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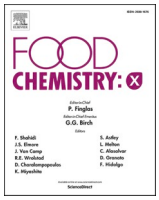

Non-beany flavor soymilk fermented by lactic acid bacteria: Characterization, stability, antioxidant capacity and *in vitro* digestion

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ABSTRACT

LAB fermentation could reduce the beany flavor, the sensitization of soymilk and improve the digestibility of soymilk, easy to be accepted by consumers. This study evaluated the characterization, stability, *in vitro* digestion and antioxidant capacity of soymilk fermented by different Lactic acid bacteria (LAB). The results showed that fat content of *L.plantarum*-S (0.77 g/100 mL) was the lowest, which proved that *L.plantarum* had a significant effect on lipid degradation, the protein content of *L.delbrueckii*-S (23.01 mg/mL) was higher. *L.delbrueckii*-S and *L. paracasei*-S were more acceptable to people, as well as high overall ratings. *L.paracasei* fermented soymilk has better suspension stability and smaller particle size. The fermented soymilk showed higher free amino acids (FAA) content, peptide content and stronger antioxidant activity than soymilk after digestion. The soymilk fermented by *L. plantarum* contained higher FAA content and *L.delbrueckii* contained the highest peptide content compared with other strains. *L.acidophilus*-S and *L.rhamnosus*-S showed stronger DPPH scavenging rate and FARP, which were 57.03 % and 52.78 % stronger than unfermented soymilk, respectively. These results may be provided a theoretical basis for the strain screening of fermented soymilk.

1. Introduction

In recent years, “Functional” and “nutrition” have become the mainstream of people’s definition of healthy food and are respected by more and more people. Soymilk is a soybean extract, which is an alternative to animal dairy products and a good source of protein for people with lactose intolerance. In contemporary world, more and more people are pursuing vegetarianism and some healthy non-dairy products (Chaminda et al., 2017). Soymilk has high nutritional value and contains 8 kinds of essential amino acids, among which lysine is extremely rich. Other advantages of soymilk include no cholesterol, reduce cholesterol, prevent cardiovascular and breast cancer and other diseases, but also has the function of lowering blood fat, antioxidant (Jayachandran & Xu, 2019). However, soymilk is restricted by the consumer market due to its beany flavor (Andres, Tenorio, & Villanueva, 2015). What’s more, traditionally developed soy milk contains a lot of insoluble fiber (Li et al., 2020), resulting in poor stability and difficulty to store.

Fermented foods with lactic acid bacteria (LAB) have antifungal effect (Shi & Maktabdar, 2021) and regulate intestinal flora (Li et al., 2021). Through LAB fermentation, the unsaturated fatty acids in soymilk are degraded into small molecules of alcohol, acid and other flavor substances, reducing the beany flavor, easy to be accepted by consumers (Blagden & Gilliland, 2010). Fermentation can also improve the digestibility of soymilk (Rui et al., 2016a), and reduce the sensitization of soymilk (Aguirre, Garro, & Giori, 2008). Soybeans fermented by LAB showed strong antioxidant activity and ACE inhibitory activity (Voss, Monteiro, Jauregi, Valente, & Pintado, 2020).

The characteristics of lactic acid bacteria play a crucial role in the quality of fermented products (Vlahopoulou, Bell, &Wilbey, 2010). Studies had reported that fermented soymilk can be produced by different strains, including *Lactiplantibacillus plantarum* (Lee et al., 2018), *Streptococcus thermophiles* with *Lactobacillus helveticus* (Champagne, Tompkins, Buckley, & Green-Johnson, 2010), and *Lacticaseibacillus paracasei* (Menezes et al., 2018), but the differences in stability, sensory and digestion of soymilk produced by different strains have not been studied.

In this study, soymilk was fermented with different strains, including *Lactobacillus acidophilus* (*L.acidophilus*), *Lactiplantibacillus plantarum* (*L. plantarum*), *Lacticaseibacillus casei* (*L.casei*), *Lacticaseibacillus rhamnosus* (*L.rhamnosus*), *Leuconostoc mesenteroides* (*L.mesalococcus*), *Lactococcus lactis* (*L.lactis*), *Lacticaseibacillus paracasei* (*L.paracasei*), *Streptococcus thermophilus* (*S.thermophilus*) and *Lactobacillus delbrueckii* (*L.delbrueckii*).

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The stability of fermented soymilk was studied by particle size, centrifugal precipitation rate and suspension stability. The digestive characteristics were also studied by content of soluble protein, FAA and peptide. DPPH scavenging rate and FARP were analyzed for evaluating antioxidant capacity of soymilk fermented by LAB. The aim of this study is to develop a healthy and high quality fermented soymilk and provide theoretical basis for the development of fuctional fermented soymilk.

2. Materials and methods

2.1. Materials

Non-beany soybeans were purchased from Jiamusi city in Heilongjiang Province. MRS was purchased from Haibo Biotechnology Co., Ltd., (Shandong, China). Porcine pancreatin and α-amylase were purchased from Yuanye Biotechnology Co., Ltd., (Shanghai, China). Pepsin (porcine gastric mucosa, ≥250 U/mg) was purchased from Solarbio Science and Technology Co. Ltd., (Beijing, China). Trypsin was purchased from yuanyeBio-Technology Co. Ltd., (Shanghai China). Bile salt was purchased from Aobox Biotechnology Co. Ltd., (Beijing, China). Other reagents were analytical grade.

2.2. Strains

Lactococcus lactis, *Lacticaseibacillus paracasei*, *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus*, *Leuconostoc mesenteroides*, *Streptococcus thermophiles and Lactobacillus delbrueckii* were stored in College of Food Science (Northeast Agricultural University). Strains were cultured at 37 ℃ for 12 h.

