J. Agr. Sci. Tech. (2020) Vol. 22(1): 147-158

Evaluation of Fatty Acids and Volatile Compounds in Iranian Ghee by Head Space-Solid Phase Microextraction Coupled with Gas Chromatography/Mass Spectroscopy

S. H. Erfani[1], M. Ghavami[1*], Sh. Shoeibi[2], A. Zand-Moghaddam[3], and H. Rastegar[2]

ABSTRACT

Ghee, a nutritional dairy product in Iranian culture, can be easily produced on a small scale. This study was undertaken to analyze fatty acids and volatile compounds of collected ghee samples from different ghee production sites of Iran (Ilam, Kermanshah and Hamedan) using HeadSpace Solid Phase MicroExtraction (HS-SPME) technique. According to the results, palmitic and oleic acids were the dominant fatty acids in all the samples investigated. Further, it might be concluded that compounds such as dodecane, acetone, butyric acid, hexanoic acid, 2-pentanone, 2-heptanone, and 2-undecanone, which are present and might have accumulated as the results of oxidative, hydrolytic, or microbial activities, contribute to the flavor of ghee. Lactones, which are produced at high temperatures, were not collected in any sample except the Hamedan sample (< 1%). Low thermal processing in the ghee production prevented the formation of off-flavor volatile compounds. The qualitative and quantitative parameters determined in this study might be useful in assessing the quality of the ghee and may help the industry to improve its commercial production.

Keywords: Butterfat, Dairy product, Flavor of ghee, HS-SPME-GC/MS.

INTRODUCTION

Ghee as a traditional butterfat in cooking recipes plays important role in Iranian diet. According to the report of Food and Agriculture Organization Statistical (FAOSTAT, 2014), the annual ghee production in Iran is 204,344 tons. It contains fat-soluble vitamins, essential fatty acids, flavoring agents, and offers a remarkable source of energy, which can be produced from a variety of full-fat milk (sheep, cow, and camel) (Rajorhia, 1993; Urbach and Gordon, 1994). Approximately, saturated fatty acids comprise two thirds of ghee's fatty acids, majority of which is composed of long-chain fatty acids, while the rest of this composition consists of monounsaturated fatty acid with a minor fraction of polyunsaturated fatty acids (Mehta, 2013). However, ghee is mainly used in cooking and frying, it can be considered as one of the ingredients of confectionery products. Different producers are involved in important sites of ghee production of Iran including Kermanshah, Ilam, and Hamedan, based on the traditional or modern techniques. In this regard, the main four production techniques can be summarized as milk butter method (with

[1] Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Islamic Republic of Iran.
[2] Food and Drug Laboratory Research Center, Food and Drug Organization, Tehran, Islamic Republic of Iran.
[3] Department of Food Science and Technology, Ahvaz Branch, Islamic Azad University, Ahvaz, Islamic Republic of Iran.
* Corresponding author; email: mehrdad_ghavami@yahoo.com

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

highest level of flavor agents), creamery butter method, direct cream method, and prestratification method (Munro *et al*., 1992; Ganguli and Jain, 1972). In the traditional practice of ghee production in Iran such as milk butter method, the whole milk is turned into yoghurt by inoculation of Lactic Acid Bacteria (LAB) and the resulting yoghurt is then fed to a churn to form butter, followed by the formation of ghee in the final stage of processing. The percentage and quality of milk's fat as well as production conditions correspond to flavor of ghee, which is the key factors for its marketing and consumer acceptance. The quality of ghee is determined by some parameters such as peroxide value, flavor, and acidity. The quality of ghee in storage duration has been measured by acid and peroxide value. The temperature of clarification is the most important factor that controls the intensity of flavor of ghee. Ghee produced at 120ºC or at higher temperature has intense flavor, which is usually referred to as cooked or burnt ghee, while ghee prepared at around 110ºC has milder flavor (Nadeem *et al*., 2013). The volatile compounds of ghee are carbonyls, Free Fatty Acids (FFAs) and lactones, which readily evaporate at room temperature and trigger the smell sensation once reaching olfactory receptors (Angerosa, 2002; Wadodkar *et al*., 2002). The conventional techniques for extraction of volatile compounds include distillation, solvent extraction, supercritical CO_2 extraction, and different absorptive extraction techniques (Hashemi *et al*., 2009; Chen *et al*., 2008). Simultaneous Distillation-Extraction (SDE) is a widely used method for isolation of volatile compounds. Among them, Solid Phase MicroExtraction (SPME) offers numerous advantages over traditional methods such as no toxic solvents, optimized temperatures and short process times, cheap, selectivity, and fast direct extraction of organic compounds (Arthur *et al*., 1992). In this context, in the sample preparation technique, samples are concentrated, extracted, and injected into the Gas Chromatography (GC) device in just one phase. The extractor fiber matrix comprised of fused silica with a thin polymer coating (micrometer thickness) for the extraction phase (Bola and Tan, 2015; Zhu *et al*., 2009). In this technique, analytes are first absorbed by the fiber coating, which is then desorbed by GC heat following extraction and concentration (Zhao *et al*., 2007). The quality and quantity of analytes absorbed by the SPME fiber can be varied by changing experimental conditions, fiber composition, absorption ratio, and distribution coefficient (Yang and Peppard, 1994). Moreover, fibers mixed with DiVinylBenzene/Carboxen/PolyDiMethylSiloxane (DVB/CAR/PDMS) coatings can also be utilized to increase analyte absorption and distribution (Marsili, 2007). Therefore, the extraction time, fiber composition, ghee concentration, headspace volume, and the GC conditions (*e.g.*, injector desorption temperature and injection depth) can be optimized (Camarasu, 2000). The HeadSpace SPME (HS-SPME) technique is a special extraction method that can trap the formed aromatic compounds at the headspace, which in turn improves the extraction and absorption.

