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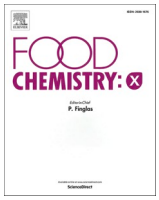

Effect of different strains on quality characteristics of soy yogurt: Physicochemical, nutritional, safety features, sensory, and formation mechanism

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ABSTRACT

The purpose of the study was to explore effect of four different strains on quality characteristics of soy yogurt. The results showed that four strains were all related to the genus *Lactobacillus* and N1 was *Lacticaseibacillus rhamnosus*, N2 was *Lacticaseibacillus paracasei*, N3 was *Lacticaseibacillus plantarum*, and N4 was *Lacticaseibacillus acidophilus*. The result analysis of biochemical, sensory, nutritional, functional and safety properties of fermentation process and end products showed that the soy yogurt fermented with L. *rhamnosus* N1 had the highest isoflavone content and the lowest phytic acid content; the soy yogurt fermented with L. *paracasei* N2 had the highest content of free amino acids and oligosaccharides, the lowest content of trypsin inhibitors; the soy yogurt fermented with *L. plantarum* N3 had the lowest oil content; the soy yogurt fermented with L. *acidophilus* N4 had optimal functional properties. In summary, N4 was suitable as a fermentation strain for soymilk.

1. Introduction

The consumption of dairy product was limited due to lactose intolerance, risk of allergy, and cardiovascular diseases due to the high content of saturated fat. Food industry had exchanged dairy with plant-based dairy alternatives to prevent some diseases (Huo, Yang, & Li, 2023; Zhu, Thakur, Feng, et al., 2020). Plant based foods have gained popularity as representatives of healthy foods. Consumers give several reasons for increasing their consumption of these next-generation plant-based foods, including their perceived healthful, environmental and sustainable benefits, animal welfare concerns, and desirable quality attributes (McClements & McClements, 2023).

Soymilk, a convenient and healthful soy food, has been gradually accepted as an alternative to cows' milk in worldwide markets due to equivalent protein and carbohydrate content as well as lower fat content. Meanwhile, soymilk devoid of cholesterol may help preventing cardiovascular diseases. However, the application of soymilk is limited due to the undesirable flavor of soybean components, flatulence caused by oligosaccharides, trigger an allergy, and increased gastrointestinal burden (Huo et al., 2023; Zheng, Fei, Yang, Yu, & Li, 2020). Fermentation with food grade safe strains is a promising strategy to improve flavor and increase digestion, absorption, and functionality of soymilk. For example, Peng et al. (2022) prepared the soy yogurt using lactic acid bacteria (LAB) and kombucha bacteria and found that the content of hexanal was reduced and new flavor compounds were generated. Huang et al. (2022) investigated the functional properties of soy yogurt with LAB and indicated that fermentation enhanced the antioxidant capacities and digestive enzyme inhibitory activities.

The research and product development of soy yogurt mainly focuses on investigating the effects of fermentation strains and fermentation process on the quality of soy yogurt and its health functions. The mostly used strains for fermenting soymilk were LABs, which are diversely and markedly different in growth rate, acid production capacity, probiotic properties, and fermentation performance. Hati, Patel, and Mandal

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(2017) studied the growth characteristics of 8 kinds of LAB in soymilk and found that *S. thermophilus* grew and produced the fastest acid, and the pH dropped from 6.93 to 4.43 after 24 h, followed by L. *rhamnosus* and *L. bulgaricus*, the number of live bacteria was 7.70 lg cfu/mL ~ 9.25 lg cfu/mL, and the slowest growing ones were L. *acidophilus* and *L. helveticus*. The effects of *L. lactis* RQ1066 fermentation on physicochemical properties, anti-nutritional factors, antioxidant capacities, amino acids composition, and sensory evaluation of mung bean milk was investigated and found that the mung bean milk could be used for fermentation substrate of L. *lactis* RQ1066 and the nutritional and functional properties of soymilk was changed (Li et al., 2023). These results demonstrated that fermentation strains are the key factor to determine the quality characteristics of soy yogurt. Therefore, screening more dominant strain with improved quality characteristics have become crucial in soy yogurt manufacturing practices.

The post-fermentation stage is the biotransformation of soymilk by enzymes synthesized bacteria to improve the soy yogurt flavor, however, organic acid tended to accumulate during post-fermentation, decreasing stability. Refrigerated temperature (2–10 °C) during storage and transportation is the foremost technique for preventing post-acidification. Moreover, screening suitable strains is another strategy to improve the quality of soy yogurt. Ge et al. (2024) investigated the impact of different inoculum ratios of these 2 strains on fermentation time and post-acidification and determined that the ratio of 19:1 (*S. thermophilus* CICC 6038 and Lb. bulgaricus CICC 6047) is optimal for achieving favorable fermentation performance and enhanced post-acidification. However, current research about post-fermentation has focused on milk yogurt, the effect of post-fermentation on the quality characteristics of soy yogurt was further investigated.

Therefore, during our surveying bacterial diversity of folk fermented food, four strains, designated N1, N2, N3 and N4, were isolated from folk fermented yogurts at Inner Mongolia. Four strains are further taxonomically characterized using a polyphasic approach to clarify its taxonomic status in the present study. The change of physicochemical properties, nutritional composition, functional and safety features of soy yogurt with the four strains was investigated during fermentation and post-fermentation. Meanwhile, the mechanism of the four bacteria preparing yogurt has also been further explored. This study will provide a technical support for the directional fermentation technology of soy yogurt.

2. Materials and methods

2.1. Materials and strains

Soybean were obtained from a supermarket in Beijing, China. The pure cultures of strain N1, N2, N3 and N4, isolated by our lab before, were stored as a suspension in 15% (*w/v*) sterile glycerol at −80 °C and routinely cultivated in de Man, Rogosa and Sharpe (MRS) broth (Difco Laboratories, Detroit, MI, USA) at 37 °C under microaerobic conditions before proceeding with subsequent investigations. All other chemicals used were at least of analytical grade (Beijing Chemical Reagent Co., Beijing, China).

2.2. Morphologic observation and phylogenetic analyses

The colony morphologies of four LAB strains on MRS plate were observed and taken pictures with camera. Gram staining was performed according to the methods described by Moyes, Reynolds, and Breakwell (2009). The morphologies of four strains were observed using transmission electron microscopy (H-7650, HITACHI, Tokyo, Japan). Genomic DNAs were extracted using a bacterial genomic DNA Mini kit (TaKaRa Bio) according to the manufacturer's protocol. The gene encoding 16S rDNA was amplified, cloned and sequenced following a previously described procedure (Wang, Liu, & Zhang, 2020). The evolutionary history was inferred using the Neighbor-Joining method (Saitou, 1987). The evolutionary distances were computed using the p-distance method in the units of the number of base differences per site. This analysis involved 24 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 882 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura, Stecher, & Kumar, 2021).

