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Chemical composition, fatty acid composition, and volatile compounds of a traditional Kurdish fermented cereal food: Tarkhineh

Nima Mohammadi [a,*], Nikoo Ostovar [b]

[a] Department of Biological Sciences, Faculty of Science and Engineering, University of Limerick, V94 T9PX Limerick, Ireland
[b] Department of Food and Drug, University of Parma, Parco Area delle Scienze 49/A, 43124, Parma, Italy

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ABSTRACT

Tarkhineh is a traditional Kurdish fermented food made from the wheat meal (bulgur) and Doogh and is extensively consumed by people from all walks of life. The strategies of chemical composition (ash, protein, crude fat, salt), headspace solid-phase microextraction, and gas chromatography-mass spectrometry (HS-SPME-GC/MS) were applied to analyze Tarkhineh. The amount of ash and protein was relatively high, while the amount of fat was low. The average ash, protein, fat, and salt contents of the samples were 5.37%, 10.45%, and 1.46%, 4.87% respectively. A total of 9 fatty acids were identified in Tarkhineh, comprised of 34.42% unsaturated and 65.66% saturated fatty acids. Palmitic acid (48.58%), oleic acid (30.42%), stearic acid (6.29%), myristic acid (5.13%), and linoleic acid (4.1%) were the major fatty acids. Furthermore, 13 volatile compounds were identified. Hexadecane (53.42%), tetracosane (9.68%), and tetradecane (9.34%) were the most common aroma volatile compounds in Tarkhineh. This study provides new information on Tarkhineh that will be useful in explaining its unique properties.

1. Introductıon

Ever since ages ago one of the best and most cost-effective ways of food production and preservation has to be fermentation. Fermentation is a natural biotechnological process that can decrease food contamination by improving the antimicrobial properties of the final product with components such as ethanol, organic acids, and bacteriocins (Özdemir et al., 2019; Shiferaw Terefe & Augustin, 2020). Fermentation also improves the flavor, scent, texture, and shelf life of the end-product, particularly in foods that spoil quickly, and improves the foods' organoleptic properties, the ability for digestion of carbohydrates and proteins, and access to minerals and vitamins (Erbaş et al., 2006; Gan et al., 2017).

During this process, antimicrobial items presented by lactic acid bacteria (LAB) regularly constrain the growth of spoilage organisms and pathogens. There are LAB strains with probiotic features in fermented foods containing dairy and cereals (Sharma et al., 2020). Probiotic bacteria can promote people's health due to their anti-pathogenic properties (Minj et al., 2021). They usually act as fermentation starters. Due to probiotics, fermented foods have a positive effect on urinary tract symptoms in women, lactose intolerance, traveler's diarrhea, gut function, antibiotic-associated diarrhea, infantile diarrhea, inflammatory bowel disease (IBD), helicobacter pylori gastritis, colorectal cancer (CRC), irritable bowel syndrome (IBS), infant health, immune function, atopic dermatitis, and atopic disease, reducing blood cholesterol levels, osteoporosis, atherosclerosis, allergies and obesity (Hasan et al., 2014; Manzoor et al., 2022; Şanlier et al., 2019).

Tarkhineh, also known as, Tarkhowana or Doowina in Kurdish, is a traditional food product created in the western provinces of Iran such as Kermanshah, Kurdistan, Lorestan, and Hamedan (Meybodi et al., 2016), Other countries, including Turkey, Iraq, and Egypt, eat food similar to Tarkhineh (Moradi et al., 2022). 'Tarhana' (Turkey) and 'Trachano[*s*], 'Trachana's, 'Trakahano[*s*]' and 'Tarhonaya[*s*]' (Greece) are other spellings of this kind of food. The same traditional fermented milk is known as 'xinochondros' on the island of Crete and 'chackla' on the island of Lesbos, while it is known as 'kushuk' in Iraq, 'kishk' or 'khisk' in Lebanon, Jordan, Egypt, and Syria. Vegetables, Salt, herbs, yeast, spices, and fruits are all optional components. Tarkhineh is a kind of food with a wide range of compositions, shapes, and nutritional value. This is due to differences in the types of grain and milk products used, as well as differences in the proportions in which these basic ingredients are blended. Fermentation, heating, shape, and fragmentation are some of the methods of preparation (Cappers, 2018). The key ingredients are Doogh (Iranian fermented milk drink), wheat flour, and several kinds of vegetables, making it a strong source of minerals, vitamins, and free amino acids, so it is suitable for children, the elderly, and patients. (Moradi et al., 2022; Sakin-Yilmazer, 2017). There are also some amino acids in Doogh such as methionine and lysine which are not in cereal, instead some minerals