This study was aimed to analyze the physicochemical properties and the fatty acid profile of bovine ghee from main production centers of Iran (*i.e.*, Kermanshah, Ilam, and Hamedan) and its flavor-inducing volatile compounds using the HS-SPME-GC/MS technique. The quality and quantity of volatile compounds and their sources are also discussed and reported.

MATERIALS AND METHODS

Raw Milk

Whole milk was collected from sixty forage-fed cows including twenty cows from Javanrud Farms (Kermanshah, Iran), twenty cows from Nahavand Farms (Hamedan, Iran) and twenty cows from Abdanan Farms (Ilam, Iran). The milk was collected from the cattle with similar feeding practices and

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

breed in the morning during the summer months (July-August, 2017). The collected milk was stored in the cold room overnight in order to keep the premium quality.

Ghee Production

Raw milk samples from each region were heated to 85°C for 5 minutes as the primary thermal processing. They were then cooled down to 32±3°C as the recommended temperature of lactic starter inoculation, followed by 10 to 16 hours incubation at the same temperature. At the end of this period, the prepared yogurt samples were fed into the churn and churned at 15°C until the butter formation. Buttermilk was discarded after 30 minutes, and butter grains were washed with cold water to remove the remaining buttermilk. The resulting butter was kept in steel containers to evaporate the remaining water at 104°C, and also to form the volatile compounds corresponding to flavor. Afterward, final ghee samples were placed into plastic containers and kept at 4°C (Munro, 1992).

Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters were prepared according to the procedure recommended by ISO 12966-2:2011 (ISO, 2011). About 10-50 mg of the sample was dissolved in 1 mL isooctane and methylated with 2 mL of 0.2M sodium methoxide. The fatty acid methyl ester (FAME) was injected to an ACME 6000M GC equipped with 60 m DM-2330 (Young Lin Instrument Co., Ltd) capillary column (0.25 mm ID×0.2 µm df, Courtaboeuf Cedax, France) and Flame Ionization Detector (FID). The initial temperature was kept at 50°C for 1 minute and was raised to 180°C with a 8°C min^{-1} slope. The Helium stream velocity was 1 mL min^{-1} and its purity was 99.999%. The relative retention time of the ghee sample was compared to the external FAME standards (FAME Mix C4-C24, Supelco, Sigma-Aldrich), and the fatty acids were identified.

HS-SPME Conditions

A certain amount of ghee was removed from the refrigerator and was melted for 5 minutes in a water bath. A three mL sample was taken and poured into special 10 mL glass containers with SPME fibers and silicon/polytetrafluoroethylene caps. They were then covered fully with aluminum sheets. SPME fiber with DVB/CAR/PDMS 50/30 µm coating was purchased from Supelco (Park, Bellefonte PA, USA). The containers were kept for 24 hours in an oven (Binder, Germany) at 60°C to allow the formation of volatile compounds in the headspace. After that, the SPME fiber was placed at headspaces and samples were kept at 60°C in a circulating water bath for 60 minutes (Cole-Parmer Instrument Co., USA) for absorption of volatile compounds onto the stationary phase of the fiber. The fiber was carefully removed from the headspace and transferred to GC/MS for desorption measurement (Henneberry, 2016).

Analysis of Volatile Compounds by GC/MS

Before extraction and concentration, the SPME fiber was coupled with the GC injector for 4 hours at 260°C to provide the optimal conditions for absorption of volatile compounds. Since absorption and desorption of volatile compounds by fiber is a highly sensitive reaction, the experiments were performed at 40, 60 and 100°C. The volatile compounds were absorbed on the fiber only at 60°C. For desorption, the SPME fiber was inserted inside the GC injector inlet (Hewlett-Packard 6890) linked to a mass spectrometer (HP - 6890). Volatile compounds were thermally desorbed and were directly transferred to a 5% phenyl dimethyl siloxane HP-1MS column (60 m×0.25 mm ID×0.25 µm df). The

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

temperature program of the oven was 60°C for 3 minutes and then increased to 220°C at the rate of 4°C min^{-1}. Detector and injector temperatures were 260 and 250°C, respectively. Helium gas (99.999% purity) was used as a carrier with a flow rate of 1 mL min^{-1}. The fiber was kept for 5 minutes in the GC injector. Mass spectra were determined at 70 eV using spectrometer detector. The mass spectra were compared against information in the Wiley and NIST database. The relative percentage of each compound was calculated as the ratio of the area under the peak curve of each volatile compound to the total area.

RESULTS AND DISCUSSIONS

Identification of Fatty Acid

The fatty acids compositions of samples are presented in Table 1. The Saturated Fatty Acid (SFA) content of samples from Ilam, Kermanshah, and Hamedan was reported as 63.3, 60.4 and 64.85%, respectively. While, the monounsaturated fatty acid content for Ilam, Kermanshah, and Hamedan samples was 30.1, 31.37, and 29.7%, respectively. Oleic acid was the dominant MonoUnsaturated Fatty Acids (MUFAs). In addition, the total PolyUnsaturated Fatty Acids (PUFAs) in the samples from Ilam, Kermanshah and Hamedan were 3.89, 4.01 and 3.06%, where linoleic acid had the highest concentration among all PUFAs.

