Study on physicochemical properties, fatty acids, texture, antioxidant and antibacterial activities of ghee from different regions

Y. Tian,1,2 B. Ding,2 Z. R. Ma,1 J. T. Yang,2 G. T. Ding,1 and H. N. Liu1,2*
1China-Malaysia National Joint Laboratory, Biomedical Research Center, Northwest Minzu University, Gansu Lanzhou 730030, China
2College of Life Science and Engineering, Northwest Minzu University, Gansu Lanzhou 730030, China

ABSTRACT

Due to the lack of basic information on Chinese ghee and the increasing demand of consumers for natural oils, this study aims to explore and distinguish the quality characteristics of ghee in different regions of China. Ghee samples from 16 regions of Qinghai Province, Gansu Province, Xinjiang Uyghur Autonomous Region, and Tibet Autonomous Region were selected and their physicochemical properties, fatty acids, texture, antioxidant and antibacterial activities were determined. The results showed that: (1) The physicochemical properties of ghee were different from different regions, but the freshness and fat content are generally high. The results of iodine value and saponification value suggest that the fatty acid composition is good; (2) The unsaturated fatty acid/saturated fatty acid content of ghee in Tibet and Xinjiang ranges from 63.05% to 79.13%, which is better than that in other regions; (3) Gansu Diebu ghee has the highest hardness (40.69 N); (4) Ghee from different regions has good antioxidant activity, DPPH free radical scavenging activity is 30.45% to 58.06%, ABTS free radical scavenging activity is 41.14% to 65.53%, and has varying degrees of inhibition on gram-positive bacteria. In addition, yak ghee, cattle-yak ghee and cow ghee have better fatty acid composition and antibacterial ability than scalper ghee. The results of this study distinguish the differences in the quality characteristics of yak ghee in different geographical regions. Therefore, it can provide a theoretical basis for the origin tracing and quality-oriented improvement of yak ghee.

Key words: yak ghee, different regions, quality characteristics, fatty acid, antioxidant activity

INTRODUCTION

The yak is a key species in the alpine pasture ecosystem of the Qinghai-Tibet Plateau in China (Dan Xue et al., 2016). Yak milk has been demonstrated to have higher nutritional value than regular cow’s milk, including rich fat (5.29%–8.73%), protein (3.45%–4.27%), and some mineral content (Kaur et al., 2017; Singh et al., 2022). Most yak milk is used to make yak ghee, an important energy source against cold and altitude (Liu et al., 2011).

Yak ghee is a milk fat product, which is made from yak milk as raw material and extracted milk fat by traditional processes (Neupaney et al., 2003). Its fat content can reach 87.7% (Agyare and Liang, 2021). As a high-fat product, its saturated fatty acid content accounts for about two-thirds of the total fatty acids, especially myristic acid, palmitic acid, and stearic acid content is higher, which makes consumers reduce the consumption of yak ghee and other foods rich in SFA for a long time. But recent studies have suggested that eating a moderate amount of foods rich in SFA, such as yak ghee, is very necessary for our health (Agyare and Liang, 2021). In addition, yak ghee provides some functional fatty acids, found in milk fats, and yak ghee is a major dietary source of CLA, which has been linked to health benefits such as anticancer, antiatherosclerosis, and inhibition of osteoporosis (Moatsou and Sakkas, 2019). Jing et al. also reported that yak ghee is rich in fatty acids and healthy, containing fatty acids such as oleic acid, linoleic acid, arachidonic acid, and other fatty acids that affect health (Jing et al., 2019). The excellent fatty acid composition implies that ghee has a healthy eating quality, which also makes ghee have better texture characteristics (Karaku et al., 2022).

According to local herdies, yak ghee can be stored for a long time without pasteurization in barrels or sticking to the walls of houses and maintains the flavor at an acceptable level (Neupaney et al., 2003). This is not only due to the low temperature and low oxygen content in the plateau, but also the fat-soluble vitamins such as vitamin A and vitamin E present in milk fat can act as natural antioxidant factors in conjunction with the oxidase system to protect ghee from oxidation (Guo et al., 2014; Smesny et al., 2015). In recent years, some studies have also been conducted to enhance the antioxidant
capacity of ghee by adding natural substances, thereby increasing its cargo life. For example, Kapadiya et al. evaluated the effect of 14 herbs such as betel leaves and curry leaves as antioxidants on the antioxidant capacity of ghee separately (Kapadiya and Aparnathi, 2018). Patel and Balakrishnan (2021) were evaluating the antioxidant potential of nonconventional plant sources such as tamarind seeds and catechu seeds in ghee (Patel and Balakrishnan, 2021). Agyare et al. (2022) enriched Goji Berry carotenoids by ultrasound-assisted extraction and high shear dispersion techniques and added to yak ghee and found that the antioxidant properties of experimental group ghee were enhanced compared with control group ghee during microwave heating and storage (Agyare et al., 2022). In addition, yak ghee is also considered to have special properties that distinguish it from other mammalian milk fat, and nomads use it to protect the skin from insect bites and the adhesion of pathogenic bacteria (Guo et al., 2014). Ghee is also used in Indian medicine to heal wounds (Singh et al., 2022). These properties imply that yak ghee has good application potential in the cosmetics and biomedical industries.

