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Reduction of Tannin on Red Kidney Bean, Yellow Soybean, and Black Soybean Through Peaghurt Fermentation

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Abstract

Legumes are valuable food sources; however, their nutritional quality and utilization are often compromised by tannins, antinutrients that diminish nutrient bioavailability. Various processing methods, including soaking, boiling, germination, dehulling, roasting, and fermentation, have been applied to reduce tannin levels, with fermentation emerging as one of the most effective biological approaches. In this context, peaghurt—a novel probiotic beverage produced from fermented bean milk using lactic acid bacteria (LAB)—has gained attention due to its ability to modify physicochemical properties, including tannin content. This study assayed the efficacy of LAB starter combinations comprising *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, and *Streptococcus thermophilus* in reducing tannin levels during peaghurt fermentation. Peaghurt was produced using a two-factor randomized block design (RBD) involving four LAB starter combinations and three bean milk substrates, each conducted in triplicate. Tannin content was quantified using UV–Vis spectrophotometry, and the data were analyzed using multivariate analysis of variance (MANOVA) followed by Tukey's post hoc test. All LAB combinations significantly reduced tannin levels (12.79–46.18%), with the highest reduction observed in yellow soybean peaghurt fermented using the combined starter of *L. bulgaricus*, *S. thermophilus*, and *L. plantarum* (46.18%). This reduction is primarily ascribed to tannase activity synthesized by LAB during fermentation.

Keywords: Bean nutrition, Fermentation, LAB starters, Probiotic, Tannin reduction

INTRODUCTION

Legumes, such as red kidney beans (*Phaseolus vulgaris*), yellow soybeans (*Glycine max* var. *yellow*), and black soybeans (*Glycine max* var. *black*), are nutrient-dense foods rich in proteins (20–40%) and dietary fiber (14–33%), along with vitamins, minerals, and complex carbohydrates (Maphosa & Jideani, 2017), highlighting their strong potential as substrates for functional food development, driven by the burgeoning consumer interest in plant-based probiotic products (Cichońska & Ziarno, 2021). However, their nutritional potential is often constrained by naturally occurring antinutritional factors (ANFs), most notably tannins (Samtiya et al., 2020).

Tannins, water-soluble polyphenolic compounds, exist at varying concentrations across legume species (Ojo, 2022), including black soybeans, red kidney beans, and yellow soybeans, where they readily form complexes with nutritionally important dietary proteins and mineral elements including iron, zinc, and calcium (Gilani et al., 2012). These interactions substantially impair nutrient digestibility and absorption (Goksen et al., 2025), reducing mineral bioavailability by approximately 20–50% (Luo YuWei & Li Jing, 2016) and ultimately diminishing the nutritional quality of legume-based foods (Maphosa & Jideani, 2017). This limitation is especially critical for probiotic bean milk beverages, where both consumer acceptance and nutritional efficacy depend heavily on minimizing tannin content (Senanayake et al., 2023).

Numerous traditional and modern food processing methods have been explored for decreasing tannin content in legumes, including soaking (Ojo, 2022), germination (P. U. Rao & Deosthale, 1982), dehulling (B. S. N. Rao & Prabhavathi, 1982), roasting (Garrido-Galand et al., 2021), pressure cooking, and boiling (Das et al., 2022). While these methods offer some efficacy in leaching or degrading tannins, fermentation has emerged as a particularly dynamic and comprehensive biological process (Senanayake et al., 2023). Previous research indicates that fermentation, through the enzymatic action of microorganisms like LAB, can actively catalyse the hydrolysis of complex tannins into smaller, less astringent, and more bioavailable phenolic compounds (Sabater et al., 2024). Studies have demonstrated that various LAB strains, including *L. plantarum*, are effective in degrading tannins and can significantly reduce their levels in fermented legume products (Sabater et al., 2024). For instance, fermentation has been shown to reduce tannin levels in red haricot beans (Kitum et al., 2020) and soybean silages (Gao et al., 2022). The use of co-cultures of LAB has also been identified as advantageous for enhancing the fermentation process and ANF reduction (Mefleh et al., 2022). Specific LAB species such as *L. bulgaricus*, *L. plantarum*, and *S. thermophilus* have been recognized for their roles in fermenting plant-based substrates and influencing nutritional profiles (Cichońska & Ziarno, 2021). Furthermore, fermentation not only contributes to improved digestibility and bioavailability of nutrients—potentially increasing beneficial bioactive compounds such as isoflavones and anthocyanins, especially in soybean-based products (Khosravi & Razavi, 2021)—but also induces significant physicochemical transformations in peaghurt, a probiotic fermented bean milk product involving LAB activity, including alterations in tannin composition (Noorhadi, 2025). Therefore, optimized strategies integrating specific LAB consortia are urgently needed to reduce tannins while enhancing the functional properties of legume-based probiotic beverages.