* Corresponding author.
E-mail address: Nima.Mohammadi@ul.ie (N. Mohammadi).

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such as Cu, Fe, and Mn which are not in milk can find them in wheat grout (slightly cooked fracture wheat) (Tabatabaei-Yazdi et al., 2012).

The production of Tarkhineh needs to use starter cultures to have foreseeable and manageable fermentation (Mashak, 2016). It should be noted that according to the taste of consumers in aromatic substances in Tarkhineh, the choice of starter cultures should be such that it provides the desired aroma (Özdemir et al., 2018). The microflora is conducive to the expansion of the aroma by the synthesis of alcohol compounds and carboxylic acid throughout the fermentation, or other aromatic items such as esters and aromatic materials which are the consequences of amylolytic, lipolytic and proteolytic enzyme activities of the microbes (García-Cano et al., 2020). In the fermentation process of Tarkhineh, both chemical and enzymatic reactions and the interaction of microorganisms affect the sensory traits of the final product. Moreover, using yeast improves the sensory properties of Tarkhineh. Compounds like ethyl alcohol, propanol, isobutanol, diacetyl, 2,3-butanediol, and 2-heptanone that yeasts produce during sourdough fermentation enhance the aroma of the product. Also, different types of LAB impact the creation of volatiles in the sourdough structure (Özdemir et al., 2018).

Drying the fermented Tarkhineh dough is traditionally done under sun emission. Temperature plays a vital role in Tarkhineh fermentation because this process is mostly carried out by microorganisms found naturally in raw materials which comprise a diverse range of microflora, including LAB. Furthermore, the existence of salt can control this process and enhance the preservation of good-quality of Tarkhineh at various temperatures (Özdemir et al., 2019). To produce industrial Tarkhineh, hot air at 60–65 °C is used to dry the product for 5–6 h, or until it reaches a moisture content of 10%. Tarkhineh can be preserved for 1–2 years in a dry and cool environment (Singh et al., 2021). Fig. 1 depicts a general overview of Tarkhineh production.

Although the literature indicates several studies on microbial and antimicrobial properties of Tarkhineh (Vasiee et al., 2014) and also the methods of drying (Ghaitaranpour et al., 2017), no published studies has yet investigated the characterization of fatty acid profile and aroma of Kurdish Tarkhineh and this traditional food has received less attention in the area of food science and technology. Hence, the purpose of this research was to analyze the ash, protein, fat, salt content, fatty acid composition, and volatile aromatic components of Tarkhineh.

2. Materials and methods

2.1. Sample collection

All 30 samples of Tarkhineh were collected from the Kermanshah province of Iran between November and December 2021. They were between the ages of 3 and 6 months. The samples were mixed and ground in a household mill to produce a fine homogeneous powder. The powder was stored in a plastic bag at 4 °C until use.

