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Smoked Circassian Cheese Flavour and Sensory Perspective

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Abstract-The objective of this study was to determine the effects of natural smoke and liquid smoke application methods on flavor and sensory perspective of smoked Circassian cheese. Smoked Circassian cheeses were the most appreciated in sense of sensorial properties such as texture/appearance and aroma, however, there was significant difference on colour properties with reference to smoke application technique. 19 volatile compounds were considered as contributing to the odor of Circassian cheese. The predominant aroma components of smoke applied cheeses were determined as butanoic acid, 1,2 benzenedicarboxilic acid, diethyl ester, ethyl hexanoate, hexanoic acid.

Keywords- *Circassian Cheese, Smoking, Flavour, Sensory*

I. INTRODUCTION

Cheese is an important and substantial food in the Turkish diet and breakfast. The most popular cheese types in Turkey are White, Kasar and Tulum and traditional cheeses such as Circassian, Abhazian, Mihalic and Civil [1]. Circassian cheese is a full-fat cheese that originated in Northern Caucasia. It is produced from goat, sheep or cow milk, or a mixture of these. Traditional Circassian cheese is produced by small dairy farms and industrial plants. It is generally produced in the Marmara region of Turkey. Circassian cheese is consumed fresh, mature or smoked. Generally beech and oak trees are used to produce the smoked type. Smoked Circassian cheese has a very long shelf life relative to fresh Circassian cheese [2].

Smoking, which is mostly used for the preservation of meat and fish, is a method that is also applied to some types of cheese, such as Cheddar, Gouda and Swiss. In Turkey, Circassian, Abhazian and Kasar cheese are smoked [3]. Foods today are smoked for sensory qualities rather than for preservation. Sensory characteristics are one of the important quality attributes that a consumer uses to judge the acceptability of a product. Smoking is utilized in the development of new products as well, particularly for the characteristic colour, taste and flavour that it brings to the foods. Two smoking methods are used: natural or liquid smoking. In addition to developing the sensory properties of the cheese by smoking, the shelf life of the cheese is also extended by the bactericidal and bacteriostatic effects of the firewood fumes [3; 4].

In natural smoking, non-resinous trees such as oak, hornbeam, maple, walnut, mahogany and beech are used. Resinous trees are associated with negative taste qualities; thus, non-resinous trees are preferred for use in smoking [3]. All food processing involving thermal treatments at high temperature and/or direct contact with combustion gases, such as smoking, may be responsible for high polycyclic aromatic hydrocarbons (PAHs) levels in processed foodstuffs. However, with smoking process (over 425 °C), there is a risk of formation of toxic PAHs. PAHs, which are composed of two or more aromatic rings with strong lipophilic character, constitute a widespread group of contaminants. PAHs are formed by the incomplete combustion or pyrolysis of organic material. With traditional smoking methods, the smoke comes in direct contact with cheese, and toxic PAHs are formed. Temperature, humidity, type of wood, oxygen concentration during smoke generation and type of smoke generators can influence PAH formation. Most of the studies on the occurrence of PAHs in smoked cheese mainly refer only to benzo[a] pyrene. Benzo[a]pyrene (C20H12; BaP) is a five-ring PAH which is an effective mutagen and carcinogen, and is the most often determined compound as a surrogate for all PAHs. Although natural smoking is a better preservative than liquid smoking, liquid smoking has begun to be used industrially for smoking cheese because benzo[a]pyrene is formed during natural smoking. Liquid smoking techniques are designed to obtain smoke compounds that do not contain carcinogens. Smoke is obtained by combustion of wood below 350 °C, resolved into its fractions by condensation through distillation, followed by removal of the PAHs [5; 6; 7].

Cheese is a very complicated physical, biochemical, and microbiological matrix. The biochemical changes occurring during ripening including the metabolism of glycolysis, lipolysis, and proteolysis. Proteolysis plays a important role on the (i) texture of cheese curd, due to breakdown of the protein network, decrease in aw through water binding by liberated carboxyl and amino groups and increase in pH (ii) contribution to characteristic flavour and also off-flavour (e.g., bitterness) of cheese through the formation of peptides and free amino acids, FAA; (iii) liberation of amino acids for secondary catabolic changes (e.g. deamination, decarboxylation, transamination, desulphuration, catabolism of aromatic compounds such as phenylalanine, tyrosine, tryptophan and reactions of amino acids with other compounds); and (iv) changes to the cheese matrix [8].

