



# Physical and Chemical Characteristics of Combined Polyherbal Yogurt

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## ABSTRACT

Yogurt has become a popular dairy product and is considered a promising candidate for functional food intake. Innovations in yogurt production continue to develop, increasing public interest in yogurt consumption by using herbal plants. The combination of active ingredients from multiple herbal plants may provide higher effectiveness compared to single herbal plants. Lactic acid fermentation in herbal plants significantly increases phenolic and flavonoid levels. This study aims to analyze the effect of polyherbal (green tea, fenugreek seed, and cinnamon) on yogurt with physical and chemical parameters. This study was conducted with the stages of making polyherbal extract, preparing polyherbal yogurt, and analyzing physical (viscosity) and chemical (pH, total titrated acid (TTA), total phenolic content, and total flavonoid content) using a completely randomized design with four different polyherbal levels (0%, 1%, 2%, and 3%) in triplicates. Physical and chemical characteristics data were summarized using analysis of variance. The lactic acid fermentation process in yogurt form on polyherbal had a significant effect on chemical characteristics, such as pH, TTA, total phenolic and flavonoid contents. The best treatment level in this research is 3% polyherbal extract addition because it contains the highest total phenolic and flavonoid.

**Keywords:** fermentation, *Lactobacillus bulgaricus*, polyherbal, *Streptococcus thermophilus*, yogurt

## INTRODUCTION

Public awareness of the nutritional value and health benefits of food has increased significantly over time. People now seek more than just basic sustenance to relieve hunger. This shift is supported by numerous studies on functional food. According to BPOM (2011), functional food is defined as processed food that contains one or more components known to have specific physiological functions, based on scientific research, and is proven to be safe and beneficial to health. Functional foods encompass items that contain active compounds offering health benefits beyond the basic nutrients present in the food. Among various processed livestock products, yogurt has gained popularity as a dairy product and is considered a promising option for functional food intake.

The decrease in pH due to the fermentation process makes most proteins can be digested properly by proteolytic enzymes, so that overall digestibility and bioavailability increase (Fishberg and Machado 2015). According to the Food and Drug Administration (FDA), yogurt is generally produced by two lactic acid bacteria (LAB) species: *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Freitas 2017). Studies show

that *L. bulgaricus* produces large amounts of certain amino acid compounds and peptides that trigger the growth and acid production of *S. thermophilus*, while *S. thermophilus* produces formic acid that triggers the growth of *L. bulgaricus* (El Abbassy and Sitohy 1993). Milk as the main raw material of yogurt certainly makes yogurt a rich source of nutrients, namely proteins that provide essential amino acids, vitamins and minerals, calcium, and phosphorus (McKinley 2005). Until now, innovations in yogurt production continue to develop to increase public interest in yogurt consumption, one of the innovations made is the addition of herbal plants to yogurt products.

Yogurt is a dairy product created through the fermentation of milk by LAB cultures. This fermentation process effectively extends the shelf life of milk by inhibiting the growth of pathogenic bacteria (McKinley 2005). Additionally, the decrease in pH during fermentation allows most proteins to be properly digested by proteolytic enzymes, thereby enhancing overall digestibility and bioavailability (Fishberg and Machado 2015). According to the Food and Drug Administration (FDA), yogurt is typically produced using two LAB species: *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Freitas 2017). Research indicates that *L. bulgaricus* generates substantial amounts of specific amino acids and peptides that promote the growth and acid production of *S. thermophilus*. Conversely, *S. thermophilus* produces formic acid, which fosters the growth of *L. bulgaricus* (El Abbassy and Sitohy 1993). As a primary ingredient in yogurt, milk serves as a rich source of essential nutrients, including proteins that provide vital amino

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acids, vitamins, minerals, calcium, and phosphorus (McKinley 2005). Ongoing innovations in yogurt production aim to enhance public interest in yogurt consumption, including the incorporation of various herbal plants into yogurt products.

Humans have relied on herbal plants from nature for a long time, especially for medicinal purposes before the advent of commercial drugs. Research has shown that these herbal plants contain compounds with biological functions that can help prevent and treat certain diseases (Kumontoy *et al.* 2023). Ulfah (2006) suggested that the effectiveness of several active ingredients found in combined herbal plants is often greater than that of a single herbal plant, as the pharmacological effects can work together synergistically. This understanding has led to the practice of utilizing polyherbal formulations, which combine various herbs such as green tea, fenugreek seeds, and cinnamon. Green tea, one of the three types of tea that originate from Indonesia, is derived from the *Camellia sinensis* plant. It is produced without undergoing fermentation (Fajar *et al.* 2018). The primary component of green tea is catechin polyphenols, particularly epigallocatechin-3-gallate (EGCG), which constitutes 50–80% of the total catechins, along with other compounds like epicatechin-3-gallate (ECG) and epicatechin (EC) (Dewi 2008). Cinnamon, a spice plant that can also serve as traditional medicine, includes species native to Indonesia, such as *Cinnamomum burmannii* (Saksina 2020). The dominant compound in cinnamon is cinnamaldehyde, which makes up about 70% of its content (Anggraeni 2018). Similarly, fenugreek (*Trigonella foenum-graecum*) is used as a spice and medicinal plant, particularly its seeds, which contain 23–26% protein, 6–7% fat, and 58% carbohydrates, as well as around 25% dietary fiber (Nursetiani and Herdiana 2018; Wani and Kumar 2016).