2.2. Chemical analysis

The amount of ash was determined according to the official methods of analysis according the procedure reported in the literature (AOAC, 2005). Furthermore, total nitrogen (TN) and protein nitrogen (PN) were determined following the Kjeldahl method (AACC, 1983). The Soxhlet method was used to determine the total fat content of ground samples (Das et al., 2016). The Volhard method was used to determine salt content in ground samples. All solvents and chemicals were of analytical grade and from Merck (Darmstadt, Germany),

2.3. Determination of fatty acids profile

GC/MS was used to evaluate the fatty acid content as methyl esters of fatty acids (FAMEs). A 0.10 g of the oil sample extracted was weighed and dissolved in 2 mL of heptane in a 5-mL screw-top test vial. A 250 μL of 2 N methanolic KOH solution was poured into the vial, which was then tightened with a screw cap. The vial was shaken vigorously for 15 s. The mixture was allowed to stand until the supernatant was clear. The upper layer was separated and GC/MS analysis was performed immediately (Montserrat-de la Paz et al., 2014). FAMEs were analyzed using a 6890 GC/5973 MSD (Agilent, USA). An HP-1MS capillary column (60 $m \times 0.25$ mm, 0.25 μm) was used, and helium was used as a carrier gas at 1 mL min^{-1}. Electron impact energy was 70 eV. The initial column temperature was 180 °C, which was maintained for 10 min before being set to rise at a rate of 1.5 °C/min from 180 °C to 200 °C. The temperature of the injector was 210 °C, and the temperature of the detector was 250 °C (Al-Bachir, 2017; Mohammadi & Ostovar, 2022b). A comparison of the retention time between the FAME and the standard peak assisted in the identification of FAMEs.

Table 1
The chemical composition of Tarkhineh.

Ash (%)*	Protein (%)*	Fat (%)*	Salt (%)*
5.37±0.07	10.45±0.06	1.46±0.09	4.87±0.15

* Values are Means ±standard deviations of triplicate determinations.

2.4. Headspace volatile analysis

Solid-phase microextraction (SPME) technique was used to extract the volatile compounds from Tarkhineh, which were then evaluated using GC/MS. This approach is solvent-free, inexpensive, quick, and only requires a small amount of sample. It is also sensitive enough for quality control of final products. Briefly, 3 g of samples were ground and placed in a 25 mL glass vial, and then packed. The samples were warmed up for 15 min at 80 °C to increase the extraction of volatiles in the Tarkihneh matrix. After that, a 65 μm Divinylbenzene/Polydimethylsiloxane fiber (Supelco, Bellefonte, PA, USA) was inserted into the vial and exposed to the headspace for 15 min at 80 °C to extract volatile compounds (Wang et al., 2016). An HP GC 6890 −5973 Mass Spectrometry System (HP, USA) was used to analyze the extracted volatiles. The extracted volatiles were desorbed by introducing the fiber into the injection port and keeping it there for 2 min at 250 °C, with helium gas at a flow rate of 1.0 mL/min Khosravi Zanjani et al., Zojaji and Bakhoda (2015); Mohammadi and Ostovar (2022a) The temperature program was conducted by Hayaloglu (Hayaloglu, 2009) technique with some modifications. The oven temperature was held at 60 °C for 3 min after the fiber was inserted and then raised to 250 °C at a rate of 7 °C/min, where it was held for 3 min. An HP-1MS capillary column (60 $m \times 0.25$ mm, film thickness 0.25 μm) was used to separate volatile compounds. The Wiley 257 mass spectrum database and a homologous series of normal alkanes (C_8–C_{24}) were compared with the MS spectra of the constituents to identify them. A comparison of retention indices of the identified compounds with those of authentic compounds and literature data confirmed their identification (Adams, 1995).

2.5. Statistical analysis

All data were conducted in triplicate and analyzed by SPSS Software v.22.0 (SPSS Science, Chicago, IL, USA). The data were expressed as mean ± SD.

