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RESEARCH **Open Access**

Probiotic Edamame Yoghurt: A Study on Sensory, Microbiological, and Physicochemical Properties of Fermentation Using Mixed Cultures

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Abstract
One of the primary risk factors for cardiovascular diseases (CVDs) that caused deaths is hypercholesterolemia, which can be identified by high levels of total cholesterol, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Dietary interventions, involving the consumption of soy protein, have been indicated to be effective in reducing LDL cholesterol levels. Edamame, a kind of soybean, is a potential source of soy protein that could be processed into functional foods. The aim of the research was to evaluate the chemical and sensory properties of edamame yoghurt fermented with lactic acid bacteria and probiotic bacteria, potentially contributing to a cholesterol-lowering benefit. The research methodology was a two-factor Randomized Block Design (RBD). The edamame yoghurt was made by combining edamame juice, skim milk powder, and sugar, followed by heat (80-85 °C for 15 minutes), and cooling to 45 °C. The mixture was then inoculated with a combination of lactic acid bacteria (Lactobacillus bulgaricus and Streptococcus thermophilus) and probiotic bacteria (Bifidobacterium bifidum and Lactobacillus acidophilus). Fermentation proceeded at 37 °C for 7 hours. The pH, total titratable acidity, lactic acid bacteria count, and soluble protein content were analysed. Sensory properties were evaluated using preference and hedonic tests. The results exhibited that the combination of bacterial cultures significantly affected ($p<0.05$) pH, total soluble protein, and sensory properties of the yoghurt. Bifidobacterium bifidum led to higher acidity pH (3.73-3.78), while Lactobacillus acidophilus resulted in the lowest titratable acidity (0.55-0.63%). The combination of Lactobacillus bulgaricus and Streptococcus thermophilus improved flavor, texture, and fermentation efficiency. The yoghurt's texture was smooth due to proteolytic activity by lactic acid bacteria. Edamame yoghurt's green color fades during fermentation, and its aroma is less favoured according to panelists, but its creamy texture and mildly acidic taste make it appealing overall. Edamame yoghurt, a source of soy protein fermented with lactic acid and probiotic bacteria, shows potential for cholesterol reduction.

Keywords: Edamame, Lactic Acid Bacteria, Probiotics, Yoghurt.

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1. INTRODUCTION

Hypercholesterolemia is the second-highest risk factor for CVDs, after hypertension, with a prevalence of 19.4%. This condition is characterized by elevated levels of total cholesterol in the blood, as well as high Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL), accompanied by an increase in triglyceride (TG) levels. Nationally, the prevalence of hypercholesterolemia among individuals over 15 years old reached 35.9% in 2013, rising to 39.5% in 2023 (Badan Penelitian dan Pengembangan Kesehatan, 2013; Badan Penelitian dan Pengembangan Kesehatan, 2019).

For a long time, LDL cholesterol has been the primary target for therapy in individuals with hypercholesterolemia. Hypercholesterolemia can be addressed through lifestyle changes that include selecting food sources to lower LDL cholesterol, one of which is the consumption of soy protein. Edamame, a variety of soybean, is high in protein and contains 18 essential amino acids necessary for the body. Additionally, edamame is cholesterol-free, low in saturated fat, and rich in fibre, vitamins A, E, and K, as well as folate, choline, and manganese. Consumption of edamame products has been shown to reduce triglycerides in rats with type 2 diabetes mellitus and lower blood pressure in hypertensive patients (Ajeng et al., 2022; Valensia et al., 2024). Edamame must be processed immediately after harvesting into frozen ready-to-eat products. Currently, various processed edamame products are available, such as dried edamame, edamame chips, and edamame cakes. These products require long processing times and high temperatures, which can damage their nutritional composition, particularly the protein. Therefore, a simpler processing method is needed to preserve the nutrients in edamame, and one recommended method could involve it into yoghurt.

Fermenting yoghurt using Streptococcus thermophilus and Lactobacillus bulgaricus not only gives a changed characteristic texture and flavour (Chen et al., 2017) but also provides health benefits. Lactic acid bacteria, like Bifidobacterium bifidum and Lactobacillus acidophilus, have shown lowering the cholesterol through cholesterol assimilation, bile acid co-precipitation, and bile salt deconjugation (Agolino et al., 2024). A recent study suggested that probiotic yoghurt can effectively reduce total cholesterol and LDL levels in hypercholesterolemia people (Caponio et al., 2020; Pourrajab et al., 2020). Additionally, protein hydrolysis during the fermentation process of edamame milk results in peptides that have antioxidant activity and have shown potential as an anti-hypertensive agent by inhibiting ACE-converting enzyme (Anggraeni et al., 2022).

