Journal of Agritechnology and Food Processing
Volume 5, issue 2 (December 2025)
ISSN 2809-3607

Effect of boiling time on protein content, total soluble solids, and sensory quality of probiotic soy milk (*Glycine max L.*)

Muhammad Faisal[1]*, Hafasa[1], Siti Malika Azizatul Fitri[1], Maharani Kusumaningtyas[1]
[1]Department of Agricultural Product Technology, Faculty of Agricultural Science and Technology, Sumbawa University of Technology, Indonesia
*corresponding author: muhammad.faisal@uts.ac.id

ABSTRACT

This study aimed to analyze the effect of different boiling time on protein content, total soluble solids (TSS), and sensory characteristics, including taste and aroma, of probiotic soy milk (*Glycine max L.*). The experiment was conducted using a Completely Randomized Design (CRD) consisting of four boiling time treatments of 5, 10, 15, and 20 minutes, each replicated three times, resulting in a total of 18 experimental units. The observed parameters included protein content, total soluble solids (TSS), and sensory evaluation performed by trained panelists using a hedonic test (five-point scale). Scores ranged from 1 ("dislike extremely") to 5 ("like extremely"), where higher values indicated greater sensory preference. The collected data were statistically analyzed using Analysis of Variance (ANOVA), and when a significant difference was observed ($p < 0.05$), the analysis was continued with Duncan's Multiple Range Test (DMRT) at a 5% confidence level. Data processing was performed using SPSS version 25. The results showed that boiling time had a significant effect on protein content, total soluble solids, and hedonic scores for taste and aroma attributes. The 15-minute boiling treatment produced the highest protein content (3.11%), optimal TSS value (8.9 °Brix), and the highest acceptance scores for both taste and aroma. Therefore, boiling at 95–100°C for 15 minutes can be considered the optimal condition to produce probiotic soy milk with the best balance between protein stability, total soluble solids concentration, and sensory quality.

Keywords: boiling time; sensory quality; probiotic soy milk; protein content; total soluble solids

INTRODUCTION

Soy milk is a plant-based beverage that has gained increasing popularity due to its high-quality plant protein, lactose-free composition, and the presence of bioactive compounds such as isoflavones, which play an important role in maintaining human health (Han et al., 2021). In addition to its nutritional benefits, soy milk is also considered more environmentally friendly than cow's milk, as its production process requires fewer natural resources and generates lower greenhouse gas emissions (Coluccia et al., 2022; Geburt et al., 2022). The growing public awareness of healthy eating habits and functional foods has further driven the demand for soy-based products, including probiotic fermented soy milk (Bisson et al., 2025; Granato et al., 2010; Kumari et al., 2022; Valero-Cases et al., 2020).

However, consumer acceptance of soy milk remains limited due to its distinct sensory characteristics, particularly the beany flavor and aroma, which are disliked by many consumers. This undesirable aroma is primarily caused by the activity of lipoxygenase, an enzyme that oxidizes unsaturated fatty acids and produces volatile compounds responsible for the unpleasant odor (G. Ji et al., 2022; Yang et al., 2016). Moreover, the presence of antinutritional compounds such as trypsin inhibitors, tannins, and phytic acid can reduce protein digestibility and impart a bitter taste to the product (Samtiya et al., 2020). Therefore, an appropriate processing method is required to inactivate the enzymes responsible for the beany aroma and to reduce antinutrient levels without damaging the main nutritional components.

One of the most common methods used to improve the quality of soy milk is the boiling process. This thermal treatment serves to inactivate lipoxygenase and reduce antinutrient content, while also improving microbiological stability and extending the product's shelf life (Guerrero-Beltránet al., 2009; Yan et al., 2024). Furthermore, boiling affects the viscosity, color, and flavor of soy milk (Liu et al., 2023). However, excessive boiling duration can lead to protein denaturation and trigger the Maillard reaction, resulting in reduced protein content, color changes, and the development of burnt flavor notes. Therefore, determining the optimal boiling duration is crucial to maintaining the balance between nutritional quality and sensory characteristics of the product.

In addition to thermal treatment, the application of fermentation technology through the incorporation of probiotic cultures represents a promising innovation to enhance the functional value and sensory quality of soy milk. Fermentation using probiotic bacteria such as *Lactobacillus plantarum* and *Bifidobacterium bifidum* has been reported to increase antioxidant activity, reduce the beany flavor, and improve the bioavailability of isoflavones through the conversion of glycoside compounds into aglycones(Delgado et al., 2019; Rani & Pradeep, 2017). The fermentation process also generates volatile compounds such as lactic acid, diacetyl, and acetoin, which contribute to more desirable aroma and flavor characteristics (Coolbear et al., 2022; Tian et al., 2019).

Beyond improving sensory properties, probiotic fermentation also influences the physicochemical characteristics of soy milk, including pH, total soluble solids (TSS), and viscosity. The viable population of *L. plantarum* can reach 8–9 log CFU/mL after optimal fermentation, indicating the strong potential of this product as a functional food (Hasan et al., 2023; Santos et al., 2019). However, the success of fermentation is strongly influenced by the initial condition of the raw material, particularly the effects of prior heat treatment. Excessive boiling may reduce the availability of substrates and nutrients required for probiotic growth, thereby inhibiting their proliferation (Upadhyaya, 2024; Yerlikaya, 2014). Therefore, optimization of boiling temperature and duration is necessary to achieve a balance between enzyme inactivation, protein stability, and substrate availability for probiotic development (Anjum et al., 2022).

Several studies have also reported that the addition of prebiotics such as inulin or fructooligosaccharides (FOS) can enhance probiotic viability during storage while improving the texture and viscosity of soy milk (Santos et al., 2019; X. Zhang et al., 2020). Physicochemical factors such as total soluble solids, pH, and protein composition play a crucial role in determining consumer acceptability of probiotic soy milk products (Hasan et al., 2023; Liu et al., 2023). Total soluble solids reflect the dry matter content in soy milk, which is directly associated with texture, viscosity, and emulsion stability (Z. Sun et al., 2022).

The close relationship between thermal treatment and the fermentation process makes boiling a key factor in determining the final quality of probiotic soy milk. This process should effectively inactivate enzymes responsible for off-flavors, reduce antinutrient levels, maintain protein stability, and preserve optimal conditions for probiotic growth. Previous studies have shown that the combination of appropriate boiling and probiotic fermentation can produce soy milk with improved flavor, higher protein content, and enhanced antioxidant activity (Kwok et al., 2002; Sebastian, 2018)

Based on this background, the present study aimed to analyze the effect of boiling duration on protein content, total soluble solids, and organoleptic quality (taste and aroma) of probiotic soy milk. This research is expected to contribute to a better understanding of the relationship between thermal treatment and both the nutritional and sensory quality of probiotic soy milk.