Despite substantial advances in legume fermentation research, a critical knowledge gap persists regarding the synergistic effectiveness of specific LAB starter combinations—particularly *L. bulgaricus*, *L. plantarum*, and *S. thermophilus*—in reducing tannin content during peaghurt fermentation. In particular, systematic and comparative investigations remain largely underexplored on how different legume substrates, including red kidney bean, yellow soybean, and black soybean milks, respond to defined LAB consortia within a peaghurt fermentation system, thereby limiting the optimization of starter selection and formulation for legume-based probiotic beverages.

Given the background information, the aim of this systematic investigation is to ascertain the efficacy of specific LAB starter combinations—*L. bulgaricus*, *L. plantarum*, and *S. thermophilus*—in reducing tannin content during peaghurt fermentation across three distinct legume substrates: red kidney bean milk, yellow soybean milk, and black soybean milk.

METHOD

The research was conducted on January 09th – January 31st, 2023, at the Microbiology-Genetics Laboratory and Chemistry Laboratory of Universitas Nasional, Jakarta. The process undertaken in this study comprised four steps: bean milk preparation; stock and starter preparation; peaghurt fermentation; and tannin content assessment of peaghurt.

Material and Equipment

In this study, the tools used included Erlenmeyer flask (Conical flask), volumetric flask, rack and test tube, Ose needle, spatula, measuring cylinders, blender, vortex mixer, cheesecloth, digital scale, analytical balance, watch glass, stove, incubator, Bunsen burner, autoclave, water bath, refrigerator, Laminar Air Flow (LAF), volumetric pipette, Pasteur pipette, rubber bulb (pipette bulb), micropipette, microtip, quartz cuvettes, UV-Vis spectrophotometer and UV-Vis software.

Meanwhile, the materials used in the study were kidney red bean, yellow soybean, and black soybean from Induk Kramat Jati Market East Jakarta, sugar, aquades (distilled water), de Man Rogosa Sharpe Broth (MRSB) medium and agar bacteriological, calcium carbonate ($CaCO_3$), sodium carbonate (Na_2CO_3) 20%, tannic acid, Folin Ciocalteu reagent, pure culture of *L. plantarum* from IPBCC (Bogor Agricultural Institute Culture Collection), and commercial starters of *L. bulgaricus* and *S. thermophilus* from Setia Guna Bogor Chemical Shop.

Research Design

A randomized complete block design (RBD) with a two-factor (4 × 3) factorial arrangement was employed, replicated in triplicate. The first factor treatment comprised four LAB starter combinations: A_1 (*L. bulgaricus* + *S. thermophilus*), A_2 (*L. bulgaricus* + *L. plantarum*), A_3 (*S. thermophilus* + *L. plantarum*), and A_4 (*L. bulgaricus* + *S. thermophilus* + *L. plantarum*). The second factor treatment involved three types of bean milk substrates: K_1 (red kidney bean), K_2 (black soybean), and K_3 (yellow soybean). The data obtained were analyzed operating two-way multivariate analysis of variance (MANOVA). Post hoc analysis applying the Tukey test was conducted upon the detection of significant differences among treatments.

