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# Nutritional composition of ghee of various animal origins produced in some silk road countries

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ABSTRACT

This study aimed to investigate the fatty acids, vitamins, and minerals of ghee from various animal milk from different countries, such as India, Kazakhstan, Kyrgyzstan, Mongolia, and Turkey. Ghee consists of 98.9% of fat, irrespective of the animal source. Among the short- and medium-chain saturated fatty acids, all samples contain butyric (C4:0), caproic (C6:0), caprylic (C8:0), and capric acid (C10:0). Mare and goat ghee additionally contain undecanoic acid (C11:0). Major fatty acids were myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and monounsaturated fatty acid was oleic acid (C18:1 *cis* 9). Notably, camel and mare ghee have the highest values of polyunsaturated fatty acids. Regarding minerals, the average levels were 5.48 mg/100 g for calcium, 5.04 mg/100 g for potassium, and 23.6 mg/100 g for phosphorus. Ghee also contains β-carotene at an average of 392 μg/100 g, vitamin A at 606 μg/100 g, and vitamin E at 1650 μg/100 g. The aforementioned results underscore the variation in the nutritional composition of ghee according to its geographical origin and source.

## 1. Introduction

Ghee (clarified butter) is a food product obtained by heating milk cream at high temperatures. It is a high-calorie dairy product with a colour that varies from white to yellow. Currently, the cream is obtained by separating milk using a cream separator. Milk cream is melted at over 100 °C to remove moisture and isolate it from the non-fat solids. It is heated until the milk proteins begin to turn brown. After cooling to room temperature, a clear upper layer of the fat is carefully decanted from the solid brown sediment. Ghee can be stored in a cool place for at least 2 years (Sharma, 1990, Iskakova & Smanalieva, 2020). Currently, ghee production is mostly carried out in factories using industrialised methods (Forero et al., 2023).

The ancient Indian society widely consumed ghee during the time of Lord Krishna, about 3000 BC (Rai et al., 2016). Almost all pastoral communities prepare ghee, which is also known as *samneh* in the Middle East, *nigour kibe* or *nitir kibe* in southern and eastern Africa, *murcchita ghrta* or *makhan* in India, *sirme* in western Africa, ghee *orghyu* or *mor* in Nepal and Bhutan, *tarkhineh* in Kurdistan (Degen, 2007), *maa* in Tibet (Rai et al., 2016), and *sary mai* in Kazakhstan and Kyrgyzstan (Iskakova & Smanalieva, 2020).

Ghee has been used in traditional medicine in the Middle East, India, and Asian and African countries since ancient times and is considered "the best diet." It improves body energy, combats flatulence, reduces stress, boosts intellect, provides nutrients, increases strength in the cold and patience with thirst in the heat, and extends one's life over generations (Sserunjogi et al., 1998). Therefore, ghee is a common ingredient in many Ayurvedic, Tibetan, and Mongolian remedies. Traditional medicine recommends ghee for treating inflammatory dermatological, eye, and ulcer diseases. Besides, cow ghee is claimed to support memory, recall, and learning—the three facets of mental functioning (Sharma, 1990). The Kyrgyz people used ghee to smear the child's mouth after birth (Smanalieva et al., 2022). Some research shows that ghee stimulates gastric secretion, has a beneficial effect on digestion, reduces the inflammation that causes liver and breast cancer (Rani & Kansal, 2011), and colorectal cancer (Giovannucci & Willett, 1994), regulates

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detoxification (Chinnadurai et al., 2013), improves the immune system, and relieves mental stress (Sserunjogi et al., 1998). Additionally, researchers have found that α-linolenic acid (18:3 n–3) reduces cholesterol and triglyceride levels, improves eyesight, reduces weight, and has anti-inflammatory effects (O'Reilly et al., 2020). All these health effects of ghee are associated with this composition since ghee is a significant food source of essential fatty acids (linolenic acid, linoleic acid, and arachidonic acid) and fat-soluble vitamins (A, D, E, and K) (Kumar et al., 2010).

Vitamin A is essential for the formation and function of the heart, lungs, eyes, and other organs, as it supports cell growth and differentiation (Blaner, 2020). Basic evidence suggests that provitamin A, β-carotene, exhibits antidepressant properties, which may be due to decreased levels of tumour necrosis factor-α (TNF-α) and interleukin-6, as well as increased levels of brain-derived neurotrophic factor, as explained by Kim et al. (2016). Additionally, Zhang et al. (2022) reported in a meta-analysis that dietary intakes of vitamin A and β-carotene were negatively associated with depression. Another fat-soluble vitamin found in ghee is vitamin E. According to Traber and Vitamin. (2006), vitamin E is involved in immune function and has been shown primarily by *in vitro* cell studies, cell signalling, regulation of gene expression, and other metabolic processes.

Calcium is important for the development of bones and teeth, giving them structure and hardness; it also plays a role in the metabolism of vitamin D. One essential micronutrient found naturally in a wide variety of meals is potassium. Potassium, as an intracellular cation, has a strong relationship with sodium, the main regulator of extracellular fluid volume, including plasma volume (Stone et al., 2016). Phosphorus, an essential mineral of the human body, is a component of bones, teeth, DNA, and RNA (Huskisson et al., 2007).

Fatty acids (FAs), either as parts of larger molecules or acting individually, perform a variety of functions in cells, from structural "building blocks" of cell membranes to energy providers and signalling molecules. Some organisms require certain physiologically essential fatty acid compounds that cannot be synthesised anew or in sufficient amounts to meet the metabolic, somatic, and reproductive functions of the body (de Carvalho & Caramujo, 2018). In the human body, FAs mainly occur as esters with various alcohols. FA molecules are classified by their chain length: short-chain (< 6 carbon atoms) (SCFA), medium-chain (6–12 carbon atoms) (MCFA), long-chain (13–21 carbon atoms) (LCFA), and very long-chain fatty acids (> 21 carbon atoms) (VLCFA) (Řezanka & Sigler, 2009). Fatty acids in the same class may have different biological effects. The most widely discussed health impacts of FAs relate to cardiovascular complications and obesity; however, influences on immune function, gut microbiota, cancer, and epilepsy have been revealed (Şafak et al., 2020).

Many publicly available food composition databases (FCDB) have limited information about the fatty acid composition and other nutrients of ghee. The fatty acid composition data for ghee in many FCDBs were borrowed from plain, salted butter. Besides, there is a dearth of scientific information about the nutritional differences in ghee from various animal sources. Since different animal sources lead to different milk compositions, it is hypothesised that this may also affect the nutrient content of ghee, especially regarding the fatty acid composition. Therefore, this study aimed to investigate the fatty acid profile, and vitamin and mineral contents of ghee samples to understand the differences in the composition of ghee from various animal sources and produced in different countries.