METHODOLOGY

Materials and Equipment

The materials used in this study included soybeans, palm sugar, and probiotic cultures as the main ingredients. In addition, several analytical-grade chemicals were utilized, including concentrated sulfuric acid (H_2SO_4, 98%), potassium sulfate (K_2SO_4), copper sulfate ($CuSO_4$), titanium dioxide (TiO_2), sodium hydroxide (NaOH, 40%), boric acid (H_3BO_3, 4%), hydrochloric acid (HCl, 0.1 N) or sulfuric acid (H_2SO_4, 0.1 N), bromocresol green (BCG, 0.1%), methyl red (MR, 0.1%), and distilled water. Additional materials included antifoam or paraffin, lint-free tissue, mineral water, and supporting materials such as sample label papers, hedonic test evaluation forms, tissues, and small plastic cups. The equipment used in this study included a pan, gas stove, blender, sieve, storage bottles, spectrometer, thermometer, basin, analytical balance, Kjeldahl digestion flask, digestion block, Kjeldahl distillation apparatus, 250 mL Erlenmeyer flask, 50 mL burette, volumetric and graduated pipettes, measuring cylinders, volumetric flasks, glass funnels, condenser, clamps and racks, 400°C thermometer, safety equipment, refractometer, measuring cups (100–250 mL), stirring spoons, funnels, and trays used to carry samples to the panelist table. Additional equipment included evaluation sheets, stationery, chairs, and panelist tables.

Experimental Design

This study employed a Completely Randomized Design (CRD) with a single factor, namely boiling duration. The treatments consisted of four levels: P1 (15 minutes of boiling), P2 (20 minutes of boiling), P3 (25 minutes of boiling), and P4 (30 minutes of boiling). Each treatment was replicated three times, resulting in a total of 12 experimental units.

Data analysis

The collected data were statistically analyzed using Analysis of Variance (ANOVA) at a 5% significance level. When significant differences among treatments were observed, Duncan's Multiple Range Test (DMRT) was applied to determine which treatment had the most significant effect. All statistical analyses were performed using SPSS software version 25.

Research Stages

The research was carried out in several stages, including preparation, processing, fermentation(Fathurohman et al., 2020; Sebastian, 2018), and analysis. The preparation stage involved cleaning and soaking soybeans to obtain high-quality raw materials. In the processing stage, soybeans were blended with water at a ratio of 2:1, filtered to remove the pulp, then the filtrate was boiled gradually from 50°C until it reached 100°C. The soy milk was then reheated at 60°C, followed by the addition of 8% palm sugar as a sweetener and carbon source. In the fermentation stage, the cooled soy milk was inoculated with 1 g of Lactobacillus sp. per 100 mL of soy milk (1% w/v). The inoculated soy milk was incubated at room temperature (approximately 28 ± 2°C) for three days to allow optimal probiotic growth. After fermentation, the product was cooled and stored at 2.7°C to maintain its quality and probiotic viability during storage. Similar incubation at ambient temperature has been reported to support the growth and metabolic activity of *Lactobacillus* spp. in soy-based beverages while maintaining acceptable sensory and physicochemical characteristics (Carneiro et al., 2022; Ruiz de la Bastida et al., 2023). The final stage involved analyzing the parameters of protein content(AOAC International, 2019), total soluble solids (TSS) (Mahmoud et al., 2023), and sensory properties(Fathurohman et al., 2020), including aroma and taste.

RESULTS AND DISCUSSION

Protein Content Analysis

Figure 1. provides information on the relationship between protein content and boiling time of probiotic soy milk. The results indicate that the protein content of probiotic soy milk decreased with increasing boiling time. The 15-minute boiling treatment yielded the highest protein

content (3.11%), while boiling for 30 minutes reduced it to 2.31%. This trend shows that proteins lose their secondary and tertiary structures when they are heated too much, which makes them less soluble in the liquid phase. Protein denaturation occurs due to the disruption of hydrogen bonds and the weakening of hydrophobic interactions that maintain the native conformation of proteins (X. Zhang et al., 2012).

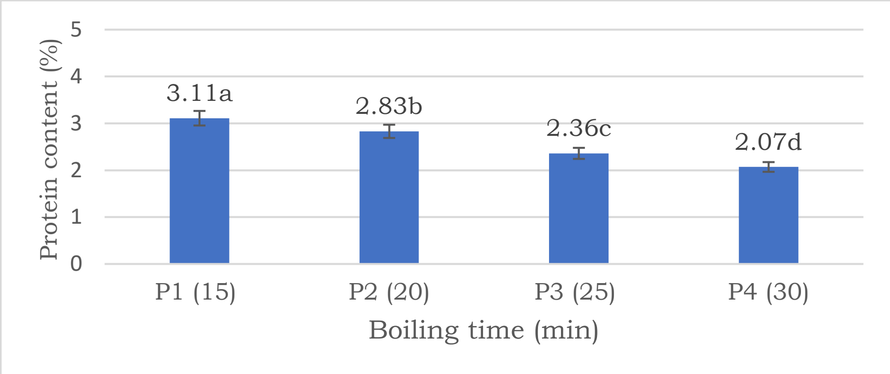

Figure 1. Relationship between protein content and boiling duration of probiotic soy milk. Different superscript letters indicate significant differences among treatments according to Duncan's Multiple Range Test (DMRT) at a 5% significance level.

The main storage proteins in soybeans, glycinin (11S) and β-conglycinin (7S), are globulin fractions most susceptible to heat treatment. Studies have shown that β-conglycinin denatures at approximately 70–75°C, while glycinin denatures at around 90–95°C. Heating beyond these thresholds leads to the unfolding of protein conformations, promoting aggregation and precipitation that reduce protein solubility (Y. Ji et al., 2024). Furthermore, during the boiling process, a Maillard reaction occurs between free amino groups (such as lysine and arginine) and reducing sugars, forming protein–carbohydrate complexes. This reaction decreases the measurable nitrogen content and reduces the biological value of the protein by lowering the availability of essential amino acids (X. Zhang et al., 2012).

Moderate heat treatment (95–100°C for 10–15 minutes) has been shown to preserve soluble protein fractions while creating an ideal substrate for the growth of lactic acid bacteria, particularly Lactobacillus casei and Lactiplantibacillus plantarum. Partially denatured proteins can even enhance substrate accessibility to probiotic proteases, thereby accelerating protein hydrolysis into bioactive peptides with antioxidant properties and improving the digestibility of soy milk (Letizia et al., 2023; Y. Sun et al., 2023; Wang et al., 2013).