2.3. Preparation of fermented soymilk

Soybean was soaked in water at 1:3 (W/V) at 4 ℃ for 13 h and then added 0.3 % $NaHCO_3$ solution and kept it warm at 80℃ for 5 min. The mixture (Soybean: water = 1:8 (W/V)) was ground for 3 min, filtered and sterilized at 105℃ for 15 min (Wang, Chen, Wan, & Guo, 2019). LAB was washed twice with PBS buffer at 2603 g for 10 min and then 3 % (V/V) LAB was added into soymilk and the mixture was cultured for 8 h. Fermented soymilk were obtained by demulsification and homogenization. LAB fermented soymilk was denoted by LAB-S. For instance, *L. lactis*-S stands for *Lactococcus lactis* fermented soymilk.

2.4. pH and chemical composition analysis

pH was measured by a PHSJ-3F Laboratory pH Meter (Shanghai, China). The content of total solid was measured by drying method, fat content was determined by Soxhlet extraction (Kirk, Sawyer, & Harold, 1991). Protein concentration was measured by a BCA Protein Assay Kit (Solarbio, Beijing, China). The number of LAB was obtained by plate counting.

2.5. Sensory evaluation

Sensory evaluation was performed according to the methods described previously (Menezes Ramos, Dias, & Schwan, 2018). Ten laboratory staff (4 men, 6 women) were invited and trained. Four of them were selected as sensory evaluators for the experiment. The color (30), texture (30) and taste (40) of fermented soymilk were evaluated. The overall impression (color, texture, taste) was based on a percentage scale.

2.6. Stability of fermented soymilk

2.6.1. Particle size

The sample was diluted 100 times with deionized water, and average particle size was determined by the Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK) at 20℃. The sample had a refractive index of 1.35.

2.6.2. Suspension stability

According the method of Ni et al. (Ni et al., 2019), the suspension stability of sample was measured at 660 nm by a UV–Vis spectrophotometer (UV-2600, Hitachi, Tokyo Japan) after centrifuged at 1006 g for 30 min.

2.6.3. Centrifugal precipitation rate

The sample was centrifuged at 2603g for 15 min. The centrifugal precipitation rate was calculated as follows (Luo, 2009):

$$Centrifugal\ precipitation\ rate\ (\%) = \frac{Mass\ after\ centrifugation\ (g)}{Mass\ before\ centrifugation(g)} \times 100$$

2.7. In vitro digestion

2.7.1. Simulated digestion

In vitro simulated digestion were performed according to the method described previously (Wang et al., 2018).

Simulated oral digestion: Simulated saliva (290 mg/L α -amylase, 89.6 g /L KCl, 175.3 g/L NaCl, 88.8 g/L NaH_2PO_4, 20.0 g/L KSCN, 57.0 g/L Na_2SO_4, 84.7 g/L $NaHCO_3$, 2.0 g/L Urea, pH 6.8). The 20 mL sample was mixed with 6 mL simulated saliva and placed in a flask with 40 mL distilled water. The sample was stirred and cultured for 5 min at 37 ℃ and taken out 10 mL. The reaction was terminated with 100 μL trifluoroacetate (TFA, 10 % V/V) solution. Sample was centrifuged at 1000 g for 10 min. The supernatant was simulated oral digestion sample.

Simulated Gastric digestion: Simulated gastric juice (15 mg pepsin, pH 2). Sample was added in simulated gastric juice and kept warm and stirred for 2 h at 37 ℃ and taken out 10 mL. The reaction was terminated with 100 μL TFA (10 % v/v) solution. Sample was centrifuged at 1000 g for 10 min.

Simulated intestinal digestion: Simulated intestinal juice (5 mL mixture of trypsin (8 mg/mL) and bile salt (50 mg/mL), pH 6.5). The sample was stirred at 37 ℃ for 4 h. The reaction was terminated with 100 μL (TFA, 10 % V/V) solution. Sample was centrifuged at 1000 g for 10 min, and the supernatant was taken for analysis.

2.7.2. Soluble protein content

The soluble protein content was measured using the Coomassie Brilliant Blue method. 0.5 mL sample was mixed with 0.5 mL water, then 5 mL Coomassie bright blue solution was added, and the absorbance was measured at 550 nm by a UV–Vis spectrophotometer (UV-2600, Hitachi, Tokyo Japan). BSA solutions were used as standards.

2.7.3. Free amino acid content

The content of free amino acid (FAA) was determined by the Yang's method (Yang & Li, 2021a). 1 mL ninhydrin solution (2.0 %), 1 mL acetic acid buffer (2 mol/L, pH 5.4) and 1 mL digestive solution were quickly mixed, and cultured at 100 ℃ for 15 min. After cooling, 3 mL ethanol (60 %) was added, and the absorbance was measured at 570 nm. Leucine solutions were used as standards.

2.7.4. Peptide content

Peptide content was measured according to Yang's method (Yang et al., 2021) with some modifications. The sample (50 μL) was added into 2 mL the reagent. The reagent (50 mL) consisted of 25 mL borax (100 mmol/L), 2.5 mL sodium lauryl sulfate (20 % w/w), o-phthalaldehyde solution (OPA, 40 mg dissolved in 1 mL methanol) and β-mercaptoe-thanol (100 μL). The reaction was carried out for 2 min at 25℃, and the absorbance at 340 nm was analysed. Leucine was used as standards.

2.8. Antioxidant capacity

2.8.1. DPPH free radical scavenging activity

DPPH free radical scavenging activity was determined by a previous method with some modifieds (Guo, Lin, Guo, Zhang, & Zheng, 2017). The sample was diluted 10-fold (2 mL) and mixed with 2 mL DPPH-ethanol solution (1×10^{-4} mol/L) and incubated at 25℃ for 30 min in the dark. Absorbance was measured at 517 nm.

$$DPPH\ free\ radical\ scavenging\ rate\ (\%) = \left(1 - \frac{A_i - A_j}{A_0}\right) \times 100$$

where A_i represents the absorbance of sample, Aj represents the absorbance of the blank group (DPPH solution was replaced with absolute ethanol), and A_0 represents the absorbance of control group (the sample was replaced absolute ethanol).