Research Procedures

Bean Milk Preparation

Red kidney beans, yellow soybeans, and black soybeans underwent thorough washing followed by soaking in water for 8–12 h (Wardani & Wardani, 2014). Post-soaking, the beans were rinsed and subjected to blanching in hot water maintained at approximately 80^{0}C for 15–30 min to reach a semi-soft consistency (Putri, 2019). The blanched beans were subsequently

pulverized with boiled water in a 1:4 (beans:water) ratio (Fathurohman et al., 2020), yielding a slurry that was filtered to obtain the bean milk. Sucrose was incorporated at 4% (w/v), after which the bean milk was heated under agitation until boiling prior to dispensing into 200 mL Erlenmeyer flasks (Ashari, 2008).

Preparation of LAB Stock and Starter Cultures

MRS broth (100 mL total) was aliquoted into 10 tubes. For MRS semi-solid medium, 0.25 g bacteriological agar was added to 50 mL MRS broth distributed across 5 tubes, each with a 1–2 cm layer of $CaCO_3$ at the base, prior to sterilization. A 0.2 mL aliquot of *L. plantarum* culture was then inoculated into 100 mL sterile MRS broth and incubated at 37^{0}C for 24 h. The resulting rejuvenated *L. plantarum* culture from MRS broth was subsequently inoculated into the MRS semi-solid medium to establish the stock culture. Thereafter, 2–3 loopfuls from the *L. plantarum* stock culture were inoculated into sterile MRS broth and incubated at 37^{0}C for 24 h. This was followed by inoculation of 10% (v/v) *L. plantarum* culture into 200 mL bean milk, which had been pasteurized in a water bath at 85^{0}C for 15 min; the *L. plantarum* starter was then incubated at 37^{0}C for 24 h. *L. bulgaricus* and *S. thermophilus* starters were employed directly in their commercial form.

Peaghurt Fermentation

Bean milk was pasteurized in a water bath at 80–85^{0}C for 15 min and subsequently cooled to room temperature. The cooled bean milk then served as the fermentation substrate and was inoculated with 2% (v/v) of each LAB starter in accordance with the research design, prior to incubation at 37^{0}C for 24 h. The resultant peaghurt was subsequently analyzed for tannin reduction.

Tannin Content Test

Maximum wavelength determination

The maximum wavelength was identified through the peak wavelength feature on the Labdex Double Beam Spectrophotometer LX242DS device application. The maximum wavelength value was determined based on the highest absorbance value recorded when reading the tannic acid sample at a predetermined concentration (10 ppm) within the wavelength range of 400 – 800 nm. The 10 ppm concentration of tannic acid was obtained from 0.01 g of tannic acid weighed and added to aquades in a 100 mL volumetric flask. Subsequently, 10 mL was taken from this solution, and then diluted with aquades in a 100 mL volumetric flask for a 10 ppm tannic acid solution stock. The result of measuring the maximum wavelength of the tannic acid standard was 780 nm, which would be used in making the standard curve and determining the total tannin content (Mulyani et al., 2022 with modifications).

Standard curve preparation

Serial dilutions of the 10 ppm tannic acid stock solution were prepared at concentrations of 0.5, 1, 2, 4, and 8 ppm, each adjusted to a final volume of 10 mL. Aliquots of 1.0 mL from each dilution were then transferred to 10 mL volumetric flasks pre-filled with 7.5 mL aquades.

Subsequently, 0.5 mL Folin–Ciocalteu reagent and 1.0 mL of 20% Na_2CO_3 solution were added to each flask, the contents were homogenized, and the mixtures were left in a dark place for approximately 30 min. Absorbance values were measured at 780 nm using a UV–Vis spectrophotometer, and a calibration curve was constructed for the tannic acid standards (Kharismawati et al., 2009 with modifications).

