Research article

Metabolite profiling of different Iranian traditional yogurts using an untargeted metabolomics approach

Reza Vaseghi Bakhshayesh [a], Bahman Panahi [b], Mohammad Amin Hejazi [a], Yousef Nami [a],*

[a] Department of Food Biotechnology, Branch for Northwest & West Region, Agricultural Biotechnology Research Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Tabriz, Iran
[b] Department of Genomics, Branch for Northwest & West Region, Agricultural Biotechnology Research Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Tabriz, Iran

ABSTRACT

We used gas chromatography-mass spectrometry (GC-MS) with an untargeted metabolomics approach to look at the metabolite profiles of traditional Iranian yogurts made from cow, goat, buffalo, and sheep milk. Results showed that different animal milks significantly influenced physicochemical properties and fatty acid (FA) composition, resulting in diverse metabolites. Over 80 % of all the fatty acids in the yogurt samples were saturated. The main fatty acids found were myristic acid (C14:0), palmitic acid (C16:0), and oleic acid + petroselenic acid (*cis*-9 C18:1 + *cis*-6 C18:1). In total, 36 metabolites, including esters, aldehydes, alcohols, and acids, were detected. Some important metabolites that changed yogurt profiles were 2-heptanone, methyl acetate, 2-propanone, butyl formate, and 4-methyl benzal. Associations between metabolite profiles and milk compositional traits were also observed, with statistical models showing a strong correlation between metabolite profiles and FA content. This study is the first to explore the impact of different animal sources and regions in Iran on the metabolome profiles of traditional yogurts. These results give us useful information about how metabolites differ between species and can be used to make new dairy products based on milk compositions and metabolites, which will help with future formulations of autochthonous starters.

1. Introduction

Dairy products are essential for a balanced diet due to their high-quality protein and abundant micronutrients. Yogurt, a beloved fermented dairy product, has experienced remarkable growth in the market over the past few decades. Nomadic Middle Easterners possibly discovered yogurt, which has been consumed by various civilizations for thousands of years. In Iran, like in other areas, traditional fermentation methods were transmitted between generations, resulting in a diverse range of fermented milk and dairy products that differ in terms of milk variety, processing techniques, and quality attributes [1]. The production methods of traditional yogurt differ across the country, mainly depending on the type of milk used, the fermentation technique employed (such as back-slopping or spontaneous fermentation), and the type of fermentation used (either lactic or lactic-yeast fermentation). Other factors that affect the production process include whey removal (in the case of strained yogurt) and the inclusion of grains in the final

Table 1
The milk composition and other parameters.

Type & Location	Milk composition (%) and other parameters							
	Fat	SNF	Density	Lactose	Salts	Protein	pH	Conductivity
Cow/Kermanshah	2.63 ± 0.00[g]	7.45 ± 0.01[b]	31.43 ± 0.01[de]	4.09 ± 0.04[h]	0.62 ± 0.01[g]	2.72 ± 0.01[i]	6.75 ± 0.01[d]	5.36 ± 0.02[d]
Cow/Zahedan	3.43 ± 0.01[fg]	8.30 ± 0.01[ab]	29.55 ± 0.03[ef]	4.57 ± 0.02[c]	0.69 ± 0.00[de]	3.05 ± 0.06[h]	6.95 ± 0.08[b]	4.52 ± 0.03[g]
Cow/Khorasan Razavi	4.05 ± 0.01[efg]	10.75 ± 0.01[a]	38.57 ± 0.00[a]	5.91 ± 0.02[a]	0.89 ± 0.01[a]	3.94 ± 0.06[g]	6.19 ± 0.05[k]	5.94 ± 0.03[c]
Buffalo/Sarab	8.66 ± 0.01[bcd]	9.07 ± 0.00[ab]	28.68 ± 0.00[ef]	4.07 ± 0.02[h]	0.65 ± 0.01[f]	4.26 ± 0.02[ef]	6.40 ± 0.03[i]	3.66 ± 0.01[i]
Buffalo/Ghilan	11.77 ± 0.04[a]	8.98 ± 0.03[ab]	25.69 ± 0.00[g]	4.01 ± 0.01[i]	0.62 ± 0.03[fg]	4.20 ± 0.05[f]	6.73 ± 0.05[de]	7.76 ± 0.01[b]
Buffalo/Khouzestan	11.04 ± 0.02[ab]	8.98 ± 0.03[ab]	26.29 ± 0.00[g]	4.02 ± 0.01[i]	0.63 ± 0.03[fg]	4.20 ± 0.05[f]	6.81 ± 0.07[c]	9.35 ± 0.03[a]
Sheep/Zanjan	6.78 ± 0.03[cde]	9.17 ± 0.02[ab]	30.66 ± 0.01[de]	4.12 ± 0.01[g]	0.68 ± 0.01[e]	4.33 ± 0.05[e]	6.24 ± 0.06[j]	5.03 ± 0.03[f]
Sheep/Mianeh	6.02 ± 0.00[def]	9.24 ± 0.02[ab]	31.58 ± 0.01[de]	4.15 ± 0.01[f]	0.680 ±0.01[e]	4.36 ± 0.00[de]	6.60 ± 0.07[g]	3.40 ± 0.02[j]
Sheep/Kermanshah	9.23 ± 0.00[abc]	10.98 ± 0.01[a]	35.75 ± 0.01[ab]	4.93 ± 0.00[gb]	0.79 ± 0.00[gb]	5.17 ± 0.01[a]	6.62 ± 0.02[f]	4.51 ± 0.02[g]
Goat/North Khorasan	8.26 ± 0.01[bcd]	10.13 ± 0.01[ab]	33.22 ± 0.03[bcd]	4.55 ± 0.00[gc]	0.73 ± 0.00[gc]	4.77 ± 0.01[b]	6.71 ± 0.01[e]	3.63 ± 0.02[i]
Goat/Yasuj	7.62 ± 0.01[cd]	9.52 ± 0.01[ab]	34.68 ± 0.00[bc]	4.28 ± 0.00[gd]	0.71 ± 0.02[cd]	4.50 ± 0.02[c]	7.27 ± 0.02[a]	4.15 ± 0.01[g]
Goat/Hormozgan	7.92 ± 0.01[cd]	9.45 ± 0.01[ab]	32.50 ± 0.00[cd]	4.24 ± 0.00[ge]	0.69 ± 0.02[de]	4.47 ± 0.03[cd]	6.43 ± 0.05[h]	5.23 ± 0.01[e]

Values are expressed as means from three replicates ± SD; values with different superscript letters in the same columns show significant differences according to Tukey's pairwise comparison ($P < 0.05$).
SNF: solid-not-fat.

product [1]. Iranian yogurts are mainly made from cow, buffalo, sheep, or goat milk, each of which significantly influences the quality of the final product. Local manufacturers are the leading producers in the yogurt and sour milk products category. This suggests that each city in Iran has its own yogurt producers who cater to demand within a limited geographical area, typically near their factories. However, there are a few major suppliers with nationwide distribution.

Yogurt has a higher content of group B vitamins, minerals, and proteins compared to milk. Although different animal milk types have relatively similar compositions, each has its own superior characteristics. It is known that milk from different animals, like cows, sheep, buffalo, and goats, has different amounts of protein and fat. This can make starter cultures act in different ways, which can lead to the formation of low-molecular-weight compounds in fermented dairy products [2]. For instance, compared to cow milk, goat milk has minimal to no αs1-casein, smaller fat globules (3.5 μm compared to 4.5 μm in cow milk), and a higher proportion of medium-chain fatty acids (30–35 % versus 10–15 % in cow milk) [3,4].