The flavor profiles of cheese are complex influenced by three major metabolic pathways: lipid catabolism, lactose catabolism, and protein catabolism. Milk fat is also essential for the development of the correct flavour in cheese during ripening. While aldehydes, ketones, fatty acids (FA), lactones, and esters are produced by lipid catabolism, lactose catabolism produces organic acids, alcohols, aldehydes, and dicarbonyls. However peptides, amino acids, Strecker aldehydes, and sulfur compounds are formed by protein break down [9; 10].

There is very limited information on Circassian cheese in the literature. Studies report only a limited number of data points on its composition and properties [11; 12]. There is limited literature discussing smoked Circassian cheese by Aydinol and Ozcan [7], but research on the properties of smoked Turkish White cheese [13] and Kasar cheese [14] has been published. The present study was undertaken to investigate the effect of smoking methods on the flavour and sensory perspective of smoked Circassian cheese.

II. MATERIALS AND METHODS

A. Materials

The starter cultures (*Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*) were provided by the DSM Food Specialties Limited Company Scotland and were used after being activated. The smoking flavour agent (Smokez Supreme Poly C-5) was supplied by the GMT Company (Istanbul, Turkey).

B. Cheese manufacturing processes

The production steps for smoked Circassian cheese made from control cheese (C) and naturally smoked cheese (NS) are shown in Figure 1. The cheese was smoked in a commercial oven using oak smoke at 20–30 °C for 16 h. The liquid smoked cheeses were prepared with a 1% (LS1) and with a 2% (LS2) smoking flavour agent solution.

Circassian cheeses were submerged in a solution of the smoking flavour agent for 3 min, and then kept in a cloth for 12 h at 25 °C. After 12 h, the solution of smoking flavour agent was applied again for 3 min, and the cheese was then kept in a cloth. All cheeses were vacuumed in a polyethylene package (7). Circassian cheeses were made with three experiments each repeated two times at different days.

C. Methods

Color measurement was performed on the upper sides five points of day samples using a colorimeter MSEZ-4500L Hunter Lab (Virginia, USA) and was reported as the L^* (whiteness), a^* (intensity of red to green) and b^* (intensity of yellow to blue) values of the samples [15]. In addition, total color difference (ΔE), hue angle (h^*) and color intensity (Chroma), were calculated using the following Eqs. (1-3), where L_0, a_0 and b_0 are the control values for cheeses. Where ΔL is the difference of lightness (L^*-L_0); Δa is the difference of redness (a^*-a_0); and Δb is the difference of yellowness (b^*-b_0). All measurements were carried out in triplicated.

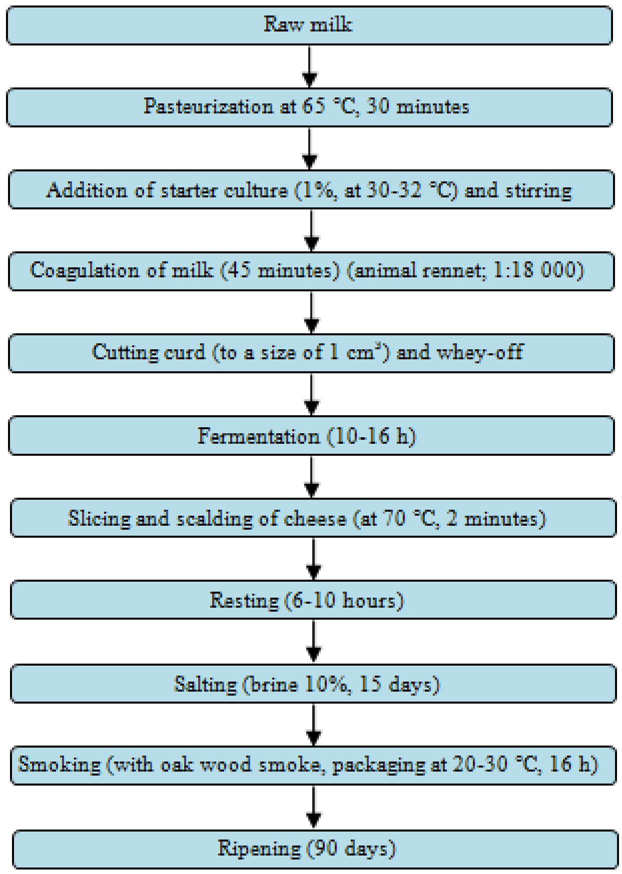

Figure 1. The production of Circassian cheeses

$$\text{TCD } (\Delta E^*) = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