Therefore, this study aims to explore and distinguish the quality characteristics of ghee in different regions of China, and the study selects ghee from 4 provinces, autonomous regions, and 16 regions as the research objects, and determines the detailed data of ghee’s physicochemical properties, fatty acids, texture, antioxidant and bacteriostatic activities in different regions, and compares the composition and characteristics of different types of ghee. The correlation between geography and ghee quality can be derived to provide a theoretical basis for targeted improvement of ghee, and this study provides important information for dairy processors and marketers.

**MATERIALS AND METHODS**

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

**Material**

A total of 16 commercially available ghee samples were obtained from Qinghai Province, Gansu Province, Xinjiang Uygur Autonomous Region, and Tibet Autonomous Region. These included 12 yak ghee samples, 2 cow ghee samples (Ili and Urumqi), 1 cattle-yak ghee sample (Shigatse), and 1 scalper ghee sample (Lhasa). Cattle in all regions are traditionally managed. Except for supplementary feeding in winter, the rest of the time is in natural pasture grazing. All ghee samples were returned to the laboratory in sealed packages and stored at −20°C in a refrigerator for testing. Ghee samples were numbered and named according to their origin. Information such as their altitude is shown in Table 1.

**Table 1. Sources and types of ghee in different regions**

<table>
<thead>
<tr>
<th>Province and autonomous region</th>
<th>Region</th>
<th>Type</th>
<th>Serial number</th>
<th>Altitude (m)</th>
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<tr>
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<td>QG</td>
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<td>Yak ghee</td>
<td>QX</td>
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<td>Yak ghee</td>
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<td>Luqu</td>
<td>Yak ghee</td>
<td>GL</td>
<td>3,500</td>
</tr>
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<td>Cow ghee</td>
<td>XY</td>
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<td>Yak ghee</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>Lhasa</td>
<td>Scalper ghee</td>
<td>ZL</td>
<td>3,650</td>
</tr>
</tbody>
</table>
**Physicochemical Properties Assay**

The moisture (Method Ca 2D–25), iodine (Method Cd 1d–92), saponification (Method C3–25), and acidity (Method Cd 3d–63) of ghee were determined according to AOCS standards, the protein was determined by the Kjeldah method (AOCS, 2003), and ether extract by Soxhlet extraction with diethyl ether for fat content (AOAC International, 2006).

**Fatty Acids Assay**

Ghee fatty acids were determined by forming FAME to determine fatty acid composition according to the method of Karakus et al. (2022) and Bai et al. (2013). Samples (0.3–0.5 g) were soaked with 1.5 mL of 0.5 N methanol NaOH at 115°C for 7 min. After cooling, 2 mL of boron trifluoride was added and heated at the same temperature for another 5 min. After the tubes cooled, 2 mL of isooctane and 3 mL of saturated NaCl solution were added and mixed for 30 s. The samples were allowed to separate the organic phase, then they were entered into (Agilent) GC analysis. The injection temperature and detector temperature were 250°C, and the oven temperature was held at 45°C for 3 min. Subsequently, the temperature was increased up to 175°C at 13°C/min and held at that temperature for 27 min. It was then increased up to 215°C at 4°C/min and held for 35 min. The carrier gas was helium (purity ≥ 99.99%), and all peaks were obtained by mixing with a standard FAME mixture (Supelco 37component FAME mix) were compared, and the relative content of each component, expressed as a percentage of the total fatty acids, was determined by area normalization. Each sample was injected 3 times by a GC autosampler (Bai et al., 2013; Karakus et al., 2022).

**Texture Properties Assay**

The texture characterization of ghee was based on the approach of Peng et al. with minor modifications to determine the hardness, consistency, and viscosity of ghee. A TA. XT-2 texture analyzer was used at 20°C with a conical probe, which was inserted into the sample at a depth of 25 mm and a speed of 2.0 mm/s, and then the force applied to the probe was automatically recorded. The assay was performed 3 separate times (Peng et al., 2018).

**Antioxidant Activity Assay**

*DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay.* With a few minor adjustments, the DPPH technique was developed from the test of Gaber et al. (2021). The antioxidant activity of ghee samples was assessed using DPPH as an inhibitor. Mix the ghee sample with DPPH's methanol solution in a 1:8 (v:v) ratio, then add deionized water to make the mixture of 10.0 mL and incubate for 30 min at room temperature in the dark. As a blank control, deionized water was used in place of the sample methanol extract to estimate the absorbance value at a wavelength of 517 nm. The inhibition rate of DPPH radicals, which is a manifestation of DPPH’s scavenging action, can be computed as follows:

\[
\text{radical scavenging rate (\%)} = \frac{A_1 - A_2}{A_1} \times 100,
\]

where:
- \(A_1\) is the absorbance of the DPPH solution by itself;
- \(A_2\) is the sample's absorbance after 30 min of testing.