During the fermentation process, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* convert lactose, the main carbohydrate in milk, into lactic acid. This acid production lowers the pH and causes milk proteins to coagulate, forming a viscous, gel-like structure [5]. *L. bulgaricus* is mostly responsible for the production of acid, while *S. thermophilus* is primarily responsible for the flavor and texture of yogurt. In addition to the mentioned strains, other lactic acid bacteria, such as *Leuconostoc mesenteroides* and *Lactiplantibacillus plantarum,* can also be present in traditional yogurts [6]. In the fermentation process of yogurt, lactic acid bacteria (LAB) break down carbohydrates, proteins, and fats. These reactions are called glycolysis, proteolysis, and lipolysis, and they happen because LAB is active. During these catabolic processes, a variety of volatile and non-volatile metabolites like carbohydrates, organic acids, peptides, amino acids, and other low-molecular-weight molecules are generated [7]. During milk fermentation, LAB produces approximately 400 volatile chemicals. These volatile molecules are among the most important modulators of yogurt flavor. As a result, volatile organic molecules are an unavoidable indicator of customer acceptance and purchase intent [8]. Some of the flavor-linked metabolites are carbonyl compounds (like acetaldehyde), diketones (like diacetyl [2,3-butanedione] and 2,3-pentanedione), volatile carboxylic acids (like butyric and acetic acids), and other organic compounds. Furthermore, carbonyl compounds make up the majority of the volatile organic chemicals that contribute to the flavor of regular yogurt (e.g., acetaldehyde, acetone, 2-butanone, diacetyl, and ethyl acetate). On the other hand, the volatile elements in yogurt can change during storage, depending on the culture, mix formulation, and storage conditions [7,9]. Therefore, comprehending the metabolic processes of starter strains during fermentation requires understanding both volatile and non-volatile metabolite profiles.

Metabolomics stands out as one of the most promising tools for examining metabolite changes in fermented dairy products, enabling detection of metabolites (up to 1500 Da) even in minute quantities [10]. Metabolomics can be classified into untargeted and targeted metabolomics, depending on the specific objectives and characteristics of the investigation. Untargeted metabolomics aims to collect information on a diverse array of chemicals, providing more extensive coverage. Due to the presence of a wide range of volatile flavor compounds in food products, untargeted metabolomics is a more suitable approach for analyzing food samples than targeted metabolomics. Untargeted metabolomics focuses on detecting dynamic changes across several metabolites, while targeted

metabolomics emphasizes specific metabolites [11]. Using non-targeted metabolomics techniques helps us understand and measure metabolites, giving us a quick look at what's going on in a biological system [12,13].

The utility of GC-MS in the analysis of metabolite profiles in milk from various species has been well established [14–17]. These studies have revealed that variations in milk composition due to geographical location, milk types, traditional production methods, and microflora can influence the volatile components in yogurts. However, to the best of our knowledge, the fatty acid and metabolite profiles of Iranian traditional yogurt have not been investigated through metabolomics approaches. The goal of this study was to look at fatty acids, volatile and non-volatile metabolites, and a non-targeted metabolomics approach using GC-MS.

2. Materials and methods

2.1. Chemicals and reagents

All solvents and chemicals used were GC/MS grade from Sigma Aldrich, USA. Nonadecanoic acid (purity >95 %) was used as an internal standard.

2.2. Sample collection

Twelve samples of traditional yogurts made from different ruminants, along with the fresh milk used to produce them, were collected from native producers in various regions of Iran (as listed in Table 1). Due to the limitation of allocated funds, we selected several samples from the same regions, and after pooling the samples from the same regions, they were subjected to metabolomics analysis. The samples were collected in sterilized tubes and stored at a temperature of −18 °C until analysis, which was conducted 48 h after collection. In the traditional Iranian yogurt-making process, milk of all types is boiled and condensed to about two-thirds of its original volume. Once the milk has cooled down to a temperature of 40–45 °C, the previous batch of yogurt is used to inoculate the new batch through a process known as back-sloping. After 12 h, the fermentation process is terminated, resulting in a natural yogurt with a firm consistency and cooked flavor. All these operations are manually conducted in rural settings, using traditional methods that have been passed down through generations and without the use of any machinery. It is worth noting that sheep, goats, and buffalos are primarily raised in rural areas, grazing on pasture fodder. Additionally, due to the presence of specific breeds in certain locations, all yogurt samples were collected after weaning their lambs and kids.

2.3. Analysis of yogurt–produced milk composition

We measured the fat, proteins, lactose, solid, not fat (SNF), density, salts, pH values, and conductivity of milk samples for making yogurt using a Milkoscan Lactoscan MCC W V1 (Milkotronic, Bulgaria), following the ISO 9622:2013 (IDF 141:2013) procedure. The measurements were carried out at 25 °C and in three replicates to assess reproducibility.

2.4. Profiling of fatty acids

2.4.1. Sample preparation
The analysis of fatty acids (FA) in yogurt samples was performed by gas chromatography [18]. For this purpose, yogurt fat was extracted with a mixture of methanol and methylene chloride in a ratio of 1:9, followed by the evaporation of the solvent under a vacuum at 40 °C [19]. The extracted fats were methylated using the Sukhija and Palmquist methods [20]. All extractions were carried out in triplicate.

2.4.2. Gas chromatography
A gas chromatography (GC) machine (Shimadzu GC-17 AAF, V3, 230 V series; Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector (FID) and a molten silica capillary column (SP-2380, 100 m × 0.25 mm; Supelco Inc.) was used to measure the fatty acids. This experiment used helium gas as the carrier and 250 °C injector and detector temperatures. The column temperature program is set as follows: The initial temperature (40 °C for 1 min) was reached at 240 °C at a rate of 5 °C per minute, and then the final temperature was maintained for 10 min. To determine the retention time, a mixture of standard fatty acids and purified acids (Sigma-Aldrich Chemicals, USA) was used. Nonanoic acid was also selected as an internal standard. The relative metabolite levels in each sample were normalized for fat-free dry matter.

2.5. Profiling of volatile compounds

2.5.1. Preparation and purging of samples
Ten grams of finely homogenized yogurt were combined with 10 g of dehydrated sodium sulfate and placed in a suitable U-shaped vial. The 4460 A automatic refining system and a sample trap condenser (O.I. Analytical, College Station, TX, USA) with a Tenax trap were used to extract volatile compounds. A flow of 40 ml/min of completely pure helium was used as the purification gas; the purification was controlled by a thermal sleeve for 20 min at 40 °C. The compounds collected in the non-cold trap were adsorbed at 220 °C for 2 min. The valves were kept at 180 °C, while the transmission line was kept at 120 °C. After adsorption, it was washed by purification at 220 °C for 40 min. All extractions were carried out in triplicate.

2.5.2. Refining and trapping of samples

The 4460 A was connected to an HP 6890 Series gas chromatographic system coupled to a selective mass detector (Hewlett-Packard, Palo Alto, CA, USA). Data were collected and analyzed using a Vectra XM 5/166 PC and a Hewlett-Packard Enhanced ChemStation G1701AA. The HP-INNOWax with a 60-m, 0.25-mm-diameter hair column coated with cross-linked polyethylene glycol (0.25-mm film thickness) was used to separate the volatile compounds. The separation conditions were as follows: Helium was a carrier gas with a constant of 1 ml per minute (min), a split ratio of 30:1, and an injection port at 200 °C. The furnace's temperature program was as follows: 32 °C for 7 min. The furnace operated at 32 °C for 7 min, then increased the temperature at a rate of 6 °C per minute until it reached 220 °C, and finally maintained this temperature for 5 min. Lne (from the gas chromatograph to the mass spectrometer) was kept at 220 °C. The detector operated in scan mode (3 scans per second) and total chromatogram mode (ion) from 19 to 250 amu. Ionization was performed by electronic ionization at eV 70. The ion source was kept at 220 °C, quadrupled to 108 °C, and calibrated automatically. Peak identification was performed by comparing the MS spectrum with the HP Wiley 275 library and the spectrum of injected standards, as well as the shelf life of the standards, if any.

2.6. Data analysis

Before we did a clustering analysis of the metabolome data, we used principal component analysis (PCA) to get a sense of how the samples were distributed, find outliers, and find features that were not quite right. After that, Partial Least Squares Discriminant Analysis (PLS-DA) and Ward's method were used to find volatiles and fatty acids that could tell the difference between yogurt samples from different species and regions. Using the algorithms implemented in the Vegan package of R software version 4.2.1, we obtained variable importance in projection (VIP) scores to highlight and project the importance of each studied metabolite in discriminating profiles in the studied samples. In this study, we focused on VIP scores in the PLS-DA predictive component, considering only those metabolites with VIP values greater than 1 as a discriminant between the classes. The models' quality was assessed using the cumulative parameters R2X, R2Y, and Q2Y, and the models were deemed significant only if the difference between R2Y and Q2Y was less than 0.50.