2.8.2. Ferric reducing antioxidant power (FRAP)

Antioxidant activity was measured using an iron reducing antioxidant capacity (FRAP) assay by Total Antioxidant Capacity Assay Kit (Beyotime Biotechnology, Shanghai, China). The FRAP working fluid consists of 2, 4, 6-tris(2-pyridyl)-S-triazine (TPTZ) diluent, TPTZ solution and detection buffer. 180 μL FRAP working fluid was added into a 96-well plate and absorbance at 593 nm was analyzed by a Infinite M200 PRO Enzyme standard instrument (Tecan, Grdig, Tyrol Austria). Ferrous sulfate solution was used as the standard solution for calibration, and the linear regression equation was $Y = 0.2482\ X + 0.0641$ ($R^2 = 0.9986$).

2.9. Statistical analysis

All experiments were carried out in duplicate at least, and data were reported as the means ± SD. Statistical analyses were performed by the software SPSS 26 (Chicago, USA). Data were analysed with one-way analysis of variance (ANOVA) and Tukey's range test, significance was taken at $P < 0.05$.

3. Results and discussion

3.1. Characterization of soymilk fermented by lactic acid bacteria

3.1.1. Acidification profiles during fermentation

Table S1 showed the changes of pH of soymilk during fermentation from 0 h to 10 h. It can be observed that *S.thermophiles*-S and *L.acidophilus*-S had higher pH and weaker acid production capacity than other samples during fermentation ($P < 0.05$), in addition, *L.mesenteroides*-S had a rapid pH decrease. All strains reached the isoelectric point of soybean protein about at 8 h, so the fermentation time of soybean milk should not exceed 8 h. Maria et al. used chickpea extract and coconut extract as fermentation substrates for LAB, and The pH of the sample was 4.8 after nine hours of fermentation (Mesquita, Leandro, Alencar, & Botelho, 2020). Another study also showed that when using corn as the fermentation substrate for LAB, the pH was the minimum 5 (Menezes Ramos, Dias, & Schwan, 2018). Compared with other media, the pH of soymilk fermented by LAB decreased rapidly, and the LAB showed stronger acid-producing ability in soymilk. The experimental results showed that soymilk was a good substrate for LAB fermentation.

3.1.2. Chemical composition and viable counts of fermented soymilk

The Chemical composition of soymilk fermented by LAB was shown in Table 1. Solids content can affect the quality of soymilk (Cai & Chang, 1997). The solids in soymilk were composed of dietary fiber, protein, fat and other substances. There were no significant difference in total solids among strains ($P > 0.05$). It can be seen that LAB fermentation has no significant effect on total solid content of soymilk. In the fermentation process, LAB can degrade fat or lipids into free fatty acids, so that the content of total fat is reduced and the content of free fatty acids is increased (You et al., 2015). *S.thermophiles*-S (1.46 g/100 mL) had the highest fat content, indicating that *S.thermophiles* did not decompose fat significantly. The fat content of *L.plantarum*-S (0.77 ± 0.015) was the lowest, which proved that *L.plantarum* had a significant effect on lipid degradation. Protein content was an important reflection of the nutritional value of fermented soymilk. The protein content of *L.delbrueckii*-S (23.01 mg/mL) and *L.mesenteroides*-S (22.25 mg/mL) was the highest, which had higher food value. The viable counts of LAB was significantly different ($P < 0.05$), *L.rhamnosus*-S and *S.thermophiles*-S had the highest total number of LAB, up to 10^9 CFU/mL. LAB showed strong growth in soymilk, and soymilk could be used as an excellent substrate for LAB fermentation.

3.1.3. Sensory evaluation

Table 1 also showed the sensory effects of different strains on fermented soymilk. There were significant differences among fermented soymilk of different strains on sensory ($P < 0.05$). *L.mesenteroides*-S tasted the worst, with the lowest score for flavor and taste. The reason may be that the pH of *L.mesenteroides* dropped too fast during the fermentation process (Fig. S1), resulting in excessive organic acids and other substances. The sour taste of fermented soymilk was not accepted

Table 1
Characterization of Soymilk fermented by Lactic acid bacteria.

strain	Chemical composition			Viable counts lg (CFU/mL)	Sensory evaluation			
	Total solids (g/100 mL)	Fat content (g/100 mL)	Protein content (mg/mL)		Appearance	Texture	Flavor and taste	Overall impression
L.rhamnosus-S	10.10 ± 0.3^{a}	1.27 ± 0.02^{ab}	21.11 ± 1.05^{ab}	9.12 ± 0.042^{a}	22.75 ± 2.40^{b}	22.00 ± 1.41^{abc}	32.75 ± 1.79 ^{ab}	77.50 ± 5.68^{b}
S.thermophilus-S	10.10 ± 0.1^{a}	1.46 ± 0.295^{a}	13.39 ± 1.98^{c}	9.06 ± 0.039^{ab}	23.50 ± 0.50^{ab}	20.00 ± 2.12^{c}	34.50 ± 0.87 ^{ab}	78.00 ± 2.35^{b}
L.casei-S	10.25 ± 0.25^{a}	1.31 ± 0.22^{ab}	16.43 ± 4.44^{c}	8.93 ± 0.047^{c}	24.25 ± 1.48^{ab}	23.251.48^{bc}	34.25 ± 1.92 ^{ab}	81.75 ± 4.71^{ab}
L.delbrueckii-S	10.25 ± 0.45^{a}	1.20 ± 0.265^{ab}	23.01 ± 1.24^{a}	8.99 ± 0.009^{bc}	26.00 ± 1.23^{a}	24.50 ± 1.12^{ab}	36.75 ± 1.30 ^{a}	87.25 ± 2.77^{a}
L.paracasei-S	10.00 ± 0.9^{a}	1.09 ± 0.07^{ab}	20.52 ± 2.06^{ab}	8.80 ± 0.017^{d}	25.75 ± 0.83^{ab}	25.75 ± 0.43^{a}	36.25 ± 0.83 ^{ab}	87.75 ± 1.79^{a}
L.mesenteroides-S	9.60 ± 0.3^{a}	1.1 ± 0.05^{ab}	22.25 ± 2.45^{a}	8.99 ± 0.044^{bc}	22.75 ± 1.79^{b}	22.00 ± 1.58^{abc}	31.25 ± 1.30^{b}	76.00 ± 3.53^{b}
L.plantarum-S	10.15 ± 0.005^{a}	0.77 ± 0.015^{b}	15.16 ± 1.58^{c}	8.97 ± 0.003^{bc}	24.25 ± 1.92^{ab}	24.00 ± 1.23^{abc}	33.75 ± 2.78 ^{ab}	82.00 ± 5.24^{ab}
L.acidophilus-S	10.00 ± 0.3^{a}	1.04 ± 0.11^{ab}	9.14 ± 0.24^{d}	8.97 ± 0.001^{bc}	22.75 ± 1.92^{b}	23.25 ± 1.22^{abc}	33.25 ± 2.77 ^{ab}	79.25 ± 4.44^{b}
L.lactis-S	10.30 ± 0.2^{a}	0.98 ± 0.14^{ab}	17.80 ± 1.56^{bc}	8.75 ± 0.006^{d}	23.75 ± 1.09^{ab}	21.50 ± 1.66^{bc}	33.25 ± 3.56^{ab}	78.50 ± 4.98^{b}