$$h^* = \tan^{-1}(b^*/a^*) \quad (2)$$

$$\text{Chroma } (C^*) = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

The sensory properties of the cheese samples were evaluated by using the method of Ozcan and Kurdal [1]. The cheeses were evaluated at 0, 30, 60 and 90 d after manufacturing by a ten-member panel (5 males and 5 females) from the Food Engineering's staff that was familiar with Circassian cheese. Samples of Circassian cheese were placed in white plastic cups coded with letters. The samples were tempered by holding them at ambient temperature (20±1 °C), and the samples were then presented to panelists in a random order for testing. Water was provided for mouth washing between samples. The trial cheese batches were analyzed for sensory attributes of texture and appearance, taste, odour, colour, aroma intensity, and saltiness using a hedonic scale of 1 to 4 (unsatisfactory/excellent). For assessment of texture and appearance, the scale was described as: 1, soft; 2, normal; 3, hard; 4, very hard. The scale for taste, odour, colour was: 1, bad; 2, sufficient; 3, good; 4, very good. The scale for aroma intensity was: 1, slightly; 2, moderate; 3, good; 4, strong. Intensity of saltiness was scored as: 1, insufficient; 2, slightly salty; 3, normal; 4, very salty.

The volatile flavor components of cheese, was determined by GC-MS method at 90 days of storage. Cheese samples stored at -20 ° C until analysis. 5 g sample of grated cheese mixed with 5 g Na_2SO_4. The mixture was put into a headspace vial with a teflon coated stir bar and closed. The sample was stirred at 40 °C for 20mins on hotplate. When the temperature reaches equilibrium in the sample solution SPME headspace injection (100 m), carboxin PDMS (75µm) (Sigma-Aldrich, USA) SPME fiber was applied. It was sampling at 80 ° C for 30 min, and manual injection process is performed.

The identification of flavor compounds was performed using an Agilent gas chromatograph (6890N series) equipped with a mass selective detector (GC-MS, Agilent 5973 inert series) at 90 days of storage. The GC-MS ion source temperature was set at 200°C. The mass spectrometer operated in electron impact mode with an electron impact energy of 70 eV and collected data at a rate of over a range of 45 to 450 m/z. The eluted identification of flavor compounds was performed by comparing their retention times and LRI values previously reported on the literature. Mass spectra from NIST and WILEY and also reference chemicals run at the same working conditions and GC-MS. The retention indices of unknown compounds were determined via sample injection with a homologous series of n-alkanes. The GC-MS conditions were the same as described above. The analyses of flavor compounds were performed on an Agilent 6890 gas spectrometer coupled to a 5793N mass selective detector. Flavor compounds were separated using a HP5M column (non-polar column). The carrier gas was helium with a flow of 1 mL/min. The temperature programmed was isothermal at 40°C for 5 min and then raised at 5°C/min to 175°C and held for 10 min. Quantification of flavor compounds to quantify the volatiles, the samples were run in duplicate using a GC-MS, and the integrated areas based on the total ion chromatograms were normalized to the areas of the internal standard and averaged. The volatile concentrations in the samples were tentatively determined by comparison with the concentration of the internal standard (undecane), assuming a response factor of 1. To determine the reproducibility of the duplicate injections and which peaks varied across samples, 2-way analyses of variance (sample and injection) were performed for each volatile peak [16].

The data of the present study were analyzed statistically using analysis of variance (ANOVA) for comparing means of the variables followed by a least significant difference (LSD) test at $p \leq 0.05$ or $p \leq 0.01$.

III. RESULTS AND DISCUSSION

High L^* values of smoked Circassian cheeses shows that these cheeses have significantly more whiteness and brightness while natural and liquid smoked cheeses have darker color. When determined the color analysis, the values a showed increasing tendency to green from redness in all cheeses. Liquid smoked cheeses were determined to be closer to red. In natural smoked cheeses, the values b^* were significantly higher and more yellowness (Table 1). The L^*, a^* and b^* values of cheeses were found statistically significant at p<0.01. Redness (a^*) and yellowness (b^*) of Circassian cheeses increased during the ripening, while their brightness (L^*) decreased (data not shown).