2.3. Preparation of soymilk

Soybean were soaked overnight in water at a ratio of 1:3 (*w/v*) for 12 h. One part of the soaked soybeans was crushed in four parts of water, for 30 min using mixer grinder (Joyoung, JYLY921, China). The process included a heating step. Then soymilk was separated from solid residue using 100 mesh filtration fabric and boiled for 10 min to confirm pasteurization.

2.4. Strain activation and preparation of soy yogurt

Four strains were cultured in MRS broth at 37 °C and washed and resuspended 3 times in phosphate buffer solution (PBS, pH 7.0, 0.02 mol/L) (Hyclone, South Logan, UT, USA) The four strains were harvested in the log phase at 0.2 mg/mL. Then the cells of each strain were used to inoculate (8%, *v*/v) soymilk with 7% sterile sucrose and a certain concentration of gelatin. The fermentation was performed at 37 °C for 6 h, and then the post-fermentation was performed at 4 °C for 12 h. Samples were collected in triplicates at different time for the following analysis.

2.5. Strain acclimation

The activated bacterial suspension was inoculated into 50 mL skim milk and soymilk (skim milk: soymilk =1:0, 3:1, 1:1, 1:3, 1:5, 0:1) successively at 8% inoculum and incubated at 37 °C for 12 h, in order to strengthen the adaptability of four kinds of bacteria to soymilk. On the basis of domestication of soymilk, the experiments of gelatin tolerance of four kinds of LAB were also carried out. Four strains, after activated in soymilk, were inoculated into soymilk with a gelatin additive amount of 0.2%, 0.4%, 0.6% and 0.8% successively.

2.6. Physicochemical property analysis of soy yogurt

The pH of soy yogurt was monitored by using a pH meter (PHSJ-3F, Shanghai, China). The acidity was determined by titrating the sample with a 0.1 mol/L NaOH solution, and its value was expressed as lactic acid (%). The number of LAB was obtained by plate counting (Gao et al., 2024).

2.7. Nutritional composition analysis

Protein content was determined by Kjeldahl method, free amino acid content was determined by an amino acid analyzer, and oil content was determined by Soxhlet extraction according to the methods of Madjirebaye et al. (2022).

The content of oligosaccharides was determined by high-performance liquid chromatography (HPLC) (Agilent, Australia). The sample (2 g) were redissolved in 5 mL deionized water, performed by ultrasound for 25 min, centrifuged at 12000 ×*g* for 15 min, and filtered with 0.22 μm filter membrane. The content of oligosaccharides was measured by isocratic eluting with 65% acetonitrile. The sample passed through the amino column (5 μm, 250 mm × 4.6 mm, 30 °C) at a flow rate of 1.0 mL/min. The sucrose, raffinose, and stachyose were as the standards and determined the content and constitution of oligosaccharide.

The analysis of isoflavones referred to the method of Huang et al. (2022), with some modifications. The extracts were carried out by

suspending 1 g of different samples in 10 mL of 70% ethanol, followed by sonication (60 °C, 40 min) and centrifugation (8000 *g*, 15 min). The supernatant was then filtered using a 0.22 μm filter membrane and fixed to 10 mL. All samples were analyzed by HPLC system equipped with Agilent ZORBAX SB-C18 (4.6 × 250 mm, 5 μm). The column temperature was 30 °C. The injection volume was 10 μL. Separation was conducted at 1 mL/min with 0.1% of formic acid in water (A) and acetonitrile (B). The following gradient: 0–15 min, 80% A; 15–18 min, 75%; 18–22 min, 70% A; 22–25 min, 65% A; 25–30 min, 55% A. In addition, soy isoflavones standards (daidzin, glycitin, genistin, daidzein, glycitein, and genistein) were prepared by dissolving in methanol and gradient diluted (0.5–20 μg/mL). The concentration and peak area of the working standard were used to make the standard curve, respectively (Hati, Vij, Singh, & Mandal, 2015).

2.8. Antinutritional factors determination in soy yogurt

Trypsin inhibitor: The method of soybean trypsin inhibitor was described by Zhang and Chang (2022). The pH of soy yogurt was adjusted to 9.0 for extracting the trypsin inhibitor. The above soy yogurt was centrifuged at 4000 ×*g* and 4 °C for 10 min. The supernatant was collected and diluted appropriately. The diluted supernatant (1 mL) was mixed with Tris-$CaCl_2$ solution (1 mL), trypsin solution (0.01%, 2 mL), and BAPA solution (0.04%, 5 mL). The mixture was reacted for 10 min at 37 °C and then the acetic acid (30%, 1 mL) was added in the mixture, immediately. The absorbance of sample was measured at 410 nm. The trypsin inhibitor activity (TIA) was calculated as follows:

$$\mathrm{TIA} = \frac{A_S - A_B}{0.01} \quad (1)$$

where A_S is the absorbance of sample, and A_B is the absorbance of the blank. The One trypsin unit (U) was defined as an increase of 0.01 absorbance and trypsin inhibitor activity (TIA) was defined as trypsin units inhibited per gram of dry soymilk (U/g).

Soybean saponin: The content of soybean saponin was measured according to the method of Navarro del Hierro et al. (2018). Soy yogurt was dissolved in methanol, samples were vortexed and heated at 60 °C for 10 min. The sample were cooled in ice and the absorbance was measured at 520 nm using a UV–vis spectrophotometer. The oleanolic acid was as the standard to obtained the content of soybean saponin.

Soybean agglutinin: The soybean agglutinin in soy yogurt was extracted using NaCl solution and ultrasonic cleaner, and then the sample was centrifuged at 3000 ×*g* for 15 min. The content of soybean agglutinin was measured using ELISA kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China) according to the manufacturer's protocol. The absorbance of samples was measured at 450 nm (Liu et al., 2021).

Phytic acid: the soy yogurt (2 mL) was dispersed into the saltpeter solution (50 mL, 0.5 mol/L). The mixture was continually stirred at 30 °C and 220 rpm/min for 2 h. And then the distilled water (3 mL) and wade reagent (0.2 mL) was added to the above mixture and well-mixed. The mixture containing distilled water and wade reagent was centrifuged at 5500 ×*g* and 4 °C for 10 min. The supernatant was collected and the absorbance was measured at 500 nm.