Despite the promising potential of edamame yoghurt, some studies have reported that sensory characteristics, such as aroma, may impact its acceptance. Edamame yoghurt was less preferred by the panellists due to the aroma (Rosiana & Amareta, 2016). The starter culture used in their study came from a commercial mixed culture known to contain Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, and Bifidobacterium bifidum. Using starter yoghurt from already-made yoghurt can potentially reduce the quality of the yoghurt. Therefore, the yoghurt starter in this research used pure cultures of Lactobacillus bulgaricus, Streptococcus thermophilus, Bifidobacterium bifidum, and Lactobacillus acidophilus. The quality and flavour of yoghurt are influenced by the types of bacteria used, as each type has different abilities to break down substrates, resulting in different metabolites. The aim of the research was to evaluate the chemical and sensory properties of edamame yoghurt fermented with lactic acid bacteria and probiotic bacteria, potentially contributing to a cholesterol-lowering benefit.

2. RESEARCH METHOD

The main ingredient for making edamame yoghurt was fresh edamame from the PT. Mitratani Dua Tujuh outlet in Jember. The characteristics of the edamame included a green color, a length of 5-7 cm, and a harvest age of 65-70 days. The other materials included sugar

(Gulaku), skim milk powder (Tropicana Slim Skim Milk), and bacterial cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus* obtained from the Microbiology Laboratory of the Faculty of Agricultural Technology at the University of Jember. The bacterial cultures *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were obtained from the Microbiology Laboratory of the Faculty of Medicine at Brawijaya University in Malang.

The experimental design used was a Factorial Randomized Block Design (FRBD) consisting of 2 factors. Factor 1 was the type of LAB (A1 = *Lactobacillus bulgaricus*; A2 = *Streptococcus thermophilus*; A3 = *Lactobacillus bulgaricus* + *Streptococcus thermophilus*). Factor 2 was the type of probiotic bacteria (B1 = *Bifidobacterium bifidum*; B2 = *Lactobacillus acidophilus*; B3 = *Bifidobacterium bifidum* + *Lactobacillus acidophilus*).

The preparation of the culture was carried out by taking a loopful of the rejuvenated stock culture using a sterile inoculating needle, which was then transferred into an erlenmeyer flask containing media. The media was MRSB, made by dissolving 52.2 grams of MRSB in 1000 ml of distilled water. This solution was then incubated at 37°C for approximately 24 hours until it turns cloudy. The preparation of the starter culture involved inoculating 5% of the culture into edamame juice that had been supplemented with 5% skim milk, then incubating it at 37°C for 7 hours or until curd formation occurs. This culture was ready to be used as a starter culture for making edamame yoghurt.

The preparation of edamame juice followed the modification by Fitriyana (2017). Raw edamame was shelled and separated. After draining, the edamame seeds were thoroughly washed with running water and drained. Next, the edamame seeds were blended until smooth with a water-to-edamame ratio of 5:1 and then filtered. The resulting filtrate was called edamame juice, which was ready to be used as a substrate in the production of edamame yoghurt.

The preparation of edamame yoghurt followed the modification by (Fitriyana, 2017). The filtered edamame juice was supplemented with 5% skim milk powder and 5% granulated sugar based on the volume of the edamame juice, and then heated at 80-85°C for 15 minutes. After heating, it was cooled to 45°C. The edamame juice was then inoculated with a starter culture of LAB and probiotic bacteria in a 1:1 ratio at a concentration of 6% according to the treatment. The edamame juice was incubated at 37°C for 7 hours or until curd formation occurs, followed by analysis.

The pH measurement was conducted using a pH meter. The measurement of total titratable acidity was performed using the principle of acid base titration. The total lactic acid bacteria test was conducted using the pour plate method with sterile MRSA liquid medium (Badan Standardisasi Nasional, 2009). The analysis of soluble protein was performed using the formol titration method (Warakaulle et al., 2024).

The sensory testing included preference tests and hedonic quality assessments (Meilgaard et al., 2020). The testing was conducted by 39 panellists. The parameter for hedonic quality was color 1-10 – white-green, acid aroma 1-10 – weak-strong, texture 1-10 – runny-thick, acid taste 1-10 – weak-strong. The parameter for preference test was 1=very dislike, 2=dislike, 3=neutral, 4=like, 5=very like. The sensory testing was approved by the ethics committee under approval number 1845/PL17/2018.

