ABSTRACT

The addition of *Bifidobacterium* to goat milk has dual effects on health, for which various inherent nutrients of goat milk are retained and live probiotics are provided. We explored the effect of *Bifidobacterium animalis* ssp. *lactis* Probio-M8 (Probio-M8) on fermentation characteristics, formation of organic acid, sensory properties, and storage characteristics of fermented goat milk (with added 4.0% sucrose). Addition of Probio-M8 decreased the fermentation time and significantly increased the content of functional organic acids, such as acetic acid, and functional long-chain unsaturated fatty acids, including linoleic acid, α-linolenic acid, and docosahexaenoic acid. Furthermore, the contents of medium-chain and short-chain fatty acids, which are related to “goaty” flavor, were significantly lower in the Probio-M8 treatment compared with the control. The number of living Probio-M8 decreased from 8.27 log cfu/mL (1.80 × 10^8 cfu/mL) to 7.94 log cfu/mL (0.79 × 10^8 cfu/mL) after 28 d of storage. Titratable acidity and pH value did not differ between the control group and experimental group (containing Probio-M8). Sensory evaluation indicated a lower goaty flavor and odor in the Probio-M8 fermented milk. Our results suggest that the addition of the probiotic Probio-M8 could improve the sensory, physicochemical, and functional properties of fermented goat milk.

Key words: *Bifidobacterium animalis* ssp. *lactis* Probio-M8, fermented goat milk, long-chain unsaturated fatty acid, low goaty flavor

INTRODUCTION

Goat milk is a commonly produced milk variety worldwide, following cow milk and buffalo milk in total production (Verruck et al., 2019). Goat milk is rich in a variety of nutrients such as immunoglobulin, lipids, AA, vitamins, and carbohydrates (Sonu and Basavaprabhu, 2020). Although the composition of goat milk is similar to that of other milks in terms of moisture, protein, lipid, and lactose concentrations, structural differences affect its digestibility and nutritional value (El-Hatmi et al., 2015). Lipids are an important component of goat milk; notably, goat milk has smaller fat globules than cow milk, exhibits no natural condensation after cooling, and has shorter-chain fatty acids, all of which improve its digestibility (El-Hatmi et al., 2015). Differences in AA composition of goat milk and the secondary structure and chemistry of milk proteins also reduce its allergy potential (Clark and Mora García, 2017).

Fermented milk is the most popular food for use as a carrier of probiotic bacteria (Khorsheidan et al., 2020). In recent years, goat milk and its products have become an increasingly important source of milk for infants, the elderly, and malnourished individuals, as well as those with gastrointestinal disorders. It is also the main carrier of probiotics in nonbovine dairy products (El-Hatmi et al., 2015; Clark and Mora García, 2017; Ranadheera et al., 2018; Liu and Zhang, 2022). The development of fermented goat milk containing probiotics is recommended as an important way to provide functional fermented dairy products. The probiotic used in fermented goat milk must not only retain the nutritional components of the milk itself, but also contain numerous lactic acid bacteria (LAB). Studies have shown that the characteristic goaty flavor of goat milk becomes less noticeable, and the nutritive value of the milk increases after fermentation (Pal et al., 2017; Jia et al., 2021). Furthermore, consumption of fermented goat milk has been associated with beneficial changes in intestinal flora that also contribute to a reduction in
gastrointestinal discomfort (Slačanac et al., 2010). The preparation of probiotic fermented goat milk has been reported previously. Varga et al. (2014) added Lactobacillus acidophilus La-5 and Bifidobacterium animalis ssp. lactis Bb-12 during fermentation. Compared with 1 d, the viable count of the La-5 and Bb-12 decreased less throughout the shelf life of fermented goat milk; the viability of La-5 was 35.5% and that of Bb-12 was 21.4% after 6 wk. Chen et al. (2018) found that cells of Lactobacillus plantarum 69 in fermented goat milk survived in the gastrointestinal tract, and the fermented goat milk maintained high angiotensin-I-converting enzyme (ACE) inhibitory activity.

The relative proportions of different species of the genus Bifidobacterium in the gut vary at different stages of life, and the extracellular structure of Bifidobacterium species plays an important role in host–flora interactions (Arboleya et al., 2016; Turroni et al., 2018). In addition, regular intake of Bifidobacterium species can improve the steady state of the intestinal flora, repair the intestinal barrier, and regulate immunity. Bifidobacterium species are also involved in the production of many metabolites with potential health benefits, including short-chain fatty acids (SCFA), CLA, and bacteriocin (Bottacini et al., 2017).

There have been few studies on fermented dairy products produced using probiotics like Bifidobacterium species, especially on the effects of probiotics on fermentation properties and organic acid metabolism of fermented goat milk. Metabolomics is increasingly being used to analyze changes in organic acid content after the end of fermentation. Studies have evaluated the C4 to C10 saturated SCFA and medium-chain fatty acids (MCFA) that can cause goaty flavor (Slačanac et al., 2010; Clark and Mora García, 2017; Pawlos et al., 2021), and the polyunsaturated long-chain fatty acids (LCFA) that are important for human health (Ruiz Morales et al., 2019).