2.9. The formation mechanism of soy yogurt

Free sulfhydryl: The content of free sulfhydryl was determined by the method of Beveridge and Nakai (1974). The concentration of soy yogurt was adjusted 1 mg/mL. The sample (0.5 mL) was dissolved in PBS (4.5 mL, pH 7.0) containing 4 mmol/L EDTA, and the Ellaman reagent (100 μL) containing 4 mmol/L EDTA and 10 mmol/L DTNB was added to the mixture. The above mixture was mixed evenly and left to react for 30 min at 25 °C. The absorbance of mixture was measured at 412 nm. The content of free sulfhydryl was calculated at follows:

$$\text{Free sulfhydryl } (\mu\mathrm{mol/mg}) = 73.53 \times A \times D/C \quad (2)$$

where A is the absorbance of mixture at 412 nm, D is the dilution factor, C is the protein concentration (mg/mL), and $73.53 = 10^6/1.36 \times 10^4$ (1.36×10^4 was molar absorptivity, $M^{-1}\ cm^{-1}$).

The intermolecular force evaluation of soy yogurt: This measure was performed using selective buffers (prepared with 0.05 M PBS, pH 7.0). These buffers were able to destroy some types of bonds in the soy yogurts, the different buffer solutions were as follow:

- (S1) 0.6 mol/L NaCl solution for ionic bonds;
- (S2) 1.5 mol/L urea and 0.6 mol/L NaCl solution for hydrogen bonds;
- (S3) 8 mol/L urea and 0.6 mol/L NaCl solution for hydrophobic forces;
- (S4) 0.5 mol/L β-mercaptoethanol, 8 mol/L urea and 0.6 mol/L NaCl solution for disulfide bonds (Wang et al., 2017). The Coomassie Brilliant Blue solution (1 mL) was mixed with the PBS (5 mL) to obtain the staining solution. Soy yogurt samples (5 mL) and the above buffer solution (5 mL) were mixed and placed at 4 °C for 1 h. The mixture was centrifuged at 10000 ×*g* and 4 °C for 20 min and the supernatant was collected. The supernatant (20 μL) was mixed with staining solution (200 μL) and the mixture was incubated for 3–5 min. The absorbance was measured at 595 nm. Bovine serum albumin was used as standard protein and the protein concentration in different buffer solutions were calculated according to the standard curve. It indirectly gave the intermolecular force.

2.10. The functional properties of soy yogurt

The water holding capacity (WHC) evaluation of soy yogurt: The WHC of soy yogurt was measured according the method of Wang, Jin, Su, Lu, and Guo (2019). The mass of empty centrifuge tube (50 mL) was recorded as W1. The soy yogurt (10 mL) was placed into the above centrifuge tube and the mass was recorded as W2, and the × sample in centrifuge tube was centrifuged at 6000 ×*g* and 25 °C for 10 min. The supernatant was removed and the mass was recorded as W3. The WHC was calculated according the equation as follows:

$$\mathrm{WHC} = (W3 - W1)/(W2 - W1) \times 100\% \quad (3)$$

Texture evaluation of soy yogurt: Soy yogurt was cut to a columned sample of 10 mm height (Φ20 mm). The TPA was carried out at 25 °C with Texture Analyzer (TA. HD plus, Stable Micro Systems, UK) equipped with a 6 mm cylindrical probe. The speed before and after the test was 1.0 mm/s, the test distance was 50%, the relaxation time was 5 s, the trigger force was 5 N, and the trigger mode was automatic.

A rheometer (Physical MCR 301, Anton Paar, Austria) was used to determine the apparent viscosity of soy yogurt. The program temperature was set at 37 °C. Twenty milliliters of soymilk (with each stain, 7% sucrose, 0.8% gelatin) was added into the rheometer for time scanning. The storage modulus (G′) and loss modulus (G″) was conducted at the frequency sweep over the 0.1–100 Hz range. The loss factor (tan δ) was calculated.

2.11. Sensory evaluation

The soy yogurt fermented with four strains at the end of fermentation and post-fermentation were assessed for colour, flavor, consistency, taste and overall acceptability using a 5-point hedonic scale according to the method of Ujiroghene et al. (2019). Meanwhile, the soy yogurt sample was placed in headspace bottles. The odor of soy yogurt was determined using electronic nose equipment (PEN3, AIRSENSE, Germany), in which the sampling time was 60 s, and the cleaning time was 180 s.

2.12. Statistical analysis

All samples were measured triple in replicate measurements, the results were reported as mean ± standard deviations. The differences among the results were analyzed using Duncan Multiple Range Test at $p < 0.05$ using a statistical software SPSS (IBM SPSS Statistics, version 20.0 for windows software).

3. Results and discussion

3.1. Identifying the isolated strains

Colonies of strain N1, N2, N3 and N4 on MRS were white, circular, slightly convex and smooth with entire margins after incubation for 3 days at 37 °C. Four bacterial strains were all gram-stain-positive, rod-shaped, facultative anaerobic. Four bacterial strains showed 16S rDNA sequence similarities of higher than 99% with members of the family LAB using NCBI BLAST searching (https://blast.ncbi.nlm.nih.gov/Blast.cgi). N1 phylogenetic tree revealed that N1with *Lacticaseibacillus rhamnosus* MT539058, N2 with *Lacticaseibacillus paracasei* MT463867, N3 with *Lactiplantibacillus plantarum* OP000872.1 and N4 with *Lactobacillus acidophilus* MT645504.1 formed a same lineage with the highest 16S rDNA identity of 99.89%, 99.54%, 99.79% and 99.29%, respectively (Fig. 1). Based on the polyphasic taxonomy characterization including bacterial colony and cell phenotypic, gram staining and phylogenetic aspects, four strains belonged to the genus of the family *Lactobacillus*, for which the name L. *rhamnosus* N1, *L. paracasei* N2, *L. plantarum* N3 and L. *acidophilus* N4 were proposed (Dimidi, Cox, Rossi, & Whelan, 2019).

3.2. The physicochemical properties of soy yogurt

Fig. 2 shows the four strains growth and the physicochemical properties of soy yogurt. pH is important quality parameters of yogurt formulations that affect their coagulation and storage. The pH 4.6 was as the fermentation end point, with the extension of fermentation time, the pH value of soy yogurt fermented by four strains showed a trend of gradual decline, indicating that the organic acid was produced by probiotic microbes. The results were consistent with the report of Luo, Bao, and Zhu (2024), which also reported that sharply decreased pH values were achieved with the increasing the fermentation time. The pH value of L. *acidophilus* N4 first reached 4.6 when the fermentation time was 240 min. The other three strains basically reached the fermentation terminal point between 260 and 280 min (Fig. 2A), suggesting that the rate of producing organic acid with L. *acidophilus* N4 was higher than the other strains.