Descriptive statistical analyses, including mean and standard deviation (SD), were performed using SPSS (IBM® SPSS® Statistics 23) software. The significance of differences at the 5 % level was determined using analysis of variance (ANOVA) and t-test ($P < 0.05$). All biochemical tests were conducted in triplicate for accuracy, and the entire experiment was repeated three times (n = 6).

3. Results and discussion

In this study, we analyzed the primary components of each yogurt (Table 1). The results across different milk types revealed that Ghilan buffalo's milk had the highest fat content at 11.77 %, whereas Kermanshah cow's milk had the lowest at 2.63 %. Overall, buffalo milk had, on average, twice as much as cow milk. Interestingly, a significant variation ($P < 0.05$) in fat content was observed across all species. The high-fat content renders buffalo milk especially well-suited for the production of dairy products, such as mozzarella cheese, known for its characteristic fresh and stringy texture [21]. Regarding protein content, Kermanshah sheep's milk and Kermanshah cow's milk exhibited the highest and lowest values at 5.17 % and 2.72 %, respectively. Previous studies have indicated comparable total protein levels in buffalo, cow, sheep, and camel milk, whereas goat milk has been observed to exhibit a higher protein content [22]. The disparity in protein contents across regions is attributed to genetic variations among these animal species, as well as differences in breed and feed. Among the species, Khorasan Razavi cow's milk had the highest lactose content at 5.91 %, while Ghilan buffalo's milk had the lowest at 4.01 %. Furthermore, Khorasan Razavi cow's milk had the highest concentration of salts at 0.89 %, whereas Ghilan buffalo's milk had the lowest salt content at 0.62 %. A negative correlation between salt content and electrical conductivity was observed, indicating that the conductivity decreases as the amount of salt increases. This effect arises due to the presence of mineral electrolytes and colloidal ions, which decrease the resistance to the flow of electric current in water, the main constituent of milk.

Additionally, it was noted that Kermanshah sheep's milk exhibited a higher solid-not-fat (SNF) content compared to other species, while Khouzestan buffaloes' milk showed notably higher conductivity levels. Lactose and salts, acting as osmolytes, influence milk's freezing point. Regarding SNF content, Kermanshah sheep's milk had the highest percentage at 10.98 %, whereas Kermanshah cow's milk had the lowest at 7.45 %. Camel milk contained more SNF than cattle, sheep, and goats. The highest density for Khorasan Razavi cow's milk was 38.57 %, and the lowest for Ghilan buffalo's milk was 25.69 %. Similar significant pH variations were also observed, with a notable difference in the pH of Yasouj's goat milk (7.27). Genetic differences consistently have a significant impact on milk composition; however, other factors such as calving, age, birth order, nutrition, feeding behaviors, lactation stage, and environmental conditions also contribute. The conductivity of milk is highly correlated with the amount of mineral electrolytes such as chlorides, phosphates, and citrates. The results of the current study are in line with the findings of previous researchers [22–26].

3.1. Investigation of fatty acid profile

The raw FA profile data of the studied samples was normalized before analysis. As seen in Supplementary Fig. 1, the bell shape indicates the normal data distribution. Based on the length of the carbon chain or saturation, fatty acids can be broken down into three groups: short-chain length FA (SCFA), medium-chain length FA (MCFA), and long-chain length FA (LCFA). They can also be broken down into three types: saturated, monounsaturated, and polyunsaturated. A total of 15 analytes were investigated in this study, and 11 individual FAs (one SCFA, 4 MCFA, 3 LCFA, one MUFA, and 2 PUFA) were identified in yogurt samples. The FA profile of yogurt

samples from different regions and animals is shown in complementary Table 1. An investigation of FA profiling showed that saturated FAs form more than 80 % of the major components of total fatty acids in all yogurts from different regions and animals. The differences in FA measurements between regions for the same animal type are relatively small, indicating a consistent fatty acid profile across regions within each animal type. However, there are slight variations, such as in cows, where Khorasan Razavi's shows a slightly higher mean compared to Kermanshah.

In this study, the SCFA (C1:0–C5:0), which is crucial in the maintenance of human health, accounts for approximately 4.51 % and 4.38 % of all saturated FA in sheep and goat samples, respectively. But, the SCFA (mainly butyric acid, C4) made up less than 3 % of all the saturated FA in yogurt from cows and buffalo. This might be because the rumen absorbed propionate and butyrate and then changed them into small, volatile branched-chain fatty acids like caprylic and capric acids. Furthermore, sheep milk is distinguished by a greater amount of butyric acid (C4:0) and ω-3 FA compared to other types of milk from ruminant animals [21]. In cow samples, the percentage of medium-chain FA was approximately 26 % of the total fat, higher than in other species. Buffalo, goat, and sheep samples had similar levels of medium-chain FAs. Values recorded for caprylic acid (C8:0) in goat milk were almost 3.5 times higher than those in cow and buffalo yogurts. Goat milk has been found to be a rich source of SCFAs, which are easily digestible [27], with a relatively high content of MCFAs [28]. It is noteworthy that a higher concentration of capric acid was also recorded for cow milk; this is in contrast with the previous research [16,17,29–31]. This difference in capric acid values can be attributed to quite many known intrinsic factors, such as the stage of lactation, breed, production technology, and genotype; or extrinsic factors, e.g., feeding rate, production method, and the difference in the location where samples were collected [32–34]. Caprylic and capric acids, which come from medium-chain FA tissue metabolism due to their absorption in the rumen, are also responsible for the distinct aroma and flavor of goat yogurt [35]. The results of our study showed that lauric acid (C12:0) was found to be the most abundant among the medium-chain FAs in all of the species. Some studies have found that goat milk has a higher percentage of medium-chain FA [2,30,36]. A concentrations and profiles in final products are mostly determined by the fatty acid content of raw milk as well as production methods in dairy processing. In the current research, both buffalo (23 %) and cow (21.5 %) yogurt samples showed a higher relative percentage of myristic acid (C14:0). Interestingly, palmitic acid (16:0) was found to be the most prevalent FA, regardless of ruminant breed and type. The concentration of stearic acid (C 18:0) was significantly higher in Sarab and Khouzestan buffalo yogurt (P < 0.05) (12.96 and 12.64 %, respectively) and lower in Mianeh sheep yogurt (8.36 %). The mammary gland synthesizes myristic and palmitic acids by de novo processes, while stearic and oleic acids are obtained from plasma lipoproteins.

Various studies have proven that ruminant fats generally consist of low levels of *trans*-FA, which range from 1 % to 8 %. There are geometric and transpositional isomers of oleic acid. The main one is t11-C18:1 (vaccenic acid, t11), which is mostly found in ruminant fats as the trans isomer [37,38]. Vaccenic acid was not detected in any yogurt samples in the present study. However, the opposite trend was noticed for oleic acid and petroselenic acid (*cis*-9 C18:1+ *cis*-6 C18:1).

The combined concentration of oleic acid and vaccenic acid in goat and sheep samples was significantly higher (P < 0.05) than in buffalo and cow samples (22 and 17 times higher, respectively). In addition, North Khorasan goat yogurt had the highest concentrations of oleic acid and vaccenic acid. Linoleic and α-linolenic acids are two PUFAs that are not synthesized by the mammary glands [39]. As demonstrated in Supplementary Table 1, the percentage of linoleic acid (C18:2) in goat and sheep yogurts was not significantly different (P < 0.05), while it was significantly higher in all buffalo and cow yogurts. Significant variation was found in α-Linolenic among yogurt samples (P < 0.05). In comparison to cow and buffalo yogurts, goat and sheep yogurts had higher concentrations of α-Linolenic. The percentage difference is 3.82 % and 3.36 %, respectively. On the other hand, Hormozghan goat yogurt had the highest concentration (1.96 %). In contrast, Ghilan buffalo yogurt had the lowest content (0.57 %). It is because some breeds of cattle have a lot of cellulolytic bacteria in their rumen. These bacteria make odd-chain fatty acids [40]. This is why there are higher levels of oleic acid and α-linolenic acid [40].

