., 2006). The soluble nitrogen compounds are mainly produced by the action of the coagulant (Roseiro et al., 2003). This indicates that the starter organisms most probably do not make a direct contribution to the WSN content in cheese (Madkor et al., 2000; Hayaloğlu et al., 2005; Cinbaş and Kılıç, 2006).

Plastic (16.81 %) and skin (16.57 %) packaged cheeses showed a high ripening degree, while stomach (5.58 %) and cloth (9.34 %) ripened cheeses showed very low levels (Table 1). Such findings are in agreement with the results of Çakmakçı et al. (2011), who reported that there was no significant difference between the WSN contents of skin (26.82 %) and plastic (24.80 %) packaged Tulum cheeses. Şengül et al. (2014) found 18 % WSN content of Tulum cheeses made with calf rennet. Ceylan et al. (2007) represented that WSN contents of skin cheeses (32.89 %) were higher than ceramic pot (30.83 %) followed by plastic (27.35 %) and these results showed considerable similarity to our findings.

The greatest part of the water- soluble nitrogen (>64 %) is consisted of nitrogen that is soluble in 12 % *trichloroacetic acid* (TCA), which corresponds to medium and small-sized (600-15.000 Dalton molecular mass) peptides with 2-22 amino acids (Moatsou et al., 2002; Roseiro et al., 2003). It was reported that rennet, bacterial proteinases and peptidases are responsible for the formation of some 12 % trichloroacetic acid-soluble nitrogen (TCA-SN) (Fox et al., 1993). TCA-SN ratios of plastic cheeses were higher than skin cheeses followed by pot, cloth and stomach cheeses, respectively (Table 1). Çakmakçı et al. (2011) reported that there was no significant difference between plastic (9.65 %) and skin (10.99 %) packaged Tulum

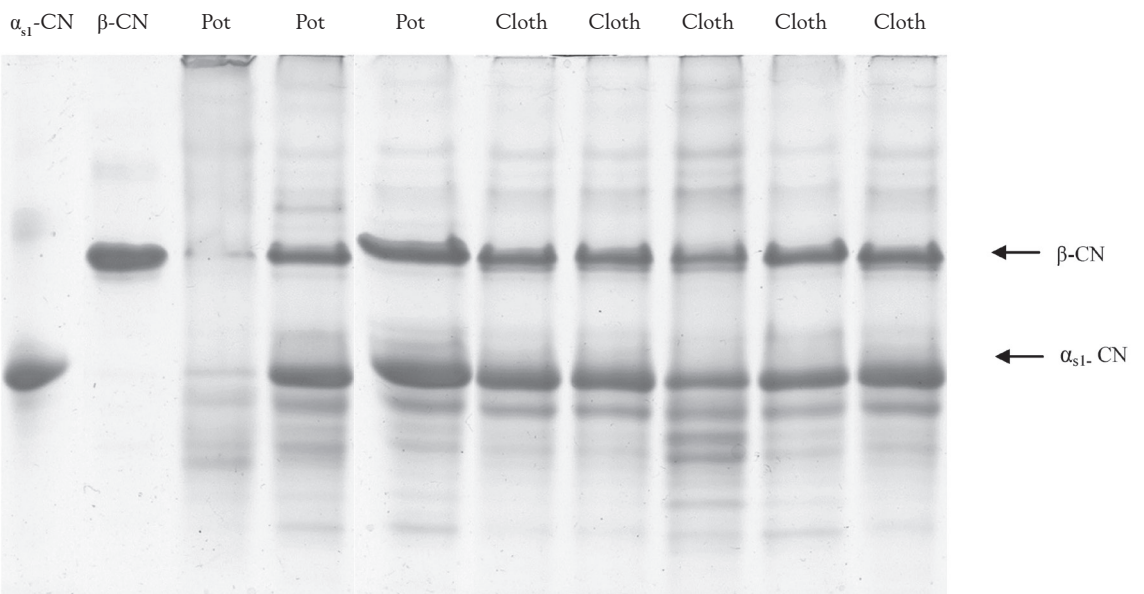

Figure 1. Ure-PAGE patterns for the caseins of pot and cloth packaged Tulum cheeses

cheeses in terms of TCA-SN contents after 120 days of ripening and, it shows considerable similarity with our results. Şengül et al. (2014) also indicated that Tulum cheeses made with calf rennet had a TCN-SN content of about 10 % after 90 days of ripening.