Total tannin content assay

Twenty-five milligrams of each treatment sample was accurately weighed and diluted to 10 mL with aquades. Subsequently, 1.0 mL of the sample solution was pipetted into a test tube containing 7.5 mL of aquades, followed by the addition of 0.5 mL Folin–Ciocalteu reagent and 1.0 mL of 20% Na_2CO_3 solution. The mixture was homogenized and left in a dark place for approximately 30 min (Andriyani et al., 2010 with modifications). The absorbance of the resulting solution was then measured at a wavelength of 780 nm using a UV-Vis spectrophotometer, and the sample concentration was determined based on the previously established standard curve employing the corresponding regression equation (Noer et al., 2018):

$$y = a + bx \quad \rightarrow \quad x = (y-a)/b$$

Description:
x = Concentration
b = Slope
y = Standard absorbance
a = Intercept (meeting point of x and y)

The results of sample concentration values obtained from regression equations were used to calculate total tannin levels using the following formula (Wachidah, 2013):

$$\text{Tannin content (mg/g)} = \frac{X \times V}{W} \times Df$$

Description:
X = Concentration of compounds in the sample solution (mg/mL)
V = Volume of sample solution (mL)
Df = Dilution factor
W = Sample weight (g)

The obtained total tannin content results were used to calculate the percentage of tannin reduction via the following formula (Setiarto & Widhyastuti, 2017):

$$\text{Reduction level of tannin content (\%)} = \frac{\text{Difference in tannin content}}{\text{Tannin content before fermentation}} \times 100\%$$

Description:
Difference in tannin content = Tannin content before fermentation – Tannin content after fermentation

RESULT

Tannin content is crucial in peaghurt, as it adversely affects sensory qualities through bitterness and astringency, nutritional value by impairing protein digestibility, and mineral

bioavailability via chelation and complexation. The total tannin value of bean milk, both before (Table 1) and after fermentation (Figure 1), can be determined using UV-Vis spectrophotometry.

Table 1. Tannin Content of Bean Milk before Fermentation

Bean Milk Types (K)	The Mean Tannin Content Value of Bean Milk before Fermentation (mg/g)
Red Kidney Bean (K1)	13.81 ± 3.54
Black Soybean (K2)	24.81 ± 4.05
Yellow Soybean (K3)	7.89 ± 1.62

The findings presented in Table 1 demonstrate that the three types of bean milk prior to fermentation exhibit divergent tannin concentrations. Notably, black soybean milk (K_2) exhibited the highest tannin content, measuring 24.81 mg/g. The total tannin content of black soybean milk (K_2) after fermentation with the respective LAB starter combinations of A_1 (*L. bulgaricus* + *S. thermophilus*), A_2 (*L. bulgaricus* + *L. plantarum*), A_3 (*S. thermophilus* + *L. plantarum*), and A_4 (*L. bulgaricus* + *S. thermophilus* + *L. plantarum*) was 21.48 mg/g, 18.69 mg/g, 16.41 mg/g, and 13.76 mg/g, respectively (Figure 1). However, based on the results of a post-hoc Tukey's test, treatment A_4K_2 (13.76 mg/g) showed no significant difference ($p > 0.05$) from treatments A_1K_1 (12.04 mg/g), A_2K_1 (10.61 mg/g), A_3K_1 (9.47 mg/g), and A_4K_1 (7.89 mg/g).