Our previous research showed that isolate Bifidobacterium animalis ssp. lactis Probio-M8 (Probio-M8), isolated from human colostrum, has an extremely high survival rate in gastrointestinal fluid and therefore has great potential as a probiotic (Liu et al., 2020). In the current study, we used Probio-M8 in combination with a commercial starter for fermentation to produce milk with both the natural benefit of being made from goat milk and the benefit of containing probiotics. We compared fermentation properties, storage properties, and organic acid metabolism of the potentially probiotic fermented milk (PFM) with fermented milk (FM; control group) without probiotic. Our results provide data and research-based evidence for the industrial production of probiotic fermented goat milk products.

**MATERIALS AND METHODS**

**Production and Sampling of Fermented Goat Milk**

Goat milk was purchased from Shepherd (Beijing) Dairy Development Co. Ltd. that was sterilized at 139°C for 2 s before purchase. The probiotic Probio-M8 and commercial starter culture PYS 010 (Streptococcus thermophilus and Lactobacillus bulgaricus, direct vat set culture containing 2 × 10^{11} cfu/g) were purchased from Jinhua Yinh Biological Science and Technology Co. Ltd. No animals were used in this study, and ethical approval for the use of animals was thus deemed unnecessary.

The goat milk (96%, vol/vol) was preheated to 60°C in a water bath; then, sucrose (4%, wt/vol) was added and stirred for 15 min until completely dissolved. The mixture was homogenized (model SPX APV2000) at 63°C and 20 MPa pressure, pasteurized at 85°C for 30 min, and rapidly cooled to 37°C (Pawlos et al., 2021) before inoculation and fermentation. The FM and PFM were inoculated with the commercial starter culture PYS 010 (0.02 g/1,000 mL), and PFM additionally received 7.00 log cfu/mL (1.00 × 10^{7} cfu/mL) of Probio-M8. The nutritional composition of goat milk before fermentation was as follows: carbohydrates: 8.00 ± 0.10 g/100 g, protein: 2.80 ± 0.05 g/100 g, fat: 3.30 ± 0.10 g/100 g, sodium: 437.00 ± 14 mg/kg, and DM: 14.70 ± 0.10%. For each treatment, 400 mL of inoculated milk was introduced into replicate 500-mL flasks and fermentation allowed to proceed at 37°C until a pH of 4.50 was achieved; then, fermentation was terminated by cooling to 20°C in a water bath, samples were ripened at 10°C for 12 h, and then the 28-d storage time began. At the termination of fermentation, the fermentation time and organic acid content were determined, and all experiments were performed in triplicate. Organoleptic evaluation was determined after 1 and 28 d of storage. The pH value, titratable acidity (TA), and viable cell counts were measured at 1, 7, 14, 21, and 28 d. The experimental design is shown in Figure 1.

**Quantifying pH and TA**

The pH of the fermented goat milk samples was determined using a pH meter (pH FE20, Mettler Toledo). Titratable acidity was determined by mixing 5.0 g of fermented milk with 40 mL of distilled water (boiled for 15 min and used after cooling) and titrating with 0.1 mol/L NaOH using 0.5% phenolphthalein as indicator (Dan et al., 2018). The volume of consumed 0.1 mol/L NaOH (V_{NaOH}) was divided by the mass (M) of the sample (5.00 g), and multiplied by 100; TA was mea-
sured in degrees Theurer (°T). The TA was calculated using the following equation:

\[ \text{TA}^{°T} = \frac{V_{\text{NaOH}} \times 100}{M} \text{ of fermented milk} \]

**Quantifying the Viable Cell Count of Probio-M8**

A 25-g sample of fermented milk was mixed with 225 mL of sterile PBS (8.0 g of NaCl/L, 0.2 g of KH₂PO₄/L, and 1.15 g of Na₂HPO₄/L; pH 7.2) in a sterilized triangular flask at low temperature and shaken to mix for 15 min before serial dilutions were prepared. The viable cell count of Probio-M8 was determined by adding 0.05% L-cysteine and 0.05 mg/mL mupirocin (Qingdao Hope Bio-Technology Co. Ltd.) in de Man, Rogosa, and Sharpe (MRS) agar (Oxoid Ltd.). Then, appropriate amounts of diluted fermented milk were applied to MRS (containing L-cysteine and mupirocin) agar plates for plate counting and incubated under anaerobic conditions at 37°C for 72 h (Liu et al., 2020).