As proteolysis continues in cheese, relatively larger molecular weight nitrogenous fractions brought about by chymosin and cell-wall proteinases are further degraded to smaller molecular weight compounds by peptidases. The formation of amino acids and peptides with molecular weight less than 600 Dalton, which are soluble in 5 % phosphotungstic acid (PTA), is strongly related to the age and flavour intensity of cheese (Moatsou et al., 2002). The phosphotungstic acid-soluble nitrogen (PTA-SN) values, which occur as a result of peptidolysis are commonly used to determine the type and the extent of proteolysis (Moatsou et al., 2002; Roseiro et al., 2003). It was observed that plastic packaged Tulum cheeses had the highest PTA-SN while the Tulum cheeses ripened in stomach packaging materials had the lowest PTA-SN (Table 1) and no significant differences were found for the PTA-SN contents ($p>0.05$) of the cheeses. The slight differences in PTA-SN contents can be linked to the lower moisture contents in the cheeses ripened in packages. Çakmakçı et al. (2011) stated that there was no significant difference between skin (4.64 %) and plastic (4.13 %) packaged Tulum cheeses.

Electrophoretical analysis in Tulum cheeses

The ratios of β-CN and α_{s1}-CN for Tulum cheeses are presented in Table 2. As it can be seen there is no statistical difference between α_{s1}-CN ratios ($p>0.05$) while the ratio of β-CN was higher in the cheeses packaged in pot and plastic. The extent of degradation of major caseins and their hydrolysis products was determined by the Urea-PAGE. Figure 1 and Figure 2 shows the electrophoretical analyses of the α_{s1}-CN and β-CN fractions and other breakdown products of experimental cheeses. α_{s1}-CN was observed with a high intensity at the end of ripening. The highest density of α_{s1}-CN was found in cheese produced in plastic package. The highest α_{s1}-CN degradation was observed in cheeses made with skin packaging material followed by pot and plastic. Such results may be attributed to the high drop of pH for skin, pot and plastic packaged cheeses. Another reason of higher degradation rate of α_{s1}-CN in skin, pot and plastic packaged cheeses may be the enhanced activity of nonstarter bacteria, depending on the decrease of pH in these cheeses. Different findings about α_{s1}-CN degradation rate in literature are possible because of the differences of milk used, manufacturing procedure and ripening conditions (Fox et al., 1993).

It was established that β-CN was more resistant to proteolysis, especially in the cheese matrix, either by calf rennet or starter enzymes, owing to

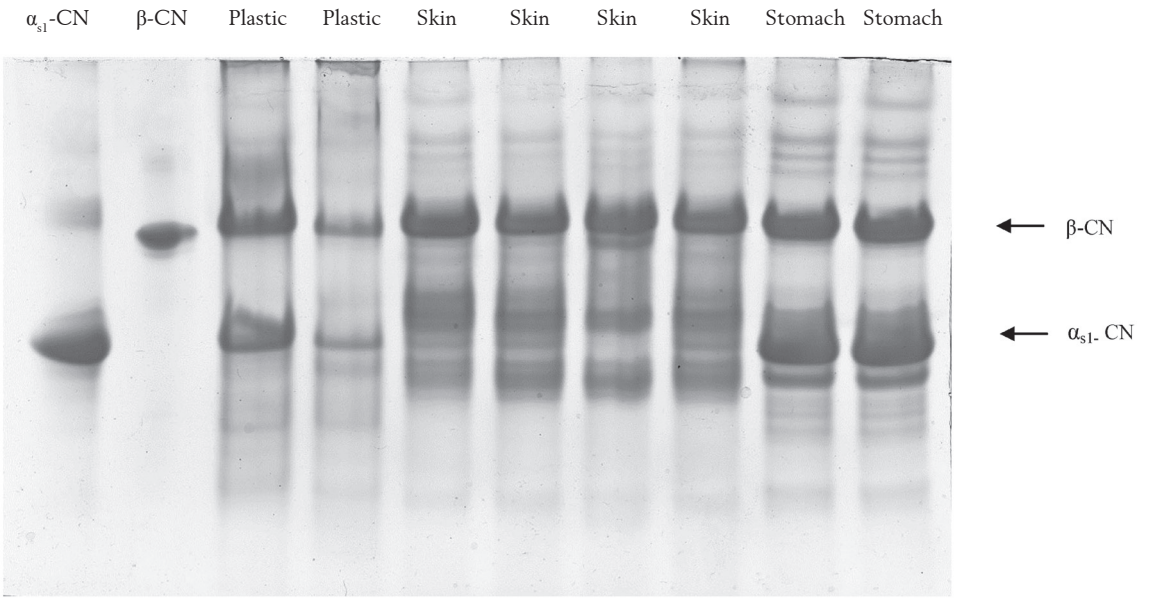

Figure 2. Ure-PAGE patterns for the caseins of plastic, skin and stomach packaged Tulum cheeses