The level of conjugated linoleic acid (CLA) was also found to be higher in goat and sheep yogurt than in other species [30]. Our results comply very well with those reported by Sarma et al., 2021 [16] and Caboni et al., 2019 [10]. It has been shown that feeding lactating dairy cows that are supplemented with grape pomace impacts the quality of both milk and the dairy products that are generated from it, generally increasing the content of polyunsaturated FA (Ianni & Martino, 2020). It was also seen that higher lipolysis by microbes and milk enzymes naturally increased free FA, especially in yogurt samples that had olive leaves added [41]. As a result, it is difficult to compare the percentage of each specific free FA in different yogurt samples because a variety of factors influence it.

The generated heatmap (Supplementary Fig. 2) illustrates the relationships among various fatty acids in the yogurt samples, revealing two distinct correlation patterns among the 11 fatty acids. The study found that butyric acid (4:0) had a positive relationship with oleic acid + petroselenic acid (*cis*-9 C18:1 + *cis*-6 C18:1) and linoleic acid (18:2). On the other hand, caproic acid (C6:0) had a positive relationship with caprylic acid (8:0). Conversely, butyric acid (4:0) displayed a negative correlation with myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and capric acid (C10:0).

The correlation analysis (Supplementary Fig. 3) revealed significant (P < 0.05) variations in metabolites across the yogurt samples. Specifically, methyl acetate showed a strong positive correlation (>0.9) with diacetyl, octadecadienoic acid, benzoic acid, and butyric acid. In the same way, 2-butane, hexyl hexanoate, 2-heptanone, and butyl formate all had strong positive relationships with furan metabolites.

Furthermore, strong negative correlations (<−0.9) were seen, showing that the presence of methyl acetate and diacetyl metabolites was opposite to that of furan, butyl formate, 2-heptanone, 4-methylbenzaldehyde, and octalactone metabolites. It was also found that the furfural metabolite had a strong negative relationship with the anethole metabolite and a strong positive relationship with the 2,5-dimethyloxan-2-yl methanol metabolite.

3.2. Volatile profile

The innately volatile elements in raw milk, which are altered by pasteurization, fermentation, processing, and storage, provide the underlying flavor of dairy products. The natural microbiome in indigenous yogurts does not significantly produce the volatile organic compounds typically found in yogurt; instead, these compounds originate from the milk. Supplementary Table 2 shows the volatile compounds identified in the traditional yogurt samples. Using GC-MS analysis, a total of 36 compounds were identified in the yogurt samples. The most predominant class was carbonyl compounds (11), followed by acids (9), alcohols (5), esters (5), aromatic compounds (3), heterocyclic compounds (2), and terpenes (1). Among all metabolites, we found 18 that differed significantly across various types and locations. Additionally, nine metabolites were detected exclusively in specific animals. There were no traces of 2-propanone, 2-butanone, methyl acetate, hexyl hexanoate, or diacetyl found in cow or buffalo yogurt, but more of these compounds were found in samples of sheep and goat milk. On the other hand, compounds such as 2-heptanone, butyl formate, furan, and 2-oxopentanedioic acid were not found in sheep and goat yogurts.

One of the most prevalent metabolites found in the various yogurt samples was 4-methylbenzaldehyde. Although aldehydes are unstable and readily convert into alcohols or their corresponding acids, they can still contribute malt, oil, or butter aroma to yogurt [7, 42]. Methylbenzaldehyde is a cherry- and fruity-tasting aldehyde found in yogurt, produced mainly from the oxidation of unsaturated fatty acids [43]. No significant differences (P > 0.05) were observed among the animals for 4-methylbenzaldehyde. However, benzaldehyde showed significant variability across different types of yogurts (cow, buffalo, sheep, and goat) and locations. Notably, Mianeh sheep yogurt exhibited the lowest benzaldehyde content (4.53 %) among all yogurt types tested.

Lipid oxidation is the most common hydrocarbon source. Significant differences were observed in hydrocarbon concentrations between the samples. Additionally, furan was found exclusively in cow and buffalo yogurt samples, constituting 1.1 % of the volatile compounds, while diacetyl was present only in sheep and goat yogurt samples. Diacetyl and acetaldehyde are the most potent flavor compounds in yogurt. In the current study, no statistically significant differences (P > 0.05) in acetaldehyde concentrations were observed across the different yogurt species. Low acetaldehyde levels can be attributed to non-starter lactic acid bacteria, distinguishing yogurt from other fermented dairy products like milk [44]. This may be due to the reduction of diacetyl to acetoin. Urbach (1995) suggests that starter bacteria can potentially reduce diacetyl to acetoin, while non-starter lactic acid bacteria further convert it to 2-butanone, thereby having a minor influence on the flavor of dairy products. Additionally, key constituents of yogurt, such as fats, proteins, and carbohydrates, can significantly mitigate actual volatile emissions [45,46]. Notably, 2-butanone, dimethyl sulfide, ethanol, and 2-propanone, all known to create off-flavors in milk and dairy products, have been documented. Ketones, like other volatile compounds, are found in yogurt [47]. *Streptococcus thermophilus* fermentation has a significant impact on ketone content. The preheating process initially accelerates milk ketone synthesis [48]. Then, during fermentation, the autoxidation reactions of unsaturated fatty acids can produce ketone molecules [48]. For example, 2-heptanone is synthesized by the β-oxidation of saturated fatty acids followed by decarboxylation or decarboxylation of β-ketoacids found naturally in milk fat. Some methyl ketones, however, were not found in all the yogurts. Interestingly, 2-propanone was only identified in sheep's milk at a higher amount.

Ketones, which give yogurt its distinctive smell, are primarily formed by the oxidation of unsaturated fatty acids, heat degradation, and microbial metabolism. They experience the greatest increase in odor emissions following oral processing [49]. The key ketones found in this study were 2-heptanone and 2,3-pentanedione. The chemical decarboxylation of 2-aceto-2-hydroxybutyrate leads to the production of 2,3-pentanedione. Acetone was found at various levels in all yogurts from different species, with a slightly sweet flavor, a small portion derived from milk, and the majority produced by lactic acid bacteria metabolism. According to Cheng (2010), acetone plays a negative role in the flavor of yogurts. 2-heptanone and 2-nonone, common ketones in cheese, were found only in cow and buffalo samples in this study.

Esters are formed by enzymatic or chemical esterification of free fatty acids and alcohols. Most esters contribute to fruity and floral odors by reducing the spiciness and bitterness of fatty acids and amines, thereby enhancing the odor [7,50]. Among the esters, ethyl acetate was predominant in the samples. Alcohols are primarily formed by the oxidation of fats and the reduction of aldehydes and ketones. The majorities of them are classified as intermediates and play a role in the synthesis of esters [51]. In our study, meso-3, 4-hexanediol was found at lower levels compared to other alcohols in all yogurt samples. Additionally, meso-3,4-hexanediol and (2,5-dimethyloxan-2-yl) methanol were notably elevated in Kermanshah cow yogurt. Despite variations in 2-methyl-1-butanethiol across different species, the concentration was twice as high in sheep and goat yogurts. It is worth noting that some aldehydes with citrus and fresh odors were converted to alcohols with greasy odors during fermentation and storage, affecting the flavor of the yogurt.