**Extraction and Quantification of Organic Acids**

Oxalic acid, tartaric acid, formic acid, malic acid, lactic acid, acetic acid, maleic acid, citric acid, succinic acid, and propionic acid were detected using HPLC (ThermoFisher U3000). The HPLC settings were as follows (Liu et al., 2022): phase column: synchronis C18; dimensions: 250 mm × 4.6 mm, 5 μm film thickness; sampling volume: 20 μL; column temperature: 30°C; flow rate: 0.5 mL/min; mobile phase: acetonitrile:water (0.05 mol/L KH₂PO₄, adjusted with H₃PO₄, pH = 2.68) = 0.5:99.5. The absorption peak was detected at 210 nm UV. Samples (2 g; precision 0.0001 g) were made up to 10 mL in deionized water, and filtered through a 0.45-μm organic filter before evaluation.

Butanoic acid, hexanoic acid, octanoic acid, decanoic acid, lauric acid, myristic acid, cis-9-myristoleic acid, pentadecanoic acid, palmitic acid, cis-9-palmitoleic acid, stearic acid, cis-9-octadecenoic acid, linoleic acid, \( \alpha \)-linolenic acid, arachidic acid, cis,cis,cis-8,11,14-linolenic acid, arachidonic acid, and docosahexaenoic acid were detected using GC-MS (ThermoFisher Trace1310 ISQ). Settings were as follows (Qamar et al., 2019):
Overall acceptability

Texture

Taste

Goaty flavor and odor

Color

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cooled to room temperature. To this, 10 to 30 mL of the flask removed from the water bath and quickly a small amount of water. Heating was then stopped, and the water bath. The reflux condenser was washed with a condenser and refluxed for 2 min at 80 ± 1°C in a solution (7 mL) was added from the top of the reflux disappeared. To this, 15% boron trifluoride methanol at 80 ± 1°C in a reflux water bath until any oil drops the reflux condenser connected. The sample was held hydroxide solution in methanol (8 mL) was added, and 2% sodium tor was used until the sample was dry, 2% sodium hydrolysate was repeatedly extracted 3 times following the ether extract was collected into a 250-mL flask. The solution was added to the separator funnel, covered, and collected in a 250-mL flask. A rotary evaporator was used until the sample was dry, 2% sodium hydroxide solution in methanol (8 mL) was added, and the reflux condenser connected. The sample was placed in a water bath at 70°C to 80°C for 20 min and oscillated every 5 min to ensure that particles attached to the flask wall were moved into suspension. After hydrolysis, the flask was removed and cooled to room temperature. The sample was then mixed with 10 mL of 95% ethanol. The hydrolysis solution in the flask was transferred to a separator funnel, and the flask and plug were washed with 50 mL of ether petroleum ether mixture. The washing solution was added to the separator funnel, covered, shaken for 5 min, and allowed to stand for 10 min. The ether extract was collected into a 250-mL flask. The hydrolysate was repeatedly extracted 3 times following the same steps described above. Finally, the separation funnel was washed with the petroleum ether mixture and collected in a 250-mL flask. A rotary evaporator was used until the sample was dry, 2% sodium hydroxide solution in methanol (8 mL) was added, and the reflux condenser connected. The sample was placed in a water bath at 80 ± 1°C in a reflux water bath until any oil drops disappeared. To this, 15% boron trifluoride methanol solution (7 mL) was added from the top of the reflux condenser and refluxed for 2 min at 80 ± 1°C in a water bath. The reflux condenser was washed with a small amount of water. Heating was then stopped, and the flask removed from the water bath and quickly cooled to room temperature. To this, 10 to 30 mL of phase column: TG-5MS (30 m × 0.25 mm × 0.25 μm); temperature program: 80°C for 1 min, raised to 200°C at 10°C/min, raised to 250°C at 5°C/min, raised to 270°C at 2°C/min, and held for 3 min. The sample temperature was 290°C, carrier gas flow rate was 1.2 mL/min, and unsplit stream sampling was used with an opening time for valve of 1 min. The MS conditions were as follows: ionization temperature: 280°C, transmission line temperature: 280°C, solvent delay time: 5.00 min, scan area: 30 to 400 amu (atomic mass units), and ionization: 70 eV. A uniform sample was weighed and placed in a 250-mL flask, and 2.0 mL of internal standard solution was added. Then, approximately 100 mg of pyrogallic acid, several zeolites, 2 mL of 95% ethanol, and 4 mL of water were added and mixed, followed by addition of ammonia (5 mL) and a final mixing. The flask was placed in a water bath at 70°C to 80°C for 20 min and oscillated every 5 min to ensure that particles attached to the flask wall were moved into suspension. After hydrolysis, the flask was removed and cooled to room temperature. The sample was then mixed with 10 mL of 95% ethanol. The hydrolysis solution in the flask was transferred to a separator funnel, and the flask and plug were washed with 50 mL of ether petroleum ether mixture. The washing solution was added to the separator funnel, covered, shaken for 5 min, and allowed to stand for 10 min. The ether extract was collected into a 250-mL flask. The hydrolysate was repeatedly extracted 3 times following the same steps described above. Finally, the separation funnel was washed with the petroleum ether mixture and collected in a 250-mL flask. A rotary evaporator was used until the sample was dry, 2% sodium hydroxide solution in methanol (8 mL) was added, and the reflux condenser connected. The sample was placed in a water bath at 80 ± 1°C in a reflux water bath until any oil drops disappeared. To this, 15% boron trifluoride methanol solution (7 mL) was added from the top of the reflux condenser and refluxed for 2 min at 80 ± 1°C in a water bath. The reflux condenser was washed with a small amount of water. Heating was then stopped, and the flask removed from the water bath and quickly cooled to room temperature. To this, 10 to 30 mL of n-heptane was added, shaken for 2 min, and then saturated sodium chloride solution added for static stratification. The upper n-heptane extraction solution was absorbed and about 3.0 to 5.0 g of anhydrous sodium sulfate was added, shaken for 1 min, and allowed to stand for 5 min; the upper solution was absorbed into the injection bottle for evaluation.