The physicochemical properties of soy yogurt during fermentation and post-fermentation were shows in Fig. 2 B—D. All the four strains could grow well in soy yogurt, the numbers of viable bacteria were all reached 10^8 in fermentation and post-fermentation stages. The number of viable bacteria of L. *rhamnosus* N1 was the highest (2×10^8 CFU/mL), followed by L. *plantarum* N3, *L. paracasei* N2, and L. *acidophilus* N4. Moreover, the pH value of soymilk fermented by L. *rhamnosus* N1 and *L. acidophilus* N4 was the lowest, near 4.20, followed by L. *paracasei* N2 and L. *plantarum* N3. Furthermore, the other two strains were also close to 0.5×10^8 CFU/mL (Fig. 2B). The titratable acidity of soy yogurt from the highest to the lowest were L. *rhamnosus* N1 with 49°T, *L. acidophilus* N4 with 47°T, *L. paracasei* N2 with 42°T and *L. plantarum* N3 with 36°T, respectively. These results demonstrated that L. *rhamnosus* N1 and *L. acidophilus* N4 were the strains producing organic acid. *L. plantarum* N3 might produce the other metabolites expect for organic acid. The acidity of each strain remained basically unchanged at the end of post-fermentation except for a slight increase in L. *rhamnosus* N1. This suggested a lack of post-acidification during post-fermentation.

3.3. Analysis of nutritional quality of soy yogurt

The quality characteristics of soy yogurt during fermentation and post-fermentation process was further monitored. The nutritional components in soymilk products determine their quality and functional aspects. The protein content of soymilk is 3.3%, the protein content of soy yogurt fermented by four strains decreased significantly. There was no significant difference in protein content among the four strains, and the protein content of each strain at the end of fermentation and post-fermentation was not significantly changed (Fig. 3A). Compared with soymilk (2.92%), the fat content of the four strains decreased significantly after fermentation and post-fermentation ($p < 0.05$). The fat content of L. *acidophilus* N4 at the end of fermentation and post-fermentation had the greatest change. *L. plantarum* N3 had the strongest adaptability to soymilk and the strongest ability to degrade fat (Fig. 3C). These results indicated that the protein and fat in soymilk were used by probiotics to maintain normal growth. However, Madjirebaye et al. (2022) found that the content of crude protein in soy yogurt increased, indicating proteases produced by bacteria. This difference might attribute the degree of protein degradation being higher than the amount produced by proteases, resulting in a decrease in crude protein content.

The content of free amino acids in soy yogurt increased significantly. Compared with the fermentation stage, the content of free amino acids in soy yogurt with L. *rhamnosus* N1 and L. *acidophilus* N4 was decreased and that with L. *paracasei* N2 and L. *plantarum* N3 was increased during post-fermentation (Fig. 3B). Compared with soymilk, the oligosaccharides content of the four strains decreased in fermentation stage and increased in post-fermentation stage. Except for that of L. *acidophilus* N4, the contents of oligosaccharides in soy yogurt fermented by L. *paracasei* N2 were higher than that in unfermented soymilk, and the contents of oligosaccharides in other L. *rhamnosus* N1 and *L. plantarum* N3 during the post-fermentation stage were lower than that in unfermented soymilk. In soymilk, oligosaccharides including sucrose, glucose, fructose, galactose, stachyose and raffinose were the carbon and energy sources for microbial cell growth (Xia et al., 2019). Yang et al. (2015) also reported that the content of polysaccharide was decreased and then increased with the increasing fermentation time (Fig. 3D). The results demonstrated that the oligosaccharide was used to the microbial cell growth during fermentation process, but subsequently polysaccharide was decreased to supplement the oligosaccharide consumption.

The benefits of soy isoflavones on the human body have been widely studied. Numerous groups have also evaluated that variations of soybean isoflavones based on processing techniques of fermentation, germination, and other biotransformation. The content of soybean isoflavones of soy yogurt with L. *paracasei* N2 was significantly decreased compared with soymilk, and there was a significant difference in the composition of soybean isoflavones between soy yogurt with L. *paracasei* N2 and soymilk. In this sample the content of genistin decreased while the content of daidzin and glycitin was increased ($p < 0.05$) (Fig. 3E & F). Except for L. *acidophilus* N4, the proportions of daidzein and genistein increased compared to soymilk (4.54%, 7.76%, respectively). Obviously, there was a higher proportion of the daidzin and genistein in L. *plantarum* N3 (5.78%, 8.13%, respectively) and L. *paracasei* N2 (6.59%, 8.19%, respectively) samples during fermentation. Therefore, the L. *plantarum* N3 was more efficient in the biotransformation of soybean glycosides to aglycones during the fermentation. These results assume that isoflavone glucosides can be converted into aglycones owing to the environmental effects such as fermentation (Hwang et al., 2021). But the content of soybean isoflavones of soy yogurt with the other strains was not significantly different ($p > 0.05$). The results demonstrated that all the strains were equally effective in transforming isoflavone glycosides into aglycones during yogurt fermentation. With N2 the reduction of isoflavone concentration suggested a chemical degradation that may be due to an alteration of chemical structure of isoflavones. Additionally, the transformation of genistin into daidzin suggested a dihydroxylation