TABLE I. COLOR PROPERTIES OF CIRCASSIAN CHEESE

Cheese Type	L^*	a^*	b^*	ΔE	h^*	C^*
C	85.69^a	-3.60^b	14.69^{bc}	-	76.23^c	15.12^b
NS	78.64^b	-3.33^b	17.73^a	7.68^a	79.36^b	18.04^a
LS1	78.18^b	-1.99^a	13.88^c	7.72^a	81.84^b	14.02^b
LS2	78.86^b	-1.56^a	15.04^b	7.14^b	84.08^a	15.12^b
Significance	**	**	**	*	**	**

Significance level: significant at p<0.01 (**), p<0.05 (*), different superscript letters on the same column indicate significant differences; C: control Circassian cheese, NS: naturally smoked Circassian cheese, LS1: 1% liquid smoked Circassian cheese, LS2: 2% liquid smoked Circassian cheese

The acceptability of food depends greatly on its sensory properties (colour, surface structure, texture, taste, flavor, etc.). The difference between structure and appearance values of Circassian cheeses was found statistically significant at p<0.01 depending on the type of cheese. Natural smoked cheeses were found more desirable by the panelists in terms of structure and appearance due to the fact that they have the characteristics of cheeses which are smoked in the wood smoke and presented to the consumers. There was a decrease in admiration in terms of structure and appearance since cheeses lose their structural rigidity during the maturation (Table 2). Proteins play an important role in cheese manufacturing. They not only give it shape and texture by coagulating but also contribute to the development of flavor by producing amino acids [17]. The breakdown of the protein network during proteolytic activity leads to textural changes in the cheese matrix. Carboxyland amine groups that are liberated during proteolysis cause a decrease in water activity by binding water molecules [18]. The fat fraction also of cheese is important for the development of typical flavour and texture. It is well known that a higher fat content leads to a less firm and more elastic body, while low-fat products tend to be harder, more crumbly, and less smooth than the characteristic cheese texture [19].

The difference between taste values of Circassian cheeses was found statistically insignificant and the cheeses were found desirable at the same rate (p>0.01). There was an increase in admiration of Circassian cheeses in terms of taste during the ripening period. The different between odour properties of Circassian cheeses was found statistically insignificant depending on the type of cheese (p<0.01). There was an increase in admiration in terms of odor properties in all 4 groups during the ripening and it was stated that natural smoked cheeses were found most desirable in terms of varieties of cheese. It was considered that the reason arises from the condensation of odor of the smoke compounds in liquid smoked cheeses during the maturation period (Table 2).

TABLE II. SENSORY PROPERTIES OF CIRCASSIAN CHEESE

Cheese Type	Texture and Appearance	Taste	Odor	Color	Aroma	Saltiness	Total Acceptability
C	2.12^{b}	2.90^{a}	2.92^{a}	3.14^{b}	2.16^{c}	2.72^{a}	2.80^{b}
NS	2.72^{a}	3.26^{a}	3.16^{a}	3.46^{a}	3.08^{a}	2.78^{a}	3.38^{a}
LS1	2.30^{b}	3.14^{a}	3.10^{a}	3.38^{a}	2.84^{ab}	2.80^{a}	3.36^{a}
LS2	2.34^{b}	2.34^{a}	3.10^{a}	3.50^{a}	2.70^{b}	2.72^{a}	3.40^{a}
Significance	**	ns	ns	*	**	ns	**

Significance level: significant at p<0.01 (**), p<0.05 (*), different superscript letters on the same column indicate significant differences; C: control Circassian cheese, NS: naturally smoked Circassian cheese, LS1: 1% liquid smoked Circassian cheese, LS2: 2% liquid smoked Circassian cheese

The difference between color properties of Circassian cheeses was found significant depending on the type of cheese (p<0.05). Cheeses which have undergone a natural and liquid smoking process were found desirable by the panelists in terms of their colour properties and it is stated that colour distribution is homogeneous in natural smoked cheeses. In liquid smoked cheeses, a uniform color could not be obtained due to the fact that the liquid smoke solution did not evenly spread on the cheese surface.

Cheese flavour is one of the most important criteria determining consumer choice and acceptance. The maximum flavor intensity values in cheeses were determined in natural smoked and 1% liquid smoked cheeses (p<0.01). Minimum flavor intensity value was found in natural smoked cheese sample. It is considered that the smoke compounds cause that natural smoked and liquid smoked cheeses have more flavor intensity compared to the control group cheeses (Table 2).

The salinity degree values were found statistically insignificant depending on the type of cheese. It is considered that the reason arising from this is that the cheese samples are taken out of the brine and undergone the smoking process in the 15^{th} day of the storage and allowed to mature under the same conditions (data not shown). The total acceptability values of Circassian cheeses showed increase during the ripening period. Natural smoked and liquid smoked cheeses were found desirable equally in general (p<0.01) (Table 2).

