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Determination of Volatile Compounds in Raw Milk with Mastitis

Mastitisli Sütlerde Uçucu Bileşiklerin Belirlenmesi

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ABSTRACT

Mastitis is a prevalent and economically significant disease in dairy cattle, affecting milk composition and quality. Subclinical mastitis (SCM) is more common and often asymptomatic. It can alter the chemical and cellular composition of milk, making it more difficult to process and potentially affecting the quality of dairy. This study aimed to investigate the volatile compounds present in raw cow's milk using Solid Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS). Milk samples were collected from various producers in Çanakkale, Biga and Yenice districts in Türkiye. From these, 10 samples that tested positive in the California Mastitis Test (CMT) were selected for analysis. A total of 49 volatile compounds were determined, including hydrocarbons (9), alcohols (4), aldehydes (3), ketones (7), acids (4), esters (11), terpenes (1), aromatic compounds (5), nitrogenous compounds (1), and other compounds (4). The most prevalent volatile components were butanoic acid, hexanoic acid, octanoic acid, and n-decanoic acid, which contribute to rancid and cheesy flavors in the milk. Other notable compounds detected included acetoin, various ketones, terpenes like d-limonene, and alcohols such as 3-methyl-1-butanol. The presence of these compounds is largely attributed to the metabolic activities of lactic acid bacteria and the degradation of fatty acids. The findings indicate that SCM significantly alters the volatile compound profile of milk, leading to the development of off-flavors that could impact the sensory quality of dairy products. The aim of this study is to provide information about the chemical changes that may occur in raw milk associated with SCM and to emphasize the importance of monitoring milk quality to ensure consumer safety and uphold high standards in dairy product quality.

Keywords: Mastitis, subclinical, CMT test, milk, volatile compound, aroma

ÖZ

Mastitis, süt sığırlarında yaygın olarak görülen ve ekonomik olarak önemli bir hastalıktır ve klinik ve subklinik formlarda ortaya çıkabilir. Subklinik mastitis (SCM), daha sık görülen ve genellikle asemptomatik olan formudur. SCM, sütün kimyasal ve sitolojik bileşiminde değişikliklere yol açarak işleme özelliklerini etkileyebilir ve patojen mikroorganizmalar ve antibiyotik kalıntıları nedeniyle halk sağlığı riskleri oluşturabilir. Bu çalışmada Katı Faz Mikroekstraksiyon (SPME) ve Gaz Kromatografisi-Kütle Spektrometrisi (GC-MS) kullanılarak çiğ inek sütünde bulunan uçucu bileşikler araştırılmıştır. Süt örnekleri, Türkiye'nin Çanakkale iline bağlı Biga ve Yenice ilçelerindeki çeşitli üreticilerden toplanmış ve California Mastitis Testi (CMT) pozitif çıkan 10 örnek analiz için seçilmiştir. Hidrokarbonlar (9), alkoller (4), aldehitler (3), ketonlar (7), asitler (4), esterler (11), terpenler (1), aromatik bileşikler (5), azotlu bileşikler (1) ve diğer bileşikler (4) dahil olmak üzere toplam 49 uçucu bileşeni belirlenmiştir. En baskın uçucu bileşenler, sütte acımsı ve peynirimsi tatlara katkıda bulunan bütanoik asit, hekzanoik asit, oktanoik asit ve n-dekanoik asit olarak belirlenmiştir. Tespit edilen diğer önemli bileşikler arasında asetoin, çeşitli ketonlar, d-limonen gibi terpenler ve 3-metil-1-bütanol gibi alkoller bulunmaktadır. Bu bileşiklerin varlığı büyük ölçüde laktik asit bakterilerinin metabolik aktivitelerine ve yağ asitlerinin parçalanmasına bağlanmıştır. Bulgular, SCM'nin sütün uçucu bileşik profilini önemli ölçüde değiştirdiğini, bu durumun da tat bozulmalarına yol açabileceğini ve süt ürünlerinin duyusal kalitesini potansiyel olarak etkileyebileceğini öne sürmektedir. Bu çalışmanın amacı, SCM ile ilişkili olarak çiğ sütte meydana gelebilecek kimyasal değişiklikler hakkında bilgi sağlamak ve tüketici güvenliğini sağlamak ve süt ürünleri kalitesinde yüksek standartları korumak için süt kalitesinin izlenmesinin önemini vurgulamayı amaçlamaktadır.