However, excessive thermal treatment (longer than 20–25 minutes) tends to accelerate irreversible denaturation and protein coagulation. Overheating induces the formation of new intermolecular disulfide bonds, resulting in protein cross-linking and aggregation, which decrease solubility and reduce the concentration of soluble proteins (Harper et al., 2022).

In addition to thermal effects, probiotic fermentation also influences protein stability and availability. The pH decrease during fermentation (reaching 4.5–5.0) leads to the precipitation of globulin proteins, while the enzymatic activity of lactic acid bacteria promotes partial hydrolysis, producing bioactive peptides. Thus, the interaction between heating and fermentation plays a critical role in maintaining the balance between protein denaturation and hydrolysis, which ultimately determines the nutritional and functional quality of probiotic soy milk (Liu et al., 2023; Mendoza-Avendaño et al., 2018)

Protein content also exhibited a positive correlation with total soluble solids (TSS), since most solid fractions are composed of proteins, carbohydrates, and lipids. Boiling for 15–20 minutes increased TSS due to the release of soluble proteins into the liquid phase, whereas prolonged heating caused a decline in TSS as a result of protein precipitation. These findings suggest that moderate heating conditions provide an optimal balance between protein stability and solubility (Sarić et al., 2019).

From a sensory perspective, partially denatured proteins contribute to the formation of volatile compounds such as aldehydes, ketones, and alcohols, which impart characteristic aroma notes to fermented soy milk. Recent studies have reported that moderate heat treatment combined with fermentation using Lactiplantibacillus plantarum enhances protein stability, solubility, antioxidant activity, and sensory quality of the final product (Aziz et al., 2023; Y. Li et al., 2025).

In conclusion, boiling at moderate temperatures (95–100°C) for 15–20 minutes represents the optimal condition for achieving a balance between protein structural stability, solubility, bioactive potential, and sensory quality in probiotic soy milk. The synergistic interaction between heat treatment and probiotic fermentation effectively maintains nutritional value, enhances functional properties, and improves consumer acceptability of fermented soy-based products.

Physicochemical Analysis (Total Soluble Solids)

Total soluble solids (TSS) represent a key parameter reflecting the concentration of dissolved components in soy milk, including protein, carbohydrate, lipid, and mineral fractions. As shown in Figure 2, the TSS values of probiotic soy milk range from 8.1°Brix to 9.8°Brix. Boiling treatment for 15 minutes resulted in a TSS of 8.9°Brix, while 30 minutes of boiling produced the highest value of 9.8°Brix. A decrease was observed between the boiling times of 15 and 25 minutes, followed by a subsequent increase at 30 minutes. This phenomenon can be attributed to water evaporation and the resulting increase in the concentration of soluble components, particularly protein and carbohydrate fractions (Nanakali et al., 2023).

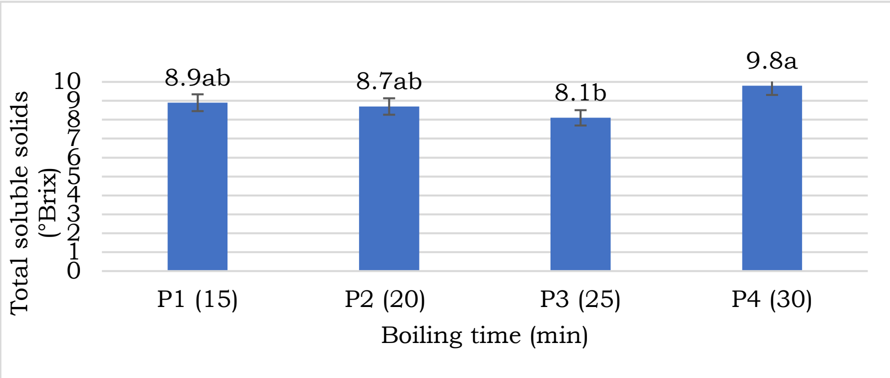

Figure 2. Relationship between total soluble solids (°Brix) and boiling duration of probiotic soy milk. Different superscript letters indicate significant differences among treatments based on Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

Heating plays an essential role in dispersing proteins and polysaccharides within the colloidal system, thereby enhancing solubility and solution viscosity. Heating up to 100°C can induce partial denaturation and dispersion of globulin proteins into the liquid phase, contributing to the increase in total soluble solids (Huo et al., 2023). This phenomenon aligns with findings indicating that moderate heating promotes the release of fine protein fractions and soluble fibers, enriching the total solids and improving emulsion stability (Letizia et al., 2023)

Furthermore, the increase in TSS during prolonged heating (25–30 minutes) may also result from the Maillard reaction between amino groups and reducing sugars. This reaction forms soluble melanoidin compounds that contribute to the measured total solids in the product (Xiang et al., 2021). However, excessive heating can lead to protein aggregation and a reduction in soluble protein content due to the formation of intermolecular cross-links between polypeptide chains (Z. Zhang et al., 2025).

The relationship between protein content and TSS is strongly correlated, as protein and carbohydrate fractions account for approximately 70–80% of the total solids in soy milk. Partial protein denaturation can enhance solubility and colloidal stability, whereas irreversible denaturation reduces the soluble fraction. Studies have shown that moderate heat treatment achieves an optimal balance between TSS enhancement and protein stability (Rana et al., 2025).

From a physicochemical perspective, TSS is also positively correlated with viscosity and emulsion stability. Higher soluble solids strengthen interparticle forces that prevent phase separation and reinforce colloidal structure (Sarić et al., 2019). Increased TSS contributes to a thicker texture and a "creamy" mouthfeel, directly influencing sensory perception and consumer acceptance of probiotic soy milk (Huo et al., 2023).

In addition to heating, probiotic fermentation also plays a crucial role in increasing TSS values. Fermentation breaks down protein and carbohydrate molecules into peptides and simple sugars, thereby enhancing the soluble fraction and viscosity of the product (Rana et al., 2024). Fermentation by *Lactiplantibacillus plantarum* has been shown to increase TSS by up to 10% while maintaining physical stability and sensory quality (Letizia et al., 2023).

Moreover, synergistic fermentation between *L. plantarum* and *L. acidophilus* can enhance protease enzymatic activity, accelerate protein hydrolysis, and improve solubility and viscosity in probiotic soy (Zhou et al., 2022). These findings suggest that fermentation following moderate heating produces a synergistic effect, optimizing both total solids content and emulsion stability.

From a sensory standpoint, increased TSS contributes to a smoother and fuller texture. Moderate to high TSS values (8.5–10°Brix) are considered ideal, as they yield a soft, non-watery texture associated with premium product quality. Recent studies have demonstrated that higher total solids content correlates positively with panelists' preference scores for fermented soy-based products (Sarić et al., 2019).

