CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Journal homepage:**http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

OVEN COOKING AS ALTERNATIVE TO SMOKING: EVALUATION OF PHYSICOCHEMICAL, MICROBIOLOGICAL, TEXTURAL AND SENSORY PROPERTIES OF CIRCASSIAN CHEESE DURING STORAGE AND DETERMINATION OF PAH CONTENTS

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Article history:
Received:
5 June 2018
Accepted:
1 November 2018

Keywords:
Characterization;
Circassian cheese;
Polycyclic aromatic;
hydrocarbons (PAHs);
Oven baked;
Smoking.

ABSTRACT
The aim of this work was to evaluate the physicochemical, microbiological, textural and sensory changes in three batches of Circassian cheese (non–smoked (AN), natural smoked (AS) and oven–baked (RO) cheese) during storage period of 90 days. Nine polycyclic aromatic hydrocarbons (PAHs) were also detected by HPLC-RD. Differences in cheese samples (total solids, fat, salt, titratable acidity, water-soluble, trichloroacetic acid-soluble and phosphotungstic acid-soluble nitrogen, hardness, gumminess, springiness, cohesiveness, chewiness and color properties) were observed depending on the manufacture procedure and smoking process. Microbial community in oven-baked cheese was found significantly lower than other samples. Use of oven for smoking enhanced the sensory properties compared to natural smoking, but batch AN had higher acceptability. Total and carcinogenic PAHs levels of oven-baked smoked cheese were remarkable lower than the levels of natural smoked cheese. Benzo[a]pyrene was detected only batch AS at the levels of 0.11 μg kg^{-1}. In this study, it was demonstrated that oven-baked smoking technique can be used as smoking technique instead of natural smoking for manufacture of Circassian cheese.

1.Introduction

Circassian cheese is a kind of traditional cheese produced from cow's, sheep's, goat's or skimmed buffalo's milk, or a mixture of these milks by circassian families in Anatolia especially the region of Sinop, Düzce, Bolu, Sakarya, Balıkesir, Bursa, Çanakkale, Biga, Hendek and Gönen (Kamber, 2008). It is also made in the ancestral regions of the Circassians in Caucasia. Circassian cheese can be consumed as fresh, ripened or dried (sundried or oven-dried) form (Ayar et al., 2015). It has typically a round shape, an average weight of 0.5 kg, and color varying from white to cream for the dried types. The change of color between the varieties can be explained with the smoked type of Circassian cheese has a light brown surface and a light yellow or cream interior, however fresh cheese has a light yellow color. The characteristic flavor profile of the cheese is slightly salty, cooked, creamy and fermented (Guneser and Yuceer, 2011) but the textural properties of the varieties show slight differences depending on soft consistency for fresh cheese and thin crust layer for dried type (Ayar et al., 2015).

Smoking process is applied to Circassian cheese in order to obtain long shelf life and higher organoleptic properties. Nowadays, Circassian cheese is mostly smoked with the aim of improving taste, color and textural properties rather than preservative impact (Ayar et al., 2015; Guneser and Yuceer, 2011). Different smoking processes have been developed to control of smoke concentration in foods, but traditional (natural) and liquid smoking techniques are mostly used for smoke of Circassian cheese (Aydinol and Ozcan, 2013; Kamber, 2008). According to Aydinol and Ozcan (2013), natural smoking method is better than liquid smoking due to better microbial quality and also natural smoked Circassian cheese received higher flavor scores. Since polycyclic aromatic hydrocarbons (PAHs) are formed during natural smoking, liquid smoking is preferred for industrial production. The number of cheesemakers using natural smoking method has gradually decreased because of highly presences of PAHs especially benzo[a]pyrene (B(a)P) and legal restrictions. PAHs are highly hydrophobic and organic lipophilic compounds with fused aromatic rings and they are formed during the incomplete combustion or pyrolysis of organic matter by different cooking process techniques such as roasting, barbecuing, grilling, frying, smoking, heating, drying, baking and cooking (Fasano et al., 2016; Singh et al., 2016). The Scientific Committee on Food (SCF) has evaluated the health risk associated with PAHs (SCF, 2002) and International Agency for Research on Cancer (IARC) has mentioned PAHs in priority pollutant list due to their carcinogenic and mutagenic properties (IARC, 2012). SCF suggested to use B(a)P as a marker of occurrence of the PAHs in foods (Lambert et al., 2012). However, four PAHs (PAH4) including B(a)P, chrysene (Chr), benzo(b)fluoranthene (B(b)F) and benzo(a)anthracene (B(a)A) has been established the most suitable indicators of PAHs in foods after September 2012 (EC, 2011) and the European Food Safety Agency (EFSA) suggested that PAH4 has been used as better indicator for the presence of PAHs in food instead of B(a)P (EFSA, 2008).

Circassian cheese is an acid–coagulated cheese and has different properties in comparison with enzyme–coagulated cheeses. Acid–coagulated cheeses, are generally used in natural smoking process, have risks of formation of PAHs. Therefore, Circassian cheese manufacturers have developed alternative practice and Circassian cheese is produced in an oven without any tree or smoked. In this case, enzyme–coagulated cheese is used at oven–baking treatment because of higher textural and sensory quality. There are limited studies about Circassian cheese (Ayar et al., 2015; Aydinol and Ozcan, 2013; Guneser and Yuceer, 2011) and there is no information about the oven–baked Circassian cheese. The aim of the present study is to investigate effects of oven–baking treatment instead of natural smoking treatment on physicochemical, microbiological, textural and sensory characteristics of Circassian cheese during storage, and PAHs contents of Circassian cheeses were also evaluated.

2. Materials and methods

2.1. Materials

Cheese productions were performed at the Karagöl Çiftliği Dairy Company (Sakarya, Turkey). Only natural smoking treatment was applied by a local cheesemaker according to traditional procedure. Calf rennet was obtained from Mayasan Company (Istanbul, Turkey) in production of enzyme–coagulated cheese. A total of 36 Circassian cheeses (three repetitions for each cheese) were manufactured as three batches independent Circassian cheeses. The samples were coded as follows: AN (acid and heat–coagulated, non–smoked cheese), AS (acid and heat–coagulated, natural smoked cheese) and RO (rennet–coagulated, oven–baked cheese). According to the experience of the producer dairy plant, the productions are restricted to these three batches because the cheese produced by the acid / heat coagulation technique is not suitable for oven-baking and if

the enzyme coagulation cheese is not baked, it is very similar to Kaşar cheese.

2.2. Manufacturing of cheeses

Raw cow's milk was filtered through a filter–line and transferred into process vat and then heated to 90 °C. Acidified whey (approximately 4%) was added into the milk, followed by obtaining of the curd formation, whey was drained. The curd was put into a plastic basket with holes then these baskets were stacked and kept for draining of the remaining whey for 12 h. After pressing, the cheese samples were put into brine solution (15% NaCl) for 24 h. Then the cheeses were kept with drying air for 24 h and after the drying treatment, non-smoked cheeses (batch AN) were vacuum–packaged. Batch AS samples were smoked with poplar tree for 36 h by using the traditional method. After cooling, the smoked cheeses were vacuum–packaged.

For manufacture of batch RO, raw cow's milk was filtered through a filter–line and pasteurized at 75 °C for 15 s. After cooling to 32 °C, the milk was coagulated with rennet enzyme. Then whey was removed from the curd and remaining whey is drained off by placing heavy blocks on the top of it. After pressing process, the curd was cut as large parts and then grated. Then, the curd was cooked at 75 °C for 3 min, and the cooked curd was kneaded and placed into molds. After waiting period for 24 h, cheeses were baked at 150 °C in a rotary electric–oven until enough browning of cheese surface and then cheese was cooled and vacuum–packaged.