## 2. Materials and methods

### 2.1. Origin of ghee samples

Indian buffalo ghee (INB) and Indian cow ghee (INC), Kazakhstan cow ghee (KZC) and Kazakhstan goat ghee (KZG), Kyrgyzstan cow ghee-1 and −2 (KGC1), (KGC2), Mongolian yak ghee preserved for 4 years (MN4Y), Mongolian cow ghee preserved for 10 years, Mongolian yak ghee (MNY), Mongolian camel ghee (MNCa), Pakistani buffalo ghee (PKB), and Turkish cow ghee (TRC) were obtained from local supermarkets, listed in Table 1. Commercially produced and homemade ghee samples were stored at –21 °C and 61% humidity before further analysis.

### 2.2. Determination of moisture and fat content

Moisture content of ghee samples (5±2 g) was determined by drying them at 105±1°C until constant weight using a halogen-infrared moisture analyser (MBG 163 L; VWR, Italy). Fat content was analysed using the butyrometer according to method GOST 5867–90, which corresponds to ISO 11870:2009 and AOAC 989.05 (AOAC, 2019; ISO 2009).

### 2.3. Fatty acid profile analysis

For the fatty acid profile analysis, the triglycerides were hydrolysed (saponified) to glycerol and free fatty acids with potassium methanolate to form fatty acid methyl esters (FAMEs). Fatty acid analysis was carried out using gas chromatography equipped with a flame ionisation detector (GC-FID; Agilent Technologies 7820 A GC System) using a ZB-FFAP capillary column (30 m × 0.25 mm, film thickness 0.25 μm; Phenomenex, Torrance, CA). The flow rate of nitrogen (carrier) gas was maintained at 2.5 mL/min temperature programme: 60 °C/3 min, 150 °C/9 min, 180 °C/19 min, 240 °C/32.33 min, and 240 °C/45 min. The temperature of the injector was 250 °C, and the sample (1 μL) was injected at a split ratio of 1:30. The FID was run at 300 °C. Pentanoic acid

**Table 1**
Ghee samples collected from different countries.

| N | Country of origin | Ghee type | Source | Obtaining data | Code |
|---|---|---|---|---|---|
| 1. | India | Ghee from buffalo milk | Local market | 2022 | INB |
| 2. | | Ghee from cow milk | Local market | 2022 | INC |
| 3. | Kazakhstan | Ghee from cow milk | Local market Astana | 15 June 2022 | KZC |
| 4. | | Ghee from goat milk | Local market Astana | 15 June 2022 | KZG |
| 5. | Kyrgyzstan | Ghee from cow milk | Lcal market Bishkek (Shoro LCC) | 18 April, 2022 | KGC1 |
| 6. | | Ghee from cow milk | Local market Bishkek (Elsut LCC) | 11 May, 2022 | KGC2 |
| 7. | Mongolia | Ghee from yak milk - new | Galuut Sum farm, Arkhangai Province, Tsakhiur Soum | 15 December, 2021 | MNY |
| 8. | | Ghee from yak milk – preserved 4 years | Erdenetsogt Sum farm, Bayankhongor Province, Erdenetsogt Soum | 24 February 2022 | MN4Y |
| 9. | | Ghee from camel milk -new | Nomadic family: Umnugobi Province, Nomgon Soum | 30 January, 2022 | MNCa |
| 10. | | Ghee from cow milk-preserved 4 years | Nomadic family: Bayankhongor Aimag, Galuut Soum | 15 May, 2022 | MN4C |
| 11. | | Ghee from mare milk | Nomadic family: Javzandulam, Uvs province, Mongolia. | 3 April, 2022 | MNHG |
| 12. | Turkey | Ghee from cow milk | Local market, LCC HAKTAN PEYNIRCILIK GIDA SAN. VE TİC. | 27 May, 2021 | TRC |

methyl ester (C5:0) and heptadecanoic acid methyl ester (C17:0) in *n*-heptane were used as internal standards. The FID signals of the sample mix were matched with the reference chromatograms of the external standard (Supelco™ 37, Sigma Aldrich). Sample quantification of fatty acid methyl esters (> 8:0) was performed by the area normalisation method, in which the amount of each sample component was estimated based on the peak area of the component relative to the total area to obtain the percentage composition. For the quantification of short-chain fatty acids, correction factors corresponding to the standard were used. Relative response factors ($R_i$) were calculated from chromatograms of the standard mixture (Formula 1).

$$R_i = \frac{A_i \cdot c_{IS}}{A_{IS} \cdot c_i} \quad (1)$$

where $A_i$ is the peak area of the corresponding FAME at ci; $A_{IS}$ is the peak area of the internal standard; $c_i$ is the concentration of the corresponding FAME (standard); and $c_{IS}$ is the concentration of the internal standard.

The content of fatty acids is calculated using Formula 2.

$$m_i = \frac{m_{IS} \cdot A_i \cdot R_i \cdot S_i \cdot 100}{A_{IS} \cdot m} \quad (2)$$

$m_i$ is the content of fatty acid in g/100 g ghee sample; $m_{IS}$ is the mass of internal standard added to the fat sample (mg); m is the weight of the ghee sample (mg); $A_{IS}$ is the peak area of the internal standard (in the chromatogram of the sample); $S_i$ (stoichiometric factor): M fatty acid methyl ester i/M fatty acid i.

### 2.4. Analysis of vitamins

High-performance liquid chromatography (HPLC, Agilent Technologies, USA) equipped with a C18 column was used to determine the β-carotene content in twelve ghee samples. Sample preparation was carried out according to Schierle et al. (2004). Five grams of ghee were weighed into a beaker, and 250 mg of butylated hydroxytoluene (BHT) were added and rinsed into a 250-mL volumetric flask with 120 mL of dichloromethane. Then 100 mL of ethanol were added and shaken. The mixture was left in the dark until it reached room temperature (ca. 2 hours). After this, the mixture was diluted to volume with dichloromethane and shaken vigorously. The aliquot was diluted in a 1 + 9 ratio with dichloromethane-ethanol (1 + 1) mixture in a 10-mL volumetric flask. The solutions were filtered through a 0.45 μm membrane, and 20 μL were injected into the HPLC system. A diode array detector at a wavelength of 456 nm was used. Identification was carried out using the retention time obtained for the reference substance β-carotene, and quantification was carried out according to the external standard method with concentration levels of 0.01, 0.5, 1.0, 2.0, 3.0, and 4 μg/mL (Schierle et al., 2004).