Both non-volatile and volatile carboxylic acids play a crucial role as flavor components in dairy products, enhancing the product's sensory characteristics [52]. Although lactic acid is not a volatile carboxylic acid, it is discussed here due to its significant role in determining the flavor of yogurt. Lactic acid contributes significantly to the scent and flavor of yogurt. It is a key taste and functionality ingredient in many fermented foods, giving yogurt its refreshingly tart flavor. Some researchers think that amino acids are the most important building blocks for most volatile fatty acids because they help make acids. Other researchers think that C2–C4 acids are mostly made by lactic acid bacteria and C4–C20 acids are mostly made by breaking down fat [7]. Nine acidic compounds—thioctic acid, octadecadienoic acid, hexanoic acid, octanoic acid, decanoic acid, and butyric acid-were identified in the volatile fraction of all yogurts. Among the nine identified acids, hexanoic acid and octanoic acid were the main acidic compounds in all yogurt types except for Khouzestan buffalo yogurt, which was different from previous reports [7,53]. Additionally, 4-methyl-3-pentanoic acid was not present in cow yogurt, while 2-oxopentanedioic acid was only detected in cow yogurts and at a significantly higher concentration (eight times higher) in Khouzestan buffalo yogurt.

Terpenes are organic compounds produced by plants as secondary metabolites. They are commonly found in shrubs, grasses, and trees, and are particularly abundant in plants from hilly areas. When animals consume a diet rich in terpene-containing vegetation,
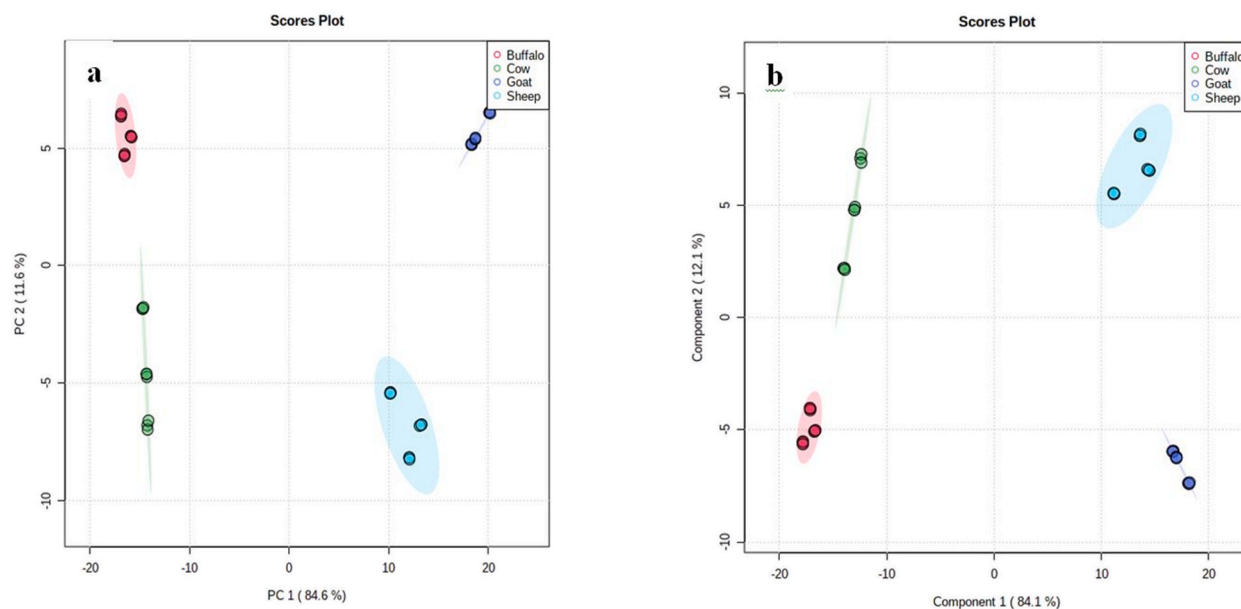
Fig. 1. (a): Scores plots of (a) Principal component analysis (PCA), (b) Partial least squares discriminant analysis (PLS-DA) of the fatty acid profile of Buffalo, Cow, Goat, and Sheep yogurt samples. Three samples were analyzed from each group. Each color symbol represents the source of sampling. The x-axis represents the correlation coefficients of principal components and FA, and the y-axis represents the correlation coefficient between principal components and FA. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

terpenes can also be present in milk and consequently in yogurts, imparting flavors reminiscent of desserts [54,55]. In the present study, limonene was detected in all samples except Zahedan cow yogurt. The highest concentrations were found in Kermanshah cow yogurt and Khouzestan buffalo yogurt, with levels nearly four times higher than those in the other yogurts. This suggests that variations in feed, environmental conditions, and microbial populations specific to each region and type of milk influence limonene levels. Anethole, a derivative of phenylpropene, is found in the essential oils of anise and fennel (Clarke, 2008). Buffalo yogurt from Zahedan (6.29 %) had the highest concentration of anethole, while cow and goat milk from Kermanshah (0.96 %) had the lowest.

3.2.1. Cluster analysis of identified metabolites

In this study, PCA was employed to understand the effects of different animals and locations on the fatty acid profiles and volatile compounds of the yogurt samples. This method allows investigators to examine all identified metabolites, describe their influence in one sample, and compare those results to effects in different samples (Worley & Powers, 2013). For fatty acid profiles, PCA showed four separate groups, with samples clustering separately on the PCA score plot, showing big differences in how they were put together (Fig. 1). Specifically, buffalo, sheep, cow, and goat yogurts exhibited distinct fatty acid profiles. The first and second components explained 84.6 % and 11.2 % of the variance, respectively, underscoring the robustness of the model. Overall, PCA captured a large proportion of the variance in FA, making the visual separation effective and meaningful. The tight clustering within each ellipse suggests that each group has low variability.

To find important metabolites that help with differentiation in the PLS-DA method, values with variable importance in projection (VIP) scores >1 were chosen to show how the FA characteristics of various samples were different. Oleic acid and petroselenic acid (*cis*-9 C18:1 and *cis*-6 C18:1) had the highest VIP score, followed by myristic acid and palmitic acid. These FA were identified as differential compounds distinguishing between animal groups, with oleic acid and petroselenic acid indicating the unique feature of goat's fatty acid profile. Significant differential FA in buffalo yogurt samples included myristic acid, palmitic acid, and stearic acid, while capric acid and lauric acid showed high VIP scores in cow yogurts. Additionally, the heat map using the Ward method highlighted significantly different FA between species (Fig. 2, panels a, b).

