The compositional and biochemical characteristics of traditional Diyarbakir Örgü cheese during the ripening period

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Abstract: This study was conducted to determine the changes in compositional and various biochemical parameters of traditional Diyarbakir Örgü cheese during storage period. For this purpose, cheese samples were collected from eight different small dairy plants. In this study, it was observed that the composition of the cheese samples during the storage period changed as follows: dry matter decreased from 55.87% to 52.29%, fat from 26.56% to 23.47%, fat in dry matter from 38.84% to 37.07%, protein from 26.56% to 23.47%; ash increased from 7.31% to 9.32%, salt from 6.31% to 8.16%, salt in dry matter from 11.40% to 15.48%, while the parameters investigated in relation to the biochemical properties of the cheese samples ranged as follows: 18.38-22.96 for SH, 5.47-5.64 for pH, 0.17-0.21% for soluble nitrogen in pH 4.6, 0.03-0.05% for soluble nitrogen in 12% TCA, 0.25-0.46% for lipolysis level and 4.19-5.61% for ripening index. In this study, the proteolysis level of the cheese during the ripening was determined electrophoretic analysis. According to the Turkish Food Codex Communiqué on Cheese (Notice No: 2015/6), DÖC is a semi-fat (25 ≤ milk fat <45) and hard cheese with the desired moisture content (up to 50%) and salt (>7.5%).

Keywords: Biochemical properties, Composition, Diyarbakir Örgü cheese, Pasta-filata cheeses

Introduction

The industrial production and marketing of traditional and regional cheeses are of a great contribution to national economies. Such cheeses are valuable export products for countries such as Germany (3.67 billion dollars), France (3.28 billion dollars) and Italy (2.67 billion dollars) (WTO, 2019). The vast majority of regional cheeses are sold in the region or country they are produced in, for which there are two main reasons. Firstly, traditional cheeses are usually produced in accordance with regional taste and secondly, most of the cheeses produced don’t have sufficient marketing opportunities in other countries.

In Turkey, most regional cheeses are produced from raw milk. This increases the risk of infection and intoxication from fresh cheeses caused by pathogenic microorganisms, especially Brucella spp. (Kaynar, 2011; Elmali and Uylaser, 2012; Hatipoglu and Celik, 2012; Kesenkas et al. 2012). Therefore, the production process of regional cheeses should be reevaluated in the context of the pasteurization of the milk and starter culture applications. In addition, cheeses of high quality and economic value should be produced in accordance with the demands of the global market. For this purpose, many studies have been carried out on traditional cheeses (Drake et al. 2001; Hort and Le Grys, 2001; Lawlor et al. 2001; Ritvanen et al. 2001; Singh et al. 2003; Caspia et al. 2006; Kucukoner and Haque, 2006; Uysal, 2008).

Diyarbakır Örgü cheese (DÖC) should be produced industrially while preserving its conventional characteristics (taste, aroma, composition etc.). DÖC is widely consumed in Diyarbakır and surrounding provinces, and is sold just above the cost in markets in cities such as Istanbul, Ankara and Izmir. The production process of DÖC is similar to that of pasta-filata cheeses such as Mozzarella, Caciocavallo and Povola dei Nebrodi. In the production of traditional DÖC, usually raw ewe milk is used. In order to produce the cheese, the milk is coagulated with liquid rennet at 29-30 °C for 54-70 minutes in a way that it should coagulate. After the coagulation, the curd which is put in the cheesecloth is pressed and fermented. Cord pulling method is used in order to test whether or not the curd is suitable for scalding. Fermented curd is submerged into the boiler (water, 78-87 °C) by placing it in perforated stainless steel pails and scalded
for 2-7 minutes. The scalded curd dough is placed on the bench, stretched threadlike by cutting in portions of 200 g on average and shaped as typical braid by weaving. Fresh cheese is left for approximately 20-30 minutes in brine (usually 12 Bé). Then, the cheese is placed into lacquered tin plates or plastic jerry cans and stored at 6±1 °C for approximately 4-6 months by adding 17-21 Bé brine. As it matures, the humidity increases and become softer. DÖC is a kind of semi fat and hard cheese (Hatişoglu, 2014, Anonymous, 2015).