2.3. Physicochemical analysis

Total solid, fat, salt, and titratable acidity (TA) were determined according to the methods described by Bradley et al. (1993). pH was measured using a pH meter (InoLab, Weilheim, Germany) calibrated with pH 4.0, 7.0 and 10.0 buffers. The total nitrogen was determined using the Kjeldahl method. The acid degree value (ADV) was determined as described by Deeth and Fitz-Gerald (1976) for evaluation of fat hydrolysis. For fat oxidation, lipid extraction from cheese was carried out according to the method of Kristensen et al. (2000) and the peroxide values were determined by a method described by Şengül et al. (2014).

2.4. Soluble nitrogen fractions

For preparation of water soluble extracts, 20 g of grated cheese was mixed with 40 mL of deionized water and homogenized with an Ultra Turrax homogenizer (WiseTis®HG–15D, Daihan, Korea) for 1 min. It was then centrifuged at 3000 x g for 30 min at 4 °C. The fatty layer was removed and the supernatant was filtered through Whatman 42 paper.

To analyses the content of water–soluble nitrogen (WSN), 10 mL of filtrate was taken and the Kjeldahl method was used (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 mL of 33% (w/v) PTA solution and 7 mL of 3.95 M H2SO4 solution were added to 10 mL of the WSN fraction. The mixture was held overnight at 4 °C and filtered through Whatman 42 paper. The nitrogen contents were determined using the Kjeldahl method (IDF, 1993) and all WSN, TCA and PTA values were expressed as the percentage of the total nitrogen content of the cheese (Jarrett et al., 1982).

2.6. Microbiological analysis

Ten g of cheese samples were homogenized in 90 mL of 2% (w/v) solution of sodium citrate (Carlo Erba, Milano, Italy) for 1 min with a stomacher (AES Chemunex, Bruz, France). The dilutions of the suspensions were prepared with Ringer's solution (Merck, Darmstadt, Germany). The following media and incubation conditions were chosen to enumerate microbial counts: YGC agar (Merck) for yeasts and moulds at 30 °C for 72 h, KAA agar (Merck) for enterococci at 30 °C for 24 h, Rogosa agar

(Merck) adjusted to pH 5.5 with acetic acid for lactobacilli at 30 °C for 5 d under anaerobiosis, M17 agar (Merck) for lactococci at 30 °C for 72 h, MRS agar (Merck) supplemented with vancomycin (30 μg mL–1; Sigma–Aldrich, Steinheim, Germany) for leuconostocs at 30 °C for 72 h, VRB agar (Merck) for coliforms at 37 °C for 24 h.

2.7. Color properties

Surface and inner colors of cheese samples were measured with a Minolta Chroma Meter (CR-400, Minolta Camera Co., Osaka, Japan) and measurements were carried out in triplicate for each cheese samples. Color was expressed according to the Commission International de l'Eclairage (CIE) as L* (lightness; 100=white, 0=black), a* (redness; +, red; -, green), and b* (yellowness; +, yellow; -, blue), hue angle (ho) and chroma. In which the chroma and hue angle were calculated as follows:

$$Hue = h_{ab}^{*} = tan^{-1}\left(\frac{b^{*}}{a^{*}}\right) \quad (1)$$

$$Chroma = C_{ab}^{*} = (a^{*2} + b^{*2})^{0.5} \quad (2)$$

2.8. Texture profile analysis

For texture analysis, Circassian cheese samples were cut using a stainless–steel cylinder knife into cylindrical discs, nearly 20±0.5 mm height and 30±0.5 mm diameter and wrapped in plastic stretch film. The samples were adjusted to room temperature (20°C) before analysis and then Texture Profile Analysis (TPA) was performed with a TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, UK) in duplicate. An aluminum cylindrical probe (P/36R) with 36 mm of diameter was attached to a moving crosshead. Pre–test, test and post–test speeds of the probe were adjusted to 1 mm/s. Samples were compressed to 25% of the original height in two consecutive cycles. Compression time and trigger force were 5 s and 25 g, respectively. Data recording and analysis were carried out with the Texture Exponent Version 4.0.13.0 software (Stable Micro Systems).

2.9. Sensory properties

Circassian cheese samples were organoleptically evaluated after ripening at 4^{o}C for 7, 30, 60 and 90 days by a semi trained panelist group (twelve assessors) at the Ondokuz Mayis University, Food Engineering Department. The assessors were informed about the characterization of Circassian cheese and trained in relate evaluation to be familiar with attributes and scoring procedures of cheese samples under study. Score card was used to evaluate flavor-aroma (on a 10 point scale), body-texture, appearance and color (on a 5 point scales) with some criteria (salty, insipid, sour, bitter, tasteless, fermented, whey and smoke for flavor; tough and dry, soft, fragile and flexible for body-texture, rough, spotted, non-uniform, porous and fissure for appearance; mat and dingy for color) that lead to reduction of score. Before the testing, cheese samples were tempered for 30 min at room temperature inside a closed dish and cut into pieces of 1.5 x 2.0 cm base and 2-5 cm long. Cheese samples were identified with three numbers and randomly presented to assessors. Assessors used water and bread to clean their plates between samples.

2.10. Determination of PAHs

The extraction and clean-up procedures of PAHs from Circassian cheese was based on the method described by Anastasio et al. (2004) and Wegrzyn et al. (2006), respectively with some modifications. Five mL of 1 M KOH etanolic solution was added to 1 g of sample previously homogenized in a Teflon centrifuge tube and then tube placed in a water bath at 80°C for 3 h. After the cooling to room temperature, 5 mL of distilled water and 10 mL of cyclohexane were added and the mixture vortexed for 5 min and then centrifuged (Sigma, Model 3K30, OsterodeamHarz, Germany) at 4000 x g for 15 min. The supernatant was poured from the tube and the sample was re-extracted with 10 mL of cyclohexane as previously described. The combined extracts were concentrated by rotary vacuum evaporator at 40°C (Buchi Rotavapor R–200, Sweden) and then dried under a nitrogen

stream. After the drying process, residue was applied to a SPE cartridge after dissolving with 2 mL of acetonitrile. For the cleanup process, firstly SPE cartridge was washed and activated by passage of 10 mL of ultrapure water followed by 10 mL of methanol and dried with air by using syringe. Two mL of eluate was applied to a SPE cartridge and dried for 1 min at the air. For the elution of analytes from the cartridge, 10 mL of dichloromethane was used. After the concentrating step under a nitrogen stream, the residue was diluted with 500 µL acetonitrile and then injected to HPLC.

The separation and identification of PAHs were performed by HPLC system from Shimadzu (Tokyo, Japan), consisted of a fluorescence detector (FLD) RF–10XL, quaternary pump LC–20AT, degassing device DGU–20A5, column oven CTO–10ASVP, auto injector SIL–10A and system controller SCL–10AVP (data station LC–20AT). The HPLC column used was a Phenomenex Envirosep–PP column (125 mm x 4.6 mm, 4.6 µm, Phenomenex). The mobile phase used for HPLC analysis consisted of acetonitrile and water at a flow rate of 1 mL/min. The linear gradient elution program was set as 0 min–85% acetonitrile + 15% water, 9 min – 100% acetonitrile. The temperature of the column during chromatographic was set at 350C and settings for PAH detection were as follows (excitation/emission wavelength): λ1=216/336 for 3.9–4.4 min (Naph): λ2=240/320 for 4.5–4.9 min (Ace):λ3=248/404 for 4.9–5.5 min (Ant): λ4=236/384 for 6.2–7.1 min (Pyr): λ5=270/388 for 8.8–11.25 min (B[a]A): λ6=250/430 for 17.5–22.5 min (B[k]F, B[a]P): λ7=295/405 for 22.9–27.2 min (DB[ah]A, B[ghi]P).