In conclusion, the duration of heating is directly proportional to TSS increase up to an optimum point of approximately 30 minutes, after which no significant improvement occurs. Moderate heating conditions (90 – 100°C for 15–20 minutes) followed by probiotic fermentation provide the best balance between soluble fraction enhancement, emulsion stability, and sensory quality. Under these conditions, elevated TSS values can serve as a reliable indicator of successful thermal and fermentative processing in producing high-quality probiotic soy milk.

Sensory Evaluation (Aroma)

Aroma is one of the primary sensory attributes that determines consumer acceptance of probiotic soy milk. Figure 3 shows that boiling for 20 minutes resulted in a slightly higher mean aroma score than the 15-minute treatment, suggesting a modest improvement in aroma intensity with longer boiling time. This finding indicates that moderate heating plays an important role in reducing the characteristic beany odor of soybeans while preserving desirable volatile compounds. Previous studies have reported that aroma preference in soy milk is strongly influenced by the stability and composition of volatile compounds formed during heating and fermentation processes (Zong et al., 2025).

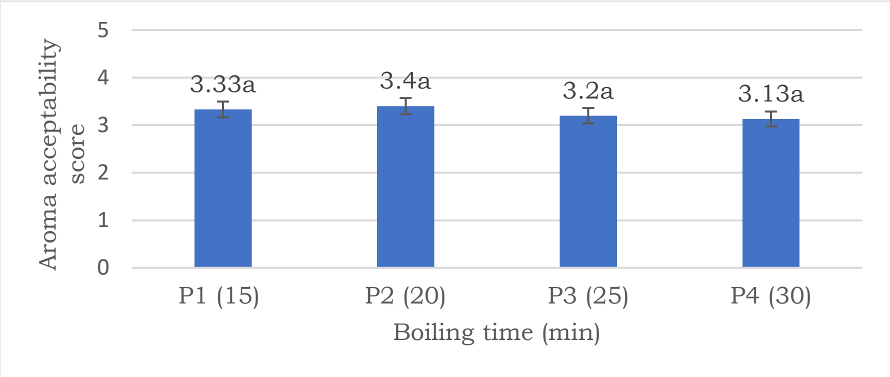

Figure 3. Relationship between aroma score and boiling duration of probiotic soy milk. Different superscript letters indicate no significant differences among treatments ($p > 0.05$).

The beany flavor of soy milk primarily originates from the activity of the lipoxygenase (LOX) enzyme, which catalyzes the oxidation of unsaturated fatty acids into short-chain aldehydes and alcohols, such as hexanal and pentanal. Heating at 95–100°C for 10–20 minutes effectively inactivates the LOX enzyme without causing excessive degradation of desirable volatile compounds (X. Li et al., 2024). This thermal treatment decreases aldehydes responsible for the beany aroma while maintaining desirable volatiles such as acetoin and 2,3-butanedione, which contribute to a creamy and smooth (Dan et al., 2019).

In addition to enzymatic inactivation, the Maillard reaction also contributes to the formation of characteristic soy milk aromas. This reaction occurs between reducing sugars and amino acids during boiling, producing volatile compounds such as furfural, maltol, and pyrazines, which impart a mild roasted aroma and enhance the aromatic complexity of the product (Z. Zhang et al., 2025a). However, prolonged heating can lead to further degradation of these compounds, resulting in the formation of long-chain aldehydes such as nonanal and decanal, which are associated with burnt and bitter notes (X. Li et al., 2024).

Probiotic fermentation also plays an essential role in the development and improvement of soy milk aroma. The metabolic activity of *Lactobacillus plantarum* generates various volatile compounds, including lactic acid, acetic acid, acetoin, and esters, which impart a fresh, slightly acidic aroma while masking the beany off-flavor of soybeans (Q. Sun et al., 2025). Fermentation by *L. plantarum* strains has been shown to reduce the concentrations of aldehydes such as hexanal and heptanal by 60–80%, which are the main compounds responsible for beany flavor (Zhou et al., 2022).

Other studies reported that combining fermentation with *L. plantarum* and yogurt cultures (*S. thermophilus* and *L. bulgaricus*) produced the most balanced aroma profile, characterized by increased acetoin content and reduced undesirable C6 aldehydes (Hidayati et al., 2021). Volatile compounds such as diacetyl, acetoin, and 2,3-butanedione contribute to creamy, sweet, and slightly acidic aromas that enhance the freshness of the final product (Zong et al., 2022).

Moreover, the interaction between the Maillard reaction and lactic acid fermentation further enriches the aroma profile of probiotic soy milk. Metabolic activity of lactic acid bacteria helps stabilize heterocyclic compounds produced through the Maillard reaction, maintaining pleasant roasted and sweet notes during storage (Z. Zhang et al., 2025b). This synergy results in an optimal aroma balance, characterized by reduced beany intensity and enhanced creamy and neutral notes preferred by panelists (Q. Sun et al., 2025).

In addition to aroma evaluation, the panelists also assessed the overall acceptability of the probiotic soy milk samples using a five-point hedonic scale, where 1 represented “dislike extremely” and 5 represented “like extremely.” This scoring system is commonly used in sensory evaluation to measure consumer preferences for specific sensory attributes such as aroma, flavor, texture, and color. A higher acceptability score indicates greater consumer preference and sensory satisfaction. The maximum score that could be given by the panelists was 5, indicating that the sample was highly liked by the evaluators. The use of hedonic scales for consumer acceptance testing in soy milk and soy-based beverages has been widely reported in previous studies (Ju et al., 2021; L. Caluza, 2019).

Overall, moderate thermal treatment (15–20 minutes at 95–100°C) followed by probiotic fermentation with *L. plantarum* provides an ideal balance between reducing beany off-flavors, forming desirable volatile compounds, and improving sensory preference. This condition promotes the development of a complex yet mild probiotic soy milk aroma characterized by creamy, slightly roasted, and neutral notes that are most favored by consumers.

Sensory Evaluation (Taste)

As shown in Figure 4, the panelists’ preference for the taste of probiotic soy milk exhibited a fluctuating pattern with increasing boiling duration. The highest mean taste acceptability score was observed in the 20-minute treatment (3.4a), followed by 30 minutes (3.1ab), 15 minutes (2.9b), and the lowest at 25 minutes (2.26c). This fluctuating trend indicates that boiling time differently affects the formation and degradation of flavor-contributing compounds, both in terms of chemical and biochemical components produced during fermentation.