Vitamin A content as retinol acetate and vitamin E content as the sum of alpha-, beta-, gamma-, delta-tocopherols, and alpha-tocopherol acetate were determined using HPLC. Samples were prepared according to Ollilainen et al. (1989). A sample weighing 2–5 g was transferred to a 25-$cm^3$ volumetric flask and dissolved in 10–15 mL of *n*-hexane. An ultrasonic bath was used to accelerate dissolution. The solution was brought to the mark with *n*-hexane. A mixture of acetonitrile, dichloromethane and methanol (50:45:5) was pumped at a flow rate of 2 mL/min. Retinoids were determined using a Zorbax 300SB-C18 column (5 μm, 4.6 × 250 mm; Agilent, Santa Clara, CA). The flow rate of *n*-hexane-2-propanol (mobile phase) (98:2) was 2 mL/min. The detector wavelength was set at 450 nm for carotenoids and 325 nm for retinoids. Standards and samples were injected into both systems through a full loop (app. 50 μL), and the column temperature in both systems was set at 30 °C (Ollilainen et al., 1989).

### 2.5. Analysis of calcium, potassium and phosphorus content

For the determination of calcium, potassium, and phosphorus content, ghee samples were prepared according to Bilandžić et al. (2014). Two grams of ghee were weighed into a PFA digestion vessel, and 1 mL of $H_2O_2$ and 6 mL of $HNO_3$ were added. To acid-digest samples, a Multiwave 3000 microwave oven (Anton Paar, Ostfildern, Germany) was utilised; 50 mL of ultra-pure water were used to dilute the digested samples. The filtrates were analysed using atomic absorption spectroscopy (Qunat-Z-ETA-T; Cortex, Russia) (AOAC 984.27, AOAC 2011.14, and ISO/CD 15151/IDF 229).

### 2.6. Statistical analysis

All the experimental measurements were conducted at least in triplicate, and the data were expressed as mean ± standard deviation using Microsoft Excel 2010. Data were analysed by one-way analysis of variance (ANOVA) to examine differences amongst the ghee varieties and origins. The data were tested at a 95% confidence interval by comparison using a Tukey post-hoc test, followed by the least-significance difference tests using SPSS (Version 21.0). All *p*-values reported were for two-tailed significance tests. A *p*-value of $< 0.05$ was regarded as statistically significant.

## 3. Results and discussion

### 3.1. Moisture, fat and vitamin content of ghee

The moisture content of ghee samples was in the range of 0.25–0.63 g/100 g (Table 2). The mean value of fat content was 98.89 g/100 g, the highest in Kyrgyzstan cow ghee-1 (KGC1), and the lowest in Mongolian camel ghee (MNCa) ($p > 0.05$). Pena-Serna & Restrepo-Betancur (2020) also reported that ghee samples from Brazil comprised a fat fraction of 98.9% of the product, less than 0.9% of non-fat solids, and 0.3% of moisture.

The mean vitamin A content in ghee samples was 606 μg/100 g. The highest vitamin A content was 718 μg/100 g in Mongolian yak ghee (MNY), and the lowest was 409 μg/100 g in Kazakhstan cow ghee (KZC). The values obtained correspond to the literature value of vitamin A for clarified butter (ghee) at 480 μg/100 g (USDA, 2023). The mean values of the content of β-carotene were 392 μg/100 g. Levels in Mongolian yak ghee preserved for 4 years (MN4Y) and in Kyrgyzstan cow ghee-1 (KGC1) were the highest, while in Mongolian yak ghee (MNY) the concentration was the lowest. The β-carotene content of clarified butter (ghee) in Australian FCDB is 480 μg/100 g (AUSNUT, 2018). Thus, different animal sources (yak and cow) or different feeds, such as natural grass industrial feeds or different grazing altitudes, affect the carotenoid levels in ghee. The recommended daily intake (RDA) for vitamin A varies by age and gender, and for adults (19–50 years), it is 700 ÷ 900 μg retinol activity equivalent (RAE) (USDHHS, 2022). Thus, 20 g of ghee can satisfy 20% of the daily intake of preformed vitamin A and provitamin A carotenoids.

Vitamin E refers to a group of fat-soluble compounds with strong antioxidant properties. The mean content of vitamin E in all ghee samples was 1650 μg/100 g, with the highest vitamin E content of 2010 μg/100 g in Mongolian yak ghee (MNY) and the lowest 1090 μg/100 g in Indian buffalo ghee (INB). The average content of vitamin E in standard cow and buffalo ghee from India was reported as 3150 and 2795 μg/100 g (Kumar et al., 2010); for comparison, butter contains 2000 μg/100 g of vitamin E (USDA, 2023). The recommended daily allowance of vitamin E for adults is 15 mg (USDHHS, 2022); therefore, 100 g of ghee can provide 11% of daily vitamin E intake.

### 3.2. Determination of minerals content

In terms of minerals, the mean value of calcium was 5.48 mg/100 g

**Table 2**
Average value of contents of fat, calcium, potassium, phosphorus and vitamins of ghee samples.

| Code | Total fat | Water | Calcium | Potassium | Phosphorus | Vitamin A | β-Carotene | Vitamin E (tocopherol) |
|---|---|---|---|---|---|---|---|---|
| | g/100 g | g/100 g | mg/100 g | mg/100 g | mg/100 g | μg/100 g | mg/100 g | mg/100 g |
| INC | 99.9±0.3 | 0.38 ±0.04 | – | – | – | 662±8 | – | 1.76±0.01 |
| KGC1 | 99.4±0.1 | 0.25 ±0.02 | 6.20±0.10 | 5.19±0.04 | 20.22±0.03 | 643±2 | 0.46±0.05 | 1.48±0.04 |
| KGC2 | 98.6±0.5 | 0.23 ±0.05 | 5.80±0.03 | 5.22±0.05 | 23.37±0.04 | 685±7 | 0.40±0.04 | 1.62±0.03 |
| KZC | 99.1±0.2 | 0.56 ±0.02 | 5.50±0.10 | 4.81±0.01 | 21.09±0.02 | 409±6 | 0.34±0.01 | 1.52±0.03 |
| MN4C | 98.3±0.1 | 0.58 ±0.05 | 3.50±0.01 | 5.18±0.05 | 23.16±0.04 | 481±5 | 0.42±0.04 | 1.65±0.01 |
| TRC | 99.0±0.4 | 0.31 ±0.02 | 5.60±0.04 | 2.99±0.01 | 20.12±0.02 | 581±9 | 0.45±0.05 | 1.63±0.02 |
| INB | 99.9 | 0.43 ±0.08 | – | – | – | 484±5 | – | 1.09±0.07 |
| KZG | 98.9±0.1 | 0.46 ±0.07 | 5.20±0.03 | 4.63±0.02 | 22.09±0.04 | 512±4 | 0.39±0.04 | 1.72±0.02 |
| MNY | 99.2±0.2 | 0.63 ±0.01 | 4.20±0.10 | 3.91±0.02 | 23.01±0.03 | 718±12 | 0.32±0.01 | 2.01±0.01 |
| MNCa | 98.0±0.1 | 0.47 ±0.05 | 6.10±0.05 | 6.05±0.03 | 26.05±0.04 | 712±7 | 0.35±0.02 | 1.57±0.02 |
| MNHG | 98.9±0.1 | 0.53±0.05 | – | – | – | – | – | – |
| Mean ± SD | 99.0±0.4 | 0.44±0.13 | 5.10±0.76 | 4.38±0.96 | 19.96±3.90 | 589±87 | 0.38±0.03 | 1.61±0.14 |
| Minimum | 98.0 | 0.23 | 3.50 | 0.94 | 0.45 | 409 | 0.32 | 1.10 |
| Maximum | 99.9 | 0.63 | 6.20 | 6.05 | 26.05 | 718 | 0.45 | 2.01 |