its structure and particularly, its tendency to associate. Plasmin, an alkaline milk protease, plays a major role in proteolysis (Stepaniak, 2004). Plasmin dissociates from casein micelles as the pH decreased (Yazıcı and Dervişoğlu, 2003) and its activity increases in the cheese (Fox et al., 1993). Breakdown of β-casein in cheeses ripened in plastic and pot materials was higher than others. The progress of the β-CN degradation in the present study was in agreement with the findings of Poveda et al. (2003), White et al. (2003) and Hayaloğlu et al. (2005). Metin (2014) stated that the percentage of α_s-CN was 43 % and β-CN 27 % in cow milk. It can be seen from the Table 2 that the percentage of α_{s1}-CN was less than β-CN in the cheeses of skin, pot and plastic. High degradation of α_{s1}-caseins was observed which implies a high ripening degree of these samples. Such results were very similar to WSN and TCA-SN contents of cheese samples shown in Table 1. Some weak bands were also observed in the region of γ-CN in stomach packaged cheeses. Densitometric evaluations of the electrophoretograms implied significant difference for β-CN concentrations between stomach and plastic packaged Tulum cheeses (Table 2). Şengül et al. (2014) reported that breakdown of α_{s1}-CN and β-CN was higher in Tulum cheeses made with of *Aspergillus niger* and *Rhizomucor miehei* than in cheeses made with calf rennet.

Table 2. β-CN and α_{s1}-CN Tulum cheeses ripened in cloth, stomach, animal skin, pot and plastic packaging materials

Casein	Packaging material types					
	Cloth	Stomach	Skin	Pot	Plastic	Mean
β-CN (%)	36.18±2.05^{ab}	31.01±0.69^{b}	39.10±4.81^{ab}	43.32±9.96^{ab}	52.16±0.97^{a}	49.60±2.52^{A}
α_{s1}-CN (%)	40.18±4.65^{a}	45.87±0.56^{a}	34.62±2.91^{a}	40.50±3.24^{a}	47.84±0.97^{a}	40.69±2.07^{A}

Means within same row followed by different lowercase letters represent significant differences ($p<0.05$)
Means within same column followed by different uppercase letters represent significant differences ($p<0.05$)

Table 3. Color values of Tulum cheeses ripened in cloth, stomach, animal skin, pot and plastic packaging materials

Variable	Packaging material types				
	Cloth	Stomach	Skin	Pot	Plastic
*L**	83.62±0.67^{a}	83.13±2.74^{a}	82.18±0.95^{a}	83.58±0.57^{a}	84.39±2.17^{a}
*a**	0.93±0.19^{a}	1.72±0.13^{a}	0.74±0.32^{a}	1.31±0.47^{a}	1.45±0.82^{a}
*b**	16.51±1.30^{a}	8.41±1.33^{b}	9.73±1.04^{b}	9.58±0.62^{b}	9.96±2.94^{b}

Means with different letters within the same column represent significant differences ($p<0.05$)

Table 4. Sensory characteristics of Tulum cheeses ripened in cloth, stomach, animal skin, pot and plastic packaging materials

Variable	Packaging material types				
	Cloth	Stomach	Skin	Pot	Plastic
Color and Appearance	7.83±0.83^{a}	6.67±0.62^{a}	8.33±0.56^{a}	7.17±1.01^{a}	7.83±0.48^{a}
Texture	8.00±0.93^{a}	7.33±0.72^{a}	7.67±0.76^{a}	7.00±0.93^{a}	8.00±0.37^{a}
Odour	7.83±0.87^{a}	6.83±0.95^{a}	7.00±0.63^{a}	8.00±1.13^{a}	7.50±0.43^{a}
Flavour	9.50±0.29^{a}	8.25±0.48^{ab}	8.00±0.58^{b}	9.25±0.48^{ab}	8.25±0.25^{ab}
Overall acceptability	8.17±0.95^{a}	7.33±0.80^{a}	7.50±0.67^{a}	7.33±1.02^{a}	7.50±0.76^{a}

Values with different letters within the same column represent significant differences ($p<0.05$)

Colour measurements in Tulum cheeses

Colour is one of the most important visual attributes in food. Quality assessment of colour may include analytical or sensory measures. L^* value is an estimation of food whiteness while a^* value is an indicator of redness. The b^* value is a measure of yellowness to blueness of the product. L^*, a^* and b^* values are shown in Table 3. According to the performed colour measurements no significant differences were noted among the experimental cheeses regarding the L^* and a^* values ($p>0.05$). However, plastic cheese had higher L^* values than cloth, pot, stomach and skin cheeses packaging material did not significantly affect the L^* value ($p>0.05$). Material types significantly ($p<0.05$) affected on the yellowness. Cloth type cheese samples showed the highest b^* values while the lowest values were detected for stomach cheese samples. Such results were in good agreement with findings of Dufossé et al. (2005) for soft cheeses. Tarakçı et al. (2011) found L^*, a^*, and b^* values after 90 days ripening as 85.80, -2.89 and 27.53, respectively. In another research L^* values ranged from 82.71 to 88.80 and b^* values ranged from 7.90 to 14.19 on tulum cheeses produced from raw goat milk (Sert et al., 2014).