3. Results and discussion

3.1. Chemical compositions

The chemical properties of the Tarkhineh sample are illustrated in Table 1. The ash content of the Tarkhineh was 5.37%. According to previous research, when salt levels increased, ash values increased directly. Other researchers reported that the Turkish Tarhana without salt contained an ash value of 1.8% (Tamer et al., 2007). The protein content

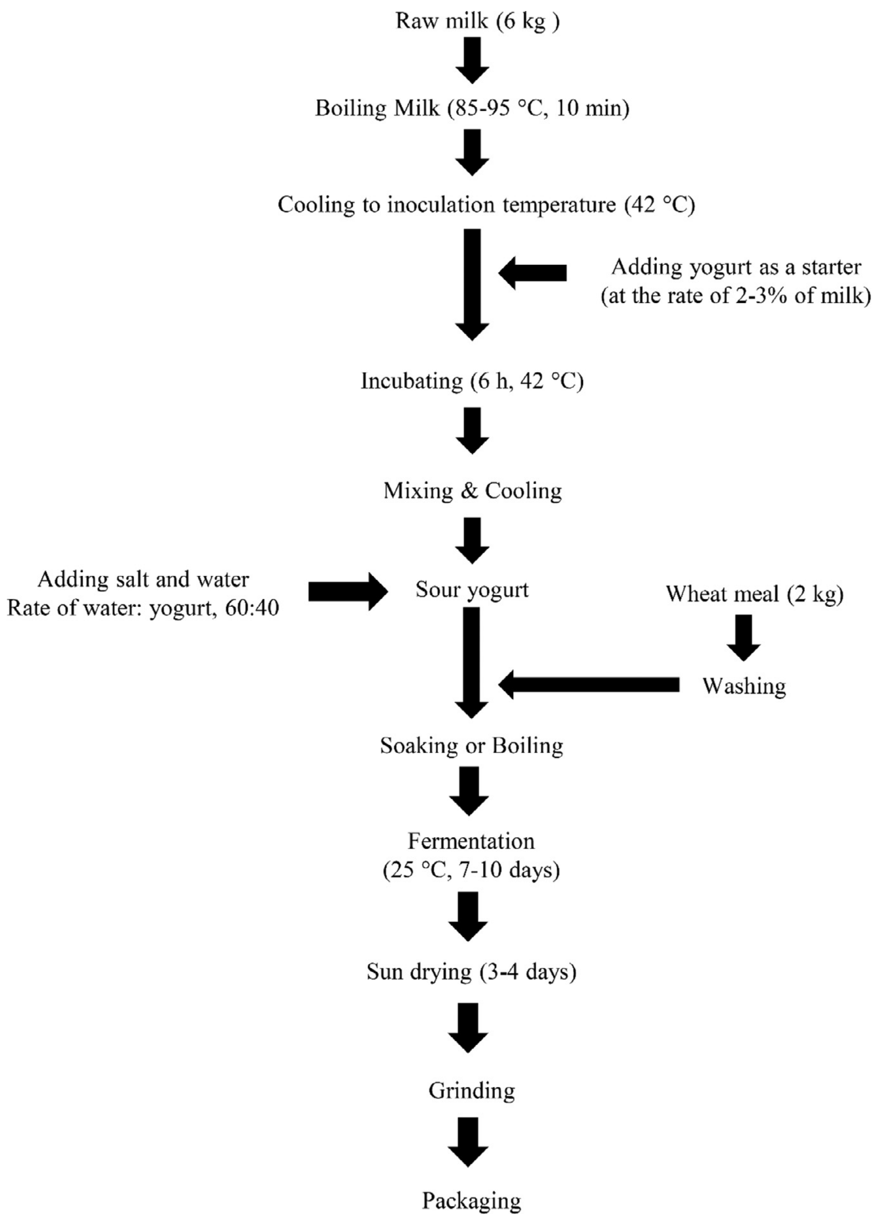

Fig. 1. Tarkhineh production flowchart.