The increase in taste acceptability from 15 to 20 minutes of boiling suggests that moderate heating provides an optimal balance between partial protein denaturation and an increase in total soluble solids (TSS). Moderate protein denaturation at 95–100°C for 15–20 minutes promotes the breakdown of complex protein structures into short peptides and free amino acids, which contribute to the development of natural sweetness and umami flavor (Z. Zhang et al., 2025b). Furthermore, the increase in TSS enhances viscosity perception (body) and improves overall flavor balance preferred by the panelists (P. Zhang et al., 2022).

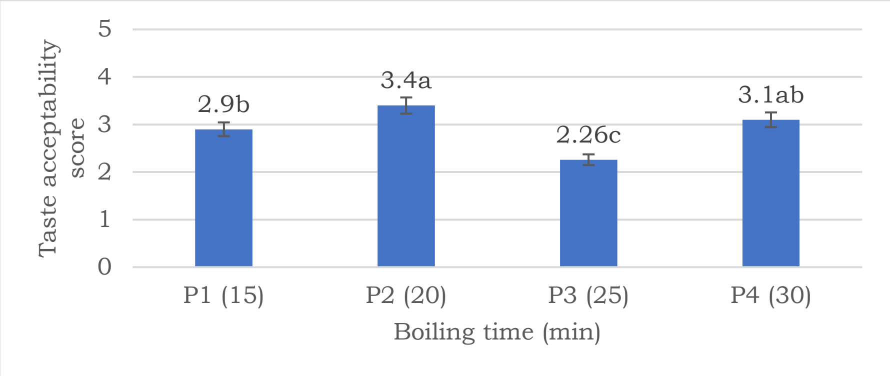

Figure 4. Relationship between taste score and boiling duration of probiotic soy milk. Identical superscript letters indicate significant differences among treatments ($p < 0.05$).

At this stage, reducing sugars and soluble proteins interact through mild Maillard reactions, forming volatile compounds such as furfural, maltol, and pyrazines that impart a subtle caramel-like aroma and pleasant sweet-savory taste, thereby increasing product acceptability (Long et al., 2025; Z. Zhang et al., 2025a).

The significant decrease in taste acceptability observed at 25 minutes indicates the negative impact of excessive heating on flavor-forming components. Prolonged heating leads to the degradation of proteins into bitter-tasting compounds and the oxidation of unsaturated fatty acids, producing aldehydes such as hexanal and nonanal, which are responsible for beany and bitter notes (Zhou et al., 2022). Additionally, excessive thermal treatment decreases the content of reducing sugars and changes the viscosity of the system, which leads to a loss of flavor freshness and sensory balance (Cui et al., 2019).

The subsequent increase in taste scores at 30 minutes suggests that extended boiling may partially remove volatile off-flavor compounds through evaporation while generating small amounts of positive volatiles, such as furfural and maltol, from advanced Maillard reactions, contributing to mild caramel and lightly roasted notes (Zong et al., 2025).

Probiotic fermentation following heat treatment also plays a crucial role in improving the overall flavor profile. The metabolic activity of *Lactobacillus plantarum* produces organic acids, acetoin, and volatile esters that create a balance between sourness, sweetness, and umami taste (Tangyu et al., 2023). Fermentation also breaks down proteins into umami and kokumi peptides, reinforcing a soft and complex flavor perception that enhances taste satisfaction (Z. Zhang et al., 2025b).

In addition to the aroma assessment, the panelists also evaluated the taste acceptability of probiotic soy milk using a five-point hedonic scale, where 1 represented “dislike extremely” and 5 represented “like extremely.” This scale is widely used in sensory evaluation studies to quantify consumer preference for specific sensory attributes such as taste, aroma, texture, and appearance (Ju et al., 2021; L. Caluza, 2019). A higher taste acceptability score indicates greater consumer liking and sensory satisfaction. The maximum score that could be given by the panelists was 5, representing samples that were highly liked by the evaluators.

The changing pattern of taste acceptability scores shows that the best boiling time is between 15 and 20 minutes, when flavor-forming reactions happen in balance without too much protein breakdown or lipid oxidation. At longer durations (>25 minutes), this balance shifts toward protein breakdown and the formation of bitter-tasting compounds, leading to decreased panelist preference. However, subsequent probiotic fermentation compensates for these negative effects by generating umami peptides and organic acids that restore flavor harmony [(Zhou et al., 2022). Thus, the optimal flavor formation in probiotic soy milk occurs at moderate boiling durations (15–20 minutes) followed by active probiotic fermentation. This condition provides an ideal balance among positive volatile compounds, smooth texture, and naturally sweet–savory taste, resulting in the most preferred sensory profile among panelists, consistent with findings from other plant-based fermented milk studies (Tangyu et al., 2023; Zong et al., 2025)

CONCLUSION

Boiling time had a significant effect on the protein content, total soluble solids (TSS), and organoleptic quality (taste and aroma) of probiotic soy milk. Boiling for 15 minutes at 95–100°C produced the best results, yielding the highest protein and TSS values, as well as the highest acceptability scores in the sensory evaluation. Extending the boiling time beyond 20 minutes led to a decrease in protein content due to excessive denaturation and a reduction in sensory quality caused by the formation of undesirable volatile compounds. Therefore, moderate boiling (15 minutes) is recommended as the optimal condition for producing probiotic soy milk with the best balance between nutritional value and flavor quality.

For future research, it is recommended to investigate the viability of probiotic bacteria following the boiling and fermentation processes to better understand the impact of thermal treatment on microbial survival. Further studies should also analyze the relationship between microbiological stability and sensory attributes, including flavor and aroma, to optimize both the functional and sensory quality of probiotic soy-based beverages. In addition, integrating molecular approaches such as proteomic or metabolomic profiling may offer more details about protein denaturation, peptide formation, and volatile compound dynamics during processing. These analyses could support the development of a more stable, nutritionally enhanced, and consumer-acceptable probiotic soy milk product.

REFERENCES

Anjum, F. M., Saeed, F., Afzaal, M., Ikram, A., & Azam, M. (2022). The effect of thermal processing on probiotics stability. In *Advances in Dairy Microbial Products* (pp. 295–302). Elsevier. https://doi.org/10.1016/B978-0-323-85793-2.00004-7

AOAC International. (2019). Official Methods of Analysis of AOAC International (21st ed.). . *Method 979.09: Nitrogen (Total) in Milk — Kjeldahl Method. Retrieved from Https://Www.Aoac.Org/Official-Methods-of-Analysis/*.