Anahtar Kelimeler: Mastitis, subklinik, CMT test, süt, uçucu bileşen, aroma

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Introduction

Mastitis is a significant economic concern in dairy cattle, as it leads to reduced milk production. It can manifest in two forms: clinical and subclinical. Subclinical mastitis (SCM) is characterized by an intramammary infection that does not present any visible clinical symptoms and occurs more frequently than clinical mastitis (Le Maréchal et al., 2011; Ibrahim, 2017; Gonçalves et al., 2018). *Streptococcus agalactiae, Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberis, and Escherichia coli* make up approximately 95% of the microorganisms causing mastitis, with other microorganisms accounting for the remaining 5% (Bray & Shearer, 1994). Several factors can affect the composition of milk, including the animal's breed, age, lactation stage, and diet. Additionally, mastitis can lead to significant changes in the chemical, cytological, and processing properties of milk (Le Maréchal et al., 2011). Milk from animals suffering from mastitis presents a public health concern due to the presence of pathogenic microorganisms and antibiotic residues. Additionally, the presence of antibiotic residues makes this milk unsuitable for further processing (IDF, 2000; Kivaria, 2006; Oliver & Murinda, 2012). Mastitis, an inflammatory reaction of the mammary gland tissue, is a condition with various causes, including infectious, traumatic, or toxic factors (International Dairy Federation (IDF), 1987; Fagiolo & Lai, 2007). However, only if these causes are better known can the condition be effectively predicted, prevented, and managed. When pathogenic microorganisms invade the mammary tissue, they proliferate, causing damage and increasing vascular permeability. The composition of the milk changes as blood components leak into the serum. The proteins, enzymes, and salts in the milk also change; casein and lactose production go down, and ion levels rise (Fagiolo & Lai, 2007; Nielsen, 2009; Leitner et al., 2011; Rovai et al., 2015a; Rovai et al., 2015b). This knowledge inspires hope for a future in which mastitis can be effectively prevented and managed. Moreover, mastitis results in significant economic losses, which include decreased milk production, changes in milk composition, wasted milk, increased costs, additional labor, treatment expenses, and veterinary services. It is estimated that mastitis causes an annual economic loss of $2 billion in the United States, $400 million in Canada, and $130 million in Australia (Aral et al., 2021). The main costs associated with mastitis can be broken down as follows: (1) reduced milk production at approximately $102 per cow, (2) discarded milk costing about $24 per cow, and (3) animal replacement at around $33 per cow. This brings the total cost of mastitis to roughly $159 per cow per year (Dalanezi et al., 2020). In a research study conducted in between 2023 November and 2024 December on endemic diseases in farms with 30 or more head of livestock in Konya (Center) province, Kadınhanı and Sarayönü districts, the economic loss costs for the farm, including treatment processes ($1 is taken as 25 TL), in mild, severe and fatal diseases caused by mastitis were determined as 1300 per cow (total 2550 TL), 2275 per cow (total 4865 TL) and 2600 TL per cow (total 46410 TL), respectively (Özdemir, 2024).

Normal metabolic processes in plant and animal tissues, specific technological processes (such as heating and cooking), and chemical reactions during storage (including photo-oxidation, hydrolysis, and lipid oxidation) can all generate aroma compounds (Reineccius, 2005). The study by Eriksson et al. (2005), which used gas chromatography-mass spectrometry to analyze the volatile compounds in milk samples with mastitis, has practical implications. The study revealed that milk from cows with mastitis contained higher levels of secondary metabolites from bacteria, including sulfides, ketones, amines, and acids. More secondary lipid oxidation products, like saturated and unsaturated aliphatic aldehydes, were in the milk from both healthy reference parts (regions) of the cows with mastitis and healthy cows. This study aimed to investigate the volatile compounds present in raw cow's milk with mastitis using Solid Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS), providing valuable insights for or food science and veterinary medicine.

Material and Methods

Material

In our previous study, raw milk samples were collected from 134 milk producers in six villages in Biga and Yenice districts of Çanakkale Province, and the somatic cell counts (SCC) in these samples were measured (Ozdikmenli Tepeli and Zorba, 2017). Based on the SCC results from this study, ten representative raw milk samples with varying SCC values were selected, as shown in Table 1. CMT scores according to Ruegg and Reinemann (2002) are also shown in Table 1. Then, volatile compound analyses of these samples were carried out using Gas Chromatography-Mass Spectrometry (GC-MS).