Data analysis was conducted using IBM SPSS Statistics version 22, employing ANOVA, followed by Duncan's post hoc test. ANOVA was used to compare the means of three or more groups to identify significant differences. If ANOVA showed significant results, Duncan’s test performed pairwise comparisons to pinpoint which group differed. Duncan’s test provided detailed information about group differences after the ANOVA test.

3. RESULTS AND DISCUSSION

The results of the chemical analysis of yoghurt are shown in Table 1. The types of lactic acid bacteria and probiotic bacteria significantly affect the pH, total titratable acidity, soluble protein, and the number of lactic acid bacteria.

The pH of edamame yoghurt with different types of LAB and probiotic bacteria is mostly below 4, ranging from 3.73 to 4.17 (Table 1). The optimum pH for yoghurt is 4.0–4.4 to avoid excessive acidic taste of the product and texture in good-quality yoghurts (Deshwal et al., 2021; Kang et al., 2019). The bacteria *Bifidobacterium bifidum* tend to produce yoghurt with a lower pH compared to *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus bulgaricus*. However, these results differ from the study by Rosiana & Amareta (2016), which showed that the average pH of edamame yoghurt fermented with a mixed commercial culture ranged from 4.39 to 5.44. Lower pH of the yoghurt act to inhibit the growth of pathogens present in the yoghurts (Matela et al., 2019).

Table 1. Results of the Chemical Analysis of Edamame Yoghurt.

Types of cid Lactic Bacteria	Types of Probiotic Bacteria	pH	Titratable Acidity (%)	Dissolved Protein (%)	Acid Lactic Bacteria (log cfu/ml)
Lactobacillus bulgaricus	*Bifidobacterium bifidum*	3.78±0.10[a]	1.04±0.31[a]	0.07±0.01[ab]	9.97±0.01[d]
	Lactobacillus acidophilus	4.17±0.17[d]	0.63±0.13[ab]	0.07±0.00[ab]	9.78±0.04[c]
	Bifidobacterium bifidum + *Lactobacillus acidophilus*	3.78±0.28[abc]	0.89±0.28[ab]	0.06±0.00[a]	10.16±0.24[de]
Streptococcus thermophilus	*Bifidobacterium bifidum*	3.73±0.05[a]	1.06±0.31[b]	0.09±0.01[b]	10.12±0.19[de]
	Lactobacillus acidophilus	4.05±0.11[bcd]	0.55±0.06[a]	0.08±0.01[bc]	9.38±0.04[ab]
	Bifidobacterium bifidum + *Lactobacillus acidophilus*	3.73±0.05[a]	0.99±0.24[ab]	0.07±0.01[ab]	9.44±0.12[ab]
Lactobacillus bulgaricus + *Streptococcus thermophilus*	*Bifidobacterium bifidum*	3.77±0.11[a]	1.04±0.28[b]	0.09±0.00[c]	9.57±0.15[b]
	Lactobacillus acidophilus	4.07±0.05[bc]	0.57±0.08[a]	0.08±0.01[bc]	9.32±0.33[a]
	Bifidobacterium bifidum + *Lactobacillus acidophilus*	3.82±0.11[ab]	0.90±0.31[ab]	0.08±0.00[bc]	10.28±0.06[e]

Note: *Data are presented as mean ± standard deviation of three replicates. Superscripts indicate significant differences within the same column based on ANOVA (p<0.05).*

Titratable acidity for yoghurt ranges from 0.55% to 1.04% (Table 1). *Bifidobacterium bifidum* is a bacterium that can produce higher levels of acid than *Lactobacillus casei*. Fermentation by products from *Lactobacillus bulgaricus* are D(−) lactic acid, peptides, amino acids, acetaldehyde (majorly); *Streptococcus thermophilus* are L(+) lactic acid, formic acid, CO_2, acetaldehyde (minor) (Deshwal et al., 2021). Lactose in milk occurs naturally in significant quantities and serves as the primary energy and carbon source for the growth of lactic acid bacteria (LAB). LAB transform lactose into lactic acid, which imparts the distinctive acidic flavor to yoghurt (Chen et al., 2017). Not only do LAB change lactose into acid, but probiotic bacteria can do the same thing. *Bifidobacterium bifidum* can produce lactic acid using lactose. The enzyme lactase produced by microbes, especially lactic acid bacteria, can break down lactose into galactose (Abdullah, 2024). The accumulation of lactic acid during fermentation can increase titratable acidity because the bacteria *Lactobacillus acidophilus* could produce organic acids in higher quantities (Melia et al., 2021).