Aziz, T., Xingyu, H., Sarwar, A., Naveed, M., Shabbir, M. A., Khan, A. A., Ulhaq, T., Shahzad, M., Zhennai, Y., Shami, A., Sameeh, M. Y., Alshareef, S. A., Tashkandi, M. A., & Jalal, R. S. (2023). Assessing the probiotic potential, antioxidant, and antibacterial activities of oat and soy milk fermented with Lactiplantibacillus plantarum strains isolated from Tibetan Kefir. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1265188

Bisson, G., Comuzzi, C., FitzGerald, J. A., Mukherjee, A., Renoldi, N., Innocente, N., Beresford, T., Mathur, H., Cotter, P. D., & Marino, M. (2025). Development of a bio-functional fermented soy beverage supplemented with microbial exopolysaccharides and its effect on the human gut microbiome *in vitro*. *Food & Function*, *16*(15), 6203–6212. https://doi.org/10.1039/D5FO01288K

Carneiro, M. da S., Ramos, G. L. de P. A., Silva, M. C., & Walter, E. H. M. (2022). Processing of soy beverages obtained from the grain, flour and powder extract and fermented by probiotics. *Food Science and Technology*, *42*. https://doi.org/10.1590/fst.79322

Coluccia, B., Agnusdei, G. P., De Leo, F., Vecchio, Y., La Fata, C. M., & Miglietta, P. P. (2022). Assessing the carbon footprint across the supply chain: Cow milk vs soy drink. *Science of The Total Environment*, *806*, 151200. https://doi.org/10.1016/j.scitotenv.2021.151200

Coolbear, T., Wilkinson, M. G., & Weimer, B. (2022). Lactic Acid Bacteria in Flavor Development. In *Encyclopedia of Dairy Sciences* (pp. 181–186). Elsevier. https://doi.org/10.1016/B978-0-12-818766-1.00019-2

Cui, S., Zhao, N., Lu, W., Zhao, F., Zheng, S., Wang, W., & Chen, W. (2019). Effect of different

Lactobacillus species on volatile and nonvolatile flavor compounds in juices fermentation. *Food Science & Nutrition*, *7*(7), 2214–2223. https://doi.org/10.1002/fsn3.1010

Dan, T., Chen, H., Li, T., Tian, J., Ren, W., Zhang, H., & Sun, T. (2019). Influence of Lactobacillus plantarum P-8 on Fermented Milk Flavor and Storage Stability. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.03133

Delgado, S., Guadamuro, L., Flórez, A. B., Vázquez, L., & Mayo, B. (2019). Fermentation of commercial soy beverages with lactobacilli and bifidobacteria strains featuring high β-glucosidase activity. *Innovative Food Science & Emerging Technologies*, *51*, 148–155. https://doi.org/10.1016/j.ifset.2018.03.018

Fathurohman, M., Aprillia, A. Y., Pratita, A. T. K., & Tenderly, V. F. (2020). Diversifikasi Produksi Susu Kedelai Berbasis Mikroalga Autotrofik Guna Meningkatkan Indeks Nutraseutikal. *Jurnal Aplikasi Teknologi Pangan*, *9*(2), 70–76. https://doi.org/10.17728/jatp.6150

Geburt, K., Albrecht, E. H., Pointke, M., Pawelzik, E., Gerken, M., & Traulsen, I. (2022). A Comparative Analysis of Plant-Based Milk Alternatives Part 2: Environmental Impacts. *Sustainability*, *14*(14), 8424. https://doi.org/10.3390/su14148424

Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G., & Faria, J. A. F. (2010). Functional Foods and Nondairy Probiotic Food Development: Trends, Concepts, and Products. *Comprehensive Reviews in Food Science and Food Safety*, *9*(3), 292–302. https://doi.org/10.1111/j.1541-4337.2010.00110.x

Guerrero-Beltrán, J. A., Estrada-Girón, Y., Swanson, B. G., & Barbosa-Cánovas, G. V. (2009). Inactivation Kinetics of Lipoxygenase in Pressurized Raw Soymilk and Soymilk From High-Pressure Treated Soybeans. *Journal of Food Processing and Preservation*, *33*(2), 143–158. https://doi.org/10.1111/j.1745-4549.2008.00234.x

Han, H., Choi, J. K., Park, J., Im, H. C., Han, J. H., Huh, M. H., & Lee, Y.-B. (2021). Recent innovations in processing technologies for improvement of nutritional quality of soymilk. *CyTA - Journal of Food*, *19*(1), 287–303. https://doi.org/10.1080/19476337.2021.1893824

Harper, A. R., Dobson, R. C. J., Morris, V. K., & Moggré, G. (2022). Fermentation of plant-based dairy alternatives by lactic acid bacteria. *Microbial Biotechnology*, *15*(5), 1404–1421. https://doi.org/10.1111/1751-7915.14008

Hasan, M., Meena, N. L., Krishnan, V., Rudra, S. G., & Dahuja, A. (2023). Impact of Storage on Probiotic Viability, Nutritional and Sensory Quality of Fermented Soymilk

Produced from Different Soybean Varieties. *LEGUME RESEARCH - AN INTERNATIONAL JOURNAL, Of.* https://doi.org/10.18805/LR-5051

Hidayati, D., Soetjipto, S., & Catur Adi, A. (2021). Characteristic and Isoflavone Level of Soymilk Fermented by Single and Mixed Culture of <i>Lactobacillus plantarum </i>and Yoghurt Starter. *Journal of Food and Nutrition Research, 9*(1), 55–60. https://doi.org/10.12691/jfnr-9-1-9

Huo, C., Yang, X., & Li, L. (2023). Non-beany flavor soymilk fermented by lactic acid bacteria: Characterization, stability, antioxidant capacity and in vitro digestion. *Food Chemistry: X, 17*, 100578. https://doi.org/10.1016/j.fochx.2023.100578

Ji, G., Li, X., Dong, Y., & Shi, Y. (2022). Composition, formation mechanism, and removal method of off-odor in soymilk products. *Journal of Food Science, 87*(12), 5175–5190. https://doi.org/10.1111/1750-3841.16370

Ji, Y., Silva, M., Lin, R., Adhikari, B., & Chandrapala, J. (2024). Gelation and Thermal Stability of Camel Milk Protein and Soy protein Blends: A Review. *Food Reviews International, 40*(10), 3816–3846. https://doi.org/10.1080/87559129.2024.2374810

Ju, S., Song, S., Lee, J., Hwang, S., Lee, Y., Kwon, Y., & Lee, Y. (2021). Development of Nano Soy Milk through Sensory Attributes and Consumer Acceptability. *Foods, 10*(12), 3014. https://doi.org/10.3390/foods10123014

Kumari, M., Kokkiligadda, A., Dasriya, V., & Naithani, H. (2022). Functional relevance and health benefits of soymilk fermented by lactic acid bacteria. *Journal of Applied Microbiology, 133*(1), 104–119. https://doi.org/10.1111/jam.15342