Organoleptic Evaluation: Pretest

Organoleptic evaluation was conducted by 30 untrained panel members (70% women and 30% men) between 23 and 41 yr of age, living in Hohhot (Inner Mongolia, China). The triangular test was used to verify each evaluator’s ability to discriminate. Specifically, each sample was restored to room temperature (20–25°C) for organoleptic evaluation. Members of the sensory panel were each provided with 3 random number-coded plastic cups (100 mL) at one time, and panel members should gargle before tasting each new sample. Samples were evaluated on a 9-point scale where 0 = does not match attribute definition and 9 = does match attribute definition). Samples were presented to panel members in random order to assess color, odor, taste, texture, and overall acceptability (Pawlos et al., 2021). Details are shown in Table 1.

Statistical Analysis

Results are expressed as means with standard deviations (SD). Principal component analysis (PCA) is widely used in metabolome data analysis, and it is an unsupervised dimensionality reduction method, which is conducive to showing the distribution characteristics of all samples in the small dimension. In the results, PCA analysis was used for the overall metabolic description of initial goat milk (IGM), FM, and PFM. Partial least squares discriminant analysis (PLS-DA) was used to visualize differences in metabolic profiles between 2 independent groups (FM and PFM) in MetaboAnalyst 5.0 (https://www.metaboanalyst.ca). R²Y is defined as the proportion of variance in the data explained by the models, indicating the goodness of

### Table 1. Definitions of the attributes evaluated in descriptive organoleptic analysis of fermented milk

| Attribute                  | Definition                                                                 |
|----------------------------|---------------------------------------------------------------------------|
| Color                      | Pure opalescent, with no other color, resembling milk and possibly lighter than milk |
| Goaty flavor and odor      | Animal-like, lingering, associated with a harsh odor and sharp taste, like the flavor of caprylic acid |
| Taste                      | Sour, sweet, and grease taste mild, no irritation, no discomfort; the sour taste was similar to lactic acid, the fat taste was similar to whole milk powder, and the sweet taste was similar to sucrose |
| Texture                    | No pale-yellow liquid (whey) present on the surface, and surface of the fermented milk was uniform |
| Overall acceptability      | Edibility or likelihood of selecting this fermented milk again |

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the fit; $Q^2$ is defined as the proportion of variance in the data that is predictable from the model, indicating predictive ability.

A one-tail Student’s $t$-test ($P$-value <0.05) was used to assess statistical significance between conditions, and the variable importance on projection (VIP) score was used to measure a variable’s importance in the PLS-DA model. Volatile fatty acids with a $P$-value <0.05 and VIP >1 were selected as being significantly different to the FM and PFM. We used the mean standard deviation method to compare the following attributes: pH value, TA value, viable cell count, and organic acid metabolic characteristics. We used Origin 2019 (Origin-Lab) and GraphPad Prism 8.0 (GraphPad Software) software. Significant differences were defined at $P < 0.05$. In our study, all experiments were performed in triplicate, and the IGM group, FM group, and PFM group were prepared.

**RESULTS**

**pH, TA, and Viable Cell Count**

Both milks were tested for pH value, TA, and viable cell count at the end of fermentation (Figure 2). The addition of Probio-M8 accelerated the fermentation speed of goat milk and shortened the fermentation time. The PFM required 8.5 h until the pH reached 4.50, and the FM group required 9 h ($P < 0.05$). There was no significant difference in TA between the 2 groups. When fermentation was halted, the viable cell count of Probio-M8 in the PFM group reached 8.27 log cfu/mL (1.80 × 10$^8$ cfu/mL). The pH value and TA of each group changed significantly with storage time, especially at 28 d compared with 1 d ($P < 0.01$). However, there was no significant difference at the same time point for the FM or PFM groups, with pH ranging from 4.30 to 4.35 and TA ranging from 85 to 90°T at 28 d. The viable cell count in the PFM group showed a downward trend, declining to 7.94 log cfu/mL (0.79 × 10$^8$ cfu/mL) at 28 d.