Table 1.
Somatic cell count (SCC) of the samples

No	Code	CMT score	SCC (cells/mL)
1	N1	Negative-1	46.000
2	N2	Negative-2	53.000
3	N3	Negative-3	99.000
4	T1	Trace-1	364.000
5	T2	Trace-2	373.000
6	T3	Trace-3	332.000
7	M1	1	573.000
8	M2	1	917.000
9	M3	2	2.114.000
10	M4	2	2.527.000

Method

Determination of some physicochemical properties of raw milk

In raw milk samples, pH was measured using the Hanna Combo (HI 98129, USA) device, DN was measured using the Funke Gerber-Cryostar (Gerber, Germany) device, and fat, lactose, protein, and non-fat dry matter (NFDM) ratios were measured using the Funke Gerber-3560 Lactostar (Gerber, Germany) device.

Volatile compounds analysis of raw milk

Three milliliters of each raw milk sample were weighed and transferred into amber bottles. After that, 1 g of NaCl and 5 µL of Internal Standard (20 µl of 2-methyl valeric acid and 5 µl of 2-methyl 3-heptanone in 5 mL of methyl alcohol) were added to a 40 mL volume vial and mixed with vortex for 15 seconds. They incubated at in a 40°C water bath (GFL, Model 1103, Burgwedel, Germany), for 20 minutes to allow the volatiles in the headspace to equilibrate. The solid phase microextraction (SPME) fiber (2 cm, 50/30 µm DVB/Carboxen/PDMS StableFlex, Supelco, Bellefonte, USA) was then inserted into the vial and left in a 40°C water bath for 15 minutes to absorb the aroma components in the headspace. Gas chromatography is equipped with a flame ionization detector (FID) and a CIS-Cooled Injection System (CIS) injection block (Agilent 6890N, Palo Alto, California, USA). All samples were injected into a polar (HP-INNOWAX, 30 mm length×0.25 mm inner diameter (i.d.)×0.25 µm film thickness; J&W Scientific) belt. The GC-MS column temperature program was at 40°C initial temperature held for 5 min, and is adjusted to reach the final temperature of 230°C with 10°C increments per min. The temperature of the injector block reached at 230°C. The waiting time at the final temperature was set to 15 min. Total analysis time was 54 min (Guneser & Karagul-Yuceer, 2011). Identification of the volatile components was performed using the Wiley Registry of Mass Spectral Data (Wiley, 2005) and the National Institute of Standards and Technology (NIST, 2008)

Statistical analysis

In order to statistically evaluate the volatile component analysis results of mastitis raw milk samples, the SPSS 22 (SPSS 2022) statistical program was used, and descriptive statistical analyses were performed. The results are given as mean and standard error. Analyses were performed in 2 replications.

Results and Discussion

Some physicochemical properties of samples with different SCC values are shown in Table 2. Although a decrease in protein value was observed as SCC values increased, many values remained within the reference limits.

Table 2.
Some physicochemical properties of raw milk samples

No		pH	Fat %	Protein %	NFDM %	Lactose %
	*Referans value**	*6.60-6.80**	*2.5-6.0**	*2.9-5.0**	*min 8.5**	*3.6-5.5**
1	N1	6.79	2.60	1.99	6.36	3.97
2	N2	6.98	3.28	2.45	7.43	4.58
3	N3	7.08	3.69	3.38	8.52	4.81
4	T1	7.06	3.42	2.73	7.58	4.49
5	T2	6.86	3.55	3.00	7.99	4.64
6	T3	6.83	3.60	3.20	8.15	4.66
7	M1	6.91	4.00	3.08	7.86	4.44
8	M2	6.74	3.58	2.69	7.52	4.51
9	M3	7.12	3.35	2.93	7.53	4.24
10	M4	6.84	3.31	2.90	7.87	4.60

*Anonim 2000, 2006, 2009 and Metin 2005. The samples are listed in ascending order according to their SCC values. NFDM: Non-fat dry matter