The chemical assay of ghee fatty acids provides information concerning the quality and flavor of ghee samples. The results can be used to evaluate, detect and investigate frauds in different ghee products. Palmitic acid was the predominant fatty acid in all the samples examined. Moreover, the presence of a particular fatty acid or the ratio of some acids might play an important role in identification or adulteration of the ghee (Palmquist, 2006). The variation in fatty acid composition might be related to differences in feeding practices, seasonal and climatic conditions, and other factors (Najafi *et al*., 2015). The results of the present investigation are in good agreement with the findings by Dorni *et al*. (2018), where the palmitic acid was the predominant fatty acid ranging from 37.14 to 41.58 %. followed by oleic acid that was between 19.49 and 25.12%. Johnson and Saikia (2009) also reported palmitic and oleic acid as the predominant fatty acids in Indian ghee.

HS-SPME Analysis of Volatile Compounds in Ghee Samples

Tables 2, 3, and 4 summarize the GC/MS results for the analysis of volatile ghee compounds. Based on the findings, a total of 17 (Ilam), 19 (Kermanshah) and 28 (Hamedan) volatile compounds were detected using MS and/or comparison with Kovats Index (KI).

The use of mixed DVB/CAR/PDMS fibers offered a better performance than simple fibers since the latter could detect fewer compounds (Chiesa *et al*., 2006). However, the aromatic compounds of ghee can vary due to differences in the feed, production season, production method, raw materials (milk, cream, butter and yogurt), butter clarification temperature, local temperature, and storage conditions (Azzara *et al*., 1992; Day *et al*., 1964; Widder *et al*., 1991). Ghee samples from these regions were produced from milk and had a wide range of volatile compounds including aldehydes, ketones, alcohols, esters, fatty acids and aromatic compounds. The main detected volatile compounds in ghee samples from all three regions were dodecane, acetone, butyric acid, hexanoic acid, 2-pentanone, 2-heptanone, and 2-undecanone.

The main acids identified in the volatile compounds of the investigated ghee were acetic acid, butyric acid, hexanoic acid, heptanoic acid, octanoic acid, decanoic acid, and tetradecanoic acid. Moreover, the highest concentration of detected acid among the three regions belonged to

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

Table 2. Volatile compounds sampled by HS-SPME and identified by GC/MS and KI for Ilam ghee.

No	Compounds	MI[a]	Peak area (%)	RT (Min)	Odor quality	Mechanism of formation
1	Dodecane	MS	0.63	17.25	Sweety	naturally occurring in milk
2	Butyric acid	MS	6.97	8.35	Buttery, sweaty, cheesy, rancid	Microbial, enzymatic
3	Hexanoic acid	MS	11.98	12.77	Pungent, musty, cheesy, rancid	Microbial, enzymatic
4	Octanoic acid	MS	0.82	16.78	Musty, pungent, rancid	Microbial, enzymatic
5	Acetone (Propane)	MS	3.82	4.82	Sweety	Metabolites of feed and silage, naturally occurring in milk
6	2-Pentanone	MS	1.77	6.12	Creamy, green, milky, soapy	Thermally induced, microbial
7	2-Heptanone	MS	28.11	9.71	Blue cheese, fruity, dairy-like	Thermally induced, microbial
8	8-Nonen-2-one	MS	2.34	14.16	Indolic, floral, honey, skatole	Mold growth/Metabolites
9	2-Nonanone	MS	18.48	14.44	Cooked	Thermally induced, microbial
10	2-Undecanone	MS	2.23	18.80	Cooked	Thermally induced, microbial
11	4-Hepten-3-one diethyl	MS, KI	0.88	26.73	Oxidized	Lipid oxidation, light abuse, Cu oxidation
12	Diethyl Phthalate	MS	1.83	24.02	Bitter, slightly fruity	Environmental pollutants, packaging
13	Cyclotrisiloxane, hexamethyl	MS	1.07	8.64	-	Fiber
14	Cyclopentasilosxane, decamethyl	MS, KI	0.89	16.43	-	Fiber
15	Di 2-ethylhexyl phtalate	MS	14.59	32.09	Bitter, slightly fruity	Environmental pollutants, packaging
16	Uknown		3.59	-	-	-

[a] MI: Method of Identification, MS: Mass Spectrum comparison using Wiley and NIST libraries, KI: Kovats Index in agreement with literature values. RT: Retention Time

hexanoic acid, which might play substantial role in both desired flavor and rancid taste of Iranian ghee. The high concentrations of short-chain fatty acids (e.g., Butyric acid, which accounted for a large quantity of volatile compounds) can be associated activities of lactic acid bacterial as well as that of lipase (Yadav and Srinivasan, 1992). The variations in the short-chain fatty acids in ghee samples might be due to high temperatures used in clarification process for butter, which causes the formation of short chain fatty acids (Wadodakar *et al*., 1996).

Among the samples examined, only Kermanshah samples had an aldehyde compound (2-methyl pentanal) the producer of a sweet buttery taste in ghee. It is formed as the result of lipid oxidation of unsaturated fatty acids during storage of butter or preparation of ghee. Due to the low concentration of USFA, the formation of this compounds is not a matter of concern. (Borle *et al*., 2001; Badings and Neeter, 1980; Grosch, 1987). Pedrotti *et al*. (2018) also reported that one of the most volatile compounds for dairy oxidative stress is aldehyde that is increased during storage (Pedrotti *et al*., 2018). The hydrocarbons formed as a consequence of oxidation of USFAs in the volatile compounds were identified as dodecane, tetradecane, hexadecane, and octadecane (Badings, 1970). However, the highest hydrocarbon diversity was found in Kermanshah samples.