*ABTS (2,2′-Azino-bis [3-Ethylbenzothiazoline-6-Sulfonic Acid]) Radical Scavenging Assay.* With a few minor adjustments, the ABTS technique was developed from the test of Gaber et al. (2021). The ABTS free radical stock solution was created by mixing 7 mmol/L of ABTS solution and 2.45 mmol/L of potassium per sulfate solution in a ratio of 1:2 by volume and letting it stand for 16 h in a darkened area. Before use, the stock solution was diluted with anhydrous ethanol to make it absorb between 0.70 ± 0.02 nm. 2.4 mL of diluted ABTS solution was added to 0.6 mL of ghee methanol extract and thoroughly blended. Utilizing deionized water as a blank, measure the absorbance at 734 nm after the reaction has been running for 6 min in the dark at room temperature. The results were expressed as ABTS cationic radical scavenging rate with the following equation:

\[
\text{ABTS cationic radical scavenging rate (\%)} = \frac{A_1 - A_0}{A_0} \times 100,
\]

where:
- \(A_0\) is ABTS and the absorbance of the sample solution;
- \(A_1\) is ABTS and the absorbance of methanol solution.

*Ferric-Reducing Power Assay.* With a few minor adjustments, the ferric-reducing power (FRAP) technique was developed from the test of Benzie et al.
(Benzie and Strain, 1996). In a test tube, 0.3 mol/L acetic acid buffer, 10 mmol/L TPTZ reagent, and 20 mmol/L FeCl₃ solution were combined in a 10:1:1 (vol: vol:vol) ratio to create the FRAP reagent. The 3.0 mL of FRAP reagent and 0.5 mL of ghee methanol extract should be combined, and the mixture for 10 min at 37°C. The absorbance at 593 nm was measured, and deionized water was used as a blank control. To compute the FeSO₄ concentration (mmol/L), the absorbance was substituted into the FeSO₄ standard curve. The FRAP value was then expressed as FeSO₄ concentration. Calibration curves of ferrous sulfate solutions with various doses in the range of 200 to 1,000 μmol/L were created.

**Reducing Power Assay.** With a few minor adjustments, the reducing power technique was developed from the test of Abedin et al. (2022). Put 0.2 mL of ghee methanol extract into a test tube, add 1.0 mL of 0.2 mol/L phosphate buffer at pH = 6.6 and 1.0 mL of potassium ferricyanide solution with 1% mass fraction, mix well, and react in a water bath at 50°C for 30 min. After cooling, 1.0 mL of trichloroacetic acid solution with 10% volume fraction was added, mixed, and centrifuged at 1,006.2 × g for 10 min at 25°C. Add 2.0 mL of supernatant to 0.4 mL of 1% ferric chloride solution, mix well, and stand for 10 min, use deionized water as blank, and measure absorbance at a wavelength of 700 nm.

**Antibacterial Activity Assay.** The inhibition activity was determined based on the method of Chen et al. (2021) with some modifications. *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC29213) were selected as the representatives of gram-negative and gram-positive bacteria, respectively. The 2 strains were inoculated on solid Luria-Bertani (LB) medium and incubated under a shaker at 37°C. Take 5 mL of the culture solution into a new 100 mL of LB liquid medium and place it in a shaker at 37°C for 24 h. Typical colonies were picked into 100 mL of liquid LB medium and incubated under a shaker at 37°C. Take 5 mL of the culture solution into a new 100 mL of LB liquid medium and place it in a shaker at 37°C for 2 h. The concentration was adjusted to C = 1 × 10⁶ cfu/mL by dilution in PBS phosphate buffer and centrifugation (M = 0.5 in a Macrobid tube) and the absorbance was measured at 600 nm (around 0.1 is appropriate). The medium was spread evenly on the surface of LB solid medium using a spreading rod, dried at room temperature for 3 min, punched evenly using a hole punch, and put into the ghee sample. The medium was stored in a refrigerator at 4°C for 2 h and then incubated in an incubator at 37°C for 12 h. The diameter of the inhibition circle (mm) was measured.

**Statistical Analysis**

Data were analyzed using one-way ANOVA. Analysis of variance was performed on all variables using SPSS 19.0 (IBM Corporation, Armonk, NY). The Tukey’s honestly significant difference test was used to compare the differences between means (P < 0.05).