DÖC, which is a traditional Anatolian cheese, is produced mostly in the Karacadag Basin situated in the South of the province of Diyarbakir. Due to the small arable areas of the basin and the presence of large meadow-pasture areas, animal husbandry, especially sheep farming, is carried out in general (Hatişoglu and Celik 2012). There were 1164.521 sheep, 157.144 goats and 128.376 cattle (domestic cows, crossbred cows, culture (non-domestic) cows and buffalo) that were being milked in the basin. Approximately 296.000 tons of cattle milk, 97.976 tons of sheep milk and 16.566 tons of goat milk are produced in the basin (Anonymous, 2019).

DÖC production is seasonally performed in portable facilities in the production area (Hatişoglu, 2014). This study is especially important as it is the first study conducted on the ripening characteristics of DÖC. In the present study, the composition and various biochemical properties of DÖC produced in small dairy farms in the Karacadag Basin were investigated.

The composition and maturity of the cheese affects the consumer’s nutritional preferences. This study is the first study conducted in the production region of traditional DÖC. The findings of the research may help to standardize and preserve the composition and biochemical properties of the cheese in the industry. Thus, it may provide basic data for the cheese to reach world markets in the near future. In addition, for researchers, this study will provide an insight into the definition and characterization of the composition and biochemical parameters of DÖC as a fieldwork.

Materials and Methods

The study was conducted in eight small-scale dairy plants, selected upon the suggestion of the Diyarbakir Provincial Directorate of Agriculture and Forestry and local cheese producers. Fresh cheese samples (4 kg) were collected four times at a week intervals from each dairy plant. The collected cheese samples were stored and ripened at 6±1 °C for 6 months.

Composition

The ratios of dry matter (DM) (gravimetric method) (Anonymous, 1989), fat (butyrometric method) (Anonymous, 1989), ash (gravimetric method) (Anonymous, 1989), salt (Mohr method) (Anonymous, 1989) and protein (Dumas method, by Leco FP 528) (Anonymous, 1989) of the cheese samples were determined on the 1st, 30th, 60th, 90th and 120th days of the ripening period.

Biochemical parameters

Titration acidity (TA) (Dagdemir et al. 2003), pH (WTW 330i, Germany) (Yazıcı and Dervişoğlu, 2003), soluble nitrogen (SN) at pH 4.6 (Dumas method, by Leco FP 528) (Gripon et al. 1975), SN in Trichloroacetic acid (TCA) (Dumas method, by Leco FP 528) (Tavaria et al. 2003) and lipolysis level (Ocak et al. 2015) analyzes were performed on the 1st, 30th, 45th, 60th, 90th and 120th days of the storage period.

Electrophoresis

The degradation level of the proteins was determined by the urea-PAGE method in accordance with the density of the protein bands obtained on the 1st, 30th, 60th, 90th and 180th days of the storage period by using vertical slab-gel electrophoresis (BioRad Laboratories Ltd. Watford, UK). The gels obtained were stained with Comassie Brilliant Blue G250 (Blakesley and Boezi 1977; Andrews, 1983; Hynes et al. 1989).

Statistical analysis

The data obtained were analyzed by the one-way ANOVA and the differences between the significant averages were determined by the Tukey multiple comparison test (Yıldız & Bircan, 1994).

Results and Discussion

Composition

The ANOVA revealed that there were statistically highly significant (P<0.01) differences between all compositional parameters (DM, protein, fat, fat in DM, ash, salt and salt in DM) of the cheese samples during storage period (Table 1). The DM, fat and protein ratios of DÖC decreased during the period. In contrast, salt, salt in DM and ash rates increased. The fat in DM rate of the cheese followed a fluctuating course throughout the period and it was determined that this ratio in ripened cheese decreased slightly compared to fresh cheese.