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

Table 1. Fatty acid composition of ghee from the western provinces of Iran.

Fatty acids as % of total fatty acids	Ilam	Kermanshah	Hamedan
Saturated fatty acids			
c4:0 Butyric	1.59±0.01	1.3±0.00	1.1±0.01
c6:0 Caproic	1.43±0.01	1.13±0.01	1.21±0.01
c8:0 Caprylic	1.06±0.02	0.86±0.02	0.89±0.00
c10:0 Capric	2.48±0.01	2.11±0.01	2.21±0.01
c12:0 Lauric	3.05±0.00	2.66±0.02	2.9±0.02
c14:0 Myristic	10.56±0.03	9.28±0.04	10.55±0.03
c15:0 Pentadecanoic	1.26±0.01	1.27±0.01	1.42±0.02
c16:0 Palmitic	29.95±0.04	30.82±0.05	33.67±0.03
c17:0 Heptadecanoic	0.73±0.01	0.74±0.01	0.84±0.02
c18:0 Stearic	10.99±0.04	9.96±0.05	9.87±0.01
c20:0 Arachidic	0.2±0.01	0.27±0.00	0.19±0.01
c22:0 Behenic	Tr	Tr	Tr
c24:0 Lignoceric	Tr	Tr	Tr
Total	63.3	60.4	64.85
Mono-unsaturated fatty acids			
c10:1 Decenoic	0.24±0.01	0.26±0.01	0.23±0.01
c14:1 Myristoleic	1.51±0.02	1.66±0.01	1.7±0.00
c15:1 cis-10 pentadecanoic	0.35±0.00	0.35±0.01	0.38±0.01
c16:1 Palmitoleic	2.98±0.01	3.23±0.01	3.61±0.03
c17:1 cis-10 Heptadecanoic	0.38±0.01	0.41±0.02	0.47±0.02
c18:1t	1.16±0.01	1.63±0.01	2.1±0.01
c18:1c Oleic	23.28±0.03	23.22±0.02	20.46±0.03
c20:1 cis-11 Eicosenoic	0.2±0.00	0.61±0.01	0.75±0.01
c22:1 Erucic	Tr[a]	Tr	Tr
c24:1 Nervonic	Tr	Tr	Tr
Total	30.1	31.37	29.7
Poly-unsaturated fatty acids			
c18:2t	0.67±0.01	0.58±0.01	0.42±0.01
c18:2c Linoleic (LA) ω6	2.78±0.02	3.43±0.02	2.16±0.01
c18:3n3 a-Linolenic ω3	0.44±0.01	Tr	0.48±0.00
c22:4n6 Arachidonic ω6	Tr	Tr	Tr
c22:2 cis 13,16 Docosadienoic ω6	Tr	Tr	Tr
Total	3.89	4.01	3.06

[a] Tr (Trace): < 0.1%.

The rank order of hydrocarbon compounds in Ilam, Kermanshah and Hamedan samples were dodecane (0.63%), tetradecane (9.30%) and dodecane (0.53%), respectively.

Among the samples from these regions, only Hamedan samples had an alcohol compound (3-pentanol). Alcoholic compounds play a significant role in the taste development in ghee. These compounds are formed by the breakdown of unsaturated hydroperoxide following the formation of alkoxy radicals. However, aliphatic alcohols can be produced by microbial enzymatic activities or lipid oxidation (Fross, 1972).

The detected ketones (methyl ketone) were acetone, 2-pentanone, 2-heptanone, 8-nonen-2-one, 2-nonanone, 2-undecanone, 4-hepten-3-one diethyl, and 2-dodecanone. The highest ketone diversity was found in Ilam samples. The concentration of 2-heptanone as dominant ketone compound was 28.11% (Ilam), 17.69% (Kermanshah), and 8.48% (Hamedan). Methyl ketones are associated with the formation of desirable flavor in ghee as their smell threshold is 10 times stronger than other ketones and have

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

Table 3. Volatile compounds sampled by HS-SPME and identified by GC/MS and KI for Kermanshah ghee.

No	Compounds	MI[a]	Peak area	RT	Odour quality	Mechanism of formation
1	Dodecane	MS	1.02	17.24	Pungency	Naturally occurring in milk
2	Tetradecane	MS	9.30	20.08	Peppery, pungency	Naturally occurring in milk
3	Hexadecane	MS	2.59	24.54	Sweety	Naturally occurring in milk
4	Octadecane	MS	0.93	27.84	Sweety	Naturally occurring in milk
5	Butyric acid	MS	6.81	8.46	Buttery, sweaty, cheesy, rancid	Microbial, enzymatic
6	Hexanoic acid	MS	9.17	12.95	Pungent, musty, cheesy, acrid	Microbial, enzymatic
7	Tetradecanoic acid Aldehyde	MS	6.18	35.04	Cheesy	Microbial, enzymatic
8	2-methyl pentanal	MS	10.32	14.44	Rancid	Lipid oxidation
9	Acetone (Propanone)	MS, KI	3.89	4.92	Sweety	Metabolites of feed and Silage, Naturally occurring in milk
10	2-pentanone	MS, KI	7.53	5.44	Creamy, green, milky, soapy	Thermally induced, microbial
11	2-heptanone	MS	17.69	9.74	Blue cheese, fruity, dairy-like	Thermally induced, microbial
12	2-Undecanone		0.94	18.79	Cooked	Thermally induced, microbial
13	Octanoic acid, ethyl ester (Ethyl octanoate)	MS	1.06	16.79	Fruity	Microbial, enzymatic
14	Diethyl phtalate	MS	16.35	23.73	Bitter, slightly fruity	Environmental pollutants, packaging
15	Diethyl phtalate	MS	2.27	24.01	Bitter, slightly fruity	Environmental pollutants, packaging
16	Pentasiloxane, dodecamethyl	MS, KI	1.09	22.90	-	fiber
17	Uknown		2.87	-	-	-