Kwok, K.-C., Liang, H.-H., & Niranjan, K. (2002). Optimizing Conditions for Thermal Processes of Soy Milk. *Journal of Agricultural and Food Chemistry, 50*(17), 4834–4838. https://doi.org/10.1021/jf020182b

L. Caluza, Prof. G. (2019). Shelf Life and Acceptability of Different Fruity Flavored Soy milk under two Types of Storage Method. *International Journal of Science and Management Studies (IJSMS)*, 107–113. https://doi.org/10.51386/25815946/ijsms-v2i1p114

Letizia, F., Fratianni, A., Cofelice, M., Testa, B., Albanese, G., Di Martino, C., Panfili, G., Lopez, F., & Iorizzo, M. (2023). Antioxidative Properties of Fermented Soymilk Using Lactiplantibacillus plantarum LP95. *Antioxidants, 12*(7), 1442. https://doi.org/10.3390/antiox12071442

Li, X., Zhao, Z., Shi, S., Li, D., Sang, Y., Wang, P., Zhao, L., Wang, F., Fang, B., Chen, S., Li, Y., Jiang, Z., Luo, J., Zhang, X., & Wang, R. (2024). Flavor properties of post-heated fermented milk revealed by a comprehensive analysis based on volatile and non-volatile metabolites and sensory evaluation. *Current Research in Food Science*, *9*, 100892. https://doi.org/10.1016/j.crfs.2024.100892

Li, Y., Zhang, W., Chen, Y., Liu, L., Wu, X., Luo, Y., & Zhang, Y. (2025). Enhanced Quality in Bean Products Through Mixed Fermentation: A Comparative Analysis of Physicochemical, Structural, and Functional Properties of Soybean Products. *Foods*, *14*(11), 1985. https://doi.org/10.3390/foods14111985

Liu, Z., Fu, Y., Liu, Y., Chen, X., Jiang, M., & Rui, X. (2023). Lactic acid bacteria fermented soy β-conglycinin: Assessment of structural conformational feature and immunoglobulin E reactivity. *LWT*, *173*, 114246. https://doi.org/10.1016/j.lwt.2022.114246

Long, Z., Yi, X., Gao, X., Wang, Y., Guo, J., Gao, S., Xia, G., & Shen, X. (2025). Combining Sensory Analysis and Flavoromics to Determine How the Maillard Reaction Affects the Flavors of Golden Pomfret Hydrolysates. *Foods*, *14*(4), 560. https://doi.org/10.3390/foods14040560

Mahmoud, A. A., Owayss, A. A., Iqbal, J., & Raweh, H. S. A. (2023). Modified Equations to Calculate Water Content and Refractive Index of Honey Based on Its Total Soluble Solids. *Journal of Food Engineering and Technology*, *12*(1), 29–33. https://doi.org/10.32732/jfet.2023.12.1.29

Mendoza-Avendaño, C., Meza-Gordillo, R., Ovando-Chacón, S. L., Luján-Hidalgo, M. C., Ruiz-Cabrera, M. A., Grajales-Lagunes, A., Ruiz-Valdiviezo, V. M., Gutiérrez-Miceli, F., & Abud-Archila, M. (2018). Evaluation of Bioactive and Anti-Nutritional Compounds During Soy Milk Fermentation with Lactobacillus plantarum BAL-03-ITTG and Lactobacillus fermentum BAL-21-ITTG. *Revista Mexicana de Ingeniería Química*, *18*(3), 967–978. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Mendoza

Nanakali, N. M., Muhammad Al-saadi, J., & Sulaiman Hadi, C. (2023). Functional and physiochemical properties of the yoghurt modified by heat lactosylation and microbial transglutaminase cross-linking of milk proteins. *Food Science & Nutrition*, *11*(2), 722–732. https://doi.org/10.1002/fsn3.3108

Rana, A., Taneja, N. K., Raposo, A., Alarifi, S. N., Teixeira-Lemos, E., Lima, M. J., Gonçalves, J. C., & Dhewa, T. (2024). Exploring prebiotic properties and its probiotic potential of new formulations of soy milk-derived beverages. *Frontiers in Microbiology*, *15*. https://doi.org/10.3389/fmicb.2024.1404907

Rana, A., Taneja, N. K., Singh, A., Dhewa, T., Kumar, V., Kumar, A., Chauhan, K., Juneja, V., & Oberoi, H. S. (2025). Synergistic fermentation of vitamin B2 (riboflavin) bio-enriched soy milk: optimization and techno-functional characterization of next generation functional vegan foods. *Discover Food*, *5*(1), 10. https://doi.org/10.1007/s44187-025-00269-x

Rani, V. U., & Pradeep, B. V. (2017). Transformation, Purification, and Quantification of Soy Isoflavone from Lactobacillus sp. and Bifidobacterium sp. In *Recent advances in Applied Microbiology* (pp. 195–211). Springer Singapore. https://doi.org/10.1007/978-981-10-5275-0_9

Ruiz de la Bastida, A., Peirotén, Á., Langa, S., Rodríguez-Mínguez, E., Curiel, J. A., Arqués, J. L., & Landete, J. M. (2023). Fermented soy beverages as vehicle of probiotic lactobacilli strains and source of bioactive isoflavones: A potential double functional effect. *Heliyon*, *9*(4), e14991. https://doi.org/10.1016/j.heliyon.2023.e14991

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition*, *2*(1), 6. https://doi.org/10.1186/s43014-020-0020-5

Santos, D. C. dos, Oliveira Filho, J. G. de, Santana, A. C. A., Freitas, B. S. M. de, Silva, F. G., Takeuchi, K. P., & Egea, M. B. (2019). Optimization of soymilk fermentation with kefir and the addition of inulin: Physicochemical, sensory and technological characteristics. *LWT*, *104*, 30–37. https://doi.org/10.1016/j.lwt.2019.01.030

Sarić, Z., Barać, M., Barukčić, I., Kostić, A., Božanić, R., & Šertović, E. (2019). Physical, Chemical, Microbiological and Sensory Characteristics of a Probiotic Beverage Produced from Different Mixtures of Cow's Milk and Soy Beverage by *Lactobacillus acidophilus* La5 and Yoghurt Culture. *Food Technology and Biotechnology*, *57*(4), 461–467. https://doi.org/10.17113/ftb.57.04.19.6344

Sebastian, A. ;Barus, T. ;Mulyono, N. ;Yanti. (2018). Effects of fermentation and sterilization on quality of soybean milk. *International Food Research Journal*, *25*(6), 2420.