The describe method was validated and performances were present in our primary study (Gul et al., 2015). Recoveries were calculated using spiked samples at three replicates each at three concentrations of PAH mix and they found in between 73.38% and 92.61%. The repeatability of method, expressed as relative standard deviation (RSD %), was comprised between 6.46% and 12.49% for all the considered individual PAHs. The limit of detection (LOD) and the limit of quantification (LOQ) for the method were lower than 0.09 and 0.27 µg kg^{-1}, respectively. The results of correlation coefficient (r^2) was better than 0.9991 for all PAHs.

2.11. Statistical analysis

Analyses of cheeses from the three experiments were performed in duplicate during storage period. Data analysis was performed with SPSS statistical package software with version 21.0 (SPSS Inc. Chicago, Illinois) and results were presented as mean ± standard deviation. One-way analysis of variance was applied to determine the differences between means with confidence level of 95% ($P<0.05$). When significant ($P<0.05$) main effect was found, the mean values were further compared using Duncan test.

3.Results and discussions

3.1. Compositional properties

The levels of total solid (TS), fat, fat in TS, salt, salt in TS, pH and titratable acidity (TA) of Circassian cheeses during storage period are shown in Table 1. Batch RO showed the highest TS values and the lowest values were determined in batch AN ($P<0.05$). Oven baking treatments in batch RO cheeses caused an increase in TS. TS values of the batch AS were higher than those of batch AN due to smoking treatment. Our TS results resemble the values of other researches (Aydinol and Ozcan, 2013; Guneser and Yuceer, 2011; Sıçramaz et al., 2017; Uysal et al., 2010). TS levels of the cheeses slightly decreased during storage period. Fat contents of the batch RO were the highest ($P<0.05$) and batch AS followed this batch depending on TS contents of the cheeses. The fat contents of cheeses didn't show significant changes during storage ($P>0.05$). The values of the fat in TS in batch RO and AN were similar and higher than those of batch AS. These results were agreed with the results of a previous report (Aydinol and Ozcan, 2013; Guneser and Yuceer, 2011). Salt contents of the

batches didn't change significantly during storage (P>0.05) and batch RO showed higher salt contents than those of other batches during storage period (P<0.05). The levels of salt in TS of the batch RO were also higher than the other batches (P<0.05). These results were similar to those of other studies (Aydinol and Ozcan, 2013; Guneser and Yuceer, 2011) and lower than results of Uysal et al. (2010). Batches AS and AN were similar in terms of slightly lower pH levels than batch RO. Similar results obtained by Ayar et al. (2015) who stated pH decreased for a greater extent for the cheese coagulated with acid whey. Additionally, Cais–Sokolińska et al. (2014) found that pH value of smoked Mozzarella cheese was significantly greater than that of unsmoked cheese. TA values of batches AN and AS were similar and they showed higher TA content than batch RO especially after day 60 due to acid and heat–coagulation of these cheeses. Sıçramaz et al. (2017) stated that the effect of smoking process on the TA value was not significant and the differences in the acidity were mainly as the result of the cheese compositions. However, in this study, batch RO was manufactured with rennet and hence, acidity value of this cheese was increased little during storage compared to acidity of batches AN and AS.

As seen in Table 1, the ADV of batch AN was significantly lower than in smoked cheeses and the highest value (2.13 meq KOH 100 g fat^{-1}) was found in batch RO (P<0.05). The higher temperature during smoking could have increased the ADVs of smoked cheeses. The ADVs increased in all cheeses during storage (P<0.05) and reached to 4.71 meq KOH 100 g fat^{-1} as the highest value in batch RO cheese.

Lipid oxidation is an important factor affecting the cheese quality especially those with a high fat content and during long periods of storage. Peroxide value of lipid extracted from samples varied from 0.86 to 1.63 meq O_2 kg fat^{-1} and the highest peroxide value detected in batch RO (P<0.05; Table 1). Smoking process caused the increase of peroxide value in cheeses and this may be associated to the increased oxidation of unsaturated fatty acids as a result of exposed to atmospheric oxygen and heating during smoking (Anvari et al., 2014). During the storage period, peroxide value was significantly increased for all cheese, however at the end of storage, it was found higher in batch RO (5.78 meq O_2 kg fat^{-1}) than others (P<0.05). The considerable differences between cheeses during storage were found due to different smoking and acid or enzyme coagulated types.

Table 1. Compositional properties of Circassian cheeses during storage.

Properties	**Samples**	**Storage time (days)**			
		7	**30**	**60**	**90**
TS (%)	*RO*	61.04 ± 0.38^{aA}	59.61 ± 1.01^{abA}	59.37 ± 0.81^{bA}	59.17 ± 1.13^{bA}
	AS	57.25 ± 0.87^{aB}	56.29 ± 0.64^{abB}	56.14 ± 0.97^{abB}	55.39 ± 0.79^{bB}
	AN	51.18 ± 1.66^{aC}	50.53 ± 0.33^{aC}	50.37 ± 0.38^{aC}	50.14 ± 0.23^{aC}
Fat (%)	*RO*	32.17 ± 1.15^{abA}	32 ± 1.01^{aA}	31.5 ± 0.25^{aA}	30.25 ± 0.25^{bA}
	AS	28 ± 0.66^{abB}	27 ± 0.5^{bcB}	26.83 ± 1.01^{aB}	26.75 ± 0.43^{cB}
	AN	25.83 ± 0.29^{abC}	26.92 ± 0.52^{aB}	25.25 ± 0.43^{bC}	26.5 ± 0.43^{aB}
Fat in TS (%)	*RO*	51.05 ± 1.57^{bA}	53.67 ± 0.84^{aA}	53.06 ± 0.37^{abA}	51.13 ± 1.23^{bA}
	AS	48.91 ± 0.87^{abB}	47.97 ± 0.71^{bB}	51.2 ± 2.58^{aA}	48.29 ± 0.49^{bB}
	AN	50.52 ± 2.06^{bA}	53.26 ± 1.23^{aA}	49.15 ± 0.61^{cB}	52.53 ± 0.86^{abA}
Salt (%)	*RO*	2.96 ± 0.18^{aA}	2.49 ± 0.26^{bA}	2.41 ± 0.22^{bA}	2.57 ± 0.37^{bA}