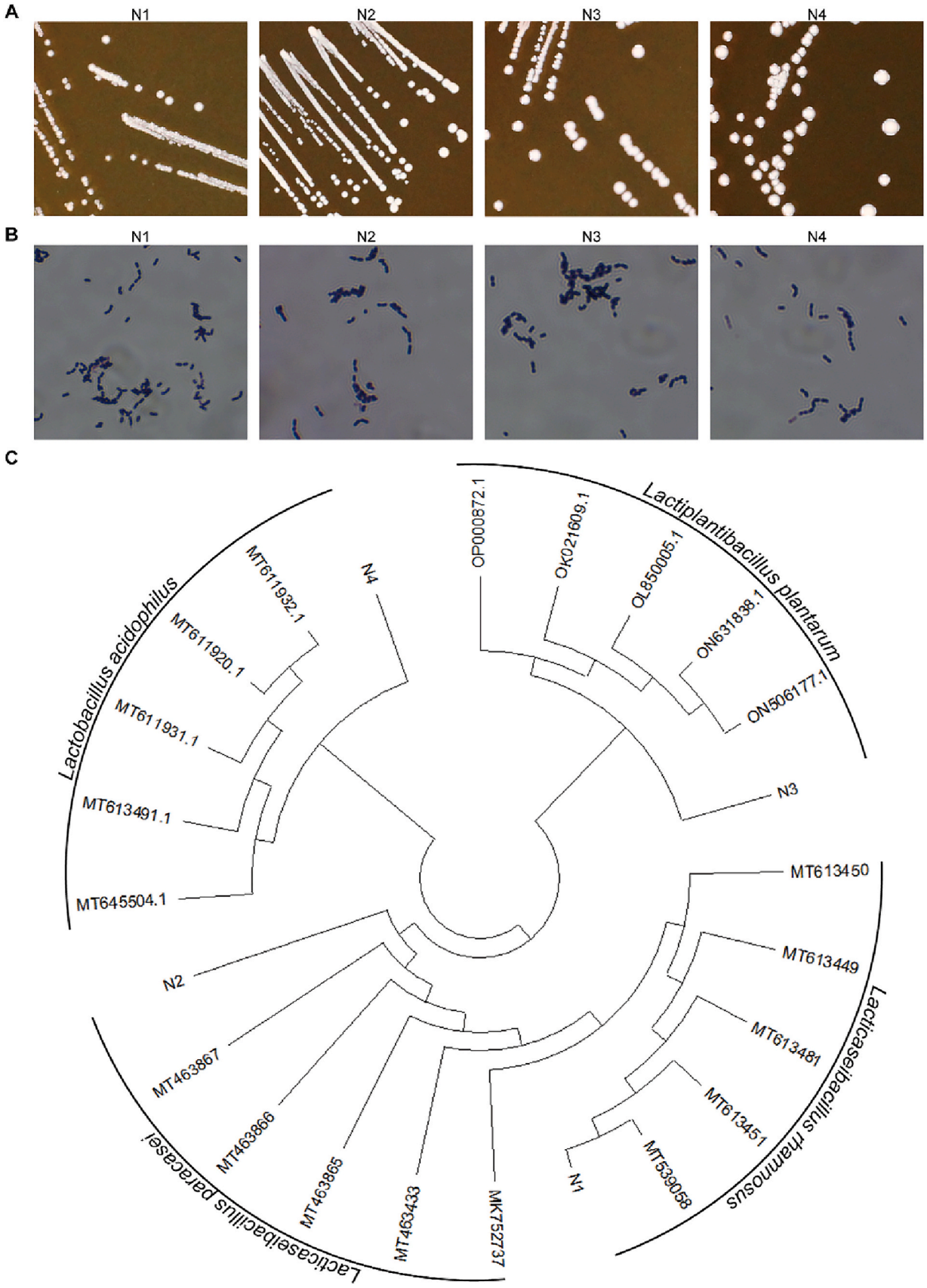

Fig. 1. Colonial morphology (A), Gram staining (B) and Phylogenetic analysis (C) of four strains. Neighbor-joining tree, based on 16S rDNA gene sequence data, showed the phylogenetic position of strain N1, N2, N3 and N4. Representatives of other related taxa within the family *Lactobacillus* were given in GenBank accession numbers of 16S rRNA sequences.

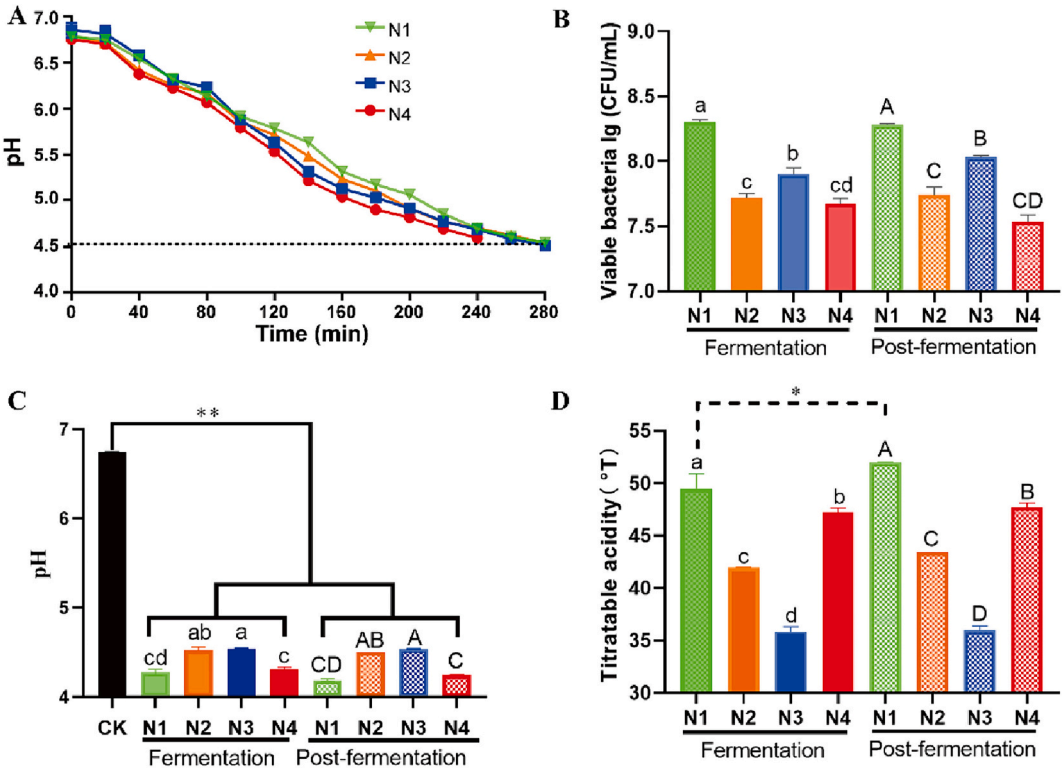

Fig. 2. The physicochemical properties of soy yogurt with four strains. (A) the pH monitoring during fermentation; (B) the number of viable bacteria; (C) the pH during fermentation and post-fermentation; (D) titratable acidity of each strain.

of one of the aromatic rings of the isoflavone skeleton (Sanjukta & Rai, 2016).

3.4. Analysis of antinutritional factors of soy yogurt

The safety features were investigated by evaluating the content of anti-nutritional factors. Trypsin inhibitor is a major and protein-based anti-nutritional factor in soybean with a molecular mass of 21 kDa, which influences protein digestion by inhibiting trypsin activity with high specificity (Hoffmann, Muetzel, & Becker, 2003). Inhibiting the activity of trypsin inhibitors was a key factor in improving the digestion property of soybean products. Compared with soymilk, the content of trypsin inhibitors of soy yogurt was decreased significantly. Soy yogurt fermented with L. *paracasei* N2, was the one with the lowest level of trypsin inhibitors (Fig. 4A), indicating that fermentation degraded the protein in soy yogurt. Silva Junior, Tavano, Demonte, Rossi, and Pinto (2012) also found that the content of trypsin inhibitor was decreased by fermentation. These results suggested that the fermentation produced enzymes which degraded trypsin inhibitors. However, the content of trypsin inhibitor of soy yogurt with L. *plantarum* N3 increased during post-fermentation, compared with fermentation.