of the Tarkhineh was 10.45%. In previous reports related to the Turkish Tarhana samples, protein values varied between 6.77 and 28.55% in the samples (Tamer et al., 2007). The amount of protein is directly related to the amount of yogurt used in the formulation. In addition to these factors, the properties of cereal and legume flour can also affect the protein content of Tarkhineh (Demir, 2014). The fat content of the Tarkhineh was 1.46%. Tarkhineh produced in Kermanshah had the lowest fat content compared to the Turkish one. Several other studies also indicated that Tarhana samples had fat contents ranging from 1.80 to 9.01% (Georgala, 2012; Mashak et al., 2014; Tamer et al., 2007). The formula of Tarkhineh is different from Turkish Tarhana in several ways. Tarkhineh is prepared from wheat meal (bulgur) instead of wheat flour, and Doogh is used in its formulation, and there are no tomatoes or onions in it (Mashak et al., 2014; Tamer et al., 2007) These findings are consistent with those reported in the literature for wheat flour used in the Turkish Tarhana samples (Georgala, 2012).

3.2. Fatty acids analysis of Tarkhineh

Nine fatty acids were identified for the first time in Tarkhineh oil (Table 2, Fig. 2). This identification was confirmed using the GC/MS technique. The predominant fatty acids in Tarkhineh oil were palmitic acid (48.58%), oleic acid (30.42%), stearic acid (6.29%), myristic acid (5.13%), and linoleic acid (4.1%), respectively, which made up 94.52% of the total fatty acid profile. A lower ratio of the total unsaturated fatty acids (ΣUFAs) to saturated fatty acids (SFAs) makes oil less susceptible to oxidation and the healthy effects of Tarkhineh are linked to the amount of its essential fatty acids. This finding is consistent with pre-

Table 2
GC/MS of the fatty acids from Tarkhineh oil.

No.	Fatty acid	Concentration (%) Mean	SD
1	Lauric acid	0.97	0.01
2	Azelaic acid	1	0.02
3	Myristic acid	5.13	0.12
4	Pentadecanoic acid	0.61	0.01
5	Palmitic acid	48.58	0.24
6	Linoleic acid	4.1	0.11
7	Oleic acid	30.32	0.31
8	Stearic acid	6.29	0.24
9	Oxiraneoctanoic acid	3.08	0.13