The analysis revealed that the following chemical groups comprised 51 identified compounds. These groups include hydrocarbons, alcohols, aldehydes, ketones, acids, esters, terpenes, and aromatic compounds. Some of these volatile compounds include ethyl alcohol, acetoin, hexane, acetic acid (vinegar), ethyl acetate, 3-hydroxy-2-butanone (sour

milk), and 3-methyl-1-butanol (fresh cheese, breathtaking, and alcoholic). The volatile compounds include butanoic acid ethyl ester, hexamethyl cyclotrisiloxane, butanoic acid (butter, cheese, sweat, acid), 3-methyl butanoic acid (sweaty, bitter, cheese, rancid), 2-methyl butanoic acid, 2,2,4,6,6-pentamethylheptane, hexanoic acid ethyl ester, hexanoic acid (sweaty, cheese, goat), d-limonene (lemon, mint), 2-nonanone (flower, fruit, peach), 2,6,10,14-phenyl ethanol (rose, flower). In general, compared to other volatile compounds, mastitis milk samples had relatively higher contents of acids such as acetic acid (vinegar), butanoic acid (cheesy), 3-methyl butanoic acid (sweaty, bitter, cheese, rancid), and hexanoic acid (sweaty, cheesy, goat). Among all volatile components, butanoic acid, hexanoic acid, octanoic acid, and n-decanoic acid were the most dominant. Butanoic acid (3583.13 µg/kg), hexanoic acid (3724.07 µg/kg), and octanoic acid (10896.32 µg/kg) were all highest in the N3 milk sample. On the other hand, butanoic acid (221.08 µg/kg) and hexanoic acid (377.64 µg/kg) were lowest in the M3 and T2 milk samples (Table 1). Lipolysis, the breakdown of lactose and amino acids, produces acids that give milk its perceivable rancid flavor. Carboxylic acids are not only vital volatile components in milk, but they are also key precursors of other compounds such as methyl ketones, alcohols, aldehydes, and esters (Yeu et al., 2015).

Milk samples with mastitis also contained aldehydes, such as hexanal, three methyl butanal, and pentanal (Table 3). Aldehydes are temporary compounds that can be formed during transamination or Strecker degradation during the catabolism of amino acids. Amino acids are converted to α-keto acids and then to aldehydes by the action of the aminotransferase enzyme. However, they are converted to alcohols and acids after a short time. Some aldehydes (such as butanal, pentanal, hexanal, octanal) are not formed during the catabolism of amino acids but by the β-oxidation of unsaturated fatty acids (Avşar et al., 2011). The Strecker metabolic pathways of lactic acid bacteria generate these compounds by decarboxylating branched-chain keto acids. Secondary oxidation of unsaturated fatty acids can also create them (Demirkol et al., 2016; Tahmas-Kahyaoglu et al., 2022). Oleic acid oxidation may generate hexanal, a well-known secondary oxidation product of linoleic acid. These compounds are responsible for the off-flavor found in milk and dairy products (Demirkol et al., 2016). The present study detected d-limonene, one of the terpenes, in different concentrations in the milk samples. However, the present study only detected this compound in four samples (N1, K5, T3, M3). The amount of d-limonene in the milk samples ranged from 0.99 to 3.04 µg/kg. Terpenes are compounds of plant origin that can pass into milk and from milk into products through grazing of dairy animals (Avşar et al., 2011). Plants naturally transfer terpenes and sesquiterpenes from forage plants to dairy products (Demirkol et al., 2016; Akgul et al., 2020; Guneser and Aydin, 2022). Further, they are crucial in determining the geographical origin (Akgul et al., 2020). Low concentrations of ketones can produce characteristic odors such as fruity, flowery, and musty. Furthermore, ketones like 2-nonanone and 2-undecanone are well-known to contribute to the aroma of dairy products (Akgul et al., 2020). Ketones are formed as β-keto acids (β-ketoacyl-coenzyme A.) after the conversion of triglycerides to free fatty acids by lipase enzyme, especially by the action of fungal (*Penicilium roqueforti*, *Penicilium camemberti* and *Geotrichum candidum*) or bacterial (*Lactococcus lactis*) enzymes. Methyl ketones are formed as a result of the decarboxylation of β-keto acids. Methyl ketones are converted to secondary alcohols by the action of lactic acid bacteria (*Lactobacillus* spp., *Leuconostoc* spp.). The formation of ketones such as diacetyl (2,3-butanedione) and acetoin (3-hydroxy-2-butanone) is not caused by fatty acids, but occurs by the breakdown of pyruvate formed as a result of lactose and citrate degradation (Avşar et al., 2011).