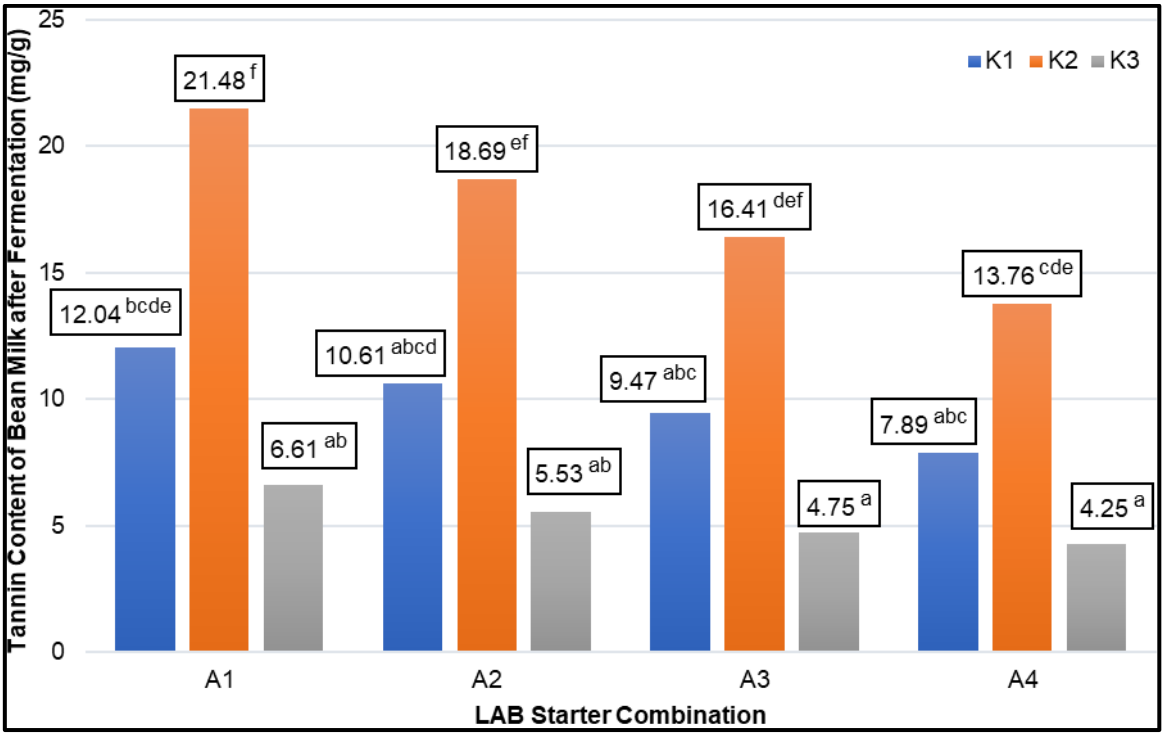


Figure 1. The mean tannin content value of peaghurt with four LAB starter combinations and three bean milk types. Numbers with a distinct minuscule letter on the graph denote statistically significant differences (α = 5%; Tukey HSD test, SPSS)

Meanwhile, Table 1 also shows that the lowest total tannin content in bean milk before fermentation was found in yellow soybean milk (K_3), measured at 7.89 mg/g. After fermentation with the respective LAB starter combinations of A_1, A_2, A_3, and A_4, the total tannin content of yellow soybean milk (K_3) decreased to 6.61 mg/g, 5.53 mg/g, 4.75 mg/g, and 4.25 mg/g, respectively (Figure 1). However, according to the results of a post-hoc Tukey test, treatment A_1K_3 (6.61 mg/g) was not significantly different ($p > 0.05$) from treatments A_4K_1 (7.89 mg/g), A_3K_1 (9.47 mg/g), A_2K_1 (10.61 mg/g), and A_1K_1 (12.04 mg/g).

The research results in Table 1 and Figure 1 indicate a decrease in tannin content between bean milk before fermentation and bean milk after fermentation (peaghurt) using various combinations of LAB starters in all three types of bean milk. This is confirmed by the data presented in Figure 2, where the highest total tannin reduction in peaghurt was observed in treatment A_4K_3, achieving a decrease of 46.18%. Significant total tannin reductions were also found in treatments A_4K_2 (44.60%) and A_4K_1 (42.92%). These findings are visually represented by the larger bar heights compared with the other treatments in Figure 2, corresponding to peaghurt fermented with the LAB starter combination of A_4 across all three bean milk substrates. This was further supported by Tukey's post-hoc test, which confirmed that the A_4 starter combination on the three bean milk substrates was significantly different ($p < 0.05$) from other treatments.