[a] MI: Method of Identification, MS: Mass Spectrum comparison using Wiley and NIST libraries, KI: Kovats Index in agreement with literature values.

higher solubility in the oil phase (Fross, 1972; Singhal and Jain, 1973). These compounds are produced by the breakdown of beta-ketoacids through thermal processes or enzymatic activities (Fross, 1972; Law, 1981). Generally, ghee has higher methyl ketone content than butter (Millia *et al*., 2008). 4-hepten-3-one diethyl, a ketone produced as the result of oxidation, has caused the formation of metallic off-flavor (Swoboda and Peers, 1977). The ketones are also present in cheese products and render special flavors (Qian *et al*., 2002; Rychlik and Bosset, 2001). Peterson and Reineccius (2003) also reported that 2-heptanone might be formed as the result of beta-oxidation of saturated fatty acids during thermal processing. Lactones produce a coconut- and peach-like aroma compounds. Only samples from Hamedan contained the delta nonalactone, delta decalactone, and delta dodecalactone in detectable concentrations more than gamma-lactone (Wadhwa and Jain, 1984). The total content of lactone compounds was below 1%. Ghee has higher lactone than butter. The smell threshold of delta-dodecalactone is much higher than delta-decalactone (Yadav and Srinivasan, 1992). Lactones are formed by hydrolysis of lactogenic glycerides into hydroxy acids through a thermal process (Alewijn *et al*., 2007). Also, the identified esters were diethyl phthalate (environmental pollution) and octanoic acid (mainly can be found in cheddar and Parmigiano Reggiano cheese samples), ethyl ester (ethyl octanoate) and the latter is produced by esterification of fatty acids and short chain alcohols by the esterase activity of lactic acid bacteria (Hosono *et al*., 1974; Carunchia-Whetstine, 2003; Ding *et al*., 1989). Lozano *et al*. (2007) also evaluated the effect of cold storage and different types of packaging in sweet cream butter and reported that

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

Table 4. Volatile compounds sampled by HS-SPME and identified by GC/MS and KI for Hamedan ghee.

No	Compounds	MI[a]	Peak area	RT	Odour quality	Mechanism of formation
1	Dodecane	MS,KI	0.54	17.26	Pungency	Naturally occurring in milk
2	Tetradecane	KI	0.14	21.22	Peppery, pungency	Naturally occurring in milk
3	Acetic acid	MS	6.95	5.35	Sour, pungent, vinegar-like, grassy	Microbial, enzymatic
4	Butyric acid	MS	20.06	8.38	Buttery, sweaty, cheesy, rancid	Microbial, enzymatic
5	Hexanoic acid	MS	29.10	12.94	Pungent, musty, cheesy, rancid	Microbial, enzymatic
6	Heptanoic acid	MS,KI	0.38	14.73	Cheesy, rancid	Microbial, enzymatic
7	Octanoic acid	MS	5.77	16.85	Cheesy, rancid	Microbial, enzymatic
8	Decanoic acid	MS	1.03	20.56	Cheesy, rancid	Microbial, enzymatic
9	3-Pentanol ketone	MS,KI	0.24	14.17	Green, mould	Lipid oxidation
10	Acetone (Propanone)	MS	4.01	4.78	Sweety	Metabolites of feed and silage, naturally occurring in milk
11	2-Pentanone	MS,KI	2.31	6.06	Creamy, green, milky, soapy	Thermally induced, microbial
12	2-Pentanone	MS,KI	5.46	6.18	Creamy, green, milky, soapy	Thermally induced, microbial
13	2-Heptanone	MS	8.48	9.71	Blue cheese, ,fruity, dairy-like	Thermally induced, microbial
14	2-Nonanone	MS	6.29	14.45	Cooked	Thermally induced, microbial
15	2-Undecanone	MS	1.85	18.81	Green, nutty	Thermally induced, microbial
16	2-Dodecanone	KI	0.35	22.64	Green, nutty	Thermally induced, microbial
17	Delta nonalactone	MS, KI	0.12	18.22	Coconut-like, peach	Thermally induced
18	Delta decalactone	MS, KI	0.43	22.33	Coconut-like, peach, flower	Thermally induced
19	Delta dodecalactone ester	MS, KI	0.07	25.97	Peach	Thermally induced
20	Diethyl phtalate	MS	1.08	24.02	Bitter, slightly fruity	Environmental pollutants, packaging
21	Cyclopentasiloxane, decamethyl	MS	0.44	16.45	-	Fiber
22	Cyclosiolxane, dodecamethyl	MS, KI	0.21	20.05	-	Fiber
23	Cyclohexanepropionic acid	MS, KI	1.52	26.74	-	Fiber
24	Uknown		3.15	-	-	-

[a] MI: Method of Identification, MS: Mass Spectrum comparison using Wiley and NIST libraries, KI: Kovats Index in agreement with literature values.

aromatic hydrocarbon (benzene, toluene, styrene) are considered major environmental contaminants because of the sources of their emission. The cyclic compounds identified were cyclotrisiloxane, hexamethyl, cyclopentasilosxane, decamethyl, di 2-ethylhexyl phthalate, pentasiloxane, dodecamethyl, and cyclohexanepropionic acid. All these compounds originated from the DVB/CAR/PDMS fiber.