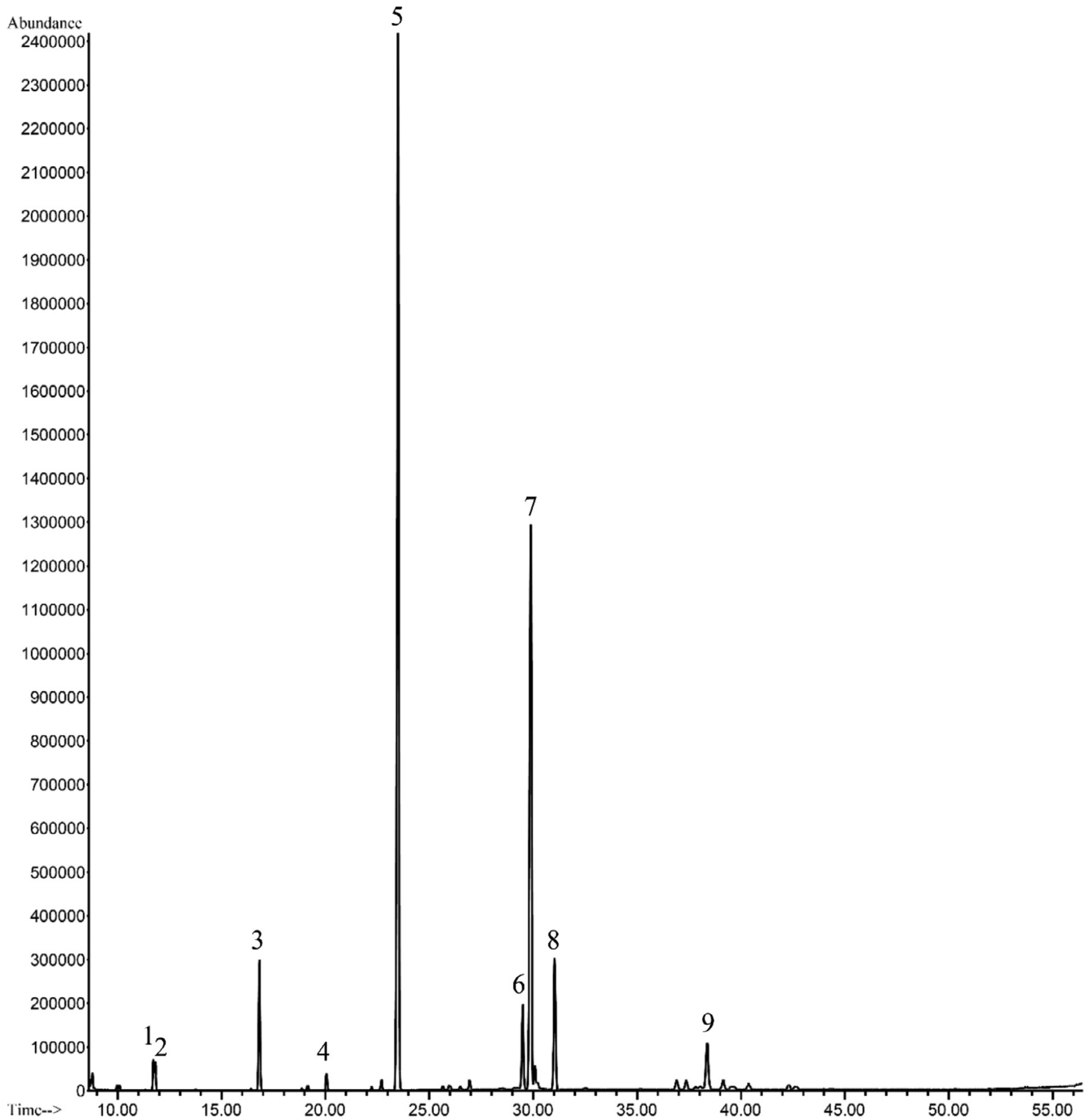

Fig. 2. GC/MS product distribution of the fatty acids from Tarkhineh. Peak numbers are listed in Table 2.

vious research on traditional Greek yogurt, which found that myristic acid, palmitic acid, and stearic acid, were the most prevalent SFAs in the fermented product, while oleic acid was the most prevalent monounsaturated fatty acid (MUFA) (Serafeimidou et al., 2012). This is due to the fact that high-melting glycerides are thought to be easily affected by lipases. Lipolysis is well recognized as one of the most important biochemical changes impacting the fatty acid profiles and shelf life of many dairy products. Furthermore, the activities of lactic acid bacteria were directly responsible for the differences in fatty acid compositions of the fermented products (Sumarmono et al., 2015). Other compounds, including oxiraneoctanoic acid, azelaic acid, lauric acid, and pentadecanoic acid, appeared with 3.08, 1, 0.97, and 0.61%, respectively. The existence of intermediate products, such as oxiraneoctanoic acid, results from incomplete hydroxylation (Sipaut et al., 2012). Also, azelaic acid is a result of the oxidation of unsaturated C18 fatty acids during the drying process (Plater et al., 2003).

3.3. Headspace volatile analysis

During fermentation, LAB produces ethanol, carbon dioxide, organic acids, acetaldehyde, and so forth, which results in low pH and a specific aroma for Tarkhineh (Çalışkan Koç et al., 2021). Table 3 shows that a total of 13 volatile components were identified in the Tarkhineh analyzed by HS-SPME-GC/MS. The heights of the peaks in the gas chromatography represent the relative concentrations of the components present in the plant (Fig. 3). The mass spectrometer is used to determine the type and structure of volatile components. It also examines the chemicals that elute at various times. These mass spectra serve as a fingerprint for the volatile component that is identified using the data library. The total peak area of the mentioned volatiles was estimated to be approximately 97.41%. Hexadecane (53.42%), tetracosane (9.68%), and tetradecane (9.34%) were the major compounds (Table 3). Based on these results, hydrocarbons were the most common volatile compound in Tarkhineh,