The average DM and protein ratios of commercial Örgü cheese were reported by Ozdemir et al. (1998) as 44.84% and 21.69%, and as 50.88% and 21.75% according to Celik and Turkoğlu (2007). Additionally, the average DM and protein ratios of traditional Lavash cheese were reported by Celik et al. (2001) as 45.80% and 17.52%. The average values of the same components of Caciocioricotta cheese were reported by Albenzio et al. (2006) as 46.43% and 16.8%. Finally, the average DM and protein values of Dil cheese were reported by Uysal (2008) as 53.78% and 23.95%.

On the other hand, the fat ratios of the samples determined in the study (Table 1) were found to be higher than those reported for
As seen from the above comparisons, the fact that all of the composition parameters of DÖC (especially DM and fat in DM) were determined to be different from those of other studies may be due to the difference in the race/breed/diet of the dairy animals and the fat content of the raw milk used in the production of the cheeses. Small-scale dairy plants do not only produce DÖC from sheep’s milk, they also use cow’s and goat’s milk (approximately 20%). Therefore, they mix the sheep, goat and cow’s milk without a fixed rate and produce DÖC from the milk of this mixture. Additionally, it was observed in the local area study that the brines used in the cheese production had different amounts of salt (17-21 Bé) (Hatipoglu, 2014).

Based on the storage period, the average ratio of fat in the DM of DÖC (37.73%) was determined to be between full-fat cheese (45%) and fatty cheese (30%) (Anonymous, 2015; Anonymous, 2006a; Anonymous, 2006b). DÖC is similar to Dil and Kashar cheeses in terms of its production technique and composition properties. Therefore, DÖC can be defined as a fatty cheese based on the cheese standards mentioned above. However, according to the Turkish Food Codex Communique on Cheese, DÖC is considered as a semi-fatty cheese (Anonymous, 2006b). DÖC is expected to be a full fat cheese type. However, DÖC cheese and butter fat are also produced at the same time in the Karacadag region.

According to the data obtained from the present study, the average ash and salt ratios of DÖC were determined to be higher than those reported by Ozdemir et al. (1998), Celik and Turkoglu (2007), and Uysal (2008). Similarly, the mean ratio of salt in DM of DÖC obtained in the study were also determined to be higher than that reported for Örgü (% 9.02 and % 13.68), Lavash (% 13.95) and Dil (% 13.23) cheeses (Ozdemir et al. 1998; Celik et al. 2001; Celik and Turkoglu, 2007; Uysal, 2008).

Table 1 The mean values of the composition parameters (%) of the samples and the groups formed during the storage period (n=30)

| SP (day) | DM     | Protein | Fat     | Fat in DM | Ash    | Salt | Salt in DM |
|----------|--------|---------|---------|-----------|--------|------|-----------|
| 1        | 55.44±1.13<sup>a</sup> | 26.56±0.64<sup>a</sup> | 21.56±0.80<sup>a</sup> | 38.84±0.99<sup>a</sup> | 7.31±0.33<sup>a</sup> | 6.31±0.27<sup>a</sup> | 11.40±0.52<sup>a</sup> |
| 30       | 55.32±1.13<sup>a</sup> | 25.84±0.64<sup>b</sup> | 20.71±0.80<sup>b</sup> | 37.38±0.99<sup>bc</sup> | 8.77±0.33<sup>b</sup> | 7.70±0.27<sup>b</sup> | 13.95±0.52<sup>d</sup> |
| 60       | 54.75±1.13<sup>a</sup> | 25.23±0.64<sup>c</sup> | 20.33±0.80<sup>bc</sup> | 37.07±0.99<sup>c</sup> | 9.19±0.33<sup>c</sup> | 8.04±0.27<sup>c</sup> | 14.72±0.52<sup>c</sup> |
| 90       | 53.78±1.13<sup>a</sup> | 24.36±0.64<sup>d</sup> | 20.19±0.80<sup>bc</sup> | 37.47±0.99<sup>c</sup> | 9.22±0.33<sup>c</sup> | 8.11±0.27<sup>c</sup> | 15.09±0.52<sup>b</sup> |
| 120      | 52.84±1.13<sup>a</sup> | 23.47±0.64<sup>e</sup> | 20.06±0.80<sup>bc</sup> | 37.88±0.99<sup>b</sup> | 9.32±0.33<sup>a</sup> | 8.16±0.27<sup>a</sup> | 15.48±0.52<sup>a</sup> |
| Mean     | 54.43±1.13 | 25.20±0.64 | 20.57±0.80 | 37.73±0.99 | 8.77±0.33 | 7.67±0.27 | 14.13±0.52 |