Sensory evaluation in Tulum cheeses

Sensory analysis is considered as an important technique to determine product quality. The first impression offood is usually visual, and a major part of the consumer's acceptance of food depends on its appearance. Often if the appearance is unattractive, a potential consumer may never experience other attributes such as flavor and texture. The appearance of foodstuffs is the only permitted way to evaluate food products. With respect to that, colour is a clue for many qualities of food such as flavour, naturalness or maturity, and drives consumers' choices. Sensory characteristics of the Tulum cheeses are presented in Table 4. However, there was no significant differences among cheese samples panelists assigned high scores on skin packaged Tulum cheeses. Cloth packaged Tulum cheeses had high scores regarding as texture, flavour and overall acceptability. Panelists assigned lowest odour scores to samples prepared with Stomach package. As similar to the present study Çakmakçı et al. (2011) indicated that skin packaged Tulum cheeses had higher scores of appearance than plastic cheeses. Ceylan et al. (2007) found that skin packaged Tulum cheeses had the highest scores of texture followed by ceramic pot and plastic while ceramic pot remarked as the highest values of flavour, odour and overall acceptability followed by skin and plastic packaging materials. Bayar and Özrenk (2011) demonstrated that Tulum cheeses packaged in skin had higher colour scores than plastic and cloth packaged Tulum cheeses.

Conclusions

Packaging materials used for ripening medium lead to have large differences in the psychochemical composition of Tulum cheeses. However, ripening was found to be extremely effective regarding the quality of the overall cheese manufacturing techniques and storage conditions. The selling points where Tulum cheese samples were submitted to consumption and package materials affected the quality of the product. The use of different packaging materials in the manufacture of Tulum cheese showed to significantly influence the ripening process of Tulum cheese. The ripening index values of the cheeses ripened in skin and plastic materials were higher than those ripened in cloth, animal stomach and pot materials. In addition, it was observed that the quality aspects of Tulum cheese were firmly related to nonstandard producing techniques, ripening and different storing places. It could be concluded that all of the analysed Tulum cheese samples had different compositions because they originated from different producers. In order to obtain a standard product all the producers should use same process method and packaging material samples.

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Utjecaj različitih načina pakiranja na neke parametre zrenja sira Tulum

Sažetak

U ovom je radu istraživan utjecaj različitih načina pakiranja (sirna marama, mišina, životinjska koža, tegla i plastična ambalaža) na Tulum sir. U svim ispitivanim uzorcima sira određivan je udio suhe tvari, pepeo, titracijska kiselost, udio soli i udio soli u suhoj tvari, udio masti, udio proteina, količina dušika topljivog u vodi (WSN), količina dušika topljivog u trikloroctenoj kiselini (TCA-SN), količina dušika topljivog u fosfor-tungastičnoj kiselini (PTA), te je načinjena elektroforetska analiza kazeinskih frakcija kao i analiza boje izražene preko L^*, a^* i b^* vrijednosti. Udio suhe tvari kretao se u rasponu 54,74-66,37 %, pepeo 3,93-4,79 %, titracijska kiselost izražena kao % mliječne kiseline 1,22-1,80 %, udio soli 2,48-3,93 %, udio soli u suhoj tvari 3,78-6,20 %, masti 21-43 %, udio proteina 17,20-23,16 %, količina WSN 5,58-16,81 %, količina TCA-SN 4,33-13,59 %, količina PTA-SN 2,06-5,12 %, omjer frakcija β-CN 31,01-52,16 %, omjer frakcija α_{s1}-CN 33,52-47,84 %. Analize čimbenika boje rezultirale su vrijednostima L*, a* i b* u rasponu 82,18-84,39, 0,93-1,72 i 8,41-16,51. Sukladno tomu, može se zaključiti da je upotreba različitih ambalažnih materijala utjecala na sastav Tulum sira, a koji je također bio usko vezan uz podrijetlo sira.

Ključne riječi: Tulum sir, ambalažni materijal, karakteristike zrenja

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