### RESULTS AND DISCUSSION

**Physicochemical Properties of Ghee in Different Regions**

The physicochemical analysis results of ghee samples in different regions are shown in Table 2. The moisture content is related to the growth of spoilage microorganisms and can affect the stability of the product (Mukisa and Kiwanuka, 2018). In this study, the moisture content of ghee was in the range of 12.14% to 16.19%, and there was no significant difference between most regions (P > 0.05). Among them, the results of the highest moisture content in the Gansu Hezuo 1 (GH1) region suggest that ghee in the region is more prone to quality degradation during storage (Jing et al., 2019). Among other types of ghee, Xinjiang cow ghee has significantly higher moisture than other types of ghee (P < 0.05).

Fat is a key indicator for evaluating the nutritional value of fats (Silva et al., 2019). The range of ghee fat content in different regions is 82.31% to 86.51%, but all regions meet the requirement of at least 80% fat content in butter reported by Codex Alimentarius in 2018, which is also similar to the result of Silva et al. (2019) reporting that the fat content of butter produced in the Azores region ranges from 82.7% to 87.9%. The fat content of ghee in Qinghai and Tibet is higher than in Gansu and Xinjiang, and Cui et al. (2016) reported that the fat content of milk fat was positively correlated with altitude. In this study, except for Qinghai Xunhua (QX), the rest of Tibet and Qinghai are located in ultra high-altitude areas (3,500–5,500 m), most of Gansu are in the high-altitude area (1,500–3,500 m), while Xinjiang is in the low-altitude area (800–1,500 m), and it is cow ghee, and the raw milk contains less fat than yak milk (Singh et al., 2022). Among the different types of ghee, cattle-yak ghee has the highest fat content, followed by yak ghee and cow ghee, and scalper ghee has the lowest.

The trend of protein content in different regions is opposite to fat content, because protein is a by-product of the ghee production process, and its content will affect the purity of ghee, and the protein content of butter is about 1.98% (Agyare and Liang, 2021). The protein content of ghee in this study was between 0.53% to 1.65%, indicating that the buttermilk was removed cleaner and the purity of oil was higher during the processing of ghee in this study (Oliver-Simancas et al., 2021). In addition, the protein of other types of ghee was significantly different (P < 0.05), among regions.
which scalper ghee had the smallest protein content (0.85%).

Table 2 also reports the iodine value of ghee. The iodine value can be used to characterize the degree of unsaturation of oils and fats, the higher the content of UFA in the oil, the greater the iodine value (Medeiros de Azevedo et al., 2020). In this study, the iodine value of ghee ranged from 33.57 to 37.91 g I/100 g, which was similar to the result that Bhatia et al. (2019) found that the iodine value of ghee was 35.38 ± 0.16. Among different regions, ghee iodine values were higher in Xinjiang and Tibet overall, and higher in Qinghai and Gansu, suggesting that these regions may have richer levels of UFA. In addition, among the different varieties of ghee, yak ghee and cow ghee had higher iodine value, followed by cattle-yak ghee, and the lowest in scalper ghee.

The acid value is a key indicator of the free fatty acid content produced by the hydrolysis of triglycerides in ghee and indicates the degree of rancidity of the oil (Agyare et al., 2022). In this study, the acid price range of ghee in different regions was 1.05 to 2.57 mg/g, which was higher than that of ghee in the Qinghai-Tibet Plateau region (1.22 mg/g) reported by Jing et al. (2019). However, except for Gansu Hezuo 1 (GH1), the rest of the ghee was less than 2.50 mg/g, indicating that the freshness of the sample as a whole was high, and no obvious rancidity occurred.

Table 2 also shows the saponification values of ghee in different regions, which to some extent can indicate the molecular weight of ghee. In this study, the saponification value of ghee reached up to 230.13 mg KOH/g, which was similar to saponification value reported in the literature for butter (231 mg KOH/g; Méndez-Cid et al., 2017). All yak ghee in Gansu and Tibet showed higher saponification values than in Qinghai, which was related to differences in fatty acid distribution (Singh et al., 2022). Among other types of ghee, cow ghee had the highest saponification value, while cattle-yak ghee and scalper ghee were smaller, but the difference was not significant (P > 0.05).

Analysis of Ghee Fatty Acid Content in Different Regions

The composition of fatty acids in milk directly affects the physicochemical properties, quality, and human health of dairy products (Erkaya et al., 2015). Table 3 shows the fatty acid composition of ghee in different regions, and a total of 27 fatty acids were identified in the samples, but there were different degrees of difference between some fatty acids (P < 0.05).