**Organoleptic Evaluation: Pretest**

The results of the organoleptic evaluation pretest are shown in Table 2. Significant differences between the PFM and FM milks ($P < 0.05$) were observed only for goaty flavor and odor and overall acceptability parameters; there were no significant differences in color, taste, or texture; similar results were observed after 28 d of storage. Unpleasant taste characteristics were significantly reduced in PFM, and the overall acceptance of fermented goat milk increased in the PFM group compared with the FM group.

**Detection of Organic Acids**

A total of 27 organic acids were detected in the IGM, FM, and PFM, shown in Supplemental Table S1 (https://doi.org/10.6084/m9.figshare.20973610.v1; Guo, 2022). A general description of the organic acid metabolites present was achieved using PCA, and the 3 groups (IGM, FM, and PFM) were separated clearly (Figure 3a). This suggests that probiotics affect organic acid metabolites, and that different starter culture combinations contribute differently to the metabolite profiles of fermented goat milk at the end of fermentation. The $t$-test (Figure 3b) of organic acid metabolites revealed significant differences ($P < 0.05$) between PFM and FM for 18 organic acid metabolites: acetic acid, octanoic acid, docosahexaenoic acid, maleic acid, arachidic acid, hexanoic acid, malic acid, cis,cis,cis-8,11,14-linolenic acid, linoleic acid, α-linolenic acid, butanoic acid, oleic acid, arachidonic acid, pentadecanoic acid, decanoic acid, lauric acid, succinic acid, and oxalic acid. We generated a heatmap (Figure 3c) of organic acid metabolic profiles of the IGM, FM, and PFM groups, and analyzed them by Spearman clustering to visualize their metabolic profiles and by hierarchal clustering to visualize the organic acid profiles. Obviously, the participation of Probio-M8 in fermentation was the main factor that distinguished the PFM group from IGM group and FM group, and it could be preliminarily judged that Probio-M8 changed the organic acid metabolism spectrum in fermented milk.

Compared with the IGM group, the concentrations of metabolites that converged in cluster A were reduced in the FM group and PFM group. Acetic acid, stearic acid, lactic acid, formic acid, citric acid, oxalic acid, succinic acid, lauric acid, butanoic acid, hexanoic acid, octanoic acid, myristic acid, and palmitic acid contents increased in the FM and PFM groups, and were concentrated in clusters C and D. The organic acid metabolites that declined in the FM and PFM groups were in cluster B: cis-9-octadecenoic acid, α-linolenic acid, linoleic acid, and docosahexaenoic acid. Cluster E contained organic acid metabolites that were significantly increased in the FM group and decreased in the PFM group: cis-9-palmitoleic acid, malic acid, decanoic acid, arachidonic acid, arachidic acid, and cis,cis,cis-8,11,14-linolenic acid.

Analysis of metabolites in the IGM, FM, and PFM groups at the end of fermentation (Figure 3d) showed significant increases in total content of organic acids in FM and PFM compared with IGM ($P < 0.01$), which derived from the action of fermentation. Total contents of organic acid metabolites were significantly higher in PFM than in FM ($P < 0.05$).
The contents of C4–C10 organic acid metabolites in IGM, FM, and PFM showed that butanoic acid, hexanoic acid, and octanoic acid were higher in FM and PFM than in IGM by the end of fermentation (Figure 4). Mean values of butanoic acid, hexanoic acid, and octanoic acid in FM were 879.93, 606.37, and 1,571.2 mg/kg, and in PFM were 723.04, 510.7, and 399.71 mg/kg, respectively. Concentrations were significantly higher in the FM group than in the PFM group ($P < 0.05$). Decanoic acid was significantly higher in the FM group (464.04 mg/kg) than in the IGM group, but there was no significant difference in decanoic acid between the PFM group and the IGM group. The proportion of these 4 organic acids of all organic acids in the IGM, FM, and PFM groups were 8.70, 11.64, and 9.49% by weight, respectively.