Flavor is developed in cheese system through a series of chemical and biochemical reactions during the cheese ripening. Cheese flavor is formed as a result of a balance created by a large number of compounds between each other [10]. During the ripening period flavor compounds are produced which are characteristic for each variety. Volatile compounds in cheese can originate from different sources. Most of them result from the metabolism of cheese microorganisms, endogenous microflora of raw milk, starters or feeding [20].

When the volatile compounds determined in the 90^{th} day of fully-mature Circassian cheeses are analyzed, a total of 19 compounds were detected. Two compound could not be identified (RT 10.04, 13.54) (Table 3). Gogus et al. [21] determined volatile components ranging from 43 to 55 in Cheddar cheese which is made ripe at mild, medium and full level. Acetic acid, 2,3- butandiol, decanal, nonanal, ethyl lactate, 3-hydroxy-2- butanone and butanoic acid were determined by them as basic compounds among these compounds. In the production of Cheddar cheese, as a starter, *L. lactis* spp. *cremoris* is used alone or together with *L. lactis* spp. *lactis*, as well as rennet enzyme [22]. As a result of the study carried out to determine the characteristic aroma substances by gas chromatography olfactometry in this cheese, it was determined that the homofuraneol forms the caramel taste; the diacetyl the buttery taste; the methional the boiled potato taste; the butanoic, propionic and hexanoic acids the cheesy and rancid taste; the ethyl butyrate and ethyl caproate the fruity taste and the 2,3- methyl butanal a sweetish taste [21; 23]. Preininger and Grosch [24] carried out a study to determine the characteristic aroma substances in Emmental cheese and in this study, it is determined that the sweetish flavor of volatile aroma substances distilled after extracted with ether is formed by methional, furaneol and homo-furaneol. In the study in which the significant aroma-active substances forming the aroma in Camembert cheese are determined, it is suggested that the butanoic and isovaleric acids are the most important acidic components. Furthermore, a mushroom-like aroma specific to this cheese is formed with 1-octen-3- ol and 1- octen-3- on [25].

It is determined that the butanoic, hexanoic, octanoic and decanoic acids form the cheesy and lipolyzed taste; the ethyl butanoate, ethyl hexanoate, ethyl heptanoate and ethyl decanoate the fruity taste; some pyrazines such as 2,3-dimethyl pyrazine, 2,6- dimethylpyrazine and trimethylpyrazine the furfural taste and the 2- furanmethanol the nutty/roasted taste, which are detected in Parmigiano-Reggiano cheese that is one of the Italian cheeses [9]. Massouras et al. [26] determined the volatile aroma compounds by using the headspace technique in the curd matured in the brine. Basic aroma substances are grouped as aldehydes, ketones, alcohols and fatty acids. Acetaldehyde, hexanal, heptonal, acetone, acetoin, acetic acid and butyric acid were substantially determined within these groups. Kaminarides et al. [27] identified the aroma substances in Halloumi cheese and determined the ethanol and acetic acid as most important substances among them. Furthermore, the alcohols, aldehydes, ketones, esters, hydrocarbons and sulfur compounds are other determined components.

Major flavor compounds in Circassian cheeses were determined such as butanoic acid, 1,2 benzenedicarboxilic acid, diethyl ester, ethyl hexanoate, hexanoic acid, hexyl acetate, octyl acetate, 1-Butanol, 2-methyl-, acetate. In the 90^{th} day of the ripening; in the control group (C) Circassian cheeses, the butanoic acid was found with the rate of 43.42% and 1,2 benzenedicarboxylic acid, diethyl ester with the rate of 19.66%

at the highest rate. In natural smoked (NS) cheeses, the butanoic acid was determined with the rate of 31.32% and 1,2 benzenedicarboxylic acid, diethyl ester with the rate of 21.13% at the highest rate. In 1% liquid smoked (LS) cheeses, the butanoic acid was found with the rate of 26.57% and 1,2 benzenedicarboxylic acid, diethyl ester with the rate of 20.37% at the highest rate.In 2% liquid smoked (LS2) cheeses, the butanoic acid was found with the rate of 30.42% and 1,2 benzenedicarboxylic acid, diethyl ester with the rate of 26.66% at the highest rate.