SD: standard deviation; –: not analysed; INC: Indian cow ghee; KZC: Kazakhstan cow ghee; KGC: Kyrgyzstan cow ghee; MN4C: Mongolian cow ghee; TRC: Turkish cow ghee; INB: Indian buffalo ghee; KZG: Kazakhstan goat ghee; MNY: yak ghee; MNCa: Mongolian camel ghee; MNHG: mare ghee.

(SE = 0.34), showing no significant regional variations (Table 2). The highest calcium content was 7.4 mg/100 g in Mongolian yak ghee preserved for 4 years (MN4Y), followed by 6.2 mg/100 g in Kyrgyzstan cow ghee-1 (KGC1) and 6.1 mg/100 g in camel ghee (MNCa). The recommended daily allowance (RDA) for calcium is 1000 mg for adult women and men (USDHHS, 2022). Thus, 100 g of ghee provides a low percentage of the daily value of calcium (0.5%).

The mean value of potassium in ghee was 5.04 mg/100 g (SE = 0.27); the highest was in Mongolian camel ghee (MNCa) and the lowest was in Kazakhstan cow ghee (KZC). RDAs of potassium for healthy individuals are 3400 mg and 2600 mg for adult males and females (USDHHS, 2022), and ghee contains 0.2% RDA of potassium. The highest value in ghee was in Mongolian camel ghee (MNCa) (26.1 mg/100 g), and Kyrgyz cow ghee (KGC2) (23.4 mg/100 g), with a mean value of 23.6 mg/100 g (SE = 1.04). For comparison, 100 g of unsalted cow milk butter contain 19 mg of phosphorus, 19 mg of potassium, and 25 mg of calcium (USDA, 2023). The RDA for phosphorus is 1250 mg for adults and children aged 4 years and older (USDHHS, 2022), so ghee contributes to a healthy diet with 3% phosphorus.

### 3.3. Fatty acid composition

In this study, a total of 32 individual FAs, including 1 short-chain fatty acid (SCFA), 7 medium-chain fatty acids (MCFA), 22 long-chain fatty acids (LCFA), and 2 very-long-chain fatty acids (VLCFA), were analysed (Table 3).

#### 3.3.1. Short-chain fatty acid (SCFA)

Many researchers have noted the absence of SCFAs in ghee and have explained that volatilisation occurs during the heat clarification of butter (Sserunjogi et al., 1998; Kirazci & Javidipour, 2008). However, in this study, short-chain fatty acid (SCFA) (C4:0) was detected in all the ghee samples. The difference in butyric acid between animal species was statistically significant ($p < 0.05$). Buffalo ghee contained the highest concentration of butyric acid (3.77 g/100 mg), followed by cow ghee (3.10 g/100 g), yak ghee (2.98 g/100 g), and goat ghee (1.88 g/100 g). Kumar et al. (2022) also found that cow ghee had almost 10% less butyric acid than buffalo ghee. The lowest amount of butyric acid found in camel ghee is 0.36 g/100 g. Studies have identified that SCFAs have beneficial effects, including anti-inflammatory (Chen et al., 2021), anti-obesity, antineoplastic, neurological, hepatological, and cardiovascular protective effects (Prentice et al., 2019; Govindarajan et al., 2011). In addition, research shows that butyric acid supports the production of killer T cells in the gut and thus a strong immune system (Chang et al., 2014). According to Skonieczna-Żydecka et al. (2018), the three most common SCFAs: acetate (C:2), propionate (C:3), and butyrate (C:4) could ultimately be used as interventional substances to target microbiota-gut-brain interactions. Tan et al. (2014) point out two important signalling mechanisms of SCFA: inhibition of histone deacetylases and activation of G-protein-coupled receptors.

#### 3.3.2. Medium-chain fatty acids (MCFA)

The medium-chain fatty acids (MCFA) such as caproic acid (C6:0), caprylic acid (C8:0), and capric acid (C10:0) were found in all ghee samples. Capric acid (C10:0) was the highest in goat ghee 2.25 g/100 g. Other researchers (Kumar et al., 2023) discovered a high content of all MCFA in goat ghee, which is undoubtedly due to the dominance of these fatty acids in goat milk that are also named after goats (caprine). The difference in capric acid between ghee samples was statistically significant ($p < 0.05$). In contrast to Kumar et al. (2023), who reported that cattle had almost 10% less caproic acid than buffalo, in our research the caproic acid in buffalo and cow ghee from India was not statistically different. In addition, Kazakhstan goat ghee (KZG) and Mongolian mare ghee (MNHG) contain a small amount of undecanoic acid (C11:0) (0.07–0.11 g/100 g). Croteau et al. (2018) found that ketones from medium-chain triglyceride compensated for the brain glucose deficit in Alzheimer's disease in direct proportion to the level of plasma ketones achieved and showed caprylic (C8:0) and capric (C10:0) acids increased total brain energy metabolism by increasing ketone supply without affecting brain glucose utilisation. MCFAs also have a direct role in inhibiting cell signalling, beyond that of energy sources and lipid metabolism. The rapid metabolism and unique transport of MCFAs have provided additional clinical benefits compared to the other substrates, such as long-chain fatty acids (LCFAs), and interest in treating metabolic and neurological disorders. MCFA serves as an energy source and regulates glucose and lipid metabolism (Roopashree et al., 2021). Fats with higher MCFAs increase fat oxidation and energy disbursement. Therefore, recently, MCFAs have become an attractive candidate for anti-obesity functional food supplements. Their easily metabolised nature makes them helpful, especially for sportsmen and the elderly. These effects are also associated with β-hydroxybutyrate, known as a ketone body.

Similarly, the energy from MCFAs can be used to metabolise other fats and lose weight; therefore, they are well-recognised for their anti-obesity properties. Since medium-chain triglycerides are metabolised differently, they provide immediate energy and control obesity. Medium-chain triglyceride has a high satiety value, preventing overconsumption of food (Kinsella et al., 2017).