Note: Different letters in same column indicated significant different ($P < 0.05$).

by the public, as previously found by Voss (Voss et al., 2020). *L.paracasei*-S had the highest texture rating ($P < 0.05$). It was stable and uniform for texture, with finer tissues and no precipitation. A previous study also found that *L.paracasei* can also improve the quality of cheese (Terpou et al., 2018). In this study, *L.delbrueckii*-S and *L.paracasei*-S had higher overall impression, due to smoother and distinctive flavor and were more acceptable to people. In addition, *L.delbrueckii* and *L.paracasei* were also widely used in fermented foods (Chourasia, Phukon, Minhajul, Sahoo, & Rai, 2022; Mesquita et al., 2020).

3.2. Stability of soymilk fermented by lactic acid bacteria

3.2.1. Average particle size

The average particle size directly affects the particle sedimentation velocity, and thus affects the stability. The average particle size of fermented soymilk was shown in Fig. 1A. The average particle size of fermented soymilk of different strains had significant difference ($P < 0.05$). Average particle size can represent the degree of protein aggregation during fermentation. The larger particle size indicated that protein aggregation was appeared during fermentation (Yang, Ke, & Li, 2021). The average particle size of *S.thermophilus*-S had no significant difference from *L.paracasei*-S or *L.plantarum*-S. It was noted that the average particle size of *S.thermophilus*-S was the least, and it could be inferred that *S.thermophilus*-S had good stability. Whereas, the average particle size of *L.acidophilus*-S was the largest, whose stability of soymilk system was poor. Correspondingly, the number of charges carried by protein micelles decreased as pH decreased, resulting in a weakening of electrostatic repulsion between particles (Mukherjee et al., 2017). This was an important reason for the instability of protein aggregates with large particle size.

3.2.2. Suspension stability

Suspension stability is an important index to judge the stability of fermented soymilk. The absorbance value reflects the suspension of small particles in soymilk. The higher the absorbance value mean the better the stability (Ni, Zhang, Fan, & Li, 2019). The different strains had significant influence on suspension stability of fermented soymilk ($P < 0.05$). The suspension stability of *L.paracasei*-S was the best (Fig. 1B), maybe they produce more extracellular polysaccharides, which make insoluble particles with smaller particle size suspended in the samples through emulsification and improve the stability of the fermented soymilk. Yang et al. showed that exopolysaccharides produced during the fermentation process of LAB have emulsifying effect (Yang & Li, 2021b). The suspension stability of *L.mesenteroides*-S was the lowest, indicating less exopolysaccharide secretion.

3.2.3. Centrifugal precipitation rate

Protein aggregation in soymilk fermented by LAB had larger particle size, and fermented soymilk was prone to sediment and decreased the stability (Yang et al., 2021). The lower centrifugal precipitation rate indicated that the macromolecules in fermented soymilk were not easy to settle and had better stability (Luo, 2009). Fig. 1C showed the influence of different strains on the centrifugal precipitation rate of fermented soymilk without stabilizer. There was no significant difference in centrifugal precipitation rate among different strains ($P > 0.05$), which may be due to the fact that the fermentation substrate is whole soymilk, which has more insoluble protein and cellulose and is easy to sedimentate (Li, Chen, Deng, Liang, & Liu, 2020). Among them, the centrifugal precipitation rate of *L.delbrueckii*-S was 51.73 %, which indicated that this fermented soymilk had good stability. However, the centrifugal precipitation rate of *L.mesenteroides*-S was 58.25 %,