The study also found ketones in the subclinical mastitis milk samples. These included 2-nonanone, which smells like dirty milk, 2-heptanone, which tastes like cheese and wax, 2-butanone, and 2-undecanone, which tastes like citrus, wax, and cream. All samples detected 2-butanone among the ketones, while some failed to detect others. So, the N3 sample had higher amounts of 2-heptanone and 2-heptanone-24.34 µg/kg than the other milk samples (Table 3). Methyl ketones are made when fatty acids are oxidized, and β-keto acids made from saturated fatty acids are decarboxylated (Guneser & Aydin, 2022).

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Table 3. presents the volatile compound profile of raw milk samples with different SCC values, as determined by the precise and reliable method of SPME-GC-MS.

Table 3.

Volatile compound profile of mastitis raw milk samples determined by GC-MS (µg/kg)

Volatile Compounds	RI	Samples (Average±std. Error)									
		N1	N2	N3	T1	T2	T3	M1	M2	M3	M4
Alcohols											
Phenyl ethyl alcohol	<500	Nd.	Nd.	Nd.	4.49±0.12	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
3-Methyl-1-Butanol	751.61	19.83±3.69	Nd.	Nd.	Nd.	5.50±0.44	Nd.	Nd.	Nd.	Nd.	Nd.
2-Ethyl-1-hexanol	1031.49	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	2.38±0.18	Nd.
Dimethyl silanediol	690.92	6.93±0.00	Nd.	8.41±2.94	4.38±0.46	6.36±0.11	3.75±0.13	Nd.	Nd.	12.42±0.00	Nd.
Esters											
Ethyl Acetate	613.43	22.36±3.28	49.41±0.70	35.42±1.63	Nd.	38.92±0.57	1.85±0.62	8.34±3.88	3.63±0.18	6.27±0.02	Nd.
Ethyle ester formic acid	611.64	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
Methyl ester butanoic acid	718.35	Nd.	1.97±0.22	14.99±5.35	0.37±0.25	Nd.	Nd.	Nd.	1.27±0.41	Nd.	1.67±0.17
Ethyl ester butanoic acid	801.83	Nd.	36.07±2.73	50.13±17.02	1.31±0.34	Nd.	Nd.	4.51±0.77	Nd.	2.35±0.07	Nd.
Ethyl ester hexanoic acid	999.81	Nd.	22.54±3.39	22.00±13.75	Nd.	Nd.	Nd.	1.15±0.15	Nd.	2.94±0.25	Nd.
Ethyl ester decanoic acid	1394.87	Nd.	Nd.	0.81±0.33	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
Methyl ester octanoic acid	1124.16	Nd.	Nd.	6.46±2.43	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
3-ethoxy-ethyl ester-2-propenoic acid	1150.28	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	2.43±0.19
4-trimethylsilyl ester benzoic acid	1161.94	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	2.13±0.47
Ethyl ester octanoic acid	1198.66	Nd.	Nd.	Nd.	0.64±0.06	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
Methyl ester decanoic acid	1321.62	Nd.	Nd.	1.32±0.54	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
Aldehydes											
3-Methyl-Butanal	645.91	12.27±0.00	1.75±0.02	1.57±0.50	Nd.	1.73±0.00	Nd.	1.77±0.00	1.07±0.06	Nd.	Nd.
Hexanal	798.86	Nd.	Nd.	Nd.	0.67±0.17	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
Pentanal	645.78	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	nd.	1.29±0.01	Nd.
Hydrocarbons											
Hexane	603.81	Nd.	Nd.	Nd.	Nd.	Nd.	1.91±0.35	Nd.	Nd.	Nd.	Nd.
Propane	652.69	Nd.	Nd.	Nd.	0.82±0.02	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
2.4-Dimethyl heptane	819.31		2.08±0.01	1.22±0.22	1.57±0.61	2.74±0.11	1.39±0.43	1.08±0.12	2.71±0.41	0.77±0.04	2.57±0.31
Decane	1000.16	Nd.	Nd.	Nd.	Nd.	Nd.	3.39±0.36	Nd.	Nd.	Nd.	Nd.
4-methyl-octane	860.22	Nd.	1.58±0.02	0.93±0.14	Nd.	2.97±0.22	1.49±0.33	1.36±0.09	2.39±0.39	Nd.	2.35±0.12
4-methyl-decane	1022.49	2.10±0.14	Nd.	Nd.	Nd.	2.73±0.01	Nd.	Nd.	Nd.	Nd.	1.27±0.23
2.6-dimethyl-nonane	1022.71	Nd.	Nd.	Nd.	1.52±0.57	Nd.	1.56±0.35	Nd.	Nd.	Nd.	Nd.
3.4-dimethyl-decane	1060.18	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	0.89±0.06
3.6-dimethyl- decane	1102.29	Nd.	Nd.	Nd.	2.37±0.91	Nd.	9.05±6.71	1.67±0.03	1.34±0.17	Nd.	Nd.