#### 3.3.3. Long-chain saturated fatty acids

Long-chain saturated fatty acids (LCFA) are associated with increased insulin resistance and have an inhibitory effect on the genes

**Table 3**
The fatty acids composition (g/100 g) of ghee.

| Fatty acids / Product code | INB | INC | KGC1 | KGC2 | KZC | MN4C | TRC | MNCa | MNHG | MNY | KZG |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Butyric acid (C4:0) | 3.78 ± 0.26 | 3.23 ± 0.07 | 3.10 ± 0.10 | 3.27 ± 0.43 | 3.13 ± 0.04 | 2.07 ± 0.92 | 2.76 ± 0.03 | 0.36 ± 0.01 | 2.02 ± 0.38 | 2.98 ± 0.07 | 1.88 ± 0.50 |
| Caproic acid (C6:0) | 1.33 ± 0.10 | 1.36 ± 0.06 | 1.59 ± 0.03 | 1.38 ± 0.16 | 1.52 ± 0.02 | 1.49 ± 0.07 | 1.28 ± 0.01 | 0.06 ± 0.00 | 1.40 ± 0.04 | 1.90 ± 0.16 | 1.67 ± 0.06 |
| Caprylic acid (C8:0) | 0.28 ± 0.03 | 0.34 ± 0.01 | 0.50 ± 0.01 | 0.38 ± 0.05 | 0.39 ± 0.01 | 0.38 ± 0.04 | 0.34 ± 0.00 | 0.03 ± 0.00 | 0.65 ± 0.01 | 0.43 ± 0.06 | 0.86 ± 0.07 |
| Capric acid (C10:0) | 0.38 ± 0.04 | 0.51 ± 0.01 | 0.08 ± 0.00 | 0.60 ± 0.08 | 0.63 ± 0.01 | 0.58 ± 0.05 | 0.56 ± 0.00 | 0.03 ± 0.01 | 1.64 ± 0.00 | 0.57 ± 0.08 | 2.25 ± 0.21 |
| Undecanoic acid (C11:0) | ND | ND | ND | ND | ND | ND | ND | ND | 0.07 ± 0.00 | ND | 0.11 ± 0.01 |
| Lauric acid (C12:0) | 1.81 ± 0.12 | 2.17 ± 0.00 | 2.25 ± 0.01 | 2.46 ± 0.26 | 3.18 ± 0.74 | 1.70 ± 0.01 | 2.35 ± 0.06 | 0.66 ± 0.02 | 3.64 ± 0.04 | 1.55 ± 0.02 | 3.71 ± 0.01 |
| Tridecanoic acid (C13:0) | 0.09 ± 0.01 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.09 ± 0.01 | 0.09 ± 0.02 | 0.23 ± 0.01 | 0.08 ± 0.00 | 0.06 ± 0.00 | 0.10 ± 0.00 | 0.07 ± 0.00 | 0.10 ± 0.00 |
| Myristic acid (C14:0) | 9.90 ± 0.40 | 9.39 ± 0.08 | 8.76 ± 0.06 | 8.59 ± 0.59 | 9.64 ± 0.68 | 7.91 ± 0.03 | 8.83 ± 0.17 | 8.61 ± 0.19 | 9.98 ± 0.09 | 7.46 ± 0.11 | 9.25 ± 0.02 |
| Pentadecanoic acid (C15:0) | 1.17 ± 0.01 | 1.05 ± 0.01 | 1.30 ± 0.01 | 1.16 ± 0.05 | 0.96 ± 0.04 | 1.86 ± 0.20 | 1.02 ± 0.01 | 1.21 ± 0.03 | 1.25 ± 0.01 | 1.10 ± 0.01 | 0.95 ± 0.02 |
| Palmitic acid (C16:0) | 33.86 ± 0.17 | 31.30 ± 0.36 | 23.71 ± 0.21 | 22.55 ± 0.57 | 27.78 ± 1.86 | 26.97 ± 2.77 | 26.55 ± 0.34 | 20.91 ± 0.39 | 27.60 ± 0.17 | 26.92 ± 0.47 | 26.89 ± 0.57 |
| Stearic acid (C18:0) | 9.65 ± 0.54 | 11.50 ± 0.01 | 12.14 ± 0.20 | 11.76 ± 0.10 | 10.17 ± 3.34 | 10.55 ± 0.19 | 10.76 ± 0.18 | 10.60 ± 0.17 | 7.87 ± 0.04 | 13.80 ± 0.16 | 7.97 ± 0.21 |
| Arachidic acid (C20:0) | 0.36 ± 0.04 | 0.37 ± 0.01 | 0.33 ± 0.01 | 0.26 ± 0.01 | 0.28 ± 0.03 | 0.52 ± 0.02 | 0.23 ± 0.01 | 0.52 ± 0.01 | 0.34 ± 0.01 | 0.39 ± 0.00 | 0.27 ± 0.01 |
| Behenic acid (C22:0) | 0.23 ± 0.01 | 0.27 ± 0.01 | 0.15 ± 0.02 | ND | 0.13 ± 0.00 | 0.34 ± 0.02 | ND | 0.29 ± 0.00 | 0.16 ± 0.00 | 0.20 ± 0.01 | ND |
| Tricosanoic acid (23:0) | 0.35 ± 0.06 | 0.25 ± 0.07 | 0.19 ± 0.00 | 0.21 ± 0.06 | 0.16 ± 0.01 | 0.32 ± 0.04 | 1.78 ± 0.31 | 1.03 ± 0.15 | 0.30 ± 0.01 | 0.29 ± 0.04 | ND |
| Lignoceric acid (24:0) | 0.30 ± 0.02 | 0.27 ± 0.01 | 0.20 ± 0.02 | 0.18 ± 0.02 | 0.18 ± 0.00 | 0.29 ± 0.01 | 0.19 ± 0.01 | 0.21 ± 0.01 | 0.16 ± 0.00 | 0.17 ± 0.01 | 0.08 ± 0.00 |
| **∑ SFA** | **63.45 ± 1.80** | **62.07 ± 0.71** | **54.39 ± 0.69** | **52.84 ± 2.40** | **58.21 ± 6.79** | **55.20 ± 4.58** | **56.71 ± 1.13** | **44.54 ± 0.98** | **57.14 ± 0.78** | **57.80 ± 1.20** | **55.95 ± 1.79** |