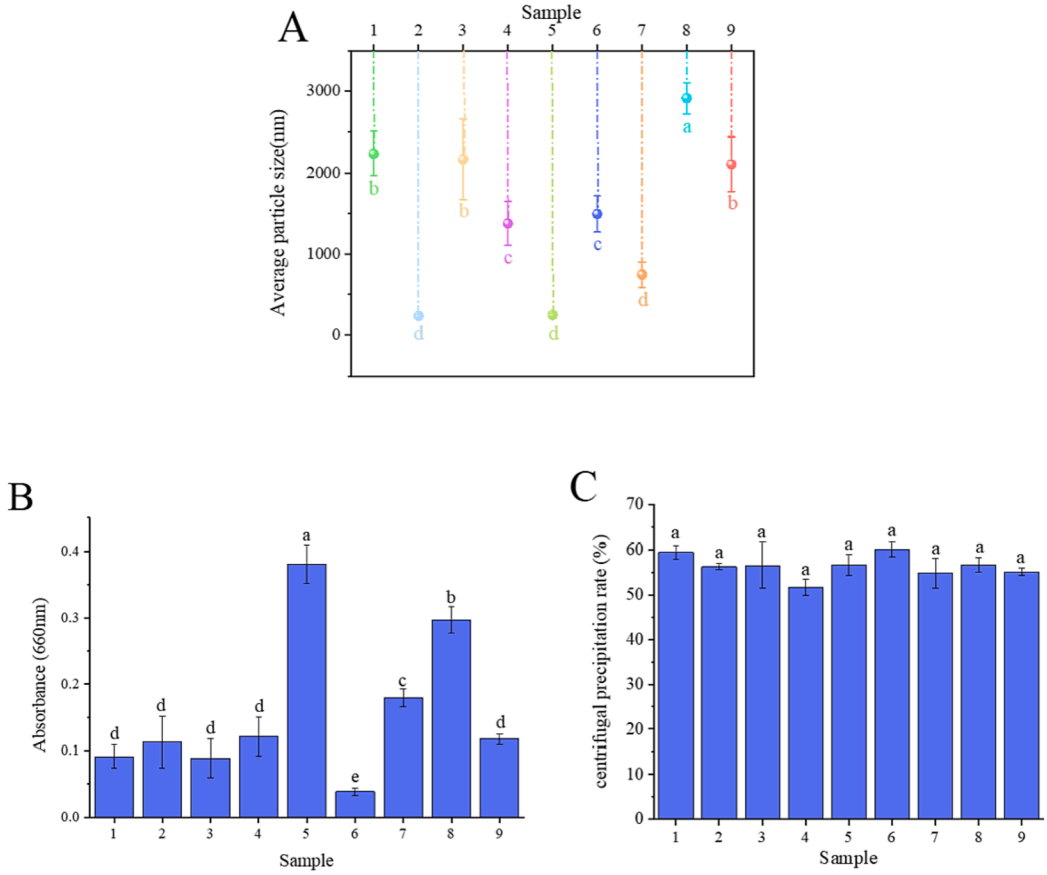

Fig. 1. The stability of fermented soymilk.(A) average particle size; (B) suspension stability; (C) centrifugal precipitation rate; Sample 1–9 represented *L.rhamnosus*-S, *S.thermophilus*-S, *L.casei*-S, *L.delbrueckii*-S, *L.paracasei*-S, *L.mesenteroides*-S, *L.plantarum*-S, *L.acidophilus*-S and *L.lactis*-S, respectively; Different letters indicated significant different ($P < 0.05$).

indicating that the stability of fermented soymilk was relatively poor.

According to the comprehensive evaluation of average particle size, suspension stability and centrifugal precipitation rate of fermented soymilk, it was found that *L.paracasei*-S had the best stability.

3.3. In vitro digestion of soymilk fermented by lactic acid bacteria

3.3.1. Soluble protein content

Soluble protein content is significantly related to factors such as pH, food form and digestive enzymes during *in vitro* digestion (Mennah-Govela & Bornhorst, 2021). Soluble protein content of fermented soymilk during digestion were shown in Fig. 2. The soluble protein content increased first and then decreased, and reached the highest value after gastric digestion, this result was similar of the finding of Rui et al (Rui et al., 2016b). In the indigested (Fig. 2A) and oral digested (Fig. 2B) samples, the soluble protein content of unfermented soymilk was significantly higher than fermented soymilk ($P < 0.05$). This was probably due to the occurrence of acid gelation during fermentation and proteins aggregate to form a gel structure, a large amount of soluble protein were preserved in the gel matrix (Guo, 2005). After oral digestion, the soluble protein content of fermented soymilk increased slightly, which may be caused by stirred effect and the destruction of the gel three-dimensional network structure (Chen, Capuano, & Stieger, 2020). The extremely low pH value in the stomach affected the interaction between soy proteins in gel, and promoted gel destruction and increased soluble protein content (Rui et al., 2016b). *S.thermophilus*-S was relatively low content of soluble protein in gastric digestion. After intestinal digestion (Fig. 2D), soluble protein content showed a downward trend under the action of intestinal digestive enzymes, this is similar to the results in Okara (soybean by-product) beverage of previous studies (Voss et al., 2020). The soluble protein contents of *S.hermophilus*-S and *L. cidophilus* -S were the highest after digestion.

3.3.2. Free amino acid content

Free amino acid (FAA) content can be used to evaluate the digestibility of fermented soymilk. FAA content of fermented soymilk increased gradually under the action of various digestive enzymes (Fig. 3). In both indigested (Fig. 3A) and oral digestion (Fig. 3B), FAA content showed the same trend as soluble protein content (Fig. 2). FAA content of fermented soymilk was significantly different after oral digestion ($p < 0.05$). After gastric digestion (Fig. 3C) and intestinal digestion (Fig. 3D), The content of FAA in fermented soymilk was gradually higher than unfermented soymilk, because protein was degraded into amino acids and peptide (Park & Kim, 2020). There were no significant difference in FAA content of *L.rhamnosus*-S, *S.thermophilus*-S, *L.delbrueckii*-S, *L.mesenteroides*-S, *L.acidophilus*-S, and *L.lactis*-S after intestinal digestion ($p > 0.05$). *L.plantarum*-S was significantly higher than other samples after gastric digestion and intestinal digestion. *L.plantarum* showed stronger protein degradation ability compared with other strains.

3.3.3. Peptide content

Gastrointestinal digestion plays an important role in peptide production by hydrolysis of proteins (González-Montoya, Hernández-Ledesma, Silván, Mora-Escobedo, & Martínez-Villaluenga, 2018). Fig. 4 showed the amount of peptides of fermented soymilk during digestion.