Table 1 shows that the average total soluble protein ranges from 0.06% to 0.09%. The lowest of total soluble protein (0.06%) is found in edamame yoghurt fermented by *Lactobacillus bulgaricus* and *Bifidobacterium bifidum*. Meanwhile, the highest of total soluble protein (0.09%) is found in edamame yoghurt fermented by *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum*. The activity of proteolytic of lactic acid bacteria has a crucial role in utilizing casein and providing essential amino acids during growth in milk. Casein will be hydrolysed into peptides by proteinases, and then intracellular peptidases will degrade it into amino acids and smaller peptides. Additionally, cellular proteolysis helps control the quality of polypeptides and regulatory mechanisms by maintaining appropriate levels of regulatory proteins and removing them when necessary. Transport systems facilitate the transfer of amino acids and peptides across the cytoplasmic membrane (Kieliszek et al., 2021). Probiotic bacteria are reported to have the ability to hydrolyse protein into peptides because they possess aminopeptidases and dipeptidases. The proteolytic activity of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* spp. *bulgaricus*, and *Lactobacillus acidophilus* was significantly higher than that of *Bifidobacterium* spp., which are not highly proteolytic in comparison to other lactic acid bacteria (Ayivi & Ibrahim, 2022). Another research study shows that pH between 5.7-6.0 will lose soymilk protein solubility. Heated soymilk cause neutralization of the negative protein complexes from proton that released by hydrolytic action of lactic acid bacteria. Fermentation of lactic acid bacteria induce acid gelation alike gelation way to that of glucano-lactone but exhibit a greater gelation pH at 6.29 (Fitriyana, 2017). Hydrolysed protein make it easier to absorb in the body than protein in milk or edamame (Sumi et al., 2023). Peptides resulting from protein hydrolysis during the fermentation process of edamame yoghurt have antioxidant activity. This short chain peptides were also disclosed the capacity to suppress ACE-converting enzyme, which shows the potential as an anti-hypertensive compound (Anggraeni et al., 2022).

Total lactic acid bacteria range from 9.32-10.28 log cfu/ml (Table 1). Yoghurt fermented with a mixture of LAB and probiotic bacteria showed the highest counts, which is beneficial for the probiotic content of the yoghurt. A high lactic acid bacteria count is essential for the health benefits associated with yoghurt, particularly for gut health. The fermentation process also leads to the production of short-chain fatty acids (SCFAs), which have prebiotic effects and further support the growth of beneficial gut microbiota (Wang et al., 2021). Moreover, LAB strains that are more acid-tolerant, like *Lactobacillus*, are better able to survive in yoghurt with a lower pH, ensuring the probiotic benefits are retained even in acidic environments (Meybodi et al., 2020).

Based on the results (Figure 1), the best combination to make yoghurt with good sensory quality is *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. A more consistent fermentation process is the result of this combination, which enhances of the taste and more preferable texture. Enhanced sensory qualities, especially in colour and aroma, also result from a combination of *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. For enhancing the overall sensory quality of the yoghurt, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* may be more valuable than *Bifidobacterium bifidum* and *Lactobacillus acidophilus* strains alone.

When not fermented, edamame juice has a light green colour. However, after being fermented into edamame yoghurt, the resulting product tends to have a faded green colour (Figure 1). The result similar with other edamame yoghurts fermented by commercial culture, the fermentation process causes the green colour in edamame yoghurt to fade to a more muted green (Rosiana & Amareta, 2016). Most colour of edamame yoghurt is liked by panellist (Figure 1). However, the green color of edamame is from the chlorophyll pigment (Yu et al., 2022). During fermentation, chlorophyll degradation occurs, which is characterized by changes in the colour of the silage from light-green to brownish-yellow (Santra et al., 2021). But no changes are found in the total chlorophyll of yoghurt added by suji leaves (Ramadhani et al., 2024).