In this study, the types of short-chain and medium-chain fatty acids were consistent between different regions.
<table>
<thead>
<tr>
<th>Item</th>
<th>QQ</th>
<th>QX</th>
<th>QH</th>
<th>GH1</th>
<th>GH2</th>
<th>GX</th>
<th>GM</th>
<th>GD</th>
<th>GL</th>
<th>XY</th>
<th>XU</th>
<th>ZS</th>
<th>ZN</th>
<th>ZR1</th>
<th>ZR2</th>
<th>ZL</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.23±</td>
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<td>1.38±</td>
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<td>—</td>
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<td>—</td>
<td>—</td>
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<td>0.10±</td>
<td>0.10±</td>
<td>0.10±</td>
<td>0.10±</td>
<td>0.10±</td>
<td>0.10±</td>
<td>0.10±</td>
</tr>
</tbody>
</table>

Table 3. The fatty acid composition of ghee 1 in different regions 2.

1. Values are expressed as percentage of total fatty acids.

2. The fatty acid composition of ghee was significantly different (p<0.05) between regions.

Continued
The fatty acid composition of ghee in different regions

<table>
<thead>
<tr>
<th>Item</th>
<th>QG</th>
<th>QX</th>
<th>QH</th>
<th>GH1</th>
<th>GH2</th>
<th>GX</th>
<th>GM</th>
<th>GD</th>
<th>GL</th>
<th>XY</th>
<th>XU</th>
<th>ZS</th>
<th>ZN</th>
<th>ZR1</th>
<th>ZR2</th>
<th>ZL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA (%)</td>
<td>67.38 ±</td>
<td>67.60 ±</td>
<td>63.85 ±</td>
<td>61.85 ±</td>
<td>78.24 ±</td>
<td>72.97 ±</td>
<td>79.13 ±</td>
<td>0.15</td>
<td>71.30 ±</td>
<td>0.56</td>
<td>78.35 ±</td>
<td>71.64 ±</td>
<td>63.05 ±</td>
<td>0.33</td>
<td>0.26</td>
<td>0.19</td>
</tr>
<tr>
<td>UFA/SFA</td>
<td>52.60 ±</td>
<td>0.26</td>
<td>0.00</td>
<td>2.60</td>
<td>1.78</td>
<td>3.09</td>
<td>1.26</td>
<td>1.32</td>
<td>0.02</td>
<td>1.80</td>
<td>1.25</td>
<td>1.29</td>
<td>1.29</td>
<td>1.19</td>
<td>1.37</td>
<td>0.00</td>
</tr>
<tr>
<td>DI14</td>
<td>0.06 ±</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.19</td>
<td>0.12</td>
<td>0.10</td>
<td>0.10</td>
<td>0.21</td>
<td>0.20</td>
<td>1.13</td>
<td>0.19</td>
<td>0.15</td>
<td>0.05</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>DI16</td>
<td>0.11 ±</td>
<td>0.16</td>
<td>0.20</td>
<td>0.15</td>
<td>0.17</td>
<td>0.10</td>
<td>0.11</td>
<td>0.10</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
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<td>0.00</td>
</tr>
<tr>
<td>DI18</td>
<td>0.53 ±</td>
<td>0.48</td>
<td>0.54</td>
<td>0.52</td>
<td>0.63</td>
<td>0.50</td>
<td>0.62</td>
<td>0.63</td>
<td>0.63</td>
<td>0.52</td>
<td>0.50</td>
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<td>0.51</td>
<td>0.51</td>
<td>0.62</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*There is a significant difference between values within different superscript alphabetical rows (*P* < 0.05). Column headings are ghee serial numbers, as shown in Table 1. Atherogenic index (C12 + 4 × C14 + C16)/UFA; DI = desaturation index; DI-14, C14:1/(C14:0 + C14:1); DI-16, C16:1/(C16:0 + C16:1); DI-18, C18:1n9c/(C18:0 + C18:1n9c). Values are the mean ± SD of 3 replicates. Dash = not detected.

In this study, the relative content of total SFA in ghee in different regions averaged 59.37%, which was similar to the result reported by Peng et al. (2008) that the saturated fatty acid content of Qinghai yak milk fat was 59.81%, it shows that during the production of ghee, the fatty acid composition of milk has only undergone a slight change (Bobe et al., 2003). Overall, Qinghai and Gansu have higher saturated fatty acid content than other regions, suggesting that yak ghee may have higher hardness in these regions, but due to the higher C16 content in QG and other regions, it may also have a role in softening the texture (Chamberlain et al., 2016). Cow ghee in Xinjiang, yak ghee in Tibet, and cattle-yak ghee in Tibet have low fatty acid content, indicating their potential for good spread ability.

Unsaturated fatty acids exhibit healthier properties than SFA (Cui et al., 2016). Oleic acid (C18:1) has been shown to be the most abundant monounsaturated fatty acid in ruminant milk (Agyare and Liang, 2021). Therefore, the outstanding result in Qinghai may be due to the richer long-chain SFA in the pastures eaten by cattle, which are metabolized and transported to the udder tissue, resulting in higher SFA (Guangxin et al., 2016).