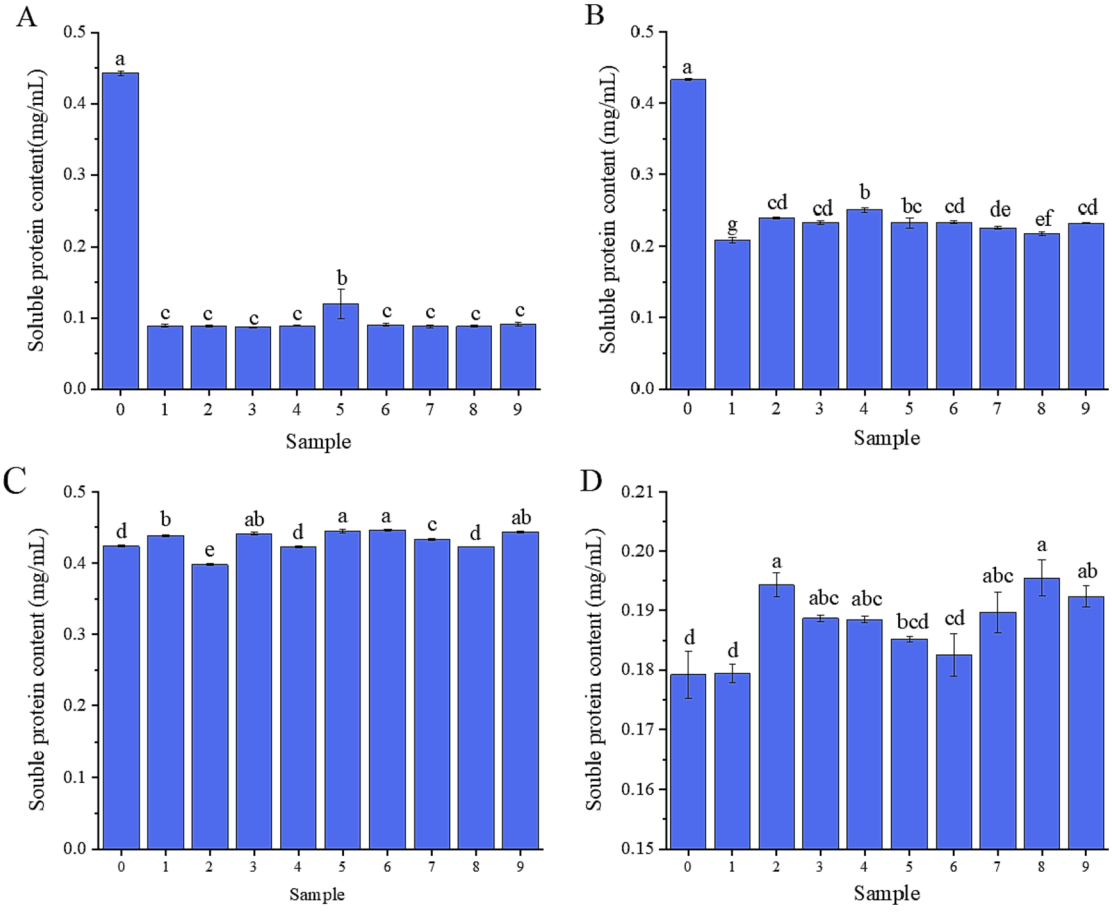

Fig. 2. Soluble protein content (A) Undigested; (B) Oral digestion; (C) After the gastric digestion; (D) After intestinal digestion; Sample 0–9 represented soymilk, *L. rhamnosus*-S, *S.thermophilus*-S, *L.casei*-S, *L.delbrueckii*-S, *L.paracasei*-S, *L.mesenteroides*-S, *L.plantarum*-S, *L.acidophilus*-S and *L.lactis*-S, respectively;Different letters indicated significant different ($P < 0.05$).