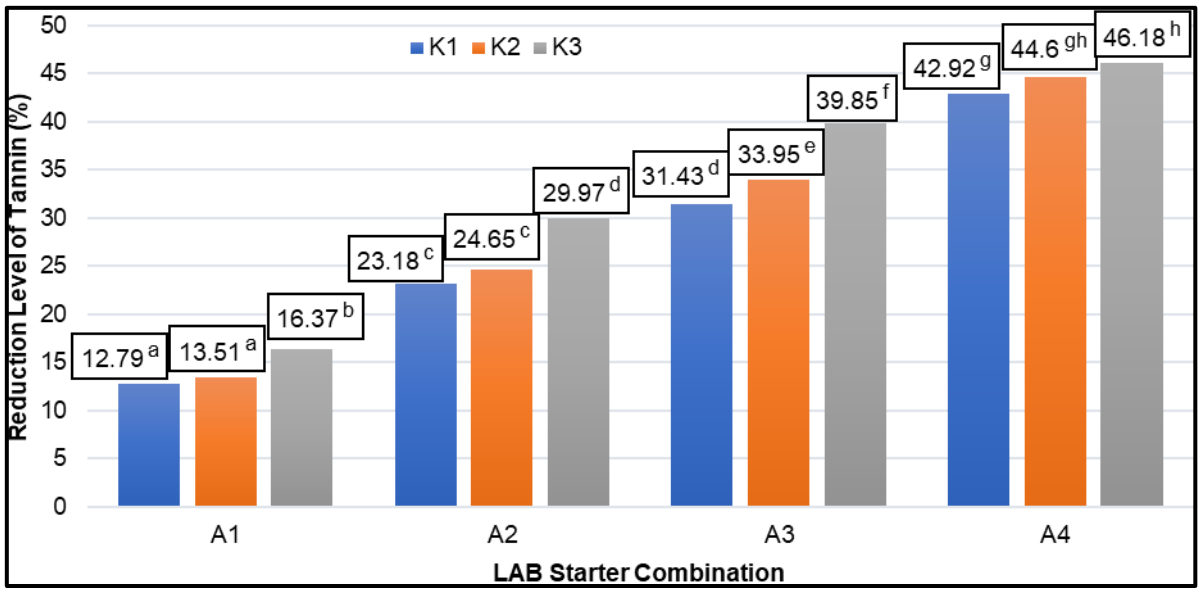


Figure 2. The mean reduction level value of tannin in peaghurt with four LAB starter combinations and three bean milk types. Numbers with a distinct minuscule letter on the graph denote statistically significant differences (α = 5%; Tukey HSD test, SPSS)

Conversely, based on the data from Figure 2, the lowest reduction level of tannin in peaghurt was observed in treatment A_1K_1, at 12.79%. Similarly low reductions were recorded in treatments A_1K_2 (13.51%) and A_1K_3 (16.37%). This is illustrated in Figure 2 by the shorter bar heights corresponding to peaghurt fermented with the LAB starter combination of A_1 across all three bean milk substrates. These observations were further supported by the results of the

Tukey post-hoc test, which confirmed that the A_1 starter combination across the three bean milk types differed significantly ($p < 0.05$) from the other treatments.

Furthermore, the results of MANOVA and Tukey's post-hoc test showed that each treatment factor, as well as the interaction between the LAB starter combinations and bean milk types, had a statistically significant effect ($p < 0.05$) on the reduction of total tannin in peaghurt. This indicates that the three bean milk types exhibit different initial tannin levels and that the tannase activity produced by the three LAB starter combinations differs significantly from one another.

DISCUSSION

The highest tannin content in peaghurt was found in the A_1K_2 treatment, which was 21.48 mg/g, while the lowest was found in the A_4K_3 treatment, which was 4.25 mg/g (Figure 1). This is attributable to the higher total tannin content in black soybean milk (24.81 mg/g) compared to yellow soybean milk (7.89 mg/g) (Table 1). This finding is supported by a study conducted by Xu & Chang (2008), which reported that black soybeans contain higher tannin levels (6.96 mg/g) than yellow soybeans (2.15 mg/g).