Sun, Q., Shi, X., Zhao, Y., Cui, R., Yao, Y., Liu, X., Wang, H., Zhang, L., & Song, L. (2025). Fermented Plant-Based Milks Based on Chestnut and Soybean: Comprehensive Evaluation of Fermentation Characteristics and Aroma Profiles Using Four Lactic Acid Bacteria Strains. *Foods*, *14*(14), 2511. https://doi.org/10.3390/foods14142511

Sun, Y., Xu, J., Zhao, H., Li, Y., Zhang, H., Yang, B., & Guo, S. (2023). Antioxidant properties of fermented soymilk and its anti-inflammatory effect on DSS-induced colitis in mice. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.1088949

Sun, Z., He, B., Chen, J., Wang, X., Wang, C., Qiao, F., & Li, W. (2022). Evaluation of Soybean Varieties for Soy Milk Based on Factor and Cluster Analysis. *Journal of Biobased Materials and Bioenergy*, *16*(4), 624–632. https://doi.org/10.1166/jbmb.2022.2211

Tangyu, M., Fritz, M., Tan, J. P., Ye, L., Bolten, C. J., Bogicevic, B., & Wittmann, C. (2023). Flavour by design: food-grade lactic acid bacteria improve the volatile aroma spectrum of oat milk, sunflower seed milk, pea milk, and faba milk towards improved flavour and sensory perception. *Microbial Cell Factories*, *22*(1), 133. https://doi.org/10.1186/s12934-023-02147-6

Tian, H., Shi, Y., Zhang, Y., Yu, H., Mu, H., & Chen, C. (2019). Screening of aroma-producing lactic acid bacteria and their application in improving the aromatic profile of yogurt. *Journal of Food Biochemistry*, *43*(10). https://doi.org/10.1111/jfbc.12837

Upadhyaya, S. (2024). Fermentation as a Method of Food Processing and Fermented Food as Probiotics: A Review. *Food Science and Nutrition*, *10*(5), 1–11. https://doi.org/10.24966/FSN-1076/100203

Valero-Cases, E., Cerdá-Bernad, D., Pastor, J.-J., & Frutos, M.-J. (2020). Non-Dairy Fermented Beverages as Potential Carriers to Ensure Probiotics, Prebiotics, and Bioactive Compounds Arrival to the Gut and Their Health Benefits. *Nutrients*, *12*(6), 1666. https://doi.org/10.3390/nu12061666

Wang, J., Wu, R., Zhang, W., Sun, Z., Zhao, W., & Zhang, H. (2013). Proteomic comparison of the probiotic bacterium Lactobacillus casei Zhang cultivated in milk and soy milk. *Journal of Dairy Science*, *96*(9), 5603–5624. https://doi.org/10.3168/jds.2013-6927

Xiang, J., Liu, F., Wang, B., Chen, L., Liu, W., & Tan, S. (2021). A Literature Review on Maillard Reaction Based on Milk Proteins and Carbohydrates in Food and Pharmaceutical Products: Advantages, Disadvantages, and Avoidance Strategies. *Foods*, *10*(9), 1998. https://doi.org/10.3390/foods10091998

Yan, F., Tong, S., Zhang, J., Zhao, Y., & Liu, P. (2024). Effects of soybean endogenous enzyme hydrolysis on the quality of soymilk after blanching. *Food Bioscience*, *57*, 103469. https://doi.org/10.1016/j.fbio.2023.103469

Yang, A., Smyth, H., Chaliha, M., & James, A. (2016). Sensory quality of soymilk and tofu from soybeans lacking lipoxygenases. *Food Science & Nutrition*, *4*(2), 207–215. https://doi.org/10.1002/fsn3.274

Yerlikaya, O. (2014). Starter cultures used in probiotic dairy product preparation and popular probiotic dairy drinks. *Food Science and Technology (Campinas)*, *34*(2), 221–229. https://doi.org/10.1590/fst.2014.0050

Zhang, P., Tang, F., Cai, W., Zhao, X., & Shan, C. (2022). Evaluating the effect of lactic acid bacteria fermentation on quality, aroma, and metabolites of chickpea milk. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.1069714

Zhang, X., Li, Y., Yang, J. J., Ma, X. Y., Jia, X. D., Li, A. L., & Du, P. (2020). The effects of inulin combined with ogalacto-oligosaccharide on the various properties of synbiotic soy cheese containing Lactobacillus acidophilus KLDS 1.0738. *Quality Assurance and Safety of Crops & Foods*, *12*(3), 46–54. https://doi.org/10.15586/QAS2019.740

Zhang, X., Qi, J.-R., Li, K.-K., Yin, S.-W., Wang, J.-M., Zhu, J.-H., & Yang, X.-Q. (2012). Characterization of soy β-conglycinin–dextran conjugate prepared by Maillard reaction in crowded liquid system. *Food Research International*, *49*(2), 648–654. https://doi.org/10.1016/j.foodres.2012.09.001

Zhang, Z., Dong, J., Zheng, L., Chen, Y., Fang, C., Chen, J., Guo, J., Sun, H., Guo, N., Fang, X., & Zhu, G. (2025a). Comparative analysis of physicochemical properties and volatile flavor compounds of a novel brown soy yogurt prepared via Maillard browning reaction. *Food Chemistry: X*, *29*, 102695. https://doi.org/10.1016/j.fochx.2025.102695

Zhang, Z., Dong, J., Zheng, L., Chen, Y., Fang, C., Chen, J., Guo, J., Sun, H., Guo, N., Fang, X., & Zhu, G. (2025b). Comparative analysis of physicochemical properties and volatile flavor compounds of a novel brown soy yogurt prepared via Maillard browning reaction. *Food Chemistry: X*, *29*, 102695. https://doi.org/10.1016/j.fochx.2025.102695

Zhou, R. Y., Huang, X., Liu, Z., Chua, J.-Y., & Liu, S.-Q. (2022). Evaluating the effect of lactic acid bacterial fermentation on salted soy whey for development of a potential novel soy sauce-like condiment. *Current Research in Food Science*, *5*, 1826–1836. https://doi.org/10.1016/j.crfs.2022.10.004

Zong, L., Lu, M., Wang, W., Wa, Y., Qu, H., Chen, D., Liu, Y., Qian, Y., Ji, Q., & Gu, R. (2022). The Quality and Flavor Changes of Different Soymilk and Milk Mixtures Fermented Products during Storage. *Fermentation*, *8*(12), 668. https://doi.org/10.3390/fermentation8120668

Zong, L., Qu, H., Wang, W., Chen, D., Wa, Y., Huang, Y., & Gu, R. (2025). Effect of key flavor compounds in fermented soymilk on sensory attributes: Integrating electronic sensory technology with GC–MS analysis. *Food Chemistry: X*, *29*, 102750. https://doi.org/10.1016/j.fochx.2025.102750