	AS	2.28 ± 0.1^{aB}	1.81 ± 0.17^{bB}	1.84 ± 0.08^{bB}	2.09 ± 0.08^{aAB}
	AN	1.77 ± 0.09^{aC}	1.97 ± 0.13^{aB}	1.81 ± 0.29^{aB}	1.91 ± 0.05^{aB}
Salt in TS (%)	*RO*	4.85 ± 0.27^{bA}	4.16 ± 0.39^{cA}	4.06 ± 0.42^{cA}	5.53 ± 0.53^{aA}
	AS	3.99 ± 0.15^{bB}	3.22 ± 0.29^{cB}	3.27 ± 0.17^{cB}	4.31 ± 0.1^{aB}
	AN	3.47 ± 0.13^{aB}	3.91 ± 0.28^{aA}	3.51 ± 0.6^{aAB}	3.79 ± 0.09^{aB}
pH	*RO*	5.77 ± 0.06^{aAB}	5.73 ± 0.05^{bAB}	5.71 ± 0.04^{bA}	5.7 ± 0.02^{bA}
	AS	5.58 ± 0.02^{aB}	5.44 ± 0.02^{bB}	5.35 ± 0.14^{bcB}	5.26 ± 0.08^{cB}
	AN	5.65 ± 0.04^{aA}	5.56 ± 0.03^{bA}	5.41 ± 0.07^{cB}	5.32 ± 0.02^{cB}
TA (%)	*RO*	0.54 ± 0.06^{bA}	0.64 ± 0.03^{bB}	0.66 ± 0.04^{abB}	0.79 ± 0.12^{aB}
	AS	0.62 ± 0.07^{cA}	0.81 ± 0.09^{bA}	0.95 ± 0.09^{aA}	1.01 ± 0.06^{aA}
	AN	0.59 ± 0.03^{dA}	0.77 ± 0.06^{cAB}	0.99 ± 0.08^{bA}	1.13 ± 0.14^{aA}
ADV (meq KOH 100 g fat^{-1})	*RO*	2.13 ± 0.36^{cA}	2.8 ± 0.13^{bA}	4.41 ± 0.21^{aA}	4.71 ± 0.35^{aA}
	AS	1.49 ± 0.19^{dB}	2.39 ± 0.21^{cAB}	2.96 ± 0.19^{bB}	3.58 ± 0.35^{aB}
	AN	0.86 ± 0.07^{dC}	1.94 ± 0.29^{cB}	2.87 ± 0.22^{bB}	3.88 ± 0.39^{aB}
Peroxide (meq O2 kg fat-1)	*RO*	1.63 ± 0.22^{dA}	2.65 ± 0.21^{cA}	4.03 ± 0.08^{bA}	5.78 ± 0.17^{aA}
	AS	0.86 ± 0.03^{dB}	1.53 ± 0.19^{cB}	2.34 ± 0.13^{bC}	3.29 ± 0.34^{aB}
	AN	0.86 ± 0.09^{dB}	2.46 ± 0.15^{cA}	3.57 ± 0.12^{bB}	5.15 ± 0.44^{aA}

TS : total solid; TA: titratable acidity; ADV: acid degree value.
$^{A-C}$ Different letters in same column indicates significant differences ($P<0.05$).
$^{a-d}$ Different letters in same line indicates significant differences ($P<0.05$).

3.2. Soluble nitrogen fractions

Proteolysis in the Circassian cheeses throughout storage period was evaluated by analyzing the proteolytic indices, including water-soluble nitrogen (WSN), trichloroacetic acid-soluble nitrogen (TCA-SN) and phosphotungstic acid-soluble nitrogen (PTA-SN). The values of WSN/TN (%), TCA–SN/TN (%), and PTA–SN/TN (%) are presented in Figure 1 during the storage of the Circassian cheeses. The values of WSN/TN (%) in cheeses showed significant increases in all stages ($P<0.05$) except batch AS on day 90. The mean WSN/TN (%) values of batches RO and AN were similar and higher than those of batch AS during storage. Guneser and Yuceer (2011) found that WSN/TN (%) levels in smoked Circassian cheese collected markets ranged between 2.30–29.35%. Uysal et al. (2010) found that the lower WSN/TN (%) was found in fresh Circassian cheese, but Circassian cheeses dried under the sun and in the stove had higher levels. S. Kalit et al. (2005) found 6.58% WSN/TN (%) in fresh Tounj cheese, which produced from raw milk and characterized by its distinct smoked flavor, and reached to 13.14% after a ripening time of 8 weeks.

The values of TCA–SN/TN (%) in cheeses were found between 0.58 to 0.78% and they showed significant increase in all storage stages ($P<0.05$). The highest value was observed in batch AN especially on day 60 and 90 (1.38 and 1.95%, respectively), whereas batch AS showed the lowest TCA–SN/TN (%) values during storage ($P<0.05$). M. T. Kalit et al. (2014), Guneser and Yuceer (2011) and S. Kalit et al. (2005) reported higher TCA–SN/TN (%) levels in Sir iz misine (cheese in a sack), Circassian and fresh Tounj smoked cheeses, respectively. The differences among the results obtained by researchers probably based on the differences in

acid sources and cheese-making procedures (Guneser and Yuceer, 2011).

Batch AN showed the highest PTA–SN/TN (%) value ($P<0.05$) and batches AS and RO were observed that they were similar. The highest value of PTA–SN/TN (%) at the end of storage reached to 0.79% for AN. The higher PTA–SN/TN (%) values (ranged between 3.33-6.26%) were found by Guneser and Yuceer (2011). In general, soluble nitrogen values of RO cheeses after the production were higher than those of other batches, and AS cheese showed the lowest soluble nitrogen values at the end of storage period. Smoking treatment caused increases in soluble nitrogen values for acid coagulated cheeses but decreases in values during storage of cheeses. Moreover, batch RO showed close values to batch AN, and higher values than those of batch AS.