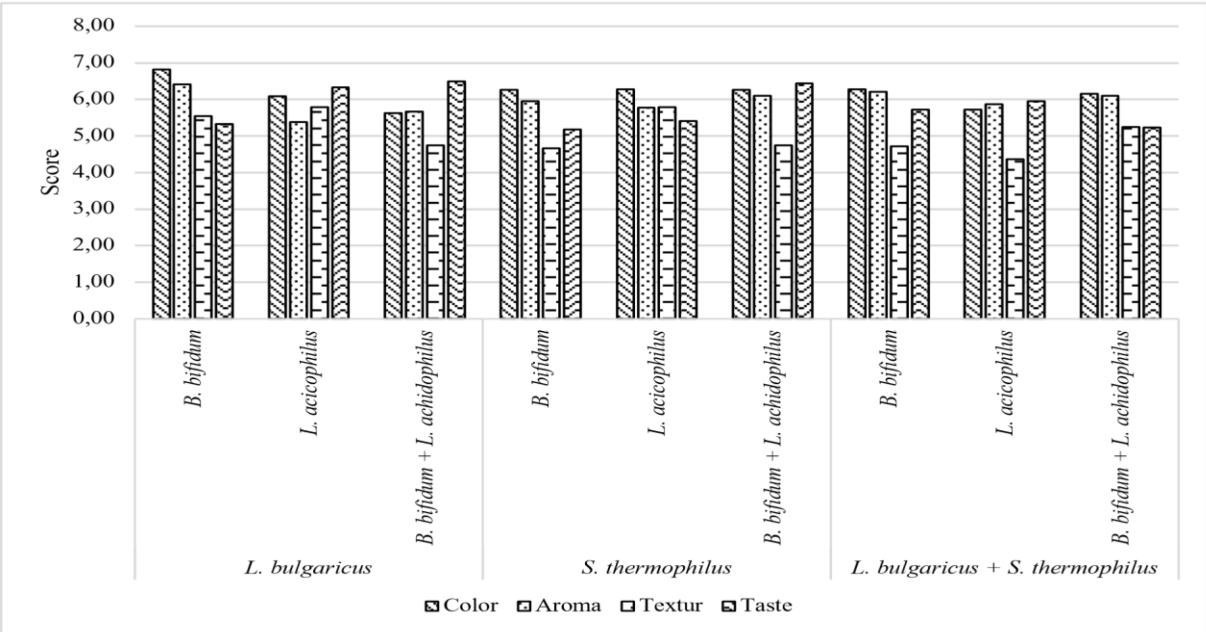

Figure 1. Sensory evaluation of edamame yoghurt. Colour 1-10 = white-green, acid aroma 1-10 = weak-strong, texture 1-10 = runny-thick, acid taste = weak-strong