Along with saponins, phytic acid exist in soybean as micro components. They are glycosidic compounds composed of a steroid or triterpenoid sapogenin nucleus with one or more carbohydrate branches. As shown in the Fig. 4B, compared with soymilk, soyasaponins of soy yogurt fermented with L. *rhamnosus* N1 and L. *plantarum* N3 showed an increasing trend. Soyasaponins of soy yogurt fermented by L. *paracasei* N2 increased in the fermentation stage, but decreased in the post-fermentation stage. The content of soyasaponins of soy yogurt fermented by L. *acidophilus* N4 decreased in the fermentation stage, but increased in the post-fermentation stage. Finally, the contents of soyasaponins in soy yogurt were higher than that in unfermented soymilk. Lai, Hsieh, Huang, and Chou (2013) also found that fermentation decreased the content of phytic acid. These results suggested that LAB and bifidobacterial might produce phytase and β-glucosidase. Phytase catalyzes the degradation of phytates. β-Glucosidase is capable of splitting sugar side chains of steroid and triterpenoid saponins and lowering water solubility for these compounds (Szakacs & Madas, 1979). Moreover, the content of phytase and β-glucosidase in the yogurt fermented with L. *rhamnosus* N1 was higher than in the other strains, leading to the lowest content of phytic acid of all strains. The decreasing in phytic acid content reduced the degree of binding with proteins and metal ions, and improved digestion rate. Compared with soymilk, soybean agglutinin of soy yogurt fermented by L. *rhamnosus* N1 and L. *paracasei* N2 decreased after fermentation, while soybean agglutinin decreased first and then increased after fermentation by L. *plantarum* N3 and L. *acidophilus* N4 (Fig. 4C). As shown in the Fig. 4D, compared with soymilk, phytic acid content in soy yogurt fermented by four strains all decreased obviously, and the phytic acid content in soy yogurt fermented by *L. rhamnosus* N1 was the lowest. However, the difference between the two stages was not significant.

3.5. The formation mechanism and functional properties of soy yogurt

The contents of free sulfhydryl group in the soy yogurt were all higher than that of soymilk and the content of free sulfhydryl group was the highest in soy yogurt fermented with L. *acidophilus* N4 (Fig. 5A). The protein content of the supernatant S3 obtained while measuring hydrophobic forces between macromolecules were the main forces in soy yogurt. The S3 protein content of soy yogurt fermented with L. *acidophilus* N4 was the highest (Fig. 5B). The results showed that hydrophobic bond and disulfide bond played a dominant role in the gel formation of soy yogurt, but the roles of ionic bond and hydrogen bond were weak. Liu et al. (2023) also reported that hydrogen bond and hydrophobic interaction were the main forces in soy yogurt gel. These results suggested that fermentation produces acid and H^+ can combine with $-COO^-$ to -COOH in proteins. Thus, the ionic bond formed between side chain carboxyl group and side chain amino group in protein

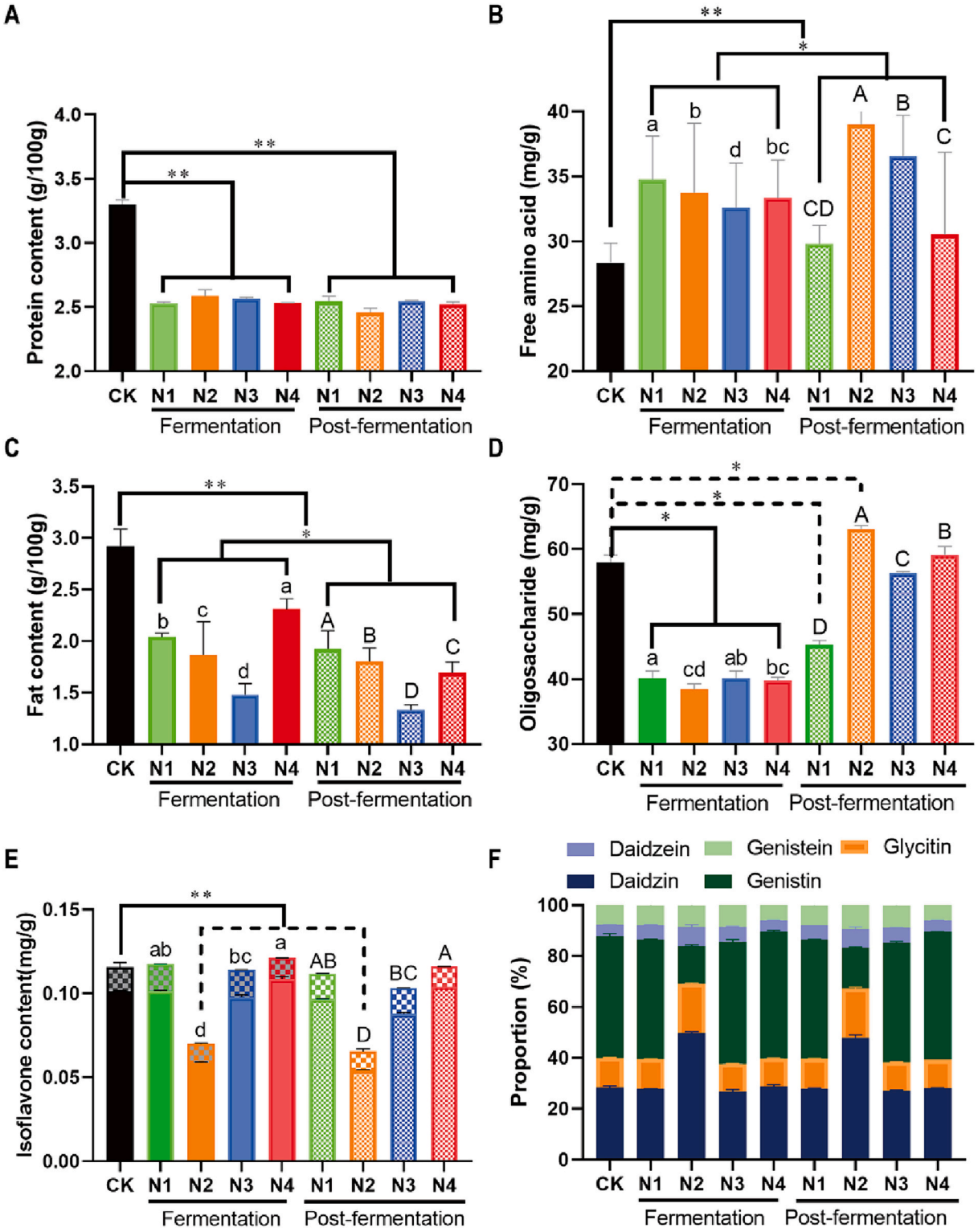

Fig. 3. The nutritional quality of soy yogurt fermented by four strains during fermentation and post- fermentation. (A) The content of protein; (B) free amino acids; (C) fat; (D) oligosaccharides; (E) soy isoflavones; (F) the proportion of each isoflavone.

molecule was reduced.