Means with different letters in the same column are significantly different (P<0.01). SP: Storage period; DM: Dry matter.

As can be seen from Table 1, the ratios of fat, protein and DM of DÖC gradually decreased during the storage period. This may be due to the degradation of proteins and lipids in the cheese matrix by enzymes and microorganisms during the storage period, and may also be due to the low molecular weight compounds formed as a result of the degradation products passing from the cheese into the brine (Grappin and Beuvier, 1997). In addition, it is thought that the amount of salt diffusing from the brine into the cheese matrix is less than the protein and lipid degradation products passing from cheese to brine.

Celik and Turkoglu (2007) reported that the DM ratio of Örgü cheese produced from raw and pasteurized milk decreased significantly during the ripening period. Moreover, the DM ratio of Dil cheese which was produced from raw, thermised, and pasteurized milk and ripened for 90 days in brine, decreased during the 90 day storage period (Uysal, 2008). In addition, Arboatti et al. (2014) reported that the DM ratio of Mozzarella cheese decreased during a 43 days of storage period.

According to the results of the present study (Table 1), the average DM ratio of DÖC (54.43%) corresponds to the DM ratio (at least 50%) specified in the legislation for Dil cheese.

During the scalding process, there was a positive relationship between the pH of the curd and the DM level of the DÖC. In contrast, it has been reported that while the pH of the curd decreased, the DM ratio of the final product increased in a study conducted on Cheddar cheese (Walstra and Jennees, 1984). This can be explained by the decrease in the water binding ability of the curd as the scalding pH approaches the isoelectric point of casein (pH 4.6).

The protein ratio of DÖC decreased significantly (P<0.01) during the storage period (Table 1). This may be due to the fact that low molecular weight nitrogenous compounds migrated from the cheese matrix into the brine. These values obtained from the
The difference between biochemical parameters of DÖC were found to be highly significant (P<0.01) in terms of storage period. The TA value of the DÖC fluctuated during the storage period (Table 2). The TA value of the DÖC fluctuated during the storage period (Table 2). The TA value of the DÖC fluctuated during the storage period (Table 2).
This was also reported in similar studies in the literature (Uysal, 2008; Celik and Turkoglu, 2007; Tarakci and Kucukoner, 2006). The mean TA value decreased up to the 45th day of the storage period and then increased (Table 2). On the 45th day of the period, the average TA value of the DÖC was the lowest (18.38 SH) and the highest mean TA value (22.96 SH) was seen on the 120th day of the period. It was reported that alkaline and neutral compounds caused by proteolysis and free acids formed as a result of lipolysis can lead to a decrease in TA (Uysal, 2008; Guven and Konar, 1997). In addition, it was reported that the acidity of the cheese is caused by lactic acid, acetic acid, butyric acid, formic acid, free fatty acids resulting from lipolysis and free amino acids formed as a result of proteolysis (Yilmaztekin, 2001).