For volatile compounds, PCA was used on the GC-MS data variables to show what was the same and what was different about the yogurt samples from different animals. This led to the identification of three separate species groups. The PCA score plots showed similar patterns for buffalo and cow samples, indicating a close relationship between their metabolites. There was no clear grouping among the cow and buffalo metabolites, suggesting that breed and location had a minimal effect on the volatile profiles of their yogurts. In contrast, goat and sheep samples formed separate clusters, demonstrating a clear distinction between these species. The first and second components accounted for 47.3 % and 27.4 % of the variation in volatile metabolites, respectively (Fig. 3a). The significant differences between the groups highlighted the substantial variability in the volatile metabolite profiles of the tested yogurts. However, there was a big difference in the amount of fatty acids found in all species in the PCA plots. This suggests that the natural microbiome system in yogurts may have a bigger effect on how they taste and how volatile their profiles are.
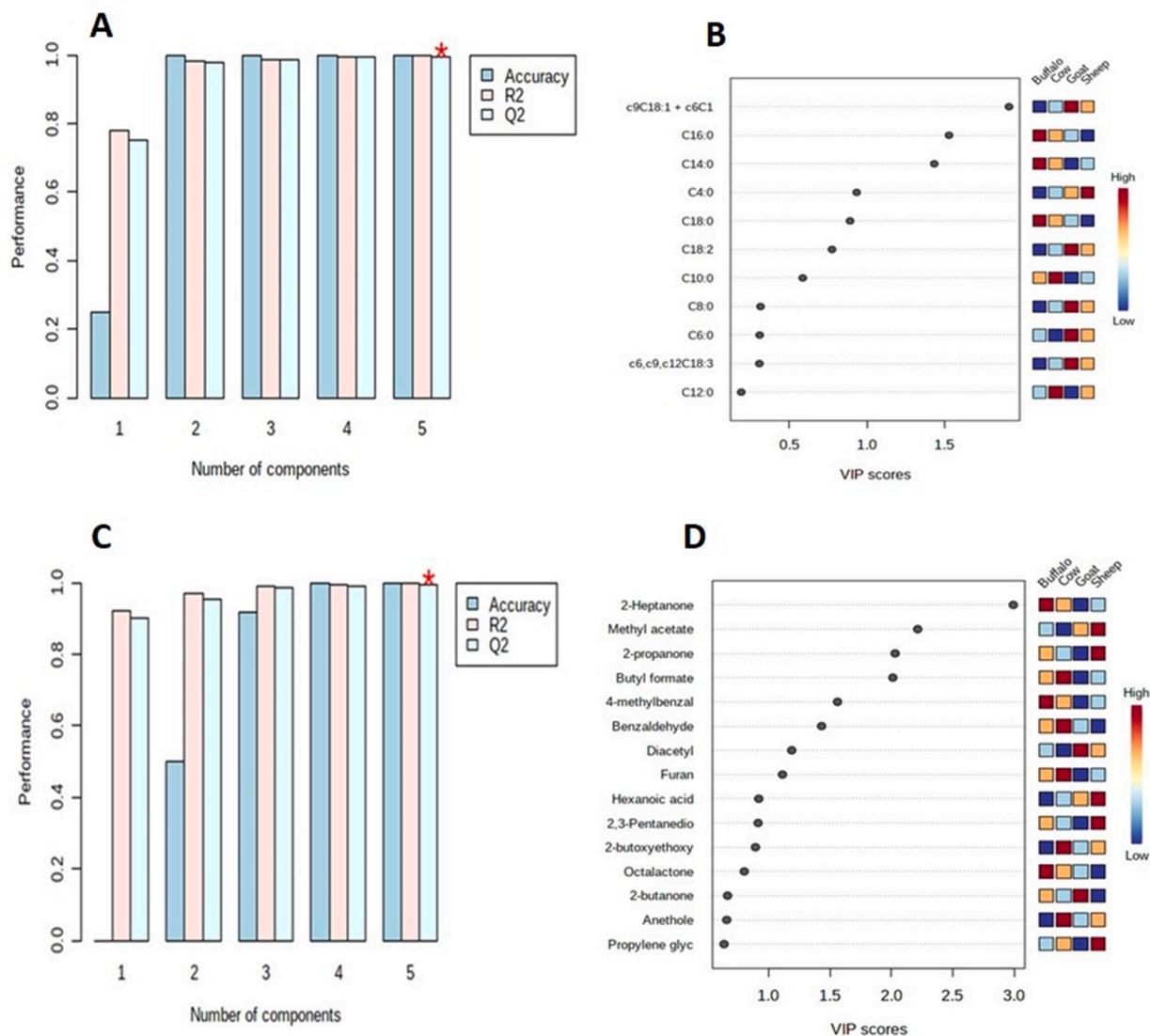
Fig. 2. (A): Validation metrics for the PLS-DA model, R2Y and Q2. **(B):** Variable importance in projection (VIP) values above 1.5 derived from PLS-DA, to compare the variability of FA between 4 species. **(C)** Plots obtained by cross-validation method (leave-one-out cross-validation [LOOCV]) applied on partial least squares-discriminant analysis (PLS-DA) of volatile data. **(D):** Variable importance in projection (VIP) values above 1.5 derived from PLS-DA, to compare the variability of volatile between 4 species.

Similar to PCA, data clustering using PLS-DA score plots of volatile compounds showed unique patterns for each species (Fig. 3b). Buffalo, cow, sheep, and goat samples formed four separate clusters with a certain distance from each other in terms of volatile metabolites. Goat and sheep yogurt samples exhibited higher variability, with a wider spread in the score plot than buffalo and cow yogurt samples. The goodness of fit (R2Y) and predictive ability (Q2) parameters were used to verify the accuracy and predictability of the PLS-DA model. The first and second components accounted for 45.8 % and 27.1 % of the variation in volatile metabolites in different samples, respectively. A comparison of the VIP method (Fig. 2; panels c, d) identified 15 metabolites with significant differences in abundance between the samples. Compounds with VIP values above 1.5 were selected based on the PLS-DA loading values. There were 15 different metabolites that were tested. Three of them were significantly different between the yogurts. These were myristic acid (C14:0), palmitic acid (C16:0), and oleic acid and petroselenic acid (cis-9 C18:1 and cis-6 C18:1).

Ward's method aims to minimize the differences within clusters by merging those with the smallest dissimilarities. The process starts with each item as an individual cluster, progressively combining similar items into existing clusters. This continues until all items are grouped into one cluster in the final stage, with each item starting with its own cluster. Once items are clustered, they remain together. The goal is to identify the optimal number of stages required to effectively cluster the items [56]. The clustering of fatty acid profiles using Ward's method, illustrated in Fig. 4, identified two primary clusters, each divided into two sub-clusters. Butyric acid
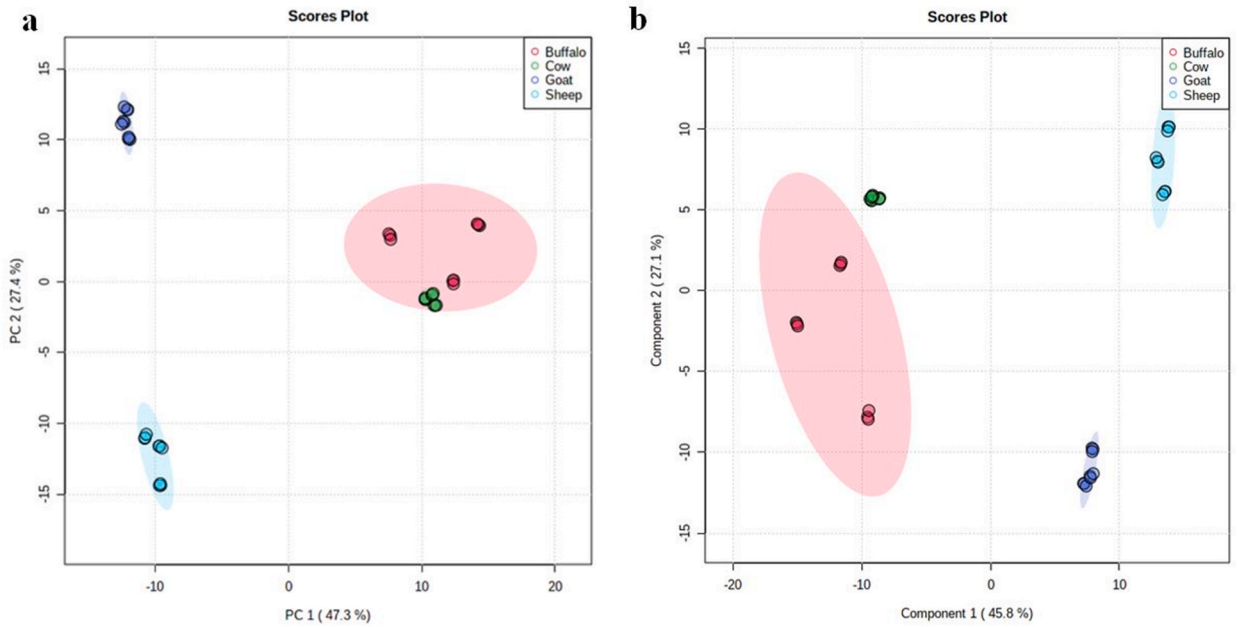
Fig. 3. (a): Principal component analysis (PCA) scores plots of 4 yogurt groups based on volatiles profile. Sample groups in this analysis: Buffalo, Cow, Goat, and Sheep yogurts. Three samples were analyzed from each group. Each color symbol represents the metabolite of one group. The x-axis represents the correlation coefficients of principal components and metabolites, and the y-axis represents the correlation coefficient between principal components and metabolites. **(b):** Partial least squares discriminant analysis (PLS-DA) for volatiles. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
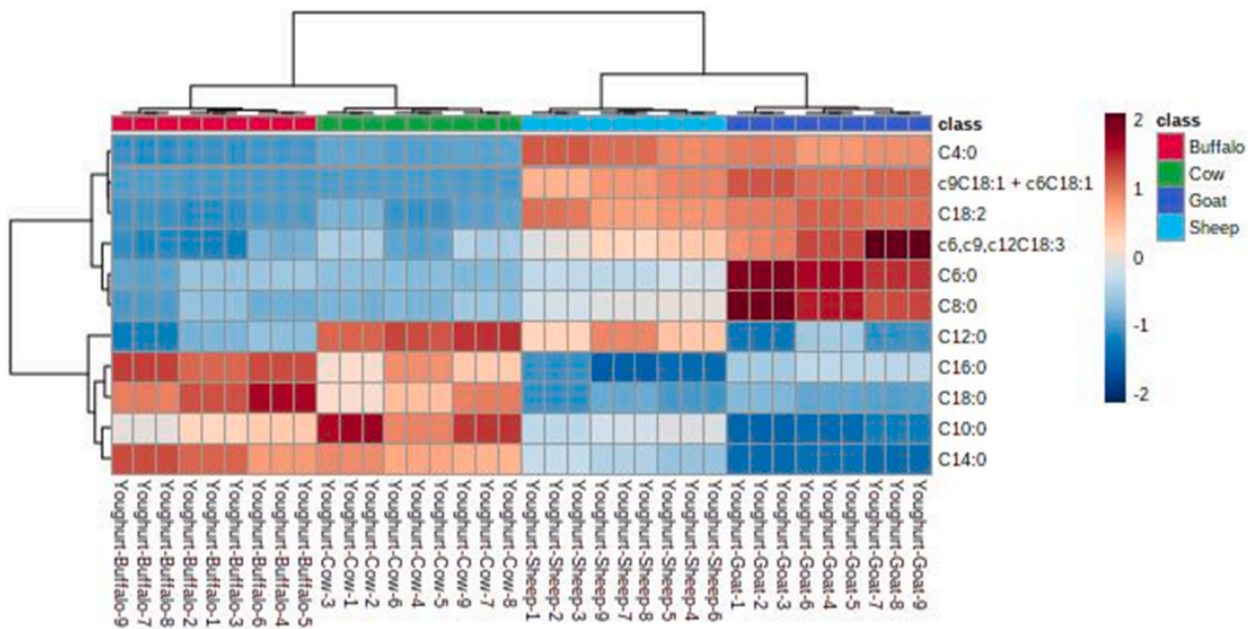
Fig. 4. Heatmap visualizing the top different FA's concentrations among the species. Colored cells correspond to concentration value (samples in column and compounds in a row). Data presented were normalized and subjected to T-test/ANOVA and features were standardized to auto-scaling.