**PLS-DA of Organic Acids in FM and PFM Groups at End of Fermentation**

We used PLS-DA to differentiate between organic acids when fermentation was terminated (Figure 5a). The $R^2_Y$ and $Q^2$ values were used to represent goodness of fit and predictive ability, respectively, verifying the accuracy and predictability of the PLS-DA model. Models with $R^2_Y$ and $Q^2$ greater $\geq 0.5$ were suitable for recognition analysis. For the PFM and FM com-
parison, the $R^2_Y$ and $Q^2$ values were 0.998 and 0.995, respectively; these values, being close to 1, indicate that the model had good accuracy and predictability. Eight organic acid metabolites simultaneously met $P < 0.05$ and VIP > 1 (Figure 5b): acetic acid, maleic acid, docosahexaenoic acid, arachidonic acid, arachidic acid, α-linolenic acid, linoleic acid, and malic acid. Metabolites with the highest concentrations in FM were arachidic acid, maleic acid, malic acid, and arachidonic acid, with concentrations of 403.23, 258.90, 1,288.28, and 655.48 mg/kg, respectively. Organic acids with highest concentrations in PFM were acetic acid, α-linolenic acid, linoleic acid, and malic acid. Metabolites with the highest concentrations in FM were arachidic acid, maleic acid, malic acid, and arachidonic acid, with concentrations of 194.43, 0.53, 49.44, and 413.29 mg/kg, respectively. Organic acids with high-concentration in PFM were acetic acid, α-linolenic acid, linoleic acid, and malic acid. Me- tabolites with the highest concentrations in FM were arachidic acid, malic acid, and arachidonic acid, with concentrations of 403.23, 258.90, 1,288.28, and 655.48 mg/kg, respectively.

**DISCUSSION**

To date, there have been few comprehensive studies on the effects of adding probiotics on the physical properties and organic acid metabolite contents of fermented goat milk, especially a combination of probiotic derived from human colostrum and goat milk, which is more easily absorbed by the human body. The probiotic properties of Probio-M8 have been studied and some clinical trials have been conducted (Liu et al., 2020). Recently clinical and nutritional studies related to Probio-M8 have been published on the volatile and nonvolatile metabolomic profiles of yogurt (Wang et al., 2021a), the regulation of gut microbiota to alleviate Alzheimer disease in the APP/PS1 mouse model (Cao et al., 2021), the treatment and prevention of acute respiratory tract infections, reduced antibiotic use, and hospital stay in young children (Mageswary et al., 2022), and on the added benefit to patients with coronary artery disease via target modulation of the gut–heart–brain axis (Sun et al., 2022). However, probiotics must exhibit more desirable fermentation characteristics or application advantages (such as fermented goat milk) before they become commercially successful strains in the dairy industry (Sharma et al., 2021).

Addition of Probio-M8 increased the number of probiotics in fermented goat milk, shortened the fermentation time, and improved the acidification rate (pH = 4.5, 8.5 h). The growth and survival ability of bacteria are important standards for functional foods based on probiotics (Xu et al., 2019). Sufficient living probiotic cells is a prerequisite for beneficial effect, so the minimum number of living cells in fermented milk should exceed 10^6 cfu/mL (Swanson et al., 2020).

The metabolic pathways of C4–C8 organic acids in goat flavor and 8 differential metabolites are shown in Figure 6, related to the fermentation processes, glucometabolic processes (Prasanna et al., 2014), AA metabolism, and lipid metabolism (Mathur et al., 2020) of LAB or probiotics. Organic acids are an important source of fermented milk flavor, and the use of more kinds and higher content of those organic acids, such as oxalic acid, lactic acid, and hexanoic acid, improves the flavor and taste of milk (Wang et al., 2021b). Goat milk has a particular flavor that has been described as having “goaty” characteristics as a result of the presence of C4–C10 SCFA and MCFA (Pawlos et al., 2020). Previous studies have shown that C4–C10 are VFA with unique health benefits for the human body (Dalile et al., 2019; Ranadheera et al., 2019; Vijay et al., 2021). It is recognized that C4–C10 VFA play an important role in regulating intestinal pH, protecting the intestinal micro-ecological balance, and regulating intestinal immunity (Dalile et al., 2019). The goaty flavor that is not favored by consumers is closely related to the high content of these fatty acids (Pawlos et al., 2021). At the end of fermentation, PF had lower levels of butanoic, hexanoic, octanoic, and capric acids than FM. Fatty acid oxidation by LAB or *Bifidobacterium* is an important decomposition and combination pathway in addition to Embden-Meyerhof-Parnas (EMP) pathway, the tricarboxylic acid (TCA) cycle, and AA metabolism during the fermentation (Chen et al., 2017). Lipids in milk are degraded to fatty acyl-CoA and acetyl-CoA by β-oxidative degradation with the lipase of LAB (Hu et al., 2022), where acetyl-CoA merges with EMP and acetyl-CoA produced by the AA metabolic pathway to produce an important central transit effect (Chen et al., 2017). Fatty acyl-CoA (butyryl-CoA and hexanoyl-CoA) is continuously metabolized by LAB or *Bifido- bacterium* lipases, to produce acids, hydrocarbons, aldehydes, alcohols, and ketones (Dan et al., 2018). Accordingly, C4–C10 (butyric acid, caproic acid, octanoic acid, and capric acid) may have 3 synthetic pathways.