| Tetradecenoic acid (C14:1, *cis* 7) | 0.63 ± 0.03 | 0.85 ± 0.02 | 0.62 ± 0.00 | 0.61 ± 0.04 | 0.40 ± 0.05 | 0.58 ± 0.01 | 0.79 ± 0.02 | 0.49 ± 0.01 | 0.66 ± 0.01 | 0.41 ± 0.01 | 0.21 ± 0.02 |
| Pentadecenoic acid (C15:1, *cis* 10) | 0.37 ± 0.01 | 0.32 ± 0.00 | ND | ND | 0.32 ± 0.01 | ND | ND | 0.37 ± 0.01 | ND | ND | 0.32 ± 0.01 |
| Palmitoleic acid (C16:1, *cis* 9) | 2.16 ± 0.01 | 1.77 ± 0.01 | 1.39 ± 0.09 | 1.43 ± 0.04 | 1.16 ± 0.11 | 1.88 ± 0.20 | 1.50 ± 0.02 | 5.99 ± 0.03 | 1.57 ± 0.01 | 1.70 ± 0.05 | 0.83 ± 0.02 |
| Heptadecenoic (C17:1, *cis* 10) | 0.36 ± 0.00 | 0.34 ± 0.01 | 0.48 ± 0.00 | 0.40 ± 0.00 | 0.43 ± 0.18 | 0.69 ± 0.01 | 0.39 ± 0.00 | 0.77 ± 0.04 | 0.46 ± 0.00 | 0.38 ± 0.00 | 0.56 ± 0.01 |
| Oleic acid (C18:1, *cis* 9) | 20.11 ± 0.93 | 20.75 ± 0.16 | 24.20 ± 0.42 | 25.11 ± 0.21 | 18.51 ± 3.19 | 19.22 ± 0.16 | 23.87 ± 0.45 | 24.52 ± 0.08 | 15.66 ± 0.08 | 16.79 ± 0.42 | 21.14 ± 0.50 |
| Gadoleic acid (C20:1, *cis* 11) | 0.36 ± 0.04 | 0.31 ± 0.06 | ND | 0.35 ± 0.01 | 0.29 ± 0.00 | 0.47 ± 0.00 | 0.35 ± 0.01 | 0.42 ± 0.01 | 0.33 ± 0.01 | 0.31 ± 0.02 | 0.15 ± 0.00 |
| Erucic acid (22:1, *cis* 13) | 0.23 ± 0.08 | 0.42 ± 0.01 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Nervonic acid (C24:1, *cis* 15) | 0.12 ± 0.01 | 0.10 ± 0.01 | 0.09 ± 0.04 | ND | 0.10 ± 0.00 | 0.13 ± 0.00 | ND | ND | 2.48 ± 0.16 | ND | 0.09 ± 0.00 |
| **∑MUFA** | **24.32 ± 1.10** | **24.84 ± 0.29** | **26.78 ± 1.05** | **27.90 ± 0.30** | **21.19 ± 3.73** | **22.97 ± 0.39** | **26.89 ± 0.50** | **32.55 ± 0.18** | **21.14 ± 0.26** | **19.57 ± 0.49** | **23.28 ± 0.57** |
| Linoleic acid (C18:2, *cis* 9. *cis* 12) | 1.37 ± 0.05 | 1.57 ± 0.00 | 2.30 ± 0.05 | 2.25 ± 0.06 | 2.24 ± 0.98 | 1.26 ± 0.00 | 2.90 ± 0.04 | 2.48 ± 0.01 | 1.64 ± 0.01 | 1.43 ± 0.01 | 2.94 ± 0.01 |
| Linoleic acid (C18:2, *trans* 9. *trans* 12) | ND | ND | ND | ND | 0.23 ± 0.09 | ND | ND | ND | ND | ND | ND |
| γ-Linolenic acid (C18:3, *cis* 6,9,12) | ND | ND | ND | ND | 0.14 ± 0.06 | ND | ND | 0.23 ± 0.01 | ND | ND | 0.11 ± 0.01 |
| α-Linolenic acid (C18:3, *cis* 9,12,15) | 0.15 ± 0.01 | 0.17 ± 0.00 | 1.54 ± 0.01 | 0.86 ± 0.01 | 0.53 ± 0.05 | 0.64 ± 0.01 | 0.42 ± 0.00 | 0.67 ± 0.00 | 0.64 ± 0.00 | ND | 0.58 ± 0.01 |
| Eicosadienoic acid (C20:2, *cis* 11.14) | 0.98 ± 0.01 | 0.99 ± 0.04 | 0.84 ± 0.13 | 0.94 ± 0.19 | 1.03 ± 0.03 | 0.87 ± 0.06 | ND | 1.29 ± 0.04 | 1.35 ± 0.01 | 0.80 ± 0.14 | 0.97 ± 0.11 |
| Eicosatetraenoic acid (C20:4, *cis* 5.8.11.14) | 0.19 ± 0.01 | 0.23 ± 0.04 | 0.17 ± 0.01 | 0.16 ± 0.02 | 0.18 ± 0.05 | 0.19 ± 0.02 | ND | 0.16 ± 0.00 | ND | 0.15 ± 0.00 | 0.23 ± 0.01 |
| Eicosapentaeonic (C20:5, *cis* 5.8.11. 14.17) | ND | ND | ND | ND | 0.16 ± 0.00 | 0.26 ± 0.00 | ND | 0.22 ± 0.01 | ND | ND | ND |
| Docosadienoic acid (C22:2, *cis* 13.*cis* 16) | 2.35 ± 0.05 | ND | ND | ND | ND | ND | ND | 2.28 ± 0.04 | 2.83 ± 0.07 | 2.25 ± 0.35 | 0.09 ± 0.00 |
| ∑PUFA | **5.03 ± 0.13** | **2.96 ± 0.08** | **4.84 ± 0.19** | **4.21 ± 0.28** | **4.51 ± 1.34** | **3.23 ± 0.09** | **3.33 ± 0.05** | **7.31 ± 0.09** | **6.46 ± 0.09** | **4.63 ± 0.50** | **4.91 ± 0.15** |
| Unknown | 7.54 | 10.25 | 12.33 | 14.83 | 16.08 | 16.66 | 12.78 | 15.70 | 13.02 | 16.62 | 10.87 |
| ∑of FA | **100.33** | **100.11** | **98.34** | **99.77** | **99.99** | **98.06** | **99.70** | **100.09** | **97.75** | **98.61** | **95.00** |