Nd: Not Detected

Table 3.
Continued

Ketones	RI	Samples (Average±std. Error)									
3-methyl-2-butanone	69..28	1.70±0.13	10.95±1.13	2.96±0.81	0.96±0.14	2.42±0.11	0.93±0.16	1.61±0.01	1.49±0.01	1.51±0.06	7.82±0.99
3-hydroxy-2-butanone	606.28	6.72±1.22	Nd.	Nd.	Nd.	5.37±0.09	Nd.	Nd.	Nd.	Nd.	Nd.
Acetoin	<500	Nd.	57.15±7.45	17.28±6.50	80.75±18.69	65.76±1.72	7.59±0.55	79.17±13.62	35.94±4.05	Nd.	64.62±0.85
2-Butanone	600.26	1.70±0.13	22.54±3.39	25.69±10.06	0.98±0.14	2.42±0.11	0.93±0.15	1.15±0.15	1.49±0.00	2.94±0.25	4.53±0.15
2-Heptanone	887.64	2.61±0.26	21.41±1.18	24.34±8.68	Nd.	13.80±1.43	27.21±5.65	4.13±0.68	3.34±0.26	Nd.	3.61±0.01
2-Nonanone	1090.30	1.91±0.06	6.72±0.89	9.17±3.27	Nd.	14.23±0.45	27.97±7.06	0.93±0.01	1.49±0.61	Nd.	Nd.
2-undecanoate	1292.15	Nd.	1.08±0.07	2.19±0.21	Nd.	Nd.	0.31±0.19	Nd.	Nd.	Nd.	Nd.
Aromatic compounds											
Methyl benzene	759.83	Nd.	Nd.	3.54±1.17	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	12.09±0.53
Toluene	760.25	1.84±0.34	8.72±0.32	Nd	6.27±1.04	9.29±0.58	6.74±1.07	5.64±0.69	16.59±2.67	5.99±0.31	
1.2.3.4-tetramethyl-benzene	1111.74	1.83±0.13	1.46±0.01	1.16±0.19	0.28±0.02	1.78±0.01	0.62±0.08	1.36±0.08	1.22±0.24	1.35±0.08	0.41±0.02
1-ethyle.2.3-dimethyl benzene	1115.63	Nd.	Nd.	Nd.	0.28±0.02	0.50±0.16	Nd.	Nd.	0.32±0.01	Nd.	1.59±0.05
1.2-dichloro-benzene	1004.94	Nd.	Nd.	Nd.	2.38±0.70	Nd.	Nd.	Nd.	Nd.	Nd.	6.02±0.30
Acids											
Butanoic acid	797.79	319.68±7.02	521.13±231.31	3583.13±13.5	234.74±22.30	267.20±7.80	Nd.	959.15±151.03	613.29±20.37	221.08±36.29	1430.35±71.09
Hexanoic acid	1002.97	1168.50±117.74	2305.04±331.01	3724.07±84.66	663.80±91.53	377.64±9.68	Nd.	2151.33±300	1880.63±88.64	692.90±41.62	2996.64±8.66
Octanoic acid	1184.70	Nd.	3335.32±20.63	10896.32±658.51	Nd.	Nd.	Nd.	764.16±30.47	860.48±147.95	Nd.	2131.49±287.93
n-Decanoic acid	1369.28	Nd.	543.47±39.08	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.	432.02±123.64
Terpenes											
dl-Limonene	102..76	2.20±0.01	Nd.	Nd.	1.52±0.57	Nd.	3.04±0.51	Nd.	Nd.	0.99±0.16	Nd.
Other compounds											
1.1.2.2-tetrachloro ethane	909.05	Nd.	Nd.	Nd.	0.47±0.18	Nd.	0.61±0.11	Nd.	Nd.	Nd.	Nd.
Tetrachloroethylene	806.98	7.99±0.26	18.93±1.6	Nd.	15.98±2.29	24.08±1.55	13.90±2.61	17.19±2.60	29.19±4.04	14.87±0.23	28.95±1.22
Hexamethyl-cyclotrisiloxane	822.19	3.96±0.53	7.53±1.32	Nd.	4.11±0.48	5.63±0.12	3.63±0.06	4.79±0.76	4.85±0.48	6.03±0.27	Nd.
Octamethyl-cyclotetrasiloxane	1005.05	Nd.	Nd.	Nd.	3.91±0.44	5.18±0.17	3.83±0.14	5.88±1.23	3.94±0.18	5.59±0.52	Nd.
Nitrogenous compound											
Methoxy-phenyl oxime-	910.95	3.39±0.43	4.37±0.71	3.87±1.44	3.25±0.21	3.86±0.04	3.39±0.08	3.54±0.45	2.99±0.49	Nd.	5.99±0.81