LAB produces acid in the fermenting process of soy yogurt, which leads to the aggregation of protein in the soymilk to form a network structure and retain water. Textural characteristics of the four kinds of soy yogurts were shown in Table 1, including hardness and cohesion. Soy yogurts fermented by fours strains had hardness values ranging from 360.57 g–494.23 g and the cohesion values ranging from 0.12 g·s –0.29 g·s, in which the hardness and cohesion of soy yogurt with L. *acidophilus* N4 was the highest. The results suggested that the interaction between proteins was stronger due to the fermentation with L. *acidophilus* N4. Meanwhile, the structure of the protein subunits changes during the fermentation process with L. *acidophilus* N4, changing the gel structure of the protein. The results might attribute that the α' was degraded and the hydrophobic groups was exposure. The more hydrophobic amino

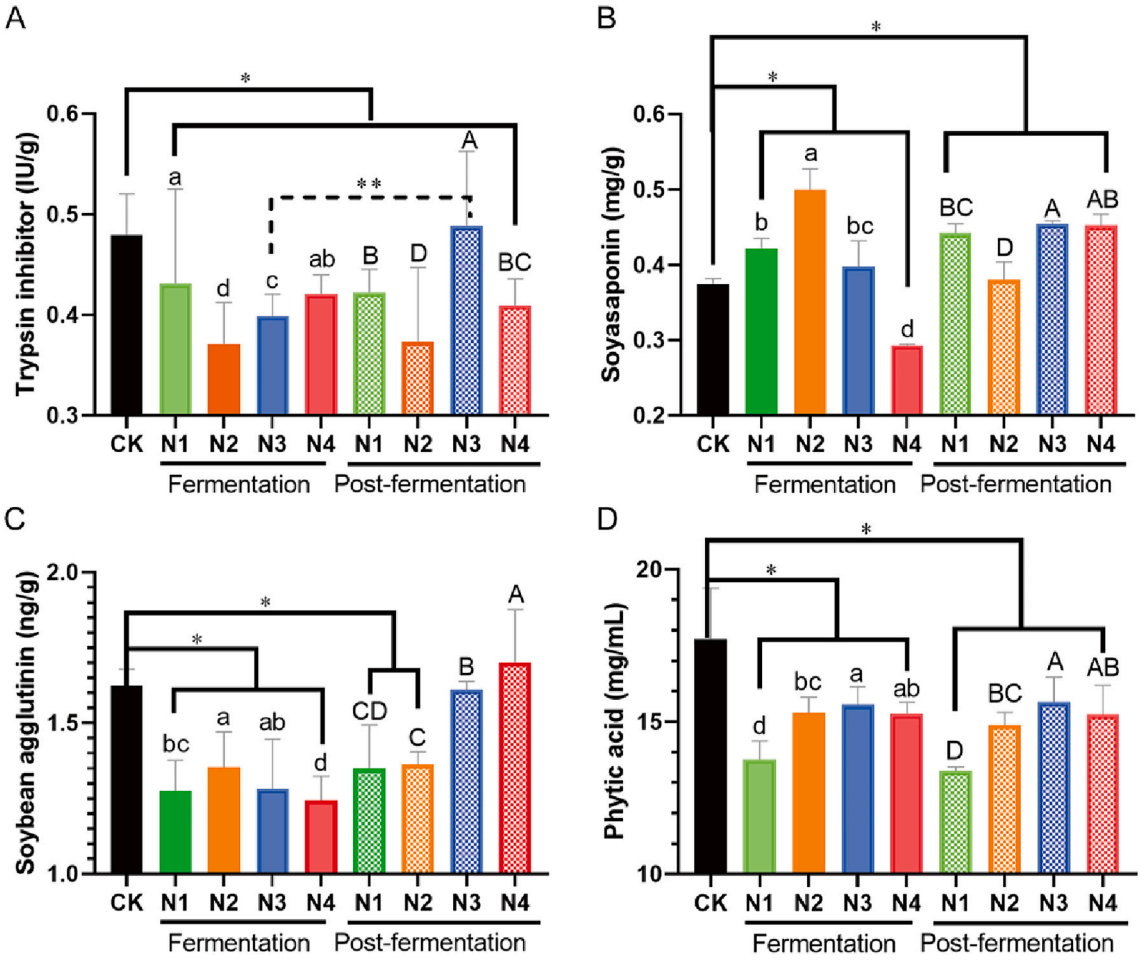

Fig. 4. Antinutritional factors of soy yogurt fermented by four strains during fermentation and post-fermentation. (A) The content of trypsin inhibitor; (B) soyasaponins; (C) soybean agglutinin; (D) phytic acid.

acids there are, the greater the degree of aggregation and the stronger the gel.

Related studies have also shown that the lack of α' sub-submissive promotes the exposure of hydrophobic groups and more hydrophobic amino acids, and promotes the aggregation of subunit, affecting the gel properties of protein.

The WHC was used to evaluate the quality of soy yogurt (Fig. 5C). The WHC of soy yogurt fermented with different LAB was significantly different ($p < 0.05$). The WHC of soy yogurt fermented with *L. acidophilus* N4 was the highest of the all soy yogurts. On the one hand, the hardness of soy yogurt with L. *acidophilus* N4 was the highest, the harder gel had a larger WHC, as harder gel undergoes less compression resulting in less water released under the same centrifugal force. On the other hand, *L. acidophilus* N4 produced more acid and form a denser network structure, thereby retaining more water in the network structure.

The dynamic rheological measurements of soy yogurt with different strains were conducted by time sweeps and frequency to observe their gelation behavior. Storage modulus (G′), represents energy stored and recovered per oscillation, indicating a material's solid-like elastic characteristics. Loss modulus (G″), refers to the energy dissipated and lost during an oscillation, indicating a material's fluid-like viscous features. The curd time of L. *acidophilus* N4 was about 90 min, and the curd times of soy yogurt with the other three strains were between 110 and 120 min (Fig. 5D). The results showed that the faster the LAB produced acid, the faster the pH decreased, the shorter the curdling time, and L. *acidophilus* N4 showed the best performance in acid production and soybean protein aggregation. However, the titratable acidity of soy yogurt with *L. acidophilus* N4 was not the highest. These results demonstrated that the L. *acidophilus* N4 changed the structure of protein to form the network structure. G' and G" represent the elastic behavior and viscous behavior of the sample, respectively. G' of all samples increased faster than G" during the fermentation process, and G' value of all samples was significantly higher than G" at the end of fermentation (Fig. 5E), indicating that the contribution rate of elasticity was higher than that of viscosity during the curd process of soy yogurt. The tan δ of soy yogurt with four strains was <1 (Fig. 5F), indicating their preponderantly solid-like properties. The results were consistent with the report of Tang, Roos, and Miao (2024), which also suggested the much higher G′ than G" of soybean protein isolate.