Myristic acid (C14), palmitic acid (C16), and stearic acid (C18) are the most abundant long-chain SFA in ghee in each region, and Erkaya et al. (2015) have reported similar results. There were statistical differences (*P* < 0.05) between different regions, among which the contents of C14 and C16 in QG were significantly higher than those in other regions, with relative contents of 9.03% and 23.56%, respectively, and the content of C18 in QX (QH) was the highest (21.23%). The C16, C18, and long-chain SFA in milk fat come from blood lipids (Agyare and Liang, 2021). Therefore, the outstanding result in Qinghai may be due to the richer long-chain SFA in the pastures eaten by cattle, which are metabolized and transported to the udder tissue, resulting in higher SFA.
fatty acid in ghee in various regions, with an average content of 22.72% in yak ghee, similar to the 17% to 22% result reported by Marquardt et al. (2018). This is followed by palmitoleic acid (C16:1). Among them, Gansu Hezuo 2 (GH2) had the highest C18:1 content (27.49%; \( P < 0.05 \)) and also showed an advantage in C16:1 content (4.14%). The content of linoleic acid (C18:2 n-6c) in PUFA ranges from 0.11% to 0.60%, which is not obvious between some regions, but the QG, Gansu Hezuo 2 (GH2), Tibet Shigatse 1 (ZR1), Xinjiang Urumqi (XU) 4 regions do not contain this fatty acid. The C18:2 n-6c content in ghee reported by Jing et al. (2019) ranged from 0.29 to 1.41 g/100 g. The concentration of linoleic acid depends on the amount of fatty acids in the feed, so the differences between the study regions and reported in the literature may be due to differences in plant composition (Cui et al., 2016). Different levels of antilinoic acid (C18:2 n-6t) were contained in the regions, especially cow ghee (\( P < 0.05 \)) in Urumqi (XU) and Ili (XY), Xinjiang, Qinghai Xunhua is the only ghee sample in different regions that contains EPA (C20:5 n-3), which proves its good health potential. In addition, an interesting result is that in addition to cattle-yak ghee, hybrid varieties of calf ghee are also rich in \( \alpha \)-linolenic acid (C18:3 n-3), \( \gamma \)-linolenic acid (C18:3 n-6), arachidonic acid (C22:4 n-6), which are believed to improve vision and prevent cancer, cardiovascular disease, and high blood pressure (Cui et al., 2016). Marquardt et al. (2018) also found that hybrid milk ghee shortening contains higher levels of functional fatty acids.

The content of total UFA in ghee in different regions ranged from 34.47% to 44.17%. Combined with UFA or SFA, most of the UFA contained in Xinjiang cow ghee, yak, and cattle-yak ghee in Tibet were higher than those in Qinghai and Gansu. Cattle breeds, grazing, feed, initial milk quality, and the processing of traditional ghee in different regions will all make the fatty acid composition of ghee in different regions. (Singh et al., 2022). Yak milk at high altitudes has been shown to be high in PUFA (Agyare and Liang, 2021). In the sampling areas of this study, most of the Tibetan areas were located in ultra high-altitude areas (3,500–5,500 m). Although Xinjiang is located at a low altitude, its high unsaturated content may be related to its feed supplementation (Guangxin et al., 2016). In addition, the results of lower PUFA in scalper ghee in Lhasa, Tibet (ZL) were consistent with their iodine value results, and also with the results of Peng et al. (2008) finding that some UFA in scalper milk were lower than that in yak milk.

The atherosclerotic index (DI) and desaturation index (DI) are used to characterize the health effects of fatty acids (Karakus et al., 2022). The atherosclerotic index showed that values of ghee in the same province and autonomous region were similar, and the difference between regions was small (\( P > 0.05 \)); the DI also showed similar results. Among them, both yak and cattle-yak ghee in Tibet showed significantly lower atherosclerotic index (\( P < 0.05 \)) than those in other regions. The 3 indicators of the DI, DI14, DI16, and DI18 all show that yak ghee in Tibet has a high desaturation capacity, especially in Shannan (ZS) in Tibet. The cow ghee from Xinjiang also has good desaturation ability, while the scalper ghee has the worst fatty acid composition among other genotypes of ghee.

### Analysis of Textural Properties of Ghee in Different Regions

Figure 1 shows the texture of ghee in different regions. In this study, the hardness of ghee varied significantly between some regions (\( P < 0.05 \)), and the main factor affecting the texture difference was fatty acids, and high SFA led to its high hardness under refrigeration conditions (Lis et al., 2021). Except for Gansu Hezuo 2 (GH2), the hardness of ghee in Gansu was generally high, among which the hardness value of Gansu Diebu (GD) was greater than 40 N, which was consistent with the result of significantly higher saturated fatty acid content in Gansu Hezuo 2 (GH2) in the determination of fatty acids. In this study, the hardness of ghee was mostly lower than that of Lee and Martini (2019), which shows that the overall spreadability of the samples in this study is good. It is worth noting that QG had the lowest ghee hardness (12.477 N), while its saturated fatty acid determination results were significantly higher than those in other regions (\( P < 0.05 \)). This may be because the region contains high levels of C16, and the addition of C16 has been reported to reduce the hardness of butter to some extent, with butter made from anhydrous milk fat produced by lactating cows with a low C16 fat source having a hardness of only 10.67 N (Chamberlain et al., 2016).