In the present study, the mean TA value was determined as 20.35 SH (Table 2). Celik and Turkoglu (2007) reported the average TA value of Örgü cheese made from raw milk and pasteurized milk which was followed by a fluctuating course during a 90 day ripening period was determined as 22.28 SH and 21.14 SH, respectively. This study revealed that TA increased until the 30th day and decreased after the 60th day. Furthermore, Uysal (2008) reported the TA of Dil cheese made from raw milk decreased until the 30th day of the ripening period and then started to increase. According to the results of studies conducted by various researchers on Dil, Örgü and Lavash cheeses, the mean TA values were found to be 22.89; 22.86, 15.11, 49.33; 22.74, respectively (Ozdemir et al. 1998; Celik et al. 2001; Turkoglu et al. 2003; Aksu et al. 1999; Kocak et al. 1997).

According to the relevant standard, the highest value of TA of the Dil cheese is 1.0% LA (44.44 SH). During the ripening period in the present study, the DÖC samples were determined to be in compliance with this standard (Anonymous, 2006a).

The average pH values of the DÖC samples increased until the 30th day of the storage period and started to decrease after the 60th day. This increase in the pH of the samples may be due to the formation of alkaline compounds as a result of proteolysis, and the decrease starting from the 60th day may be caused by the rapid increase in the lipolysis level. It was reported that the pH values of the Örgü cheese increased up to the 15th day of the ripening period and then decreased until the end of the period (Celik and Turkoglu, 2007). In another study, it was reported that the average pH values of Dil cheese increased up to the 60th day of the ripening period and decreased relatively on the 90th day of the period (Uysal, 2008). “In addition, it was stated that the pH of Mozzarella fluctuated during 43 days of storage (Arboatti et al. 2014). Similarly, it was reported that the pH values of Kashkaval cheese which was stored for 180 days fluctuated between 5.0-5.3 (Pappa et al. 2019). A similar result was stated for the Caciocavallo cheese which was matured for 150 days (Perna et al. 2014). Celik and Turkoglu (2007) reported the average pH of Örgü cheese made from raw milk and pasteurized milk was 5.07 and 5.08, respectively. In their study, Anar et al. (2000) reported that the average pH of Örgü cheese was 5.30. Uysal (2008) reported that the average pH values of Dil cheese made from raw, thermost and pasteurized milk were 5.07, 5.14 and 5.19, respectively. In a survey study, the average acidity of Dil cheese was expressed as 5.20 pH (Kocak et al. 1997). According to the results of the present study, the average pH of DÖC was higher than the values reported in the studies mentioned above.

The average lipolysis values of the DÖC samples (Table 2) were lower than the values of Örgü cheese made from raw and pasteurized milk (1.49 and 1.27 mg KOH/g-fat) reported by Celik and Turkoglu (2007), and higher than the values of the Dil cheese made from raw, thermost and pasteurized milk (0.25, 0.22 and 0.34 mg KOH/g-fat) determined by Uysal (2008).

In the present study, the lipolysis value of DÖC increased significantly (P<0.01) during the storage period (Table 2). The average lipolysis value of DÖC was 0.25 mg KOH/g-fat on the first day of the ripening period, while it was 0.46 mg KOH/g-fat on the 120th day of the period. Similarly, various studies reported that the lipolysis values of Örgü and Dil cheeses increased significantly (P<0.05) during the 90-day storage period (Uysal, 2008; Celik and Turkoglu, 2007). It has been stated that the level of lipolysis in the cheese matured in brine was affected by the