SFA – saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acid, FA – fatty acids, ND – not detected. INC – Indian cow ghee; KZC – Kazakhstan cow ghee; KGC – Kyrgyzstan cow ghee; MN4C – Mongolian cow ghee; TRC - Turkish cow ghee; INB – Indian buffalo ghee; KZG – Kazakhstan goat ghee; MNY – yak ghee; MNCa- Mongolian camel ghee; MHHG – Mongolian mare ghee.

associated with inflammation (Croteau et al., 2018). Major LCFAs of all ghee samples were myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). Investigated cow ghee samples have myristic acid (C14:0) at an average of 8.83 g/100 g, palmitic acid (C16:0) at 25.8 g/100 g, and stearic acid (C18:0) at 10.9 g/100 g. In cow ghee from Iran's western provinces, the main fatty acids were palmitic (C16:0) and oleic (C18:1), which made up 29.95–33.67% and 20.46–28.46% of the ghee, respectively (Erfani et al., 2020). Sawaya et al. (1984) reported a relatively high degree of saturation (63.6–74.1%) with C16:0 (27.6–30.5%) and C18:1 (19.6–30.1%) as the predominant saturated and unsaturated fatty acids, respectively, for ghee and butter from goat and sheep milk.

#### 3.3.4. Long-chain unsaturated fatty acids

The most abundant long-chain unsaturated fatty acid (LCUFA) was oleic acid (C18:1, *cis* 9), which was present in all ghee samples at a concentration ranging from 15.7 mg/100 g (MNHG**)** to 25.1 g/100 g of ghee (KGC2). According to Kumar et al. (2022), in cow, goat, and buffalo ghee, oleic acid (C18:1 *cis* 9) concentrations ranged from 31% to 35%, 26%, and 29% of the total fatty acid content, respectively. The next abundant LCUFA in ghee samples was α-linoleic acid (C18:2, *cis* 9.12), ranging from 1.37 (INB) to 2.94 mg/100 g (KZG). Ghee also contains essential fatty acids α-linolenic acid (C18:3, *cis* 9,12,15) and eicosapentaenoic (C20:5, *cis* 5,8,11,14,17). These well-known ω3 essential fatty acids prevent cardiovascular disease and inflammation (Simopoulos, 2008; Croteau et al., 2018). Since the recommended daily amount of α-linolenic acid for women, pregnant women and teens, and lactating women is 1.1 g, 1.4 g, and 1.3 g, respectively (USDHHS, 2022), ghee can be considered a source of α-linolenic acid. Furthermore, a small amount of γ-linolenic acid (C18:3 *cis* 6,9,12) was identified in Kazakhstan cow and goat ghee, as well as in Mongolian camel ghee. Docosadienoic acid (C22:2, *cis* 13,16), which is well-known as an omega-6 fatty acid, was found in all samples in the range of 2.7–2.83 g/100 g, except cow ghee, and the highest amount was found in mare ghee. Mongolian goat ghee (MNG) contains the highest value of 2.48 g/100 g of nervonic acid (C24:1, *cis* 15), which is component of muscles and the nervous system. Lack of LCUFAs during cerebral development can result in serious illnesses, including schizophrenia and attention deficit hyperactivity disorder (Janssen and Kiliaan, 2014).

### 3.4. The comparison of different ranges according to fatty acid profile

The differences in the fatty acid content of cow ghee samples from six countries are shown in Fig. 1. Statistically significant differences were observed among the cow ghee samples ($p < 0.05$). Indian cow ghee stands out for its content of gadoleic acid (C21:1). The cow ghee produced in Kazakhstan contains a small amount (0.23 mg/100 g) of *trans*-linoleic acid (C18:2, *trans* 9,12). Ghee samples with *trans*-C18:1 and *trans*-C18:2 fatty acids (more than 2% of total fatty acid content) were thought to be mixed with hydrogenated oils (Kirazci & Javidipour, 2008). Cow ghee stored for 4 years (MN4C) contained the highest amounts of pentadecanoic acid (C15:0), arachidic acid (C20:0), eicosapentaenoic acid (C20:5, *cis* 5,8,11,14,17), and the lowest amount of lauric acid (C12:0). These alterations in the studied fatty acids may be due to various variables, such as temperature, type of packaging, and radiation exposure (sunlight). The correlation between storage conditions and fatty acid concentration in ghee must be investigated in further research.

The comparison of the fatty acid profiles of Mongolian ghee samples from mare, yak, camel, and cow milk is shown in Fig. 2. The fatty acid spectrum of yak ghee (MNY) was not significantly different from cow ghee; the significant difference was only in the amount of stearic acid

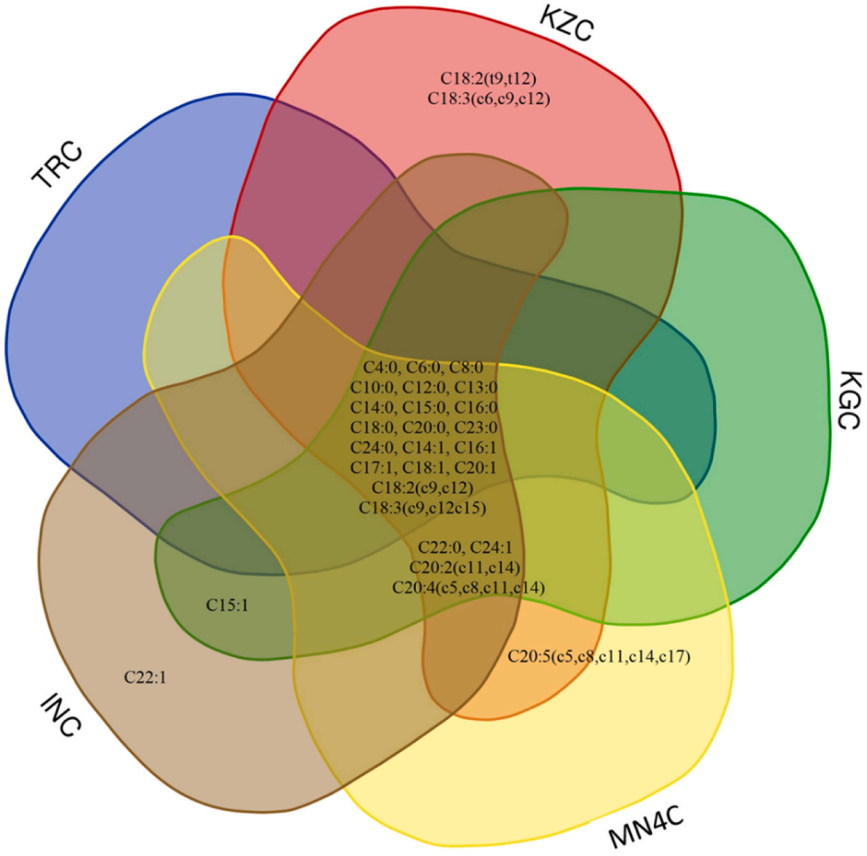


**Fig. 1.** Venn diagram of the differences in fatty acids of cow ghee samples from 7 countries: INC - *Indian cow ghee*. KZC - *Kazakhstan cow ghee*; KGC - *Kyrgyzstan cow ghee*; MN4C – *Mongolian cow ghee*, TRC - *Turkish cow ghee*.