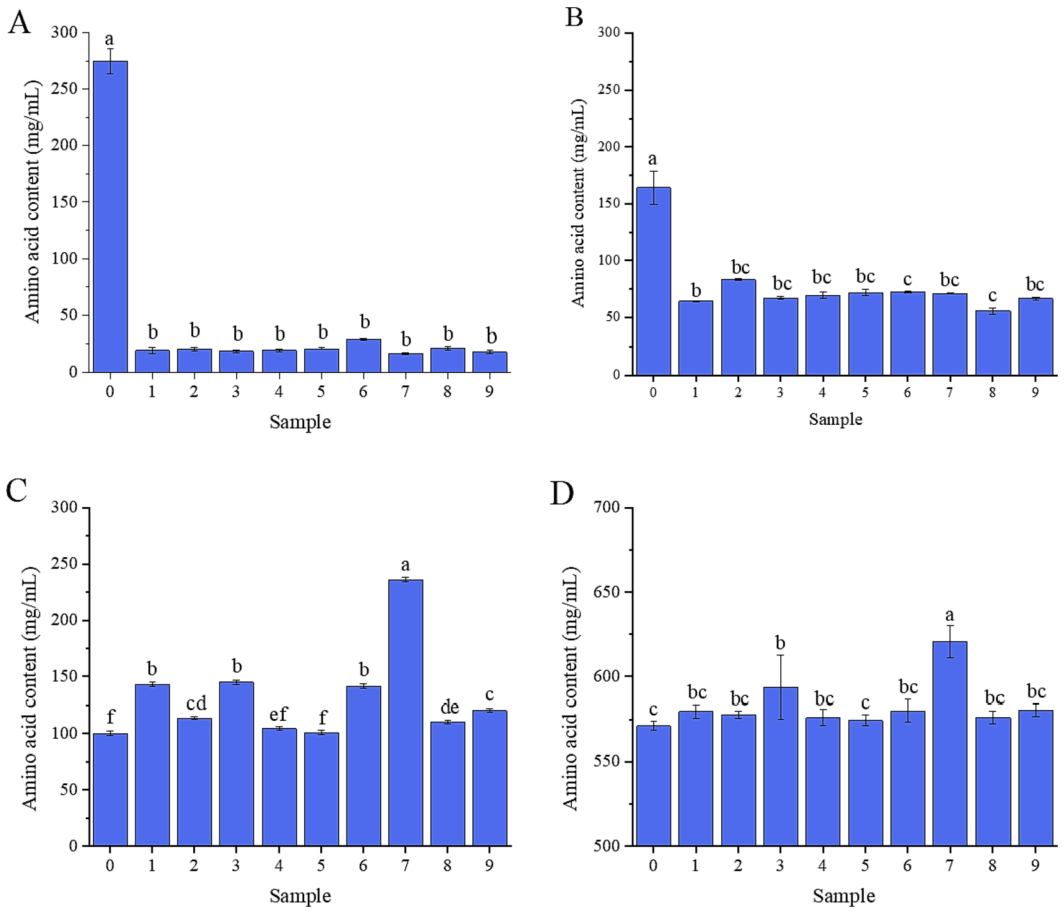

Fig. 3. FAA content (A) Undigested; (B) Oral digestion; (C) After the gastric digestion; (D) After intestinal digestion; Sample 0–9 represented soymilk, *L.rhamnosus*-S, *S.thermophilus*-S, *L.casei*-S, *L.delbrueckii*-S, *L.paracasei*-S, *L.mesenteroides*-S, *L.plantarum*-S, *L.acidophilus*-S and *L.lactis*-S, respectively;Different letters indicated significant different ($P < 0.05$).

The peptide content of fermented soymilk gradually increased, which was consistent with the change of FAA (Fig. 3), due to proteolysis during digestion. After digestion, the peptide content of fermented soymilk was significantly higher than unfermented soymilk ($P < 0.05$), indicating that LAB could degraded protein into short peptide. The peptide content of *S.thermophilus*-S was significantly lower than other samples. The results indicated that *S. thermophilus* had a poor ability to degrade protein, and the soluble protein content after digestion was still higher (Fig. 2). *L. delbrueckii*-S had the most peptide content and increased by 42.10 %.

3.4. Antioxidant capacity of soymilk fermented by LAB

Different antioxidant compounds may act through distinct mechanisms against oxidizing agents, consequently, one isolated method can hardly fully evaluate the antioxidant capacity of complex foods (Xiao et al., 2014). Table 2 showed DPPH scavenging rate and FARP of fermented soymilk. Soymilk has been observed to show more strong DPPH scavenging rate in undigested and orally digested process than fermented soymilk. Protein aggregation in fermented soymilk may hinder the release of active substances, resulting in the decrease of DPPH free radical scavenging ability (Chen Y, Capuano E, & Stieger M 2021). The phenomenon that antioxidant capacity reaches the maximum in gastric digestion and decreases in intestinal digestion is similar to the research of María Janeth, antioxidant activity may be related to the release of active substances, especially phenols (Rodríguez-Roque María Janeth, Rojas-Graü María Alejandra, Elez-Martínez Pedro, & Martín-Belloso Olga, 2013). It may enhance antioxidant capacity due to the low pH in the stomach is conducive to the release of phenols (Voss et al., 2020). What's more, the decrease in the antioxidant activity under intestinal conditions might be attributed to the fact that some substances with antioxidant activity, such as phenolic compounds, may be transformed into different structural forms with other chemical properties due to their sensitivity to higher pH values (Janeth et al., 2013).

After intestinal digestion, fermented soymilk had significantly higher antioxidant activity than soymilk ($P < 0.05$). Previous studies had shown that fermented soybean beverages hydrolyzed by protease showed stronger antioxidant activity than unfermented sample (Fernandes et al., 2017; Lee et al., 2018). The results showed LAB fermentation could enhance the antioxidant capacity of soymilk, which may be due to the increased content of total flavonoids and total phenols by LAB fermentation (Voss et al., 2020). *L.acidophilus*-S showed strong DPPH free radical scavenging ability which increased by 57.03 % and *L. rhamnosus*-S showed more strong FARP, which increased by 52.78 %.

4. Conclusion

In this study, the differences in characterization, stability, antioxidant capacity and *in vitro* digestion of fermented soymilk of different LAB strains were compared, and they were significantly different. The stability may be related to the acid-producing ability and metabolites of LAB and the aggregation degree of proteins. *L.paracasei*-S showed better stability. The decomposition of protein by LAB increased the content of FAA and peptides in fermented soymilk after digestion, *L.plantarum*-S had the highest FAA content, *L.delbrueckii*-S had the highest peptides content. All fermented soymilks exhibited the highest antioxidant capacity in gastric digestion (DPPH), LAB fermentation could enhance the