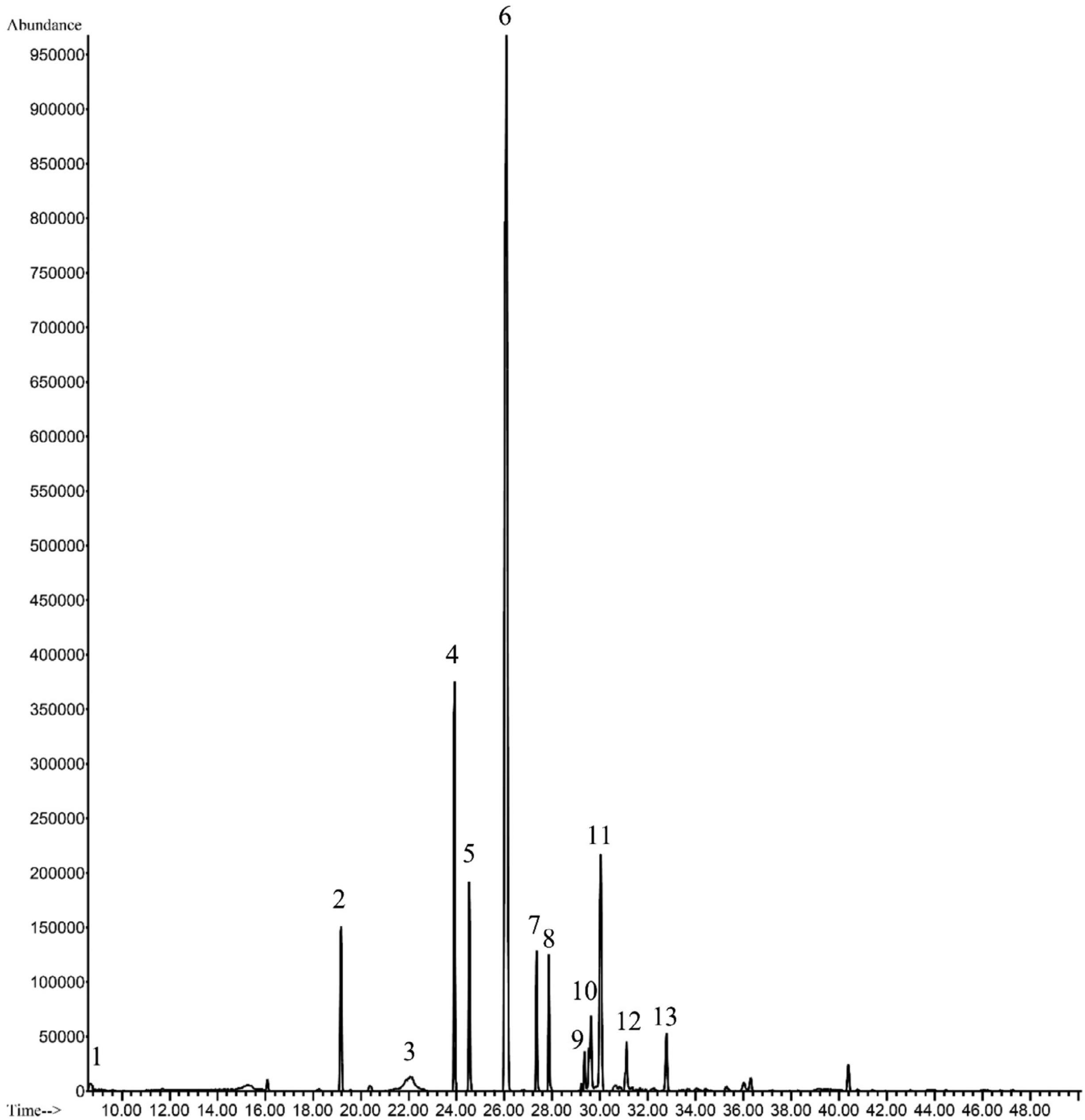

Fig. 3. GC/MS of volatile compounds extracted from Tarkhineh. Peak numbers are listed in Table 3.