The consistency of ghee is a parameter determined by many related factors, such as fat crystals, fat globules, bubbles, and droplets in the aqueous phase (Lis et al., 2021). The determination results of consistency in different regions were similar to the trend of hardness, which showed significant differences in the consistency of ghee between some regions (\( P < 0.05 \)), and higher consistency in other regions except Hezuo 2 (GH2) in Gansu. The viscosity determination showed that Gansu Hezuo 1 (GH1) and Gansu Hezuo 2 (GH2) showed significantly higher viscosity (\( P < 0.05 \)), with little difference between the remaining samples. These tiny viscosity formations may be related to differences in the processing. According to local herders, the smaller
scale of yak breeding makes its milk production insufficient to produce ghee, so herders will centrifuge the collected yak milk for the sequel and then remove buttermilk to make ghee, and this process experiences temperature difference between day and night, which may change the aggregation of crystal particles inside ghee, affecting the agglomeration network during stirring (Buldo et al., 2013). The melting and solidification temperature, as well as the solids content at a given temperature, have been shown to be highly correlated with the spread ability of ghee (Lis et al., 2021).

Analysis of Antioxidant Activity of Ghee in Different Regions

The antioxidant capacity of dairy products is maintained by a combination of antioxidant factors (Usta and YILMAZ-ERSAN, 2013), it is difficult for a single method of oxidation determination to fully and accurately reflect the oxidation resistance of a sample. Therefore, 4 indexes, DPPH, ABTS, FRAP, and reducing power, were used to obtain a more accurate evaluation of antioxidant activity (Figure 2). Both DPPH and ABTS assays are mainly based on the hydrogen supply capacity of antioxidants and are used to determine the antioxidant activity of DPPH• and cationic ABTS+ radicals that scavenge organic nitrogen, respectively (Gaber et al., 2021). The FRAP method is to characterize the antioxidant capacity of ghee by using antioxidants that can reduce Fe3+ to produce blue Fe2+. The determination of reducing power is expressed in absorbance value, the stronger the reducing ability, the greater the absorbance value, and the greater the oxidation resistance (Abedin et al., 2022).

In this study, ghee showed good antioxidant properties. Both DPPH and ABTS showed similar trends, but the free radical scavenging rate of the ABTS method was higher than that of DPPH, which was attributed to the different mechanisms of action of the 2 assays and the differences in DPPH and ABTS radical characteristics (Soleymanzadeh et al., 2019). The clearance ranges were 0.45% to 58.06% and 41.14% to 65.53%, respectively. Several antioxidant indexes showed that ghee in the Qinghai region has good antioxidant properties, which is that most of the fatty acid composition in Qinghai is SFA, and UFA have been shown to be more likely to degrade and rancid oils to a certain extent (Rahila et al., 2018). However, the DPPH radical scavenging rate of yak ghee purchased by Agyare et al. (2022) from Qinghai was 53.17%, and the scavenging rate of ABTS radical was 78.61%. The overall results are higher than those of this study because the sample
was pretreated and purified by heat filtration to obtain new yak ghee, a refining process that may have excluded the influence of certain impurities on the antioxidant factors in the ghee. The free radical scavenging activity of cow ghee in the Ili area of Xinjiang was the lowest, indicating that the antioxidant activity of cow ghee was lower than that of yak ghee, scalper ghee, and cattle-yak ghee.

The antioxidant activity of ghee is influenced by the combined action of antioxidants and antioxidant enzymes. Ghee is rich in fat-soluble vitamins such as vitamin A and vitamin E, and the conjugated diene bonds in vitamin A are effective quenchers and scavengers of lipid peroxidized radicals, hydroxyl radicals, and other free radicals (Hu et al., 2020). Vitamin E blocks the chain reaction of free radicals in dairy products and inhibits the peroxidation of lipids in dairy products and lipoprotein complexes (Wang et al., 2021). According to the results of this study, the fat content is as high as 80% and, according to the literature, vitamin A can effectively inhibit the photooxidation of dairy products with high-fat content, and vitamin E can reduce oxidative damage caused by high altitude (Guo et al., 2014). It is worth noting that the scavenging capacity of ghee radicals in the Tibetan area in this study was ZN (4,500) > ZR1 (4,325) > ZL (3,650) > ZR2 (4,325) > ZS (3,700), which was basically positively correlated with altitude, and the results reported by Cui et al. (2016) that vitamin A content was positively correlated with altitude. Therefore, the vitamin itself can play an important role in the antioxidant process of ghee.