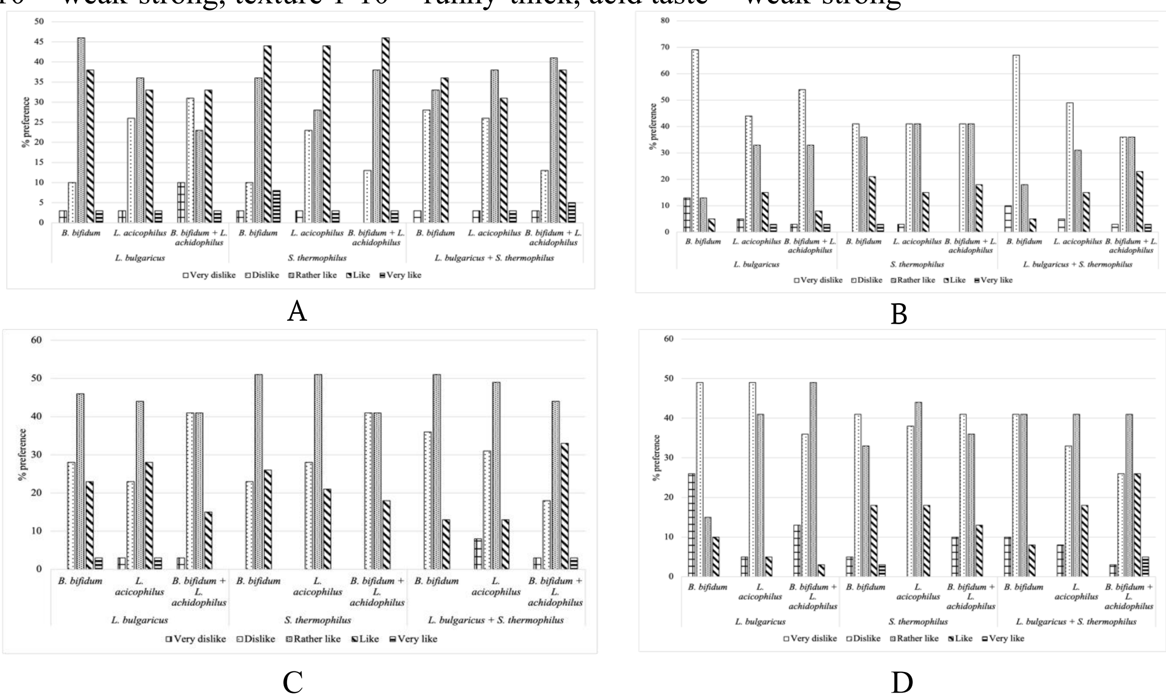

Figure 2. Preference of edamame yoghurt, A=color, B=aroma, C=texture, D=taste

The panellists responses to the aroma, according to the hedonic quality test, indicate that edamame yoghurt tends to have a sharp, tangy smell (Figure 1). During fermentation, lactic acid bacteria carry out three major biochemical conversions of milk components: (i) the conversion of carbohydrates into lactic acid or other metabolites (glycolysis), (ii) the hydrolysis of caseins into peptides and free amino acids (proteolysis), and (iii) the breakdown of milk fat into free fatty acids (lipolysis). These reactions, catalysed by microbial enzymes, also lead to the production of various flavour and off-flavour compounds in yoghurt. Additionally, some nonenzymatic reactions occur, primarily as a result of milk processing, such as after heating (Chen et al., 2017). These aroma of edamame yoghurt is disliked by panellist (Figure 2).

The panellists responses to the texture of edamame yoghurt indicate that the yoghurt tends to have a runny to thick consistency (Figure 1). This indicates that the yoghurt had a smooth and creamy texture. The production of acid during the fermentation process results in a decrease in pH and protein coagulation, which causes the yoghurt's texture to become thicker. Table 1 shows that pH of yoghurts is around 4, indicating that these yoghurts have good quality of texture. Bacteria added to fermented milk products produce organic acids, which influence the pH drop and are markers of metabolic activity. This acid contributes to the distinctive sensory qualities and acts as a natural preservative (Melia et al., 2021). Yoghurt with whey protein addition was more viscous than the yoghurt that didn't. Whey protein added to the goat's milk increased the firmness of the yoghurt and produced results comparable to those of cow's milk yoghurt. Protein aggregates, which get formed when casein micelles and denatured whey proteins interact through intermolecular disulfide bonds, may be the cause of the yoghurt's increased hardness upon adding whey protein (Bierzuńska et al., 2019).

The panellists responses to the acid taste of edamame yoghurt indicate that the yoghurt tends slightly acidic to moderately acidic. This suggests that the yoghurt had a pleasant taste that was not too acidic. In milk, lactose is the major source of energy and carbon for the growth of lactic acid bacteria. Lactic acid bacteria changes lactose into lactic acid, which provides acidic taste in yoghurt (Chen et al., 2017). *Lactobacillus bulgaricus* and *Streptococcus thermophilus* show increased performance when combine together rather than separately. The initial pH of milk supports the rapid growth of *Streptococcus thermophilus*, while the subsequent increase in acidity favors the growth of *Lactobacillus bulgaricus*, which flourish at a pH below 4.5. At the start, *Lactobacillus bulgaricus* make easier the growth of *Streptococcus thermophilus* by delivering amino acids like valine, leucine, histidine, and methionine from milk proteins. So, *Streptococcus thermophilus* supports *Lactobacillus bulgaricus* growth by bringing out small amounts of formic acid. This mutual interaction between the two species, especially in the early stages of their mixed culture, accelerates the production of lactic acid and aromatic compounds more effectively than when either species is cultured individually (Routray & Mishra, 2011).

4. CONCLUSION

The results of this study demonstrate that the type of lactic acid bacteria (LAB) and probiotic bacteria used in edamame yoghurt fermentation significantly impact its pH, titratable acidity, soluble protein content, and probiotic bacteria levels. Specifically, the combination of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* yielded higher counts of lactic acid bacteria, resulting in yoghurt with desirable probiotic benefits. The proteolytic activity of these bacteria contributed to a breakdown of proteins into peptides and amino acids, enhancing the nutritional profile and digestibility of the yoghurt. The balance between the acidity produced by different bacterial strains affected the texture and flavor, with some combinations creating a more acidic taste and others maintaining a smoother profile. These findings highlight the importance of selecting bacterial strains not only for their fermentation capabilities but also for their synergistic interactions, which can optimize both the sensory qualities and health benefits of edamame yoghurt. Further research could investigate the long-term health impacts of consuming edamame yoghurt enriched with certain bacterial strains.

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