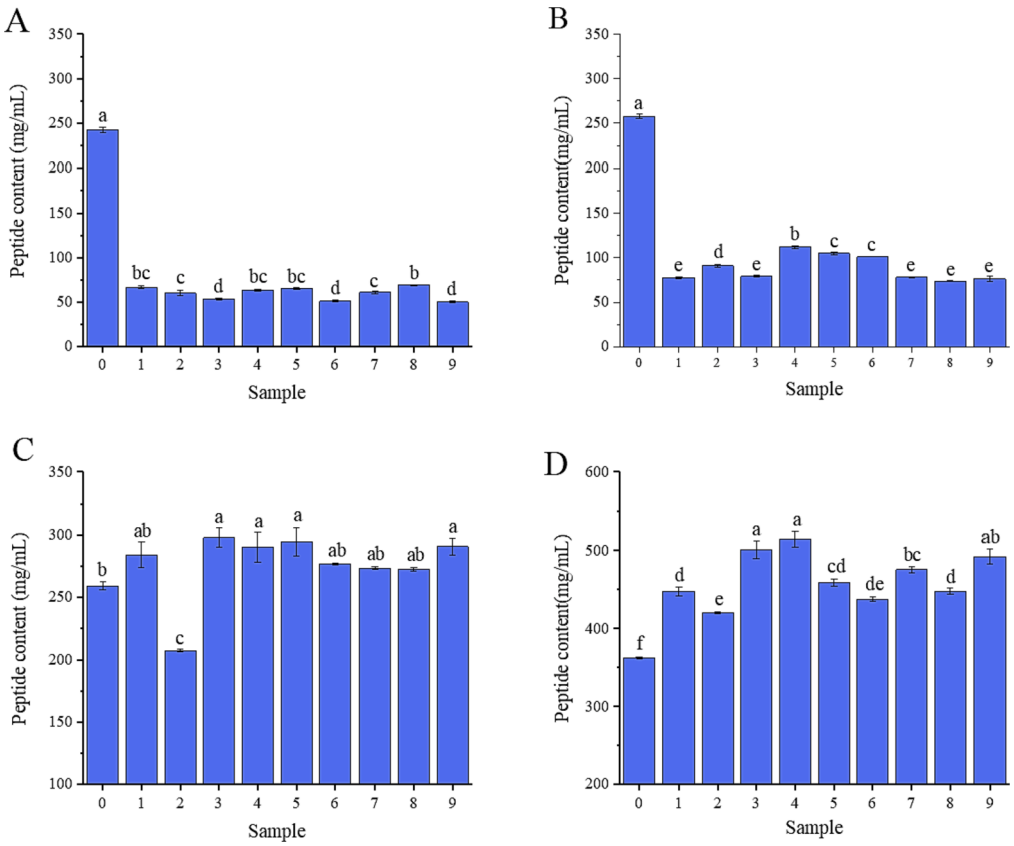

Fig. 4. Peptide content (A) Undigested; (B) Oral digestion; (C) After the gastric digestion; (D) After intestinal digestion; Sample 0–9 represented soymilk, *L.rhamnosus*-S, *S.thermophilus*-S, *L.casei*-S, *L.delbrueckii*-S, *L.paracasei*-S, *L.mesenteroides*-S, *L.plantarum*-S, *L.acidophilus*-S and *L.lactis*-S, respectively; Different letters indicated significant different ($P < 0.05$).

Table 2
Antioxidant capacity of Soymilk fermented by Lactic acid bacteria.

Sample	DPPH Scavenging Rate				FARP			
	Initial	Oral digestion	Gastric digestion	Intestinal digestion	Initial	Oral digestion	Gastric digestion	Intestinal digestion
Soymilk	46.96 ± 0.45^{a}	45.42 ± 0.16^{a}	81.90 ± 1.69^{bcd}	23.20 ± 0.85^{h}	0.45 ± 0.003^{b}	0.48 ± 0.006^{b}	0.42 ± 0.009^{cd}	0.36 ± 0.008^{d}
L.rhamnosus-S	41.87 ± 0.40^{c}	32.82 ± 0.28^{d}	68.67 ± 0.21^{e}	31.04 ± 0.21^{e}	0.38 ± 0.028^{c}	0.36 ± 0.015^{d}	0.44 ± 0.011^{bc}	0.55 ± 0.014^{a}
S.thermophilus-S	35.34 ± 0.43^{e}	27.61 ± 0.29^{f}	82.30 ± 1.72^{bc}	29.27 ± 0.21^{f}	0.65 ± 0.029^{a}	0.40 ± 0.010^{c}	0.41 ± 0.008^{de}	0.38 ± 0.009^{d}
L.casei-S	37.69 ± 0.16^{d}	41.87 ± 0.40^{b}	73.60 ± 0.57^{de}	32.59 ± 0.35^{bc}	0.34 ± 0.014^{c}	0.36 ± 0.013^{d}	0.39 ± 0.008^{f}	0.41 ± 0.009^{bc}
L.delbrueckii-S	38.95 ± 0.21^{d}	40.03 ± 0.14^{c}	72.97 ± 0.21^{e}	33.62 ± 0.35^{b}	0.38 ± 0.037^{c}	0.52 ± 0.012^{a}	0.40 ± 0.006^{ef}	0.38 ± 0.007^{cd}
L.paracasei-S	44.50 ± 0.24^{b}	38.89 ± 1.23^{c}	81.44 ± 0.28^{cd}	30.99 ± 0.43^{e}	0.33 ± 0.028^{c}	0.45 ± 0.014^{b}	0.42 ± 0.010^{cd}	0.43 ± 0.010^{b}
L.mesenteroides-S	40.84 ± 0.40^{c}	31.67 ± 0.21^{d}	74.40 ± 0.28^{cde}	32.65 ± 0.28^{bc}	0.35 ± 0.013^{c}	0.44 ± 0.011^{b}	0.46 ± 0.010^{a}	0.38 ± 0.012^{d}
L.plantarum-S	38.03 ± 0.21^{d}	28.47 ± 0.29^{ef}	83.51 ± 0.24^{b}	31.90 ± 0.43^{cd}	0.35 ± 0.019^{c}	0.37 ± 0.014^{d}	0.42 ± 0.009^{de}	0.41 ± 0.009^{bc}
L.acidophilus-S	38.20 ± 0.29^{d}	29.84 ± 0.08^{e}	96.16 ± 1.41^{a}	36.43 ± 0.37^{a}	0.36 ± 0.013^{c}	0.36 ± 0.016^{d}	0.44 ± 0.006^{b}	0.41 ± 0.010^{bc}
L.lactis-S	28.12 ± 0.81^{f}	21.19 ± 0.81^{g}	84.82 ± 6.52^{b}	26.86 ± 0.21^{g}	0.36 ± 0.013^{c}	0.33 ± 0.014^{d}	0.45 ± 0.007^{a}	0.37 ± 0.013^{d}

Note: Different letters in same column indicated significant different ($P < 0.05$).

antioxidant capacity of fermented soymilk. *L.acidophilus*-S and *L.rhamnosus*-S had the strongest antioxidant capacity. These results may be provided a theoretical basis for the strains screening of LAB-fermented soymilk and promote product innovation of fermented vegetable protein.

CRediT authorship contribution statement

Chunyan Huo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Writing – original draft. **Xiaoyu Yang:** Methodology, Visualization, Validation, Software, Writing – review & editing. **Liang Li:** Funding acquisition, Supervision, Project administration, Methodology, Visualization, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100578.

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