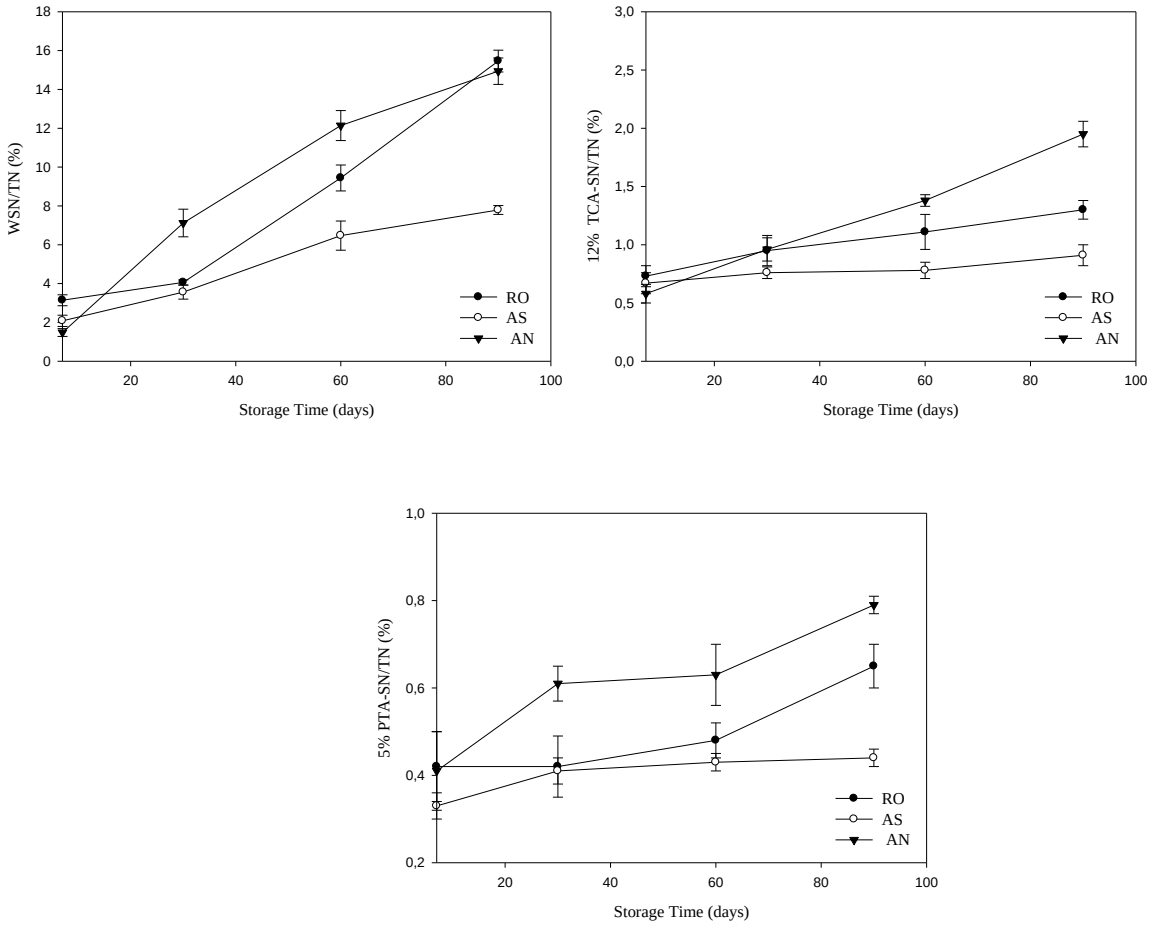


Figure 1. Soluble nitrogen fractions of Circassian cheeses during storage time

3.4. Microbiological characteristics

The counts of the lactobacilli in Circassian cheeses ranged from 5.27 to 9.12 log cfu g^{-1} during storage (Table 2). The counts of the lactobacilli showed a significant increase in only batch RO cheeses ($P<0.05$) during storage. The lactobacilli counts of batches AN and AS were similar and showed higher values than those of batch RO. The lactobacilli counts in all the batches remained constant during storage. The counts of the lactococci in batches AN and AS were also higher than those of the batch RO. Like lactobacilli and lactococci, leuconostoc had the lowest count in batch RO. The counts of the leuconostoc in all the batches did not show any important changes during storage ($P>0.05$). The counts of enterococci in batch AN were the highest ($P<0.05$), but enterococci were not found in batch RO during storage. Cooking and

oven baking treatments in batch RO cheeses caused decreasing in the lactobacilli, lactococci, leuconostoc and enterococci counts. Moreover, Majcher et al. (2011) reported that the number of bacteria inactivated owing to the increase in phenolic compounds formed during the natural smoking process, which are known to have bacteriostatic and/or bactericidal properties. The yeast and mould counts in the cheeses ranged from 3.27 to 5.42 log cfu g^{-1} during storage for all cheese samples and these results were agreed with the report of Aydinol and Ozcan (2013) and Sıçramaz et al. (2017). The yeast and mould counts in batch AN were higher than those of batches AS and RO and counts of batches AS and RO were similar (P>0.05). It was observed that cooking or baking and smoking treatments leaded to decrease in the counts of yeast and mould. While the yeast and mould counts of batches AS and RO showed tendency to decrease, batch AN exhibited increase during storage. Like enterococci, coliforms were not found in batch RO during storage due to probably cooking and baking processes. Batch AS showed decrease in the counts of coliforms during storage (P<0.05), but cheeses AN remained constant. Our coliform results were higher than those of Aydinol and Ozcan (2013) and lower than the results obtained by Sıçramaz et al. (2017).

Table 2. Microbiological composition of Circassian cheeses during storage time

Microbial groups	**Samples**	**Storage time (days)**			
		7	**30**	**60**	**90**
Lactobacilli	*RO*	5.27 ± 0.71^{cB}	6.13 ± 0.28^{bB}	7.01 ± 0.54^{aB}	7.38 ± 0.48^{aB}
	AS	9.12 ± 0.32^{aA}	8.54 ± 0.17^{aA}	8.61 ± 0.15^{aA}	8.81 ± 0.14^{aA}
	AN	8.54 ± 0.14^{aA}	8.24 ± 0.22^{aA}	8.51 ± 0.23^{aA}	8.71 ± 0.36^{aA}
Lactococci	*RO*	8.1 ± 0.62^{bB}	8.21 ± 0.57^{bB}	8.48 ± 0.65^{abB}	9.13 ± 0.32^{aA}
	AS	9.52 ± 0.29^{aA}	8.97 ± 0.17^{aA}	8.95 ± 0.53^{aAB}	9.14 ± 0.38^{aA}
	AN	9.54 ± 0.37^{aA}	9.42 ± 0.31^{aA}	9.51 ± 0.23^{aA}	9.61 ± 0.22^{aA}
Leuconostocs	*RO*	7.51 ± 0.62^{aC}	7.29 ± 0.48^{abB}	6.84 ± 0.31^{bB}	6.55 ± 0.32^{bC}
	AS	8.73 ± 0.44^{aB}	8.19 ± 0.11^{aA}	8.52 ± 0.16^{aA}	8.71 ± 0.43^{aB}
	AN	9.4 ± 0.24^{aA}	8.66 ± 0.21^{bA}	8.99 ± 0.19^{abA}	9.47 ± 0.41^{aA}
Enterococci	*RO*	<1	<1	<1	<1
	AS	4.52 ± 0.12^{cA}	4.98 ± 0.11^{bA}	5.46 ± 0.28^{cA}	5.07 ± 0.9^{bA}
	AN	4.46 ± 0.33^{aB}	4.35 ± 0.19^{aB}	4.47 ± 0.14^{aB}	4.19 ± 0.12^{aB}
Yeast and Moulds	*RO*	5.42 ± 0.15^{aA}	4.62 ± 0.45^{abAB}	4.17 ± 0.43^{bcB}	3.86 ± 0.74^{cB}
	AS	4.82 ± 0.5^{aB}	4.01 ± 0.09^{bB}	3.59 ± 0.24^{cB}	3.27 ± 0.25^{cB}
	AN	4.74 ± 0.46^{aB}	5.29 ± 0.54^{aA}	5.31 ± 0.31^{aA}	5.4 ± 0.19^{aA}
Coliforms	RO	<1	<1	<1	<1
	AS	3.24 ± 0.29^{aB}	2.28 ± 0.33^{bB}	1.55 ± 0.11^{cB}	1.54 ± 0.58^{cB}
	AN	4.39 ± 0.09^{aA}	3.72 ± 0.12^{bA}	3.81 ± 0.11^{bA}	4.01 ± 0.11^{abA}

$^{A-C}$ Different letters in same column indicates significant differences (P<0.05).
$^{a-c}$ Different letters in same line indicates significant differences (P<0.05).

3.5. Color properties

The rind and internal color properties of cheese types depend upon smoking and storage period are shown in Table 3. The color properties of rind and internal of cheese samples were mainly affected by smoking and storage period ($P<0.05$). Rind and internal L* values of batch RO were lower than other cheese samples due to cooking, kneading and oven baking process ($P<0.05$). While traditional smoking process significantly affected the rind L*value of cheese samples, there was no change at internal L* values in comparison non-smoked cheese. Similarly, Cais–Sokolińska et al. (2014) found that a significant difference related with lightness of color between non-smoked and smoked cheeses was shown only outer edge layer and at the center of non-smoked and smoked cheeses were equally light. The decrease of lightness for all cheese samples by the end of 60 d storage period was occurred and then both of rind and internal L* values of cheese samples were increased. It was probably related with the concentration of cheese components and differences of in dry matter content (Saldo et al., 2002). On the contrary, a marked increase in L* value was recorded for non-smoked Mozarella cheese but there was no significant changes of lightness for smoked cheese of the center (Cais–Sokolińska et al., 2014). The rind a* values for batch AS and RO were found more redness than AN ($P<0.05$), but internal a* values of all cheese samples were found similar. During storage, rind and internal a* values of all samples were slightly decreased. Moreover, the highest rind and internal b* value was recorded for batches AS and RO, respectively and the lowest for batch AN ($P<0.05$). During ripening period there was a little change in color of rind as more yellow by increases b* values for all cheese samples. The internal b* value only increased in batch AN during ripening, however these values of batches RO and AS were decreased at the end of 30 and 60 day of storage, respectively and then increased for both cheese samples. The degree of color saturation (chroma) was effected by a* and b* values, and the color of the rind and internal for batches AS and RO was more saturated than the others, respectively. Chroma values of cheese inside were not changed during storage period for all cheese samples. Color in the rind of smoked cheese became less saturated until the storage of 60 days and then increased. However, rind chroma value for batch AN was significantly increased during storage ($P<0.05$). The internal hue angle values for all cheese samples were found similarly and there was no difference during storage. But, the rind hue angle value for batch AS was found higher than batches AN and RO ($P<0.05$).