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**Table 2.** Organoleptic evaluation pretest of fermented goat milk

| Sensory attribute                  | Storage time (d) | Type of goat milk |
|-----------------------------------|-----------------|------------------|
|                                   | FM (n = 30)     | PFM (n = 30)     |
| Color                             |                 |                  |
| 1                                 | 7.23 ± 1.23 a   | 7.45 ± 1.10 a    |
| 28                                | 7.14 ± 1.00 b   | 7.21 ± 1.34 b    |
| Goaty flavor and odor             |                 |                  |
| 1                                 | 5.77 ± 1.30 b   | 2.64 ± 1.38 b    |
| 28                                | 6.85 ± 0.50 c   | 3.45 ± 1.13 c    |
| Taste                             |                 |                  |
| 1                                 | 7.35 ± 1.61 a   | 6.99 ± 1.88 a    |
| 28                                | 7.17 ± 1.89 a   | 7.09 ± 1.22 a    |
| Texture                           |                 |                  |
| 1                                 | 7.98 ± 0.87 a   | 7.24 ± 1.23 a    |
| 28                                | 7.89 ± 0.19 a   | 7.33 ± 0.89 a    |
| Overall acceptability             |                 |                  |
| 1                                 | 6.00 ± 0.96 a   | 7.46 ± 0.84 a    |
| 28                                | 4.84 ± 2.01 a   | 6.32 ± 1.19 a    |

**Note:** Means in the same row followed by different lowercase letters are significantly different ($P \leq 0.05$).

1Values are means ± SD.
2FM = fermented milk; PFM = probiotic fermented milk.
in fermented milk, including conversion of aldehydes, conversion of fatty acyl-CoA, and the synthesis of acetyl-CoA. Importantly, these metabolites are all associated with β-oxidation. In our results, however, the C4–C10 content in PFM was lower than that in FM; thus, Probio-M8 may improve the goaty flavor and odor of PFM by affecting the β-oxidation of fatty acids. In addition, SCFA and MCFA generate aldehydes and ketones, respectively, through the action of specific active enzymes (dehydrogenase) of LAB and probiotics (Singh et al., 2003; Dan et al., 2018) and help reduce the goaty flavor (Figure 6a). Notably, Probio-M8 contributed to this series of metabolic pathways. For the development of functional high-quality food, Probio-M8 has obvious flavor advantages, which is particularly important for the acceptability of food. Interestingly, the tasters reached a conclusion consistent with the quantitative results for fatty acid content, noting that the goaty

Figure 3. The partial least squares discriminant analysis (a), t-tests (b), heat map (c), and total content (d) of organic acid metabolites in fermented milk from the initial goat milk (IGM), fermented milk (FM), and the probiotic fermented milk (PFM) groups. Data presented are mean values of 3 independent experiments ± SD. One-tail Student’s t-test was used to assess statistical significance between conditions. PC = principal component. *P < 0.05; **P < 0.01.
flavor and odor of PFM were lower than that of FM and the overall acceptability of PFM was higher.

Probio-M8 reduced the goaty flavor of yogurt and changed the metabolic characteristics of LCFA. In FM, the metabolites with highest concentrations were arachidic acid, maleic acid, malic acid, and arachidonic acid, compared with acetic acid, α-linolenic acid, linoleic acid, and docosahexaenoic acid in PFM.

According to previous studies, acetic acid in fermented milk mainly comes from the conversion of AA, TCA metabolism, and the EMP pathway (Bintsis, 2018). However, based on our results, the acetic acid content of PFM was significantly higher than that of FM, perhaps as a result of the “bifidus” pathway as reported for *Bifidobacterium* species (Figure 6b). Fructose-6-phosphoketolase is involved in this pathway as the key enzyme. Originally, glucose is transformed to fructose-6-phosphate with hexokinase and glucose-6-phosphate isomerase (Prasanna et al., 2014). Then, fructose-6-phosphoketolase decomposes fructose-6-phosphate into erythrose-4-phosphate and acetyl phosphate. Erythrose-4-phosphate reacts with fructose-6-phosphate via a complicated mechanism to generate glyceraldehyde-3-phosphate and more acetyl phosphate. Therefore, the concentration of acetic acid significantly increased in PFM. It is undeniable that acetic acid is of health significance to the human body: it can reduce the obesity caused by diet without reducing food intake (McNabney and Henagan, 2017). Acetic acid, propionic acid, and butanoic acid account for 90 to 95% of SCFA in the colon; they are the main products of microbial fermentation and play an important role in regulating intestinal pH and protecting intestinal microbial balance (Dalile et al., 2019). Of these 3 acids, acetic acid is present in the largest proportion and is the most abundant SCFA. Acetic acid enters the TCA cycle and is transported to peripheral tissues for cholesterol metabolism and lipid formation; it may play a role in the regulation of appetite (Frost et al., 2014). Acetic acid can be produced by ethanol degradation, AA metabolism, aspartate metabolism, fatty acid biosynthesis, and pyruvate metabolism. It can regulate the production of IgA in the colon, combine with potentially harmful bacteria, and prevent them from entering and invading the mucus layer, which is beneficial to maintaining the balance of the intestinal flora, and it improves intesti-
nal barrier function when used as a dietary supplement (Takeuchi et al., 2021). Probiotic M8 is beneficial to the synthesis and accumulation of functional long-chain UFA. Linoleic acid, α-linolenic acid, arachidonic acid, and docosahexaenoic acid (DHA) are all related to the metabolism of LCFA by microorganisms (Figure 6c); of these, linoleic acid, α-linolenic acid, and DHA were significantly higher in PFM than in FM. Natural oils can be divided into n-9, n-6, and n-3 according to the number of double-bond carbon atoms closest to the methyl end of PUFA. Linoleic acid and arachidonic acid belong to the n-6 group, whereas α-linolenic acid and DHA belong to the n-3 group. Mammals lack the unsaturated double bond desaturase at the ninth carbon atom of fatty acids to the methyl terminal position (Whelan and Fritsche, 2013; Jäger et al., 2020); therefore, synthesis of linoleic acid and α-linolenic acid is limited. Importantly, linoleic acid functions to reduce serum cholesterol levels. Ingesting large amounts of linoleic acid is beneficial to people with diseases associated with high triglycerides; linoleic acid also helps to reduce serum cholesterol and inhibit formation of arterial thrombosis, prevents cardiovascular diseases such as atherosclerosis and myocardial infarc-