Proteolytic enzymes from lactic acid bacteria cause the degradation of casein and peptides, leading to production of free amino acids that contribute directly to the basic taste of cheese and indirectly to cheese flavor, as the precursors for other catabolic reactions These reactions and side-chain modification may yield keto-acids, ammonia, amines, aldehydes, acids and alcohols, which are essential contributors to cheese taste and aroma. For example, bitterness is due to hydrophobic peptides, rancidity to fatty acids, and fruitiness to esters [28; 29]. Important flavor compounds are esters, formed by condensation of an acid and an alcohol either spontanously or mediated by microbial esterases [30]. Sulphur compounds has several sulphur-containing compounds such as methanethiol (cabbage), methional, dimethylsulphide, dimethyldisulphide, dimethyltrisulphide (garlic), dimethyltetrasulphide(cabbage), carbonyl sulphide and hydrogen sulphide, which contribute to the aroma of cheese [19].

Volatiles play an important role in flavor perception of cheese. Cheeses can contain also a great number of hydrocarbons, which belong to a family of secondary products of lipid antioxidants. They do not have a major contribution to aroma in cheese, but may serve as precursors for the formation of other aromatic compounds [31; 32]. Flavoring compounds like thiols, hydrogen sulphide, esters, aldehydes, alcohols and ketones are present in the lipid phase of ripened cheese [33].

TABLE III. POTENTIAL FLAVOR COMPOUNDS IDENTIFIED IN CIRCASSIAN CHEESE RIPENED FOR 3 *MONTH*

Peak Number	RT	LRI	Flavor Compounds	C	NS	LS1	LS2
1	6.03	576	Hexanoic acid	7.97	2.90	4.38	2.43
2	6.57	614	Butanoic acid	43.42	31.32	26.57	30.42
3	6.84	657	1-Butanol, 2-methyl-, acetate	1.32	2.29	3.46	3.90
4	7.59	675	Butanoic acid, pentyl ester	0.25	3.23	0.73	0.55
5	7.81	687	2-nonenoic acid	0.40	2.25	2.84	4.94
6	8.34	695	Linalyl butyrate	0.93	3.55	0.39	0.40
7	9.38	761	2,4-Hexadienal, (E)-	0.19	2.93	2.07	2.94
8	9.70	769	2-Furanmethanol	0.14	1.20	0.38	1.01
9	10.04	788	NI	0.32	1.62	4.65	5.60
10	10.39	803	Ethyl hexanoate	14.29	11.72	18.44	12.20
11	10.82	820	Hexyl acetate	7.91	1.65	4.38	1.14
12	11.22	834	Octyl acetate	1.17	6.20	7.64	3.01
13	13.17	867	Propyl hexanoate	0.21	1.84	0.32	0.61
14	13.54	884	NI	0.19	0.61	1.38	1.86
15	16.08	1029	Butyl hexanoate	0.20	1.66	0.87	0.70
16	16.25	1045	Ethyl octanoate	0.61	1.19	0.71	1.34
17	19.89	1176	1,4-Benzodiazepin-2-one	0.62	1.34	0.23	0.16
18	26.52	1539	1,2-Benzenedicarboxylic acid, diethyl ester	19.66	21.13	20.37	26.66
19	28.23	1714	2,6-Dihydroxybenzoic acid	0.19	1.37	0.18	0.11

RT: Retention time; LRI: linear retention index, NI: not identified flavor compounds. **C:** control Circassian cheese, **NS:** naturally smoked Circassian cheese, **LS1:** 1% liquid smoked Circassian cheese, **LS2:** 2% liquid smoked Circassian cheese

Fatty acid composition, lipolytic enzymes, moisture, temperature, storage time, oxygen, and surface area all affect lipolysis. Lipolytic degradation of triglycerides of milk fat leads to the formation of FFAs, which are catabolized to volatile compounds, such as methyl ketones, thioesters, and lactones [34]. Lactose is also a major milk constituent for the formation of cheese aroma. Lactose, lactate and citrate contribute to the formation of diacetyl, acetoine, ethanol and acetate [8; 35; 36].

IV. CONCLUSION

Volatiles play an important role in flavor perception of cheese. Typical cheese aroma is the result of volatiles formed by lipolysis, proteolysis and metabolism of lactose, lactate and citrate. The results of this study confirmed that major flavor compounds were noticed as butanoic acid, 1,2 benzenedicarboxilic acid, diethyl ester, ethyl hexanoate, hexanoic acid in natural smoke and liquid smoked Circassian cheese with good characteristics, regarding to their sensory properties and flavor perspective.

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