Table 3. Color properties of Circassian cheeses during storage time

Color Properties	Samples	Storage time (days)			
		7	30	60	90
Rind L*	*RO*	37.77 ± 1.57^{abC}	35.16 ± 1.76^{bcA}	34.62 ± 2.86^{cB}	38.84 ± 1.15^{aC}
	AS	44.11 ± 2.71^{aB}	44.38 ± 1.23^{aA}	41.15 ± 0.93^{bC}	43.35 ± 2.19^{abB}
	AN	87.71 ± 0.26^{aA}	82.8 ± 1.01^{bC}	81.87 ± 0.64^{bB}	83.39 ± 0.34^{bA}
Internal L*	*RO*	75.65 ± 1.19^{aB}	66.08 ± 0.89^{cA}	65.98 ± 0.78^{cA}	63.01 ± 0.44^{bB}
	AS	87.14 ± 0.37^{aA}	83.52 ± 0.72^{bcA}	83.05 ± 0.97^{cB}	84.76 ± 0.15^{bA}
	AN	87.08 ± 0.22^{aA}	84.06 ± 0.47^{bcB}	83.02 ± 0.78^{cA}	84.73 ± 1.13^{bA}
Rind a*	*RO*	14.54 ± 0.31^{aA}	12.22 ± 1.64^{bB}	12.37 ± 1.13^{bC}	12.41 ± 1.29^{bA}
	AS	11.52 ± 0.66^{aA}	10.99 ± 0.64^{aB}	10.87 ± 0.16^{aA}	10.42 ± 0.3^{aB}
	AN	-0.84 ± 0.07^{aC}	-1.44 ± 0.1^{aA}	-1.23 ± 0.12^{aB}	-1.66 ± 0.15^{aC}
Internal a*	*RO*	-0.24 ± 0.61^{aA}	-0.42 ± 1.02^{aA}	-0.55 ± 1.07^{aA}	-0.35 ± 0.28^{aA}

	AS	-0.91±0.07^{aB}	-1.27±0.24^{aB}	-0.95±0.39^{aA}	-1.12±0.29^{aB}
	AN	-0.92±0.14^{aB}	-1.44±0.1^{aA}	-1.44±0.29^{aB}	-1.63±0.23^{aB}
Rind b*	*RO*	17.05±1.12^{aB}	12.5±1.27^{bA}	13.24±0.48^{bC}	15.72±2.44^{aC}
	AS	19.62±1.01^{aA}	18.23±1.16^{abB}	16.61±0.56^{bB}	18.34±1.44^{abA}
	AN	13.37±0.18^{aA}	15.06±1.41^{aB}	15.07±0.24^{aA}	15.79±0.17^{aC}
Internal b*	*RO*	22.12±0.53^{aA}	22.59±0.94^{aB}	22.58±1.23^{aB}	22.72±0.11^{aA}
	AS	14.32±0.39^{bB}	15.77±0.61^{abB}	15.87±0.16^{aA}	15.39±0.35^{abB}
	AN	13.81±0.64^{bB}	14.34±1.52^{bA}	14.86±0.35^{aB}	14.21±1.26^{bB}
Rind Chroma	*RO*	22.42±0.89^{aA}	18.48±2.04^{bB}	18.13±1.03^{bA}	20.03±2.68^{bA}
	AS	22.78±0.75^{aA}	21.31±0.81^{abA}	19.83±0.79^{bA}	21.11±1.36^{abA}
	AN	13.39±0.18^{bB}	15.13±1.01^{aC}	15.13±0.24^{aB}	15.88±0.17^{aB}
Internal Chroma	*RO*	22.13±0.53^{aA}	22.62±0.94^{aA}	22.60±1.27^{aA}	22.72±0.11^{aA}
	AS	15.35±0.4^{aB}	15.82±0.63^{aB}	15.90±0.19^{aB}	15.45±0.34^{aB}
	AN	13.84±0.65^{aC}	14.41±1.52^{aB}	14.93±0.04^{aC}	14.31±1.27^{aB}
Rind Hue	*RO*	0.86±0.03^{aB}	0.80±0.02^{bB}	0.82±0.04^{bB}	0.90±0.03^{aB}
	AS	1.04±0.04^{abA}	1.03±0.05^{abA}	0.99±0.02^{bA}	1.05±0.03^{aA}
	AN	-1.51±0.01^{aC}	-1.47±0.01^{aC}	-1.49±0.01^{aC}	-1.46±0.01^{aC}
Internal Hue	*RO*	-1.55±0.01^{aB}	-1.5±0.01^{aA}	-1.55±0.02^{aA}	-1.56±0.01^{aB}
	AS	-1.51±0.01^{aA}	-1.49±0.01^{aA}	-1.51±0.02^{aA}	-1.48±0.02^{aA}
	AN	-1.50±0.01^{aA}	-1.47±0.02^{aA}	-1.48±0.02^{aA}	-1.46±0.01^{aA}

$^{A-C}$ Different letters in same column indicates significant differences (P<0.05).
$^{a-c}$ Different letters in same line indicates significant differences (P<0.05).

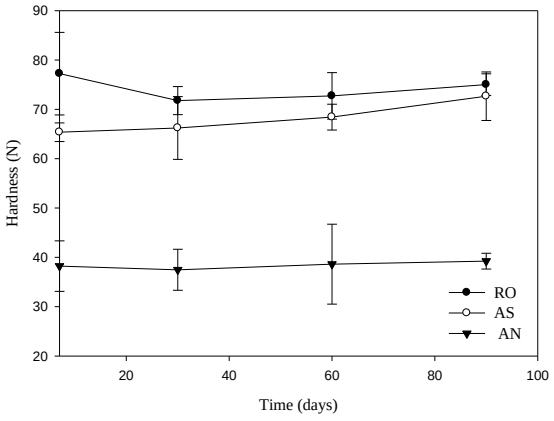
Table 4. PAH content of Circassian cheese

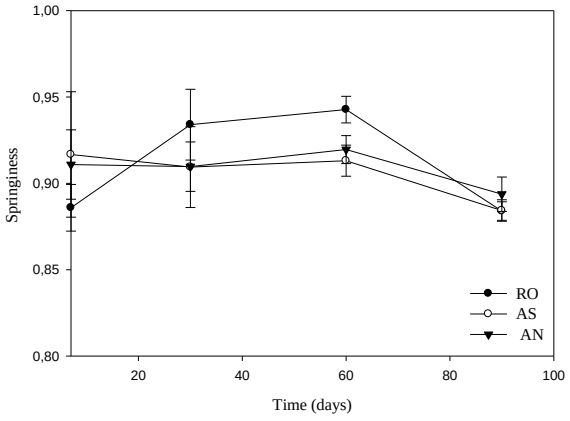
PAHs	**RO**	**AS**	**AN**
Naph	0.41±0.05	1.08±0.08	0.29±0.01
Ace	0.43±0.07	2.02±0.06	0.19±0.06
Ant	0.28±0.07	0.44±0.05	0.16±0.02
Pyr	0.29±0.06	0.86±0.04	ND
B[a]A	ND	0.15±0.01	ND
B[k]F	0.1±0.01	0.17±0.03	0.09±0.01
B[a]P	ND	0.12±0.01	ND
DB[ah]A	ND	0.1±0.01	ND
B[ghi]P	ND	ND	ND
Sum of PAHs	1.51±0.1^{b}	4.93±0.1^{a}	0.74±0.09^{c}
Sum of carcinogenic PAHS	0.1±0.01^{b}	0.53±0.04^{a}	0.09±0.01^{b}

ND: below the limit of detection
$^{a-c}$ Different letters in same line indicates significant differences (P<0. 05).