An overview of the determined volatile compounds in Iranian ghee suggests that, due to controlled process conditions, the products contain low concentrations of off-flavors. Moreover, auto-oxidation of milk fat is manifested by the presence of compounds

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

like hexanal, heptanal, 2-nonenal, 2,4-decadienal, 2,6-nonadienal, and 1-octen-3-ol, which gives a metallic, tallowy, and buttery taste (Badings, 1970; Swoboda and Peers, 1977). These compounds are the products of auto-oxidation of USFAs and lengthy storage of butter in cold storages, while none of them was identified in the samples collected from different regions of Iran.

According to previous investigations, the high temperatures during process can result in formation of lactone and cyclic compounds. Cyclic compounds like thiazoles, pyrroles and pyridines are reported in butter treated at temperatures above 150°C, and low smell threshold can create off-flavor (Shimaboto, 1986). Moreover, lactones, as off-flavor in ghee, might be formed at temperatures above 100°C. Cyclic compounds were not observed in the Iranian ghee samples, whereas lactones were only reported in Hamedan samples (< 1%).

CONCLUSIONS

The current study aimed to monitor and evaluate volatile compounds in different ghee samples from Iran by HS-SPME sampling, and GC/MS technique. In this regard, a total of 26 flavor-generating compounds were isolated and identified in ghee samples of different regions. Given the fact that feeding conditions, lactation periods, season, production and storage conditions were similar for all the ghee samples, the geographical region might be an effective factor concerning the volatile compounds and their diversity. Moreover, the application of the DVB/CAR/PDMS fiber can be considered as a useful approach in screening of functional products. Considering the results obtained, the taste and the quality of Iranian ghee produced was desirable without any off-flavors observed.

REFERENCES

1. Alewijn, M., Smit, B. A., Sliwinski, E. L. and Wouters, J. T. M. 2007. The Formation Mechanism of Lactones in Gouda Cheese. *Int. Dairy J.*, **17:** 59–66.
2. Angerosa, F. 2002. Influence of Volatile Compounds on Virgin Olive Oil Quality Evaluated by Analytical Approaches and Sensor Panels. *Eur. J. Lipid Sci.*, **104:** 639–660.
3. Arthur, C. L., Killam, L. M., Motlagh, S., Lim, M., Potter, D. W. and Pawliszyn, J. 1992. Analysis of Substituted Benzene Compounds in Groundwater Using Solid-Phase Microextraction. *Environ. Sci. Technol.*, **26:** 979–983.
4. Azzara, C. D. and Campbell, L. B. 1992. Off-Flavors of Dairy Products. In: "*Off-Flavors in Foods and Beverages"*, (Ed.): Charalambous, G. Elsevier, Amsterdam, PP. 329–374.
5. Badings, H. T. and Neeter, R. 1980. Recent Advances in the Study of Aroma Compounds of Milk and Dairy Products. *Neth. Milk Dairy J.*, **34:** 9-30.
6. Badings, H. T. 1970. Cold-Storage Defects in Butter and Their Relation to the Autoxidation of Unsaturated Fatty Acids. *Neth. Milk Dairy J.*, **24:** 145-256.
7. Bola, L. and Tan, G. H. 2015. Chemometric Approach to the Optimization of HS-SPME/GC–MS for the Determination of Multiclass Pesticide Residues in Fruits and Vegetables. *Food Chem.*, **177:** 267–273.
8. Borle, F., Sieber, R. and Bosset, J. O. 2001. Photo-Oxidation and Photoprotection of Foods, with Particular Reference to Dairy Products. An Update of a Review Article (1993-2000). *Sci. Aliment.*, **21:** 571-590.
9. Camarasu, C. C. 2000. Headspace SPME Method Development for the Analysis of Volatile Polar Residual Solvents by GC-MS. *J. Pharm. Biomed. Anal.*, **23:** 197–210.
10. Carunchia-Whetstine, M. E., Karagul-Yuceer, Y., Avsar, Y. K. and Drake, M. A. 2003. Aroma-Active Compounds in Fresh Goat Cheese. *J. Food Sci.*, **68:** 2441–2447.
11. Chen, Y., Guo, Z., Wang, X. and Qiu, C. 2008. Sample preparation. *J. Chromatogr. A.*, **1184:** 191 – 219.
12. Chiesa, L. M., Soncin, S. and Biondi, P. A. 2006. Different Fibers for the Analysis of Volatile Compounds in Processed Meat