Figure 5. Partial least squares discriminant analysis (a) and variable importance on projection (VIP) analysis (b) of organic acid metabolites at fermentation halt. The 5 horizontal lines of the boxplot respectively represent the maximum, the first quartile, the sample median, the third quartile, and the minimum. Data presented are mean values of 3 independent experiments ± SD. One-tail Student’s t-test was used to assess statistical significance between conditions. **P < 0.01; ***P < 0.001.

Figure 6. Metabolic pathway of major organic acid metabolites in the probiotic fermented milk (PFM) group. FA = fatty acid; LP = lipase; OX = oxidation; DES = desaturase; HPL, hydroperoxide lyase; F6PPK = fructose-6-phosphate phosphoketolase; ω-OX = ω-oxidation.
tion, and can participate in lipolysis and metabolism, enhance body immunity, and promote metabolism of bone tissues (Marangoni et al., 2020). α-Linolenic acid is an essential factor involved in maintenance of the human brain and nervous system. A lack of α-linolenic acid causes disorders of the nervous system including the brain and retina (Akbar et al., 2021). Arachidonic acid can be generated from the dehydrogenation of linoleic acid (Figure 6c; Hanna and Hafez, 2018). Although considered an essential fatty acid (Hanna and Hafez, 2018), it does not necessarily have to be obtained from the diet because the body can convert linoleic acid (from vegetables, nuts, and plant seeds) to arachidonic acid. Probio-M8 can promote conversion of α-linoleic acid to DHA (Figure 6c; Calder, 2016). Studies have demonstrated that dietary DHA has numerous health benefits throughout human life (Calder, 2016; Ramsden et al., 2021), including brain and eye development in fetuses and infants; prevention of early preterm delivery; prevention of cardiovascular disease; and improvements in the cognitive and eye health of adults (Li et al., 2021). The advantages of dietary DHA might be linked to the regulation of intestinal microorganisms as shown by a recent study (Fu et al., 2021). The Chinese Nutrition Society (2013) and the Food and Agriculture Organization of the United Nations (Fu et al., 2021) recommend DHA intakes for particular age groups. For example, 0.1 g of total DHA/day is recommended for children from birth to 4 yr of age and the general recommendation for adults should be increased to 0.25 to 2 g/d. Therefore, concentrations of DHA in PFM could meet the needs of different age groups. The PFM had higher concentrations of linoleic acid, α-linolenic acid, and DHA, which might be closely related to the lower C4–C10 content (Singh et al., 2003). Probio-M8 promotes accumulation of nutritional long-chain UFA and further reduces goaty flavor. Arachidic acid, malic acid, and maleic acid are also common fatty acids in food (Zielińska and Nowak, 2017; Zhao et al., 2020). Arachidic acid is an SFA, and high intake is considered to increase blood cholesterol level; the low content in PFM is more beneficial for human health (Moisès Laparra et al., 2015). Malic acid is related to the omega oxidation of milk fat and the TCA cycle (Figure 6d), so it can be produced during the energy cycle in human metabolism. Like arachidonic acid, it has been shown to be beneficial (Zhang et al., 2020) and is derived from multiple sources. Malic acid concentrations were low in both PFM and FM.

CONCLUSIONS

In this study we showed that the probiotic Probio-M8 played a vital role in improving fermentation characteristics, increasing the concentration of essential long-chain UFA such as linoleic acid, α-linolenic acid, and DHA. Probio-M8 reduced concentrations of some SCFA and MCFA, and ameliorated the goaty flavor, which improved the overall acceptability of fermented goat milk yogurt. The detailed data described here provide insight to support the use of Bifidobacterium in fermented goat milk.

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