Tannins, polyphenolic secondary metabolites ubiquitous in plants, confer a number of beneficial properties, including astringency, antidiarrheal activity, antibacterial effects, anti-inflammatory agents, and antioxidant capacity (Malangngi et al., 2012). Despite their bioactive properties, tannins negatively affect food quality by contributing to bitterness (Awika & Rooney, 2004), impairing protein digestibility through reduced protease accessibility, and inhibiting the bioavailability of essential minerals, including iron and magnesium (Goldstein & Swain, 1965), which can ultimately reduce the nutritional value of food. In relation to this statement, there is a necessity for food processing approaches can simultaneously improve the flavor and acceptability of legume-based foods while enhancing nutrient absorption through the inactivation of key antinutritional compounds such as tannins, trypsin inhibitors, and hemagglutinins (Xu & Chang, 2008).

Fermentation mediated by LAB is widely recognized as an efficient food processing strategy for reducing tannin content in food matrices, as it utilizes microbial metabolic activity to degrade complex compounds, thereby lowering antinutrient levels while simultaneously enhancing the overall nutritional quality of foods (Senanayake et al., 2023). Moreover, the results obtained from this study indicate that reductions in total tannin content were positively correlated with increased lactic acid production during the fermentation process. This phenomenon is attributed to the extracellular tannase enzyme, produced by LAB during the fermentation process, which hydrolyzes tannins into glucose and gallic acid (Setiarto & Widhyastuti, 2017). Subsequently, the resulting glucose can be utilized for lactic acid production through the glycolytic pathway.

The highest decrease in the value of peaghurt tannin levels was found in treatment A_4K_3, namely: 46.18%, and the lowest decrease in the value of peaghurt tannin levels was found in the A_1K_1 treatment, namely: 12.79% (Figure 2). This phenomenon is attributed to the higher carbohydrate (24.9 g), protein (40.4 g), and fat (16.7 g) content in yellow soybean milk, which

is more efficiently metabolized by the three LAB starter cultures compared to the composition in red bean milk (carbohydrates 56.2 g, protein 22.1 g, fat 1.1 g) (Tim Update TKPI Kemenkes, 2018). This finding aligns with Setiarto & Widhyastuti (2017), who reported that low tannase enzyme activity results from suboptimal substrate composition that inadequately supports the metabolic processes essential for LAB growth. As LAB growth accelerates, tannase enzyme activity correspondingly increases, leading to a more significant reduction of total tannin content in the substrate. Tannase (tannin acyl hydrolase), the enzyme mediating this process, catalyzes the hydrolytic cleavage of ester in hydrolysable tannins and gallic acid esters, yielding gallic acid and glucose (Aguilar et al., 2007).

In addition, each microbial species produces significantly different levels of tannase enzyme activity (Setiarto & Widhyastuti, 2017). This was revealed in the present study, where peaghurt fermented with the LAB starter combination of A_4 showed higher tannase activity than peaghurt produced using the LAB starter combination of A_1. This is attributable not only to the greater bacterial diversity in the A_4 starter combination compared with A_1, but also to the fact that *L. plantarum* exhibits higher tannase activity than *L. bulgaricus* and *S. thermophilus*. According to Rodríguez et al., (2008), *L. plantarum* isolated from wine fermentation is capable of producing tannase with high activity, which can improve the organoleptic quality of food products. This finding is congruent with the report by Oriabinska et al., (2018), who elucidated that *L. plantarum* exhibits higher tannase activity (0.024 U/mL) compared with *L. bulgaricus* (0.009 U/mL). Meanwhile, the tannase activity of *S. thermophilus* has not yet been determined and has not been previously investigated.

CONCLUSION

Overall, the results indicate that all of combinations of the LAB starters *L. bulgaricus*, *L. plantarum*, and *S. thermophilus* have the potential to reduce tannins through peaghurt fermentation by 12.79% to 46.18%. The most effective LAB starter combination for reducing tannin content was *L. bulgaricus*, *L. plantarum*, and *S. thermophilus* (A_4), achieving reductions of 42.92% in red kidney bean milk (K_1), 44.60% in black soybean milk (K_2), and 46.18% in yellow soybean milk (K_3). The reduction in tannin content during peaghurt fermentation is attributed to the activity of tannin acyl hydrolase synthesized by these LAB.

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