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

Products by Solid Phase Microextraction (SPME). *Vet. Res. Commun.*, **30(1):** 349–351.
13. Day, E. A., Lindsay, R. C. and Forss, D. A. 1964. Dimethyl Sulfide and the Flavor of Butter. *J. Dairy Sci.*, **47:** 197-198.
14. Ding, D., Zhu, M., Huang, Z., Song, Z. and Zhao, J. 1989. Aroma Constituents of Different Varieties of *Osmanthus fragrans* Flowers. In: *"11th International Congress Essential Oils Food and food Proceedings"*, (Eds.): Bhattacharygas, C., Sen, N. and Sethi, K. L. Oxford and IBH Publishing Co., New Delhi, PP. 27–35.
15. Dorni, C., Sharma, P., Saikia, G. and Longvah, T. 2018. Fatty Acid Profile of Edible Oils and Fats Consumed in India. *Food Chem.*, **238:** 9-15.
16. FAO. 2014. *Butter and Ghee Production*. Food and Agriculture Organization of United Nations. Available at: http://www.helgilibrary.com/indicators/butter-and-ghee-production/iran/; http://www.helgilibrary.com/ indicators/downloadExcel/1449/.
17. Forss, D.A. 1972. Odour and Flavour Compounds from Lipids. In: "*Progress in the Chemistry of Fats and Other Lipids"*, (Ed.): Holman, R. T. Pergamon, Oxford, PP. 177-223.
18. Ganguli, N. C. and Jain, M. K. 1972. Ghee: Its Chemistry, Processing and Technology. *J. Dairy Sci.*, **56:**19-25.
19. Grosch, W. 1987. Reactions of Hydroperoxides-Products of Low Molecular Weight. In: "*Autoxidation of Unsaturated Lipids"*, (Ed.): Chan, H. W-S. Academic Press, London, PP. 95-139.
20. Hashemi, P., Abolghasemi, M. M., Ghiasvand, A. R., Ahmadi, S., Hassanvand, H. and Yarahmadi, A. 2009. A Comparative Study of Hydrodistillation and Hydrodistillation–Solvent Microextraction Methods for Identification of Volatile Components of *Echinophora cinerea*. *Chromatogr.*, **69:** 179-182.
21. Henneberry, S., Sullivan, M. G. O., Kilcawley, K. N., Kelly, P. M., Wilkinson, M. G. and Guinee, T. M. 2016. Sensory Quality of Unheated and Heated Mozzarella Style Cheeses with Different Fat, Salt and Calcium Levels. *J. Dairy Sci.*, **69(1):** 38-50.
22. Hosono, A., Elliot, J. A. and McGugan, W. A. 1974. Production of Ethylesters by Some Lactic Acid and Psychrotrophic Bacteria. *J. Dairy Sci.*, **57:** 535-539.
23. IDF. 1997. *Anhydrous Milk Fat, Anhydrous Butter Oil or Anhydrous Butterfat, Butter Oil or ButterFat, Ghee: Standards of Identity*. FIL-IDF 68A, International Dairy Federation, Brussels, Belgium.
24. ISO. 2011. International Standard 12966-2, Animal and Vegetable Fats and Oils: Gas Chromatography of Fatty Acid Methyl Esters. Part 2. "*Preparation of Methyl Esters of Fatty Fcids*". International Organization for Standardization, Geneva, Switzerland.
25. Johnson, S. and Saikia, N. 2009. *Fatty Acids Profile of Edible Oils and Fats in India*. Report of Centre for Science and Environment, New Delhi.
26. Law, B. A. 1981. The Formation of Aroma and Flavor Compounds in Fermented Dairy Products. *Dairy Sci. Abs.*, **43:** 143–154.
27. Lozano, P. R. Miracle, E. R. Krause, A. J. Drake, M. and Cadwallader, K. R. 2007. Effect of Cold Storage and Packaging Material on the Major Aroma Components of Sweet Cream Butter. *J. Agric. Food Chem.*, **55(19)**: 7840–7846.
28. Millia, S., Escher, F. and Schlichtherle-Cerny, H. 2008. Aroma-Active Compounds of Butter: A Review. *Eur. Food Res. Technol.*, **226:** 315–325.
29. Marsili, R. 2007. The Application of Chemometrics for Studying Flavour and Off-Flavour Problems in Foods and Beverages. In: "*Sensory-Directed Flavor Analysis",* (Ed.): Marsili, R. CRC Taylor and Francis, NY, PP. 181-204.
30. Mehta, M. 2013. Consumption Pattern and Fatty Acid Composition of Ghee. *Food Sci. Res. J.*, **4(2):** 116-120.
31. Munro, D. S., Cant, P. A. E., Mac Gibbon, A. KH., Illingworth, D., Kennett, A. and Main, A. J. 1992. Concentrated Milkfat Products. In: "*The Technology of Dairy Products",* (Ed.): Early, R. Blackie and Sons Ltd, Glasgow, PP. 117-145.
32. Nadeem, M., Abdullah, M., Khalique, A., Hussain, I., Mahmud, A.and Inayat, S. 2013. The Effect of *Moringa oleifera* Leaf Extract as Antioxidant on Stabilization of Butter Oil with Modified Fatty Acid Profile. *J. Agr. Sci. Tech.*, **15**: 919-928.
33. Najafi, V., Barzegar, M. and Sahari, M.A. 2015. Physicochemical Properties and Oxidative Stability of Some Virgin and Processed Olive Oils. *J. Agr. Sci.*, **17**: 847-858.