(4:0), oleic acid + petroselenic acid (*cis*-9 C18:1+ *cis*-6 C18:1), and linoleic acid (18:2) were all in sub-cluster 1, and their presence patterns were the same across all the samples. Despite differences in these fatty acids' presence patterns in sheep samples compared to cow and buffalo samples, they showed similarities with goat samples. Sub-cluster 2 comprised caproic acid, caprylic acid, and α-linolenic acid (C18:3 (cis 9, 12, and 15), which shared a presence pattern. The presence pattern of these fatty acids in buffalo samples

Fig. 5. Heatmap plot clusters of volatile compounds in yogurt samples from diverse species.

differed significantly from goat and sheep samples but was similar to cow samples. Capric acid, myristic acid, palmitic acid, and stearic acid were all in sub-cluster 3, and their presence patterns in the samples were similar. Lauric acid, on the other hand, was in sub-cluster 4, and its presence pattern was different. Except for lauric acid, the fatty acid profiles in buffalo samples matched those in cow samples, while sheep samples were similar to goat samples. Lauric acid showed a unique presence pattern, with similarities between goat and buffalo samples and cow and sheep samples. To identify the four main groups of yogurt metabolites, a heat map using Ward's method was created, as shown in Fig. 5. This figure reveals differences in the abundance of metabolites between yogurts from different species. The metabolite profiles of goat and sheep samples are clustered into individual groups, in contrast to buffalo and cow yogurt profiles.

4. Conclusion

In the present study, the GC-MS-based metabolomics approach successfully highlighted the differences in FA and volatiles between various ruminants and regions in traditional Iranian yogurts. The milk from four different animal sources (buffalo, cow, goat, and sheep) used for yogurt production was analyzed and compared. Significant differences in the fatty acids and metabolites were observed

among the yogurts produced from these different types of milk. These differences were primarily attributed to milk composition, feed variations, environmental conditions, and region-specific microbial populations. The main FAs that set cow, buffalo, sheep, and goat yogurts apart were myristic acid (C14:0), palmitic acid (C16:0), and a mix of oleic acid and petroselenic acid (*cis*-9 C18:1 + *cis*-6 C18:1). Two-heptanone, methyl acetate, two-propanone, butyl formate, and four-methyl benzaldehyde were named as important metabolites in yogurts that are volatile compounds. In projection using the PLS-DA algorithm, variable importance indicated that myristic acid and capric acid (C:10) could be used as important indicators of FA for buffalo and cow yogurt. In contrast, samples from goats showed the highest VIP scores for oleic acid and petroselenic acid (*cis*-9 C18:1 and *cis*-6 C18:1). It was found that 2-heptanone, methyl acetate, 2-propanone, butyl formate, and 4-methyl benzaldehyde are some of the most important volatile compounds that help yogurts stand out. Overall, the connections found between metabolites and important milk traits suggest that the metabolite profile could be used as a predictive biomarker in different areas of the dairy industry, such as creating new hybrid dairy products using different types of milk and autochthonous starter cultures. Although the study was constrained to a limited number of regions in Iran due to funding and resource limitations and did not account for a higher number of samples per region or seasonal variability in milk metabolites affecting yogurts, the promising results encourage further research in this area.

CRediT authorship contribution statement

Reza Vaseghi Bakhshayesh: Writing – original draft, Methodology. **Bahman Panahi:** Validation, Software, Formal analysis. **Mohammad Amin Hejazi:** Writing – original draft. **Yousef Nami:** Writing – review & editing, Project administration, Methodology.

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.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34760.

References

[1] H. Ezzatpanah, Traditional fermented dairy products of Iran, Int. Dairy J. 147 (2023) 105773.
[2] M.P. Mollica, et al., Milk fatty acid profiles in different animal species: focus on the potential effect of selected pufas on metabolism and brain functions, Nutrients 13 (4) (2021) 1111.
[3] K. Saipriya, et al., Effect of dairy unit operations on immunoglobulins, colour, rheology and microbiological characteristics of goat milk, Int. Dairy J. 121 (2021) 105118.
[4] M. Goswami, et al., Implication of functional ingredients of goat milk to develop functional foods, J. Animal Feed Sci. Technol. 5 (2017) 65–72.
[5] R.C. Chandan, K. O'Rell, Principles of yogurt processing. Manufacturing Yogurt and Fermented Milks, 2013, pp. 239–261.
[6] M.M. İspīrlı, et al., Detrended fluctuation analysis of leakage current characteristics of polyurethane insulators, in: 2018 26th Signal Processing and Communications Applications Conference (SIU), IEEE, 2018.
[7] H. Cheng, Volatile flavor compounds in yogurt: a review, Crit. Rev. Food Sci. Nutr. 50 (10) (2010) 938–950.
[8] C. Soukoulis, et al., Proton transfer reaction time-of-flight mass spectrometry monitoring of the evolution of volatile compounds during lactic acid fermentation of milk, Rapid Commun. Mass Spectrom. 24 (14) (2010) 2127–2134.
[9] A. Ahmad, Z. Ahmed, Fortification in beverages, in: Production and Management of Beverages, Elsevier, 2019, pp. 85–122.
[10] P. Caboni, et al., A metabolomics comparison between sheep's and goat's milk, Food Res. Int. 119 (2019) 869–875.
[11] L.D. Roberts, et al., Targeted metabolomics, Curr. Protoc. Mol. Biol. (2012) 1–24 **(Chapter 30)**: p. Unit 30.2.
[12] Y. Xia, et al., A UPLC-Q-TOF-MS-based metabolomics approach for the evaluation of fermented mare's milk to koumiss, Food Chem. 320 (2020) 126619.
[13] D. Li, et al., Metabolic footprinting revealed key biochemical changes in a brown fermented milk product using Streptococcus thermophilus, J. Dairy Sci. 103 (3) (2020) 2128–2138.
[14] Y. Gu, et al., Impact of Lactobacillus paracasei IMC502 in coculture with traditional starters on volatile and non-volatile metabolite profiles in yogurt, Process Biochem. 99 (2020) 61–69.
[15] A. Murgia, et al., GC-MS metabolomics comparison of yoghurts from sheep's and goats' milk, Int. Dairy J. 96 (2019) 44–49.
[16] H. Sharma, et al., Comparative analysis of metabolites in cow and goat milk yoghurt using GC–MS based untargeted metabolomics, Int. Dairy J. 117 (2021) 105016.
[17] H. Sharma, R. Ramanathan, Gas chromatography-mass spectrometry based metabolomic approach to investigate the changes in goat milk yoghurt during storage, Food Res. Int. 140 (2021) 110072.
[18] H. Türkoğlu, Free fatty acid composition and sensory characteristics of Orgu cheese, Sci. Res. Essays 6 (7) (2011) 1555–1560.
[19] S. Garcia-Lopez, et al., Changes in the content of conjugated linoleic acid (CLA) in processed cheese during processing, Food Res. Int. 27 (1) (1994) 61–64.
[20] P.S. Sukhija, D. Palmquist, Rapid method for determination of total fatty acid content and composition of feedstuffs and feces, J. Agric. Food Chem. 36 (6) (1988) 1202–1206.
[21] A. Mohapatra, A.K. Shinde, R. Singh, Sheep milk: a pertinent functional food, Small Rumin. Res. 181 (2019) 6–11.
[22] N. Huma, et al., Characterization of milk proteins from different animal species through gel electrophoresis, Pakistan J. Zool. 50 (2018) 1601–1998.