Nd: Not dedected

Methyl ketones are made when fatty acids are oxidized, and β-keto acids made from saturated fatty acids are decarboxylated (Guneser & Aydin, 2022). Acetoin, responsible for the creamy flavor and sweet taste of dairy products, is derived from the fermentation of lactose. All milk samples (except for N1 and M3) contained compounds, albeit in varying quantities. The T1 sample had the highest amount of acetoin (80.75 g/kg), while the T3 sample had the lowest amount (7.59 g/kg) (Table 3). When homo/heterofermentative lactic acid bacteria break down citrate and lactose, they produce acetoin, a volatile compound (Guneser & Aydin, 2022). Moreover, lactic acid bacteria use α-acetolactate synthase and α-acetolactate decarboxylase enzymes in lactose or citrate metabolism to create acetoin (Demirkol et al., 2016).

Alcohols are formed through various metabolic processes, including amino acid metabolism, lactose metabolism, methyl ketones reduction, and degradation of unsaturated fatty acids. They can also be converted into ethyl alcohol by heterofermentative microorganisms, acetaldehyde, and 8-carbon unsaturated alcohols. Deamination events, influenced by oxido-reductases, are also involved in the formation of alcohols from amino acids. *Geotrichum candidum*, rich in oxido-reductases, can convert amino acids into alcohol compounds like ethanol, 2-methylpropanol, 3-methyl-butanol, and phenyl alcohol. (Avşar et al., 2011). Subclinical mastitis-affected milk samples contained alcohols such as phenylethyl alcohol, 3-methyl-1-butanol, 2-ethyl-1-hexanol, and dimethyl silanediol (Table 3). Furthermore, only phenylethyl alcohol and 2-ethyl-1-hexanol were detected in samples T1 and M3, respectively. Many metabolic pathways in dairy products produce alcohol, including lactose metabolism, methyl ketone degradation, and amino acid metabolism. Furthermore, the native milk flora significantly contributes to alcohol formation in dairy products. Even though ethanol is a precursor of ethyl esters, it only has a minor aromatic function in dairy products. Furthermore, milk widely accepts branched-chain alcohols like 3-methyl-2-butanol as off-flavoring components. This is thought to be due to the conversion of reactive aldehydes into alcohol or acid components (Akgul et al., 2020).

Esters are formed as a result of lactose fermentation and/or amino acid catabolism and alcohol (primary and secondary) that will participate in the reaction, and ester compounds are formed as a result of the esterification reaction of acid and alcohol in equal molar volumes. In addition, acidolysis and transesterification reactions play a role in the modification of esters (Avşar et al., 2011). Esters are flavor-enhancing compounds present in fermented milk. Although they contribute positively to flavor balance at low concentrations, at high concentrations, they can cause fruity taste defects (Akgul et al., 2020; Tahmas-Kahyaoglu et al., 2022). Moreover, high ethyl esters of long-chain fatty acids (C12 or above) might give the product an unpleasant, soapy, tallowy odor (Akgul et al., 2020). Eleven different ester compounds were detected in milk samples (Table 3). While multiple samples detected ethyl acetate, methyl ester butanoic acid, ethyl ester butanoic acid, and ethyl ester hexanoic acid, only one sample detected each other. Hydrocarbons, which are secondary products of lipid oxidation, directly influence scent; they also contribute to the formation of other aroma components. Low concentrations of hydrocarbons were detected in all milk samples. While multiple samples detected 2,4-dimethylheptane, 4-methyloctane, and 4-methyldecane, only one sample detected each of the other compounds. Each milk sample contained aromatic compounds, even at low concentrations. Except for samples M4 and N3, low amounts of toluene were detected. This compound may have originated from carotenoid degradation or solvent contamination (Cakmakci & Hayaloglu, 2011).