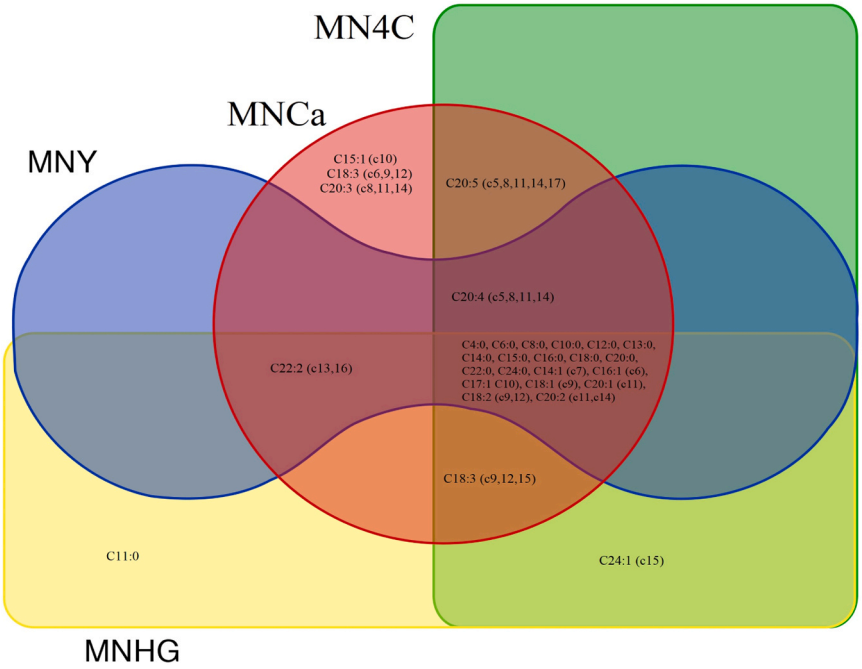


**Fig. 2.** Venn diagram of the differences in fatty acids of Mongolian ghee samples: MNHG- *mare ghee*, MNY - *yak ghee,* MNCA - *camel ghee,* MN4C - *cow ghee*.

(C18:0). Sserunjogi et al. (1998) also found that ghee from cow milk and yak milk were similar in fatty acid content. In camel ghee (MNCa), the lowest amount of saturated fatty acid (42.5 g/100 g) and the highest amount of mono- and poly-unsaturated fatty acids (32.5 and 7.28 g/100 g, respectively). Palmitoleic acid (C16:1, *cis* 9) and linoleic acid (C18:2, *cis* 9,12) were in camel ghee the highest (5.99 g/100 g and 2.48 g/100 g, respectively). Buffalo and cow ghee samples from India stood out due to their content of erucic acid (22:1, *cis* 13), and in cow ghee, their content was highest (Fig. 3). Among the ghee samples, Indian buffalo ghee (INB) had the highest concentration of palmitic acid (C16:0). These finding was in line with Upadhyay et al. (2018) and Kumar et al. (2022).

The composition of ghee varies slightly between regions and is more pronounced between animal species. Each ghee from different animal sources has its peculiarity in fatty acid composition. Camel and mare ghee have high amounts of LCUFA, especially docosadienoic acid (C22:2, *cis* 13,16). Goat ghee has a high content of MCFA, and cow ghee can supply the daily recommended value of essential α-linolenic acid. Therefore, all ghee samples can be regarded as healthy foods in an adequate amount (10–15 g) and are recommended for use in daily nutrition.

## 4. Conclusion

Ghee can be valuable in nutrition, especially for its essential fatty acids and fat-soluble vitamins. Thus, 100 g of ghee correspond to 100%

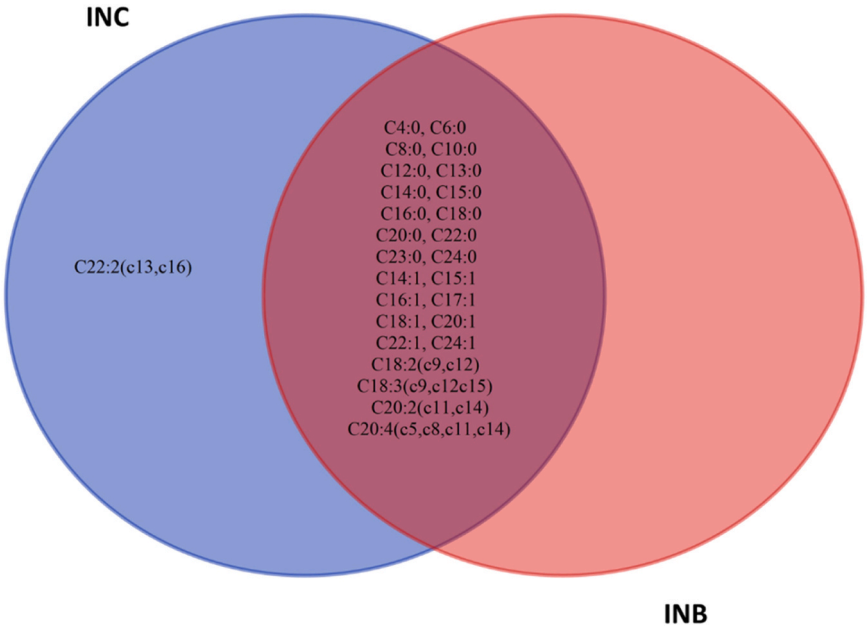


**Fig. 3.** Venn diagram of the differences in fatty acids of *Indian cow* (INC) and *buffalo (INB*) ghee samples.

of the recommended daily value of vitamin A and 11% of vitamin E. In terms of minerals like calcium, ghee is not a sufficient source, despite being a dairy product. The clarifying process removes most of the minerals. According to fatty acid analysis, buffalo ghee has the highest concentration of butyric acid, ahead of cow, yak, mare, goat, and camel ghee. This short-chain fatty acid may be responsible for the positive effects on intestinal inflammation. Among monounsaturated fatty acids, the most abundant fatty acid was oleic acid (C18:1, *cis* 9) in all samples, while Kyrgyz cow ghee (KGC2) contained the highest amount. Additionally, cow ghee (KGC2) contains the highest amount of α-linolenic acid, one of the ω3 fatty acids. Mare ghee and camel ghee contain high amounts of ω6 fatty acid docosadienoic acid and are the most valuable in terms of LCPUFA content; however, their production is most limited.

## CRediT authorship contribution statement

**Enkhtuya Vankhuu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Begzhan Kalemshariv:** Visualization, Resources. **Narangerel Choijilsuren:** Methodology, Investigation. **Janyl Iskakova:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Anne Hellwig:** Writing – review & editing, Methodology, Investigation. **Jamila Smanalieva:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Nomin-Erdene Ulambayar:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## 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.

## Data Availability

Data will be made available on request.

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