The sensory characteristics of different soy yogurt was not significantly different (Fig. 5 G & H). The contribution rates of the first principal component (PC1) and the second principal component (PC2) were 95.41% and 3.85%, respectively. The score chart showed that the clear trend of separation trend between soy yogurt sample. However, during the post-fermentation stage, the soy yogurt sample overlapped in the Fig. 5H, indicated that there were similarities in aroma composition. These results demonstrated that fermentation and post-ripening could significantly affect the flavor of soy yogurt.

4. Conclusions

The four strains from folk fermented yogurts at Inner Mongolia, designated N1, N2, N3, and N4 was all related to genus *Lactobacillus*, and

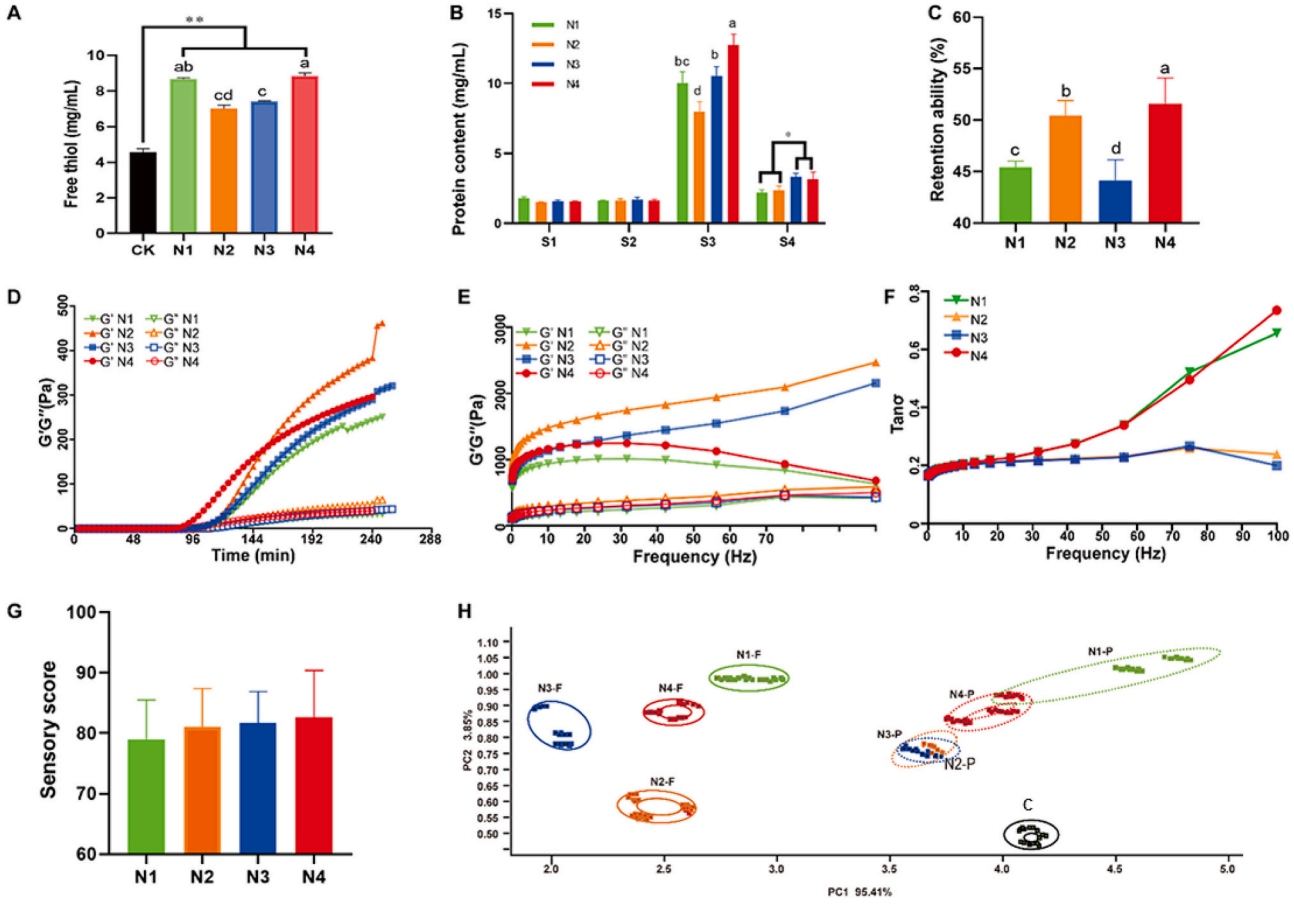

Fig. 5. The formation mechanism and functional properties of soy yogurt. (A) free sulfhydryl group; (B) intermolecular force of soy yogurt fermented by four strains; (C) water holding capacity; (D–F) rheological behavior; (G) sensory properties; (H) electronic nose.

Table 1
Textural characteristics of soy yogurt fermented by four LAB.

Measuring items	*L. rhamnosus* N1	*L. paracasei* N2	*L. plantarum* N3	*L. acidophilus* N4
Hardness (g)	404.71 ± 3.42^{b}	429.14 ± 1.65^{c}	360.57 ± 2.12^{a}	494.23 ± 4.26^{d}
Cohesion (g·s)	0.13 ± 0.001^{a}	0.12 ± 0.002^{a}	0.16 ± 0.006^{b}	0.29 ± 0.004^{c}

the four strains were identified as *Lacticaseibacillus rhamnosus N1*, *Lacticaseibacillus paracasei* N2, *Lacticaseibacillus plantarum* N3, and *Lacticaseibacillus acidophilus* N4. The soy yogurts were prepared with the four strains and the physicochemical, nutritional, safety features, sensory, and formation mechanism of soy yogurt were investigated. Fermentation was an effective way of improving soy yogurt's physicochemical properties and nutritional qualities, degrading the anti-nutritional factors. The species of fermenting strains significantly affected the quality characteristics of soy yogurt, in which the soy yogurt fermented with L. *acidophilus* N4 exhibited the higher water holding capacity and faster gel formation time. Meanwhile, disulfide bonds and hydrophobic interactions were the main forces in the formation of soy yogurt with the four strains. The research demonstrated that the four strains had their own characteristics and advantages, which laid a theoretical foundation for the directional development of specific functional soy yogurt. In the future, the correlation between bacterial metabolism and the quality characteristics of soy yogurts need to been investigated.

CRediT authorship contribution statement

Shuying Li: Writing – review & editing, Writing – original draft. **Miao Hu:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **Wei Wen:** Formal analysis, Data curation. **Pengfei Zhang:** Software, Investigation. **Wenhua Yu:** Investigation, Funding acquisition. **Bei Fan:** Methodology, Project administration. **Fengzhong Wang:** Conceptualization, Funding acquisition, Supervision.

Declaration of competing interest

The authors confirmed that they had no conflicts of interest with respect to the work described in this manuscript.

Data availability

The authors do not have permission to share data.

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