Other researchers (Mounchili et al., 2005; Eriksson et al., 2005) have also identified some of these volatile compounds in raw milk samples from cows with mastitis. Eriksson et al. (2005) identified 103 volatile compounds in milk samples suffering from mastitis. The researchers found that milk samples from cows with mastitis contained higher levels of sulfur, ketones, amines, and acids. The main parts found by researchers were ethanol, trimethylamine, 2,3-butanedione, 2-pentanone, 2-methylbutanal, methane-thiol, dimethylsulfide, and acetic acid compounds. They found that milk samples from healthy individuals contained more secondary lipid oxidation products, particularly homologous saturated and unsaturated aldehydes, than those from individuals with mastitis. In particular, the researchers linked samples containing higher levels of pentanal with reference milk samples. Researchers found that milk samples from healthy cows had higher levels of typical secondary lipid oxidation products and C4-C8 alcohols compared to milk with mastitis. Mounchili et al. (2005) first analyzed milk samples from 9 Holstein cows organoleptically using two sensory panelists, mass spectrometry/flame ionization (MSD/FFID), and olfactometric detectors. The analysis revealed 75 volatile compounds. Butane-2,3-dimethyl, pentane-3-methyl, acetic acid ethyl ester, heptane, hexane-2,5-dimethyl, hexane-2,4-dimethyl, pentane-2,3, 3-trimethyl, hexane-2,3-dimethyl, 4-methyl heptane-, 3-methyl-heptane, octane, 2-ethyl-dodecane-, ethyl benzene, 1,3-dimethyl benzene, hexanoic acid, limonene, nonanal, undecane,

octanoic acid, benzoic acid, ethyl ester, benzaldehyde ethyl, undecane-2-one, and decanoic acid were some of the parts that researchers found.

Comparative analysis with previous studies, such as those by Eriksson et al. (2005) and Mounchili et al. (2005), supports the observation that milk from animals with mastitis tends to have elevated levels of compounds, including sulfur, ketones, amines, and acids, which negatively affect the sensory qualities of the milk. These compounds are often the result of microbial activity, lipid oxidation, and other metabolic processes triggered by the mastitis infection.

Conclusion and Recommendations

In conclusion, the use of solid-phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) to analyze raw milk samples from animals with subclinical mastitis (SCM) revealed the presence of a wide range of volatile compounds. The 51 compounds identified were divided into various chemical groups, including hydrocarbons, alcohols, aldehydes, ketones, acids, esters, terpenes, and aromatic compounds. The results show that milk samples from cows with mastitis contained higher amounts of volatile acids, including butanoic acid, hexanoic acid, octanoic acid, and decanoic acid. These acids are associated with unpleasant odors, such as rotten, cheesy, and sweaty smells.

Additionally, aldehydes and ketones, particularly hexanal, 3-methylbutanal, and 2-heptanone, were detected as contributing to taste disturbances. Though in lower concentrations, terpenes such as d-limonene highlight the influence of forage plants on the milk's volatile profile. Alcohols and esters have also been found to have different effects on flavor depending on their concentration. Overall, mastitis, an inflammation of the mammary gland in dairy cattle, is a significant challenge for the dairy industry worldwide. It affects milk production, quality, and safety, while also imposing substantial economic burdens on milk producers. Early detection of mastitis is critical for maintaining milk safety, reducing economic losses, and ensuring animal welfare. Particularly in its subclinical form, it can compromise milk safety by introducing pathogenic bacteria into the milk supply. Moreover, these volatile compounds compromise the sensory qualities of milk and suggest potential health risks associated with consuming milk from animals with subclinical mastitis. This underscores the importance of early detection and management of mastitis to ensure the safety and quality of dairy products.

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