3.6. Textural properties

Textural properties of Circassian cheeses produced with different methods were evaluated for instrumental textural profile analysis (TPA) during the storage period for 90 days at 4 ^{o}C and shown in Figure 2. The batch RO had significantly highly hardness value than the others after cheese production and during storage ($P<0.05$). It may be explained by baking process for batch RO production due to applied high temperature and by higher TS contents of batch RO than other cheeses (see in Table 1). Moreover, Van Hekken et al. (2007) stated that the amount of fat and protein of the cheese also effects cheese texture. Additionally, Ayar et al. (2015) reported that the culture added and non-smoked Circassian cheese had the lowest hardness values. The hardness values decreased during storage of 30 days for the all cheese, however the decreasing of the value was significantly for only batch RO ($P<0.05$). After the storage of 30 days, hardness values significantly increased ($P<0.05$) and reached to 39.23, 72.66 and 75.02 N for batches AN, AS and RO, respectively. Similar result was obtained by García et al. (2016) found that hardness significantly increased during ripening period and this increases were probably related with the decrease in moisture content during storage. In our study, increase of hardness is probably explained by the increasing of protein in total solid (data not shown) that could affect the texture of the cheese, since moisture values of cheese samples were not significantly changed. Batch RO had higher gumminess value than other cheese samples and it was changed similar to hardness values during storage. The springiness and cohesiveness values of batch RO were found lower than other cheese samples and they were not significantly affected during storage period for all cheese ($P>0.05$). The highest chewiness value was observed in batch RO ($P<0.01$). An increasing trend for chewiness was observed during the 60 days of storage for all cheese and then it was significantly decreased ($P<0.05$). The resilience values of the all cheese samples were increased until storage of 30 days, but it was significantly decreased at the end of storage ($P<0.05$).





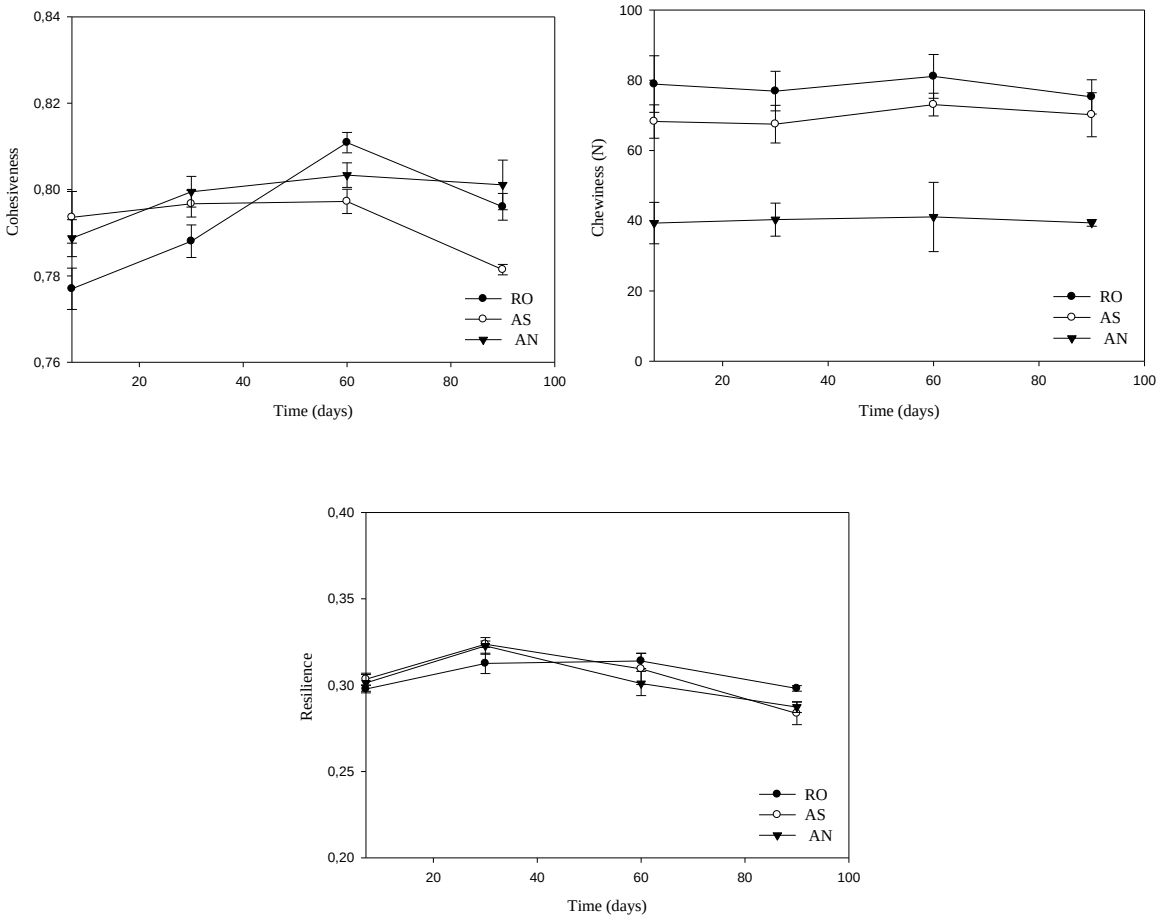


Figure 2. Textural properties of Circassian cheeses during storage time

3.7. Sensory properties

The results of sensory evaluation of Circassian cheeses are presented in Figure 3. The aroma scores of Circassian cheese samples were found higher in batch AN, followed RO and AS ($P<0.05$). Some panelists stated that higher incense aroma of batch AS was negatively affected the cheese aroma. Similarly, Atasever et al. (2003) showed that Kashar cheese smoked with traditionally had less aroma scores than non-smoked cheese and smoked with liquid smokes. However, Ayar et al. (2015) found that the incense aroma was achieved by applying the traditional method of fumigation. As expected, "whey" and "fermented" aroma, the most characteristic aroma terms of Circassian cheese, were defined for batch AN by panelists. Moreover, smoking process was decreased the "whey" or "fermented" aroma of smoked cheeses. On the contrary, Cais–Sokolińska et al. (2014) reported that smoked Mozzarella cheese was less acceptable than non-smoked cheese in related "whey" aroma. The aroma values of cheese samples were increased during storage except batch AN ($P<0.05$) and at the end of storage period, batches AS and RO were considered more acceptable than batch AN.

The texture scores were found highest for batch RO ($P<0.05$) and all texture scores of cheese samples were considered as "good". The texture of batch AN was identified as soft by panelists, but batches RO and AS were identified as slightly tough and flexible. Ayar et al. (2015) found that smoked cheeses were better than non-smoked cheeses according to average

structure scores. During storage period, texture values were increased for all cheese samples and finally batch RO was exhibited the most desirable texture.