Antioxidant enzymes have the function of effectively scavenging oxidizing free radicals and toxic lipid peroxidation products in the body or tissues. The naturally
occurring oxidase systems in milk mainly include superoxide dismutase, glutathione peroxidase (GSH-Px), and catalase (CAT), which work together to remove excess oxygen radicals produced in milk. Antioxidant enzymes enhance the antioxidant activity of ghee by alleviating and blocking the peroxidation of lipids and protecting PUFA in milk fat from being destroyed by peroxidation, so the synergistic effect between vitamins and enzyme systems also affects the antioxidant properties of ghee (Smesny et al., 2015).

Nepalese Himalayans usually protect their face and skin from the sun or cold with yak butter mixed with fresh lemon juice (Peng et al., 2008). In this study, ghee has good antioxidant activity, which is consistent with the results of Peng et al. (2008) that yak milk fat has good antioxidant properties, so it can be used as a raw material in the pharmaceutical, food, and cosmetics industries.

**Analysis of Antibacterial Activities of Ghee in Different Regions**

The bacteriostatic activity of ghee in different regions is characterized by measuring the diameter of its inhibition circle (Figure 3). All ghee samples showed an antibacterial effect only against *Staphylococcus aureus*, except Urumqi (XU), Xinjiang, which showed no inhibitory effect on gram-negative bacteria. This is similar to the findings of Medeiros de Azevedo et al. (2020), who tested the bacteriostatic activity of cocoa butter and found that it only showed inhibitory effects against gram-positive bacteria (Medeiros de Azevedo et al., 2020).

This may be due to the different cell structures of the 2 test bacteria, the cell wall structure of gram-positive bacteria (*Staphylococcus aureus*) is simpler, the cell wall is only surrounded by a peptidoglycan layer, while the cell wall of gram-negative bacteria (*Escherichia coli*) presents a multilayer structure. The cell wall has 1 layer of lipopolysaccharides in addition to peptidoglycan and phosphatic (Zhang et al., 2016). Studies have confirmed that this structure is more resistant to hydrophobic substances (Hsouna et al., 2011). Therefore, the antibacterial substances in ghee accumulate in the cytoplasmic membrane of bacteria, resulting in increased permeability of the cell membrane of gram-positive bacteria and interaction with its contents, inhibiting cell functional properties, causing cell contents to leak, and eventually leading to cell death (Cheng et al., 2013).

The diameter of the inhibition circle in GD, Tibet Shigatse 2 (ZR2), QX, Xinjiang Ili (XY), Gansu Hezuo 2 (GH2), and Gansu Luqu (GL) were greater than 10 mm, and the antibacterial effect of the remaining samples was not obvious. According to the literature, some short- and medium-chain SFA have antibacterial activity in animals and in vitro, especially against gram-positive bacteria (Pădureț, 2021). However, in this study, not all regions with outstanding bacteriostatic effects contained higher levels of short- and medium-chain SFA, indicating that there are other factors affecting the bacteriostatic ability of ghee. Kaur et al. (2017) isolated some lactic acid bacteria from yak milk and found that some strains had strong bacteriostatic ability, and lactic acid bacteria can produce metabolites with bacteriostatic activity (Ulpawahkumbura et al., 2016). Therefore, lactic acid bacteria may act as dominant bacteria to promote the good antibacterial effect of ghee, and regional differences may also be related to the activity of lactic acid bacteria. Among other types of ghee, cattle-yak ghee is the best, and scalper ghee has the worst bacteriostatic effect. In addition, the good antibacterial effect of ghee also makes it not only edible but also improved and used in the cosmetics and biomedical industries.

**CONCLUSIONS**

This is a detailed report on the quality characteristics of ghee in different regions of China. The results show that there are differences in the quality characteristics of ghee in different regions. The texture of ghee is harder in most regions of Gansu, especially in the Diebu region. Yak ghee from Tibet and ghee from Xinjiang have a better UFA/SFA content than other regions. Ghee from the Qinghai region has an overall higher antioxidant activity. All the ghee, except the ghee from...
Urumqi, showed varying degrees of inhibition of gram-positive bacteria. In addition, yak ghee, cattle-yak ghee, and cow ghee were superior to scalper ghee in terms of fatty acid composition and antibacterial activity. The research can provide a reliable theoretical basis for improving the quality of ghee in different regions and, in addition to better meeting the nutritional needs of the public and the income of marketers, it also hints at the potential of ghee in the cosmetics and biomedical industries.

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**ORCIDS**

Y. Tian https://orcid.org/0000-0001-8621-0356

B. Ding https://orcid.org/0000-0001-4880-352X

G. T. Ding https://orcid.org/0000-0001-7884-4869

H. N. Liu https://orcid.org/0000-0001-9895-8337