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

34. Palmquist, D. L. 2006. Milk Fat: Origin of Fatty Acids and Influence of Nutritional Factors Thereon. 2. In: "*Advanced Dairy Chemistry",* (Eds.): Fox, P. M. and Mcsweeney, P. L. H. Third Eds, Springer, US, PP. 43-92.
35. Pedrotti, M., Khomenko, I., Cappellin, L., Fontana, M., Somenzi, M., Falchero, L., Arveda, M., Fogliano, V. and Biasioli, F. 2018. Rapid and Non-Invasive Quality Control of Anhydrous Milk Fat by PTR-MS: The Effect of Storage Time and Packaging. *J. Mass. Spect.,* **53(9):** 753-762.
36. Peterson, D. G. and Reineccius, G. A. 2003. Determination of the Aroma Impact Compounds in Heated Sweet Cream Butter. *Flavour Fragr. J.,* **18(4):** 320–324.
37. Qian, M., Bloomer, S. and Nelson, C. 2002. Evaluation of Fat-Derived Aroma Compounds in Blue Cheese by Dynamic Headspace Gas Chromatography/Olfactometry-Mass Spectrometry. *J. Am. Oil Chem. Soc.,* **79:** 663–667.
38. Rajorhia, G.S. 1993. Ghee. **4.** In: "*Encyclopaedia of Food Science, Food Technology and Nutrition",* (Eds.): Macrae, R., Robinson, R. K. and Sadler, M. J. Academic Press Ltd, London, PP. 2186-2192.
39. Rychlik, M. and Bosset, J.O. 2001. Flavour and Off-Flavour Compounds of Swiss Gruye`re Cheese. Evaluation of Potent Odorants. *Int. Dairy J.,* **11:** 895–901.
40. Shimaboto, T. 1986. Odor Threshold of Some Pyrazines. *J. Food Sci.,* **51:** 1098-1099.
41. Singhal, O. P. and Jain, M. K. 1973. A Note on the Profile of Free Fatty Acids of Ghee Prepared through Different Methods. *Indian J. Anim. Sci.,* **43:** 1026–1027.
42. Swoboda, P. A. T. and Peers, K. E. 1977. Metallic Odor Caused by Vinyl Ketones Formed in the Oxidation of Butterfat. The Identification of Octa-1-cis-5-dien-3-one. *J. Sci. Food Agric.,* **28:** 1019-1024.
43. Urbach, G. and Gordon, M. H. 1994. Flavours Derived from Fats. In: "*Fats in Food Products',* (Eds.): Moran, D. P. J. and Rajah, K. K. Blackie Academic and Professional, London, PP. 347-405.
44. Wadhwa, B. K. and Jain, M. K. 1984. Studies on Lactone Profile of Ghee. Part IV. In: "*Variations Due to Temperature of Clarification*". *Indian J. Dairy Sci.,* **37:** 334-342.
45. Wadodakar, U. R., Murthi, T. N. and Punjrath, J. S. 1996. Isolation of Ghee Volatiles by Vacuum Degassing, Their Separation and Identification Using Gas Chromatography Mass Spectrometry. *Indian J. Dairy Sci.,* **49:** 185-198.
46. Wadodkar, U. R. Punjrath, J. S. and Shah, A. C. 2002. Evaluation of Volatile Compounds in Different Types of Ghee Using Direct Injection with Gas Chromatography-Mass Spectrometry. *J. Dairy Res.,* **69:** 163-171.
47. Widder, S., Sen, A. and Grosch, W. Z. 1991. Changes in the Flavour of Butter Oil during Storage. *Eur. Food Res. Technol.,* **193:** 32-35.
48. Yadav, J. S. and Srinivasan, R. A. 1992. Advances in Ghee Flavour Research. *Indian J. Dairy Sci.,* **45:** 338-348.
49. Yang, X. and Peppard, T. 1994. Solid-Phase Microextraction for Flavor Analysis. *J. Agric. Food Chem.,* **42:** 1925-1930.
50. Zhao, D., Tang, J. and Ding, X. 2007. Analysis of Volatile Components during Potherb Mustard (*Brassica juncea*, Coss.) Pickle Fermentation using SPME–GC-MS. *LWT - Food Sci Technol.,* **40:** 439-447.
51. Zhu, H., Lu, Z., Cai, J., Li, J. and Lirong, G. 2009. Development of a Headspace–SPME–GC/MS Method to Determine Volatile Organic Compounds Released from Textiles. *Polym. Test.,* **28:** 521–527.

[DOR: 20.1001.1.16807073.2020.22.1.9.5]

ارزیابی ترکیبات فرار و اسیدهای چرب در روغن های حیوانی ایران با استفاده از ریزاستخراج فاز جامد فضای فوقانی به همراه کروماتوگرافی گازی-طیف سنجی جرمی

س. ح. عرفانی، م. قوامی، ش. شعیبی، ا. زندمقدم و ح. رستگار

چکیده

روغن حیوانی، یک محصول لبنی مغذی در فرهنگ ایران می‌باشد که به آسانی در مقیاس کوچک تولید می‌شود. در این مطالعه آنالیز ترکیبات فرار و پروفایل اسیدهای چرب جمع آوری شده از سه منطقه در ایران با استفاده از تکنیک ریزاستخراج فاز جامد از فضای فوقانی صورت گرفت. طبق نتایج، اسید پالمیتیک و اسید اولئیک اسیدهای چرب غالب در تمام نمونه های مورد تحقیق بودند. علاوه بر این، ترکیباتی مانند dodecane، acetone، butyric acid، hexanoic acid، 2-pentanone، 2-heptanon و 2-undecanone که ممکن است در نتیجه فعالیتهای اکسیداتیو، هیدرولیتکی و میکروبی تجمع یافته باشند، سبب ایجاد طعم روغن حیوانی شدند. لاکتونها نیز که در دماهای بالا تولید می شوند، به استثنای نمونه منطقه همدان در هیچ یک از نمونه های دیگر یافت نشد (کمتر از ۱٪). اعمال فرآیند حرارتی پایین در تولید روغن حیوانی سبب جلوگیری از تشکیل ترکیبات فرار نامطلوب شد. پارامترهای کیفی و کمی اندازه گیری شده در این تحقیق ممکن است جهت پذیرش کیفی روغن حیوانی مفید باشد و می تواند در صنعت جهت بهبود تولید تجاری آن کمک نماید.

[DOR: 20.1001.1.16807073.2020.22.1.9.5]