The appearance and color properties of cheese samples were significantly affected by manufacture and smoking process ($P<0.05$). Smoking process was negatively affected the appearance and color properties. Similarly, Atasever et al. (2003) stated that Kashar cheese smoked with traditionally method had lower scores than non-smoked cheese samples. On the contrary, Ayar et al. (2015) reported that smoking process did not significantly affect to the visual properties of Circassian cheese. After the storage period, smoked cheese samples had similar scores to batch AN and all cheese samples were acceptable considering appearance and color scores.

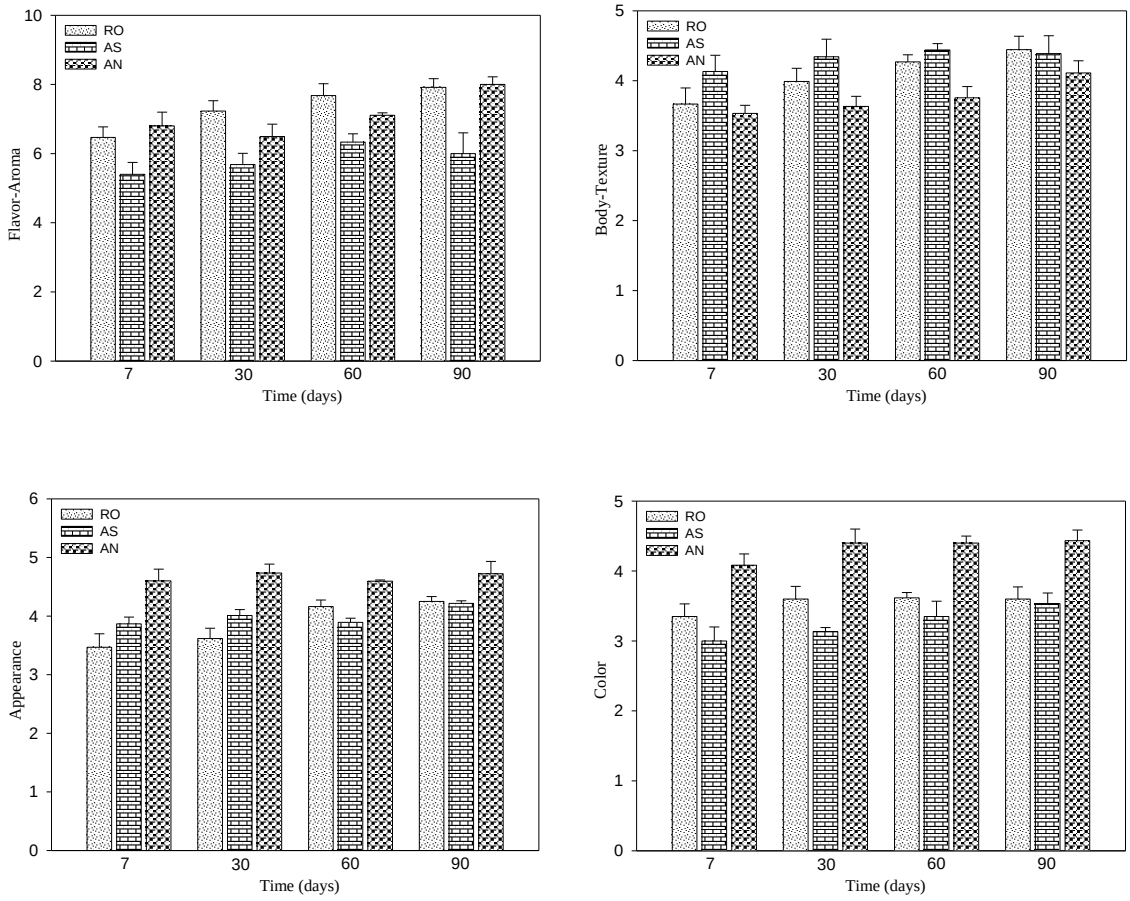


Figure 3. Sensory properties of Circassian cheeses during storage time

3.8. PAH contents

PAH contents of Circassian cheese produced and smoked with different process are shown in Table 4. The sum of nine PAHs (Naph, Ace, Ant, Pyr, B[a]A, B[k]F, B[a]P, DB[ah]A and B[ghi]P) and the sum of 5 carcinogenic PAHs (B[a]A, B[k]F, B[a]P, DB[ah]A and B[ghi]P) were found higher in batch AS (4.93 and 0.53 μg kg^{-1}, respectively) than AN and RO ($P<0.05$). Our results were much lower than other study carried out by Fasano et al. (2016) who reported that the total concentration of PAHs in the whole smoked cheese ranged from 1.1 to 176 μg kg^{-1} with mean value of 88 μg kg^{-1}. Naph and Ace showed the highest contributions of the total average PAHs as

53.25% for batch RO, 62.92% for batch AS and 66.1% for batch AN. Our results similar with those reported by Gul et al. (2015) for Circassian cheese since Naph and Ace were the most abundant of PAHs. While B[ghi]P was not detect in all samples (below the LOD), Naph, Ace, Ant and B[k]F were detected in all samples. B[a]P, could be used as marker for the occurrence of PAHs in foods (EC, 2006), was not detected (below the LOD) in batches AN and RO. But it was found in batch AS at the levels of 0.11 $\mu g\ kg^{-1}$ and the contribution of B[a]P was about 3.02%. Similarly, Aydinol and Ozcan (2013) reported that B[a]P was detected only in exterior part of the naturally smoked Circassian cheese at the level of 5 $\mu g\ kg^{-1}$. Most of studies were exhibited presence of B[a]P in naturally smoked cheese (Esposito et al., 2015; Fasano et al., 2016; Gul et al., 2015; Suchanová et al., 2008). It could be explained the deposition of PAHs containing solid particles on cheese surface during naturally smoking process (Suchanová et al., 2008). The B[a]P level of Circassian cheese in this study was below the maximum tolerable limits (5 $mg\ kg^{-1}$ for smoked meat) set by Commission Regulation (EC) No. 1881/2006 dated 19 December 2006 (EC, 2006) and the maximum permissible level of 1 $mg.kg^{-1}$ for smoked foods accepted by some European countries.

Smoking process carried out with electrically oven caused significantly increasing the level of Naph, Ace, Ant, Pyr and B[a] compared the PAH contents of non-smoked cheese. Moreover, traditionally smoking process caused significantly increase the all PAHs except B[ghi]P ($P<0.05$). Additionally, sum of nine PAHs and carcinogenic PAHs of batches AS and RO were found 2.14 and 6.67, and 1.99 and 6.12 times higher than batch AN, respectively. The traditionally smoking process could lead to the formation of toxic PAHs due to direct contact between the food and smoke (Esposito et al., 2015). However, increase of PAHs during smoking process with oven-baked could be explained by high temperature directly applied to cheese. Baking process has been increased the PAH contents in several foods such as breads, biscuits, cakes etc. reported by several studies (Iwegbue et al., 2014; Singh et al., 2016).

4. Conclusions

The results obtained from this preliminary study related with Circassian cheese smoked with different smoking processes (natural and oven) show that physicochemical, microbiological, textural and sensory properties were significantly affected the manufacture and smoking process. The use of oven for smoking caused significantly decrease the microbial counts and also decreased the PAH formation during smoking process. Carcinogenic PAHs and B(a)P detected in batch RO was considerable found lower than in batch AS. According to sensory evaluation, smoking of Circassian cheese used electrically oven was more acceptable than natural smoking process. Therefore, oven-baked smoking technique can be used as smoking process instead of natural smoking process in the dairy industry.

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Acknowledgment

This study was performed with the financial support by Ondokuz Mayis University Research Foundation (Project Number PYO.MUH.1901.11.001).