| Storage Period (day) | Acidity | Lipolysis Value (mg KOH/g-fat) | SN in pH 4.6 (%) | SN in TCA (%) | RI(%) |
|----------------------|---------|--------------------------------|----------------|--------------|-------|
| 1                    | 20.26±2.03<sup>a</sup> | 0.25±0.02<sup>b</sup> | 0.17±0.01<sup>f</sup> | 0.03<sup>f</sup> | 4.19±0.20<sup>f</sup> |
| 30                   | 18.50±2.03<sup>d</sup> | 0.29±0.02<sup>c</sup> | 0.18±0.01<sup>e</sup> | 0.04<sup>c</sup> | 4.47±0.20<sup>c</sup> |
| 45                   | 18.38±2.03<sup>d</sup> | 0.33±0.02<sup>d</sup> | 0.19±0.01<sup>d</sup> | 0.04<sup>d</sup> | 4.66±0.20<sup>d</sup> |
| 60                   | 20.41±2.03<sup>c</sup> | 0.37±0.02<sup>e</sup> | 0.19±0.01<sup>c</sup> | 0.04<sup>c</sup> | 4.86±0.20<sup>c</sup> |
| 90                   | 21.61±2.03<sup>b</sup> | 0.41±0.02<sup>b</sup> | 0.20±0.01<sup>b</sup> | 0.05<sup>b</sup> | 5.18±0.20<sup>b</sup> |
| 120                  | 22.96±2.03<sup>a</sup> | 0.46±0.02<sup>a</sup> | 0.21±0.01<sup>a</sup> | 0.05<sup>a</sup> | 5.61±0.20<sup>a</sup> |
| Mean                 | 20.35±2.03 | 0.35±0.02 | 0.19±0.01 | 0.04 | 4.83±0.20 |

Means with different letters in the same column are significantly different (P<0.01). TA: Titration acidity (SH), SN: Soluble nitrogen, TCA: 12%Trichloroacetic acid, RI: Ripening index
Fig. 1 Urea-PAGE electrophoretogram of DÖC during the storage period
Electrophoresis

The density of the bands representing the $\alpha_{s1}$-Cn was relatively low compared to the bands representing the $\beta$-Cn on the electrophoretogram of the DÖC samples (Fig 1). This can be explained by the chymosin activity which was higher than the plasmin activity in the cheese curd (Mcsweeney and Sousa, 2000). Similarly, chymosin is also known to hydrolyse $\alpha_{s1}$-Cn (Creamer and Mills, 1971; Creamer, 1976). And this situation confirms resistance of $\beta$-Cn to chymosin hydrolysis as reported by Fox et al. (1993). Due to the high salt concentration (17-21 Bé) of the brine, it was estimated that the activity of plasmin decreased and $\beta$-Cn degradation slowed down (Fallico et al. 2005). Similar results were also reported for different types of cheeses (Milanovic et al. 1998; Saldamli and Kaytanli, 1998; Katsiari et al. 2000; Pavia et al. 2000; Gobbetti et al. 2002; Kongo et al. 2009).

On the other hand, it has also been reported that high scalding temperature, high pH and low moisture level decrease the chymosin activity in the cheese matrix, while they increase the enhancing effect of plasmin activity on $\beta$-Cn (Singh et al. 2003; Kelly et al. 1996; Sousa et al. 2001). The electrophoretogram of the DÖC samples showed that $\gamma$-Cn was formed from $\beta$-Cn (Figure 1). This revealed that plasmin activity existed in the cheese (Di Cagno et al. 2003). Plasmin activity is also known to be an important parameter in the differentiation of cheese varieties (Sousa et al. 2001).

Furthermore, Carretero et al. (1994) reported that these breakdown products emerged in cheese made from goat’s milk. In addition, the effect of bacteria on the level of proteolysis in cheese was expressed (Fox, 1989). Although starter culture was not used in the production of DÖC, non-starter lactic acid bacteria in natural flora of the milk can also increase the level of proteolysis of the cheese.

Conclusions

Since DÖC is not produced within a certain standard, the fat in DM, salt in DM and DM contents of the cheese should be standardized. In addition, in order to reduce the salt content of the cheese mass, keeping DÖC in vacuum packages instead of preserving it in brine with high salt content is a more suitable option. According to the Turkish Food Codex Communiquè on Cheese (Notice No: 2015/6), DÖC is a semi-fat (25% milk fat <45) and hard cheese with the desired moisture content (up to 50%) and salt (>7.5%). Until now, industrially produced cheeses under the name of “Örgü cheese” did not represent the traditional characteristics of DÖC. For this reason, the demand for this cheese could not be met sufficiently. In conclusion, it is thought that adopting the cheese to the dairy industry without losing its natural characteristic properties will gain more importance in the near future.

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