which was comparable to the results of other researchers (Liu et al., 2017; Pizarro & Franco, 2017). They reported that hydrocarbons were the most commonly occurring volatile compounds in cereals. These hydrocarbons are probably formed through decarboxylation and carbon-to-carbon separation from higher fatty acids (Pizarro & Franco, 2017). Also, these compounds have a high threshold, but their contribution to flavor is low (Fan et al., 2018; Hao et al., 2020). In contrast, some researchers reported that the highest number of volatile compounds were related to terpenes in Turkish Tarhana made with wheat or gluten-free flour (Çalışkan Koç et al., 2021; Özdemir et al., 2019). However, another study indicated that acids were the most abundant volatile compound in Tarhana (Temiz & Tarakçı, 2017). The major difference between the volatile compounds in this study and the Turkish samples might be also due to the addition of other ingredients (pepper, onion, mint, tomato paste, and yeast) to the Turkish formulation (Çalışkan Koç et al., 2021). Likewise, since Tarkhineh is made from bulgur instead of wheat flour, it has high protein. Hence, another reason for the low number of compounds other than hydrocarbons may be the flavor-binding property of proteins (Semenova et al., 2002). Also, it might be the use of bulgur in formulation, which led to a high concentration of hydrocarbon compounds in the GC–MS profile (Yousif et al., 2018). Some reports considered that the aroma of alkanes does not generally increase as the number of carbon chains in hydrocarbons increases, but there may be differences in overall aroma characteristics (Cai et al., 2019).

4. Conclusion

The paper describes the physicochemical, fatty acid composition, and volatile compounds of Tarkhineh. The ash and protein content was relatively high, while the fat content was very low. The analysis of fatty acids showed that Tarkhineh is a good source of palmitic and oleic acid. Moreover, the major volatile compounds of Tarkhineh were hydrocarbons. A study of the physicochemical, fatty acid composition, and volatile compounds of Tarkhineh can assist consumers and producers

Table 3
Volatile compounds in Tarkhineh were detected by HS-SPME-GC/MS (means of three replicates).

No.	Compounds	KI[a]	RT[b](min)	%
1	Acetaldehyde (C_2)	800	7.92	0.65
2	Dodecane (C_{12})	1321	19.18	4.83
3	Tetradecane (C_{14})	1550	23.91	9.34
4	Pentadecane (C_{15})	1590	24.56	4.97
5	Hexadecane (C_{16})	1685	26.11	53.42
6	Nonadecane (C_{19})	1765	27.36	3.18
7	Eicosane (C_{20})	1798	27.86	2.82
8	10-Methylnonadecane (C_{20})	1851	29.35	1.2
9	Docosane (C_{22})	1858	29.53	1.28
10	Tricosane (C_{23})	1862	29.64	2.37
11	Tetracosane (C_{24})	1889	30.4	9.68
12	Heptacosane (C_{27})	1920	31.13	1.61
13	Octacosane (C_{28})	2002	32.78	2.06

[a] KI: Kovats index.
[b] RT: Retention time.

in determining the quality, shelf life, and aroma of the final product. The outcomes presented in this research offered new information on the characterization of Tarkhineh in Kermanshah. It would be beneficial to isolate the individual phytochemical constituents for further research that may lead to the discovery of novel drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Nima Mohammadi: Conceptualization, Validation, Writing – review & editing, Supervision, Project administration. **Nikoo Ostovar:** Methodology, Formal analysis, Software, Investigation, Resources, Data curation, Writing – original draft, Visualization.

Data Availability

No data was used for the research described in the article.

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