[23] I. Yasmin, et al., Characterization and comparative evaluation of milk protein variants from pakistani dairy breeds, Food Sci. Animal Res. 40 (5) (2020) 689.
[24] E. Barros, et al., Population structure and genetic variability of the Segureña sheep breed through pedigree analysis and inbreeding effects on growth traits, Small Rumin. Res. 149 (2017) 128–133.
[25] E. Ozrenk, S.S. Inci, The effect of seasonal variation on the composition of cow milk in Van Province, Pakistan J. Nutr. 7 (1) (2008) 161–164.
[26] M.P. Mollica, et al., Milk fatty acid profiles in different animal species: focus on the potential effect of selected PUFAs on metabolism and brain functions, Nutrients 13 (4) (2021).
[27] R. Jenness, Composition and characteristics of goat milk: review 1968– 1979, J. Dairy Sci. 63 (10) (1980) 1605–1630.
[28] M.R.S. Sampelayo, J.B. López, Aspectos nutricionales de la leche de cabra, Anales de la Real Academia de Ciencias Veterinarias de Andalucía Oriental (10) (1997) 109–140.
[29] B. Paszczyk, M. Czarnowska-Kujawska, Fatty acid profile, conjugated linoleic acid content, and lipid quality indices in selected yogurts available on the Polish market, Animals 12 (1) (2022) 96.
[30] A. Serafeimidou, et al., Chemical characteristics, fatty acid composition and conjugated linoleic acid (CLA) content of traditional Greek yogurts, Food Chem. 134 (4) (2012) 1839–1846.
[31] J. Sumarmono, M. Sulistyowati, Fatty acids profiles of fresh milk, yogurt and concentrated yogurt from peranakan etawah goat milk, Procedia Food Sci. 3 (2015) 216–222.
[32] O. Hanuš, et al., The effect of cattle breed, season and type of diet on the fatty acid profile of raw milk, Arch. Anim. Breed. 59 (3) (2016) 373–380.
[33] J. Kelsey, et al., The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows, J. Dairy Sci. 86 (8) (2003) 2588–2597.
[34] J. Frelich, et al., Seasonal Variation in Fatty Acid Composition of Cow Milk in Relation to the Feeding System, 2012.
[35] F. Yangilar, As a potentially functional food: goats' milk and products, J. Food Nutr. Res. 1 (4) (2013) 68–81.
[36] Y. Chilliard, et al., Optimising goat's milk and cheese fatty acid composition, in: Improving the Fat Content of Foods, Elsevier, 2006, pp. 281–312.
[37] K. Kuhnt, et al., Trans fatty acid isomers and the trans-9/trans-11 index in fat containing foods, Eur. J. Lipid Sci. Technol. 113 (10) (2011) 1281–1292.
[38] J.-N. Lim, et al., Trans-11 18: 1 vaccenic acid (TVA) has a direct anti-carcinogenic effect on MCF-7 human mammary adenocarcinoma cells, Nutrients 6 (2) (2014) 627–636.
[39] R.K. Saini, Y.-S. Keum, Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance—a review, Life Sci. 203 (2018) 255–267.
[40] B. Vlaeminck, et al., Factors affecting odd-and branched-chain fatty acids in milk: a review, Anim. Feed Sci. Technol. 131 (3–4) (2006) 389–417.
[41] F. Bennato, et al., Volatile profile in yogurt obtained from saanen goats fed with olive leaves, Molecules 25 (10) (2020) 2311.
[42] A. Hayaloglu, et al., Volatiles and sensory evaluation of goat milk cheese Gokceada as affected by goat breeds (Gokceada and Turkish Saanen) and starter culture systems during ripening, J. Dairy Sci. 96 (5) (2013) 2765–2780.
[43] L. Zhao, et al., Addition of buttermilk improves the flavor and volatile compound profiles of low-fat yogurt, Lebensm. Wiss. Technol. 98 (2018) 9–17.
[44] J.E. Friedrich, T.E. Acree, Gas chromatography olfactometry (GC/O) of dairy products, Int. Dairy J. 8 (3) (1998) 235–241.
[45] M.S. Brauss, et al., Altering the fat content affects flavor release in a model yogurt system, J. Agric. Food Chem. 47 (5) (1999) 2055–2059.
[46] D.D. Roberts, P. Pollien, Relative Influence of Milk Components on Flavor Compound Volatility, ACS Publications, 2000.
[47] M. Korachi, et al., Biochemical changes to milk following treatment by a novel, cold atmospheric plasma system, Int. Dairy J. 42 (2015) 64–69.
[48] Y. Li, L. Zhang, W. Wang, Formation of aldehyde and ketone compounds during production and storage of milk powder, Molecules 17 (8) (2012) 9900–9911.
[49] S. Mu, et al., Characterization of the relationship between olfactory perception and the release of aroma compounds before and after simulated oral processing, J. Dairy Sci. 104 (3) (2021) 2855–2865.
[50] T. Singh, M. Drake, K. Cadwallader, Flavor of Cheddar cheese: a chemical and sensory perspective, Compr. Rev. Food Sci. Food Saf. 2 (4) (2003) 166–189.
[51] I.M. Ferreira, O. Pinho, P. Sampaio, Volatile fraction of DOP "castelo branco" cheese: influence of breed, Food Chem. 112 (4) (2009) 1053–1059.
[52] M. Gueimonde, et al., Viability and diversity of probiotic Lactobacillus and Bifidobacterium populations included in commercial fermented milks, Food Res. Int. 37 (9) (2004) 839–850.
[53] S.T. Sarhir, A. Amanpour, S. Selli, Characterization of ayran aroma active compounds by solvent-assisted flavor evaporation (SAFE) with gas chromatography–mass spectrometry–olfactometry (GC–MS–O) and aroma extract dilution analysis (AEDA), Anal. Lett. 52 (13) (2019) 2077–2091.
[54] I. De Noni, G. Battelli, Terpenes and fatty acid profiles of milk fat and "Bitto" cheese as affected by transhumance of cows on different mountain pastures, Food Chem. 109 (2) (2008) 299–309.
[55] I. Poulopoulou, et al., Terpenes transfer to milk and cheese after oral administration to sheep fed indoors, J. Anim. Physiol. Anim. Nutr. 96 (2) (2012) 172–181.
[56] D. Eszergár-Kiss, B. Caesar, Definition of user groups applying Ward's method, Transport. Res. Procedia 22 (2017) 25–34.