The process of making yogurt with a polyherbal combination is described in the work of Zain and Mirdhayati (2021). In this method, the polyherbal mixture is added during the LAB inoculation stage, allowing the polyherbal ingredients to ferment as well. Research by Kustrin *et al.* (2022) indicates that the lactic acid fermentation process in herbal plants significantly enhances the levels of phenolics and flavonoids. Currently, there are no studies exploring the use of a polyherbal blend, specifically a combination of green tea, fenugreek seeds, and cinnamon, in yogurt as a functional food. This is notable because these three herbs are rich in phenolics and flavonoids, which are crucial for their antioxidant properties. Flavonoids have been shown to reduce oxidative stress levels in adipose tissue, potentially helping to combat obesity. Therefore, it is anticipated that the fermentation of yogurt will boost the total phenolics and flavonoids from the polyherbal ingredients (green tea, cinnamon, and fenugreek). This polyherbal yogurt could emerge as a functional food that appeals to consumers.

## METHODS

The research was conducted at the Laboratory of Animal Science Production, Faculty of Animal Science, IPB University, and at the Integrated Laboratory of Bioproducts (i-Lab) of BRIN in Serpong, Indonesia, from May to June 2024.

### Tools and Materials

The tools used in this research include a pot, gas stove, sieve, digital thermometer, digital scale, spoon, jar container, incubator, viscometer, pH meter, burette, Erlenmeyer flask, volumetric flask, vortex, and spectrophotometer. The materials used in this study include fresh cow's milk, polyherbal consisting of three herbal extracts (green tea, fenugreek seed, and cinnamon), lactic acid bacteria (LAB) starter (*S. thermophilus* and *L. bulgaricus*), distilled water, alcohol, pH 4 and 7 buffer solution, 1% PP indicator, 0.1 N NaOH, HCl, gallic acid standard solution, Folin–Ciocalteu reagent, 7% Na<sub>2</sub>CO<sub>3</sub>, 1.5% NaNO<sub>2</sub>, 3% AlCl<sub>3</sub>, and 1 N NaOH.

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### Polyherbal Extraction Process

The extraction process utilized the maceration method with water as the solvent. A total of 100 g of a polyherbal mixture, consisting of green tea, fenugreek seeds, and cinnamon, in powder form, was dissolved in 1 L of water (1:10 ratio). The mixture was then boiled and allowed to cool for 15 min. After cooling, the solution was filtered to remove solid particles. The filtered polyherbal macerate was then evaporated using the double boiler technique until the extract thickened. To preserve the extracted polyherbal solution, 10% honey was added (Badriyah and Fariyah 2022; modified).

### Combining Polyherbal

The procedure for making the combination of yogurt and polyherbal was carried out using a method that referred to the modified research of Zain and Mirdhayati (2021). Fresh cow's milk was pasteurized at 90°C for 3 minutes. Then, the pasteurized milk was

cooled until the milk temperature reached  $\pm 45^{\circ}\text{C}$ . Then, LAB starters *S. thermophilus* and *L. bulgaricus* were inoculated and polyherbal extract was added with four treatment levels (0%, 1%, 2%, and 3%). Next, the samples were incubated using an incubator at  $37^{\circ}\text{C}$  for 8 hours.

The procedure for preparing a yogurt and polyherbal combination followed a modified method based on the research of Zain and Mirdhayati (2021). First, fresh cow's milk was pasteurized at  $90^{\circ}\text{C}$  for 3 min. After pasteurization, the milk was cooled until its temperature reached approximately  $45^{\circ}\text{C}$ . Then, *S. thermophilus* and *L. bulgaricus* starter cultures were inoculated, and polyherbal extract was added at four treatment levels: 0%, 1%, 2%, and 3%. Finally, the samples were incubated in an incubator at  $37^{\circ}\text{C}$  for 8 h.

### Viscosity Test

The viscosity test was carried out using a viscometer. The viscometer was turned on, then the spindle was attached to the viscometer. A sample of 100 mL was poured into the viscometer measurement container, then the spindle was dipped into the sample. The number that appears was recorded as the viscosity of the sample (Wibawanti *et al.* 2018).

### pH Test

The pH was measured using a pH meter, which was first calibrated using pH 4 and 7 buffer solutions. The pH meter was turned on until stable, then the electrode was rinsed using distilled water. The electrode was dipped into the sample solution and left for a while until a stable reading was obtained. The pH value that appeared was recorded (Apriyantono *et al.* 1989).

### Total Acid Titration (TTA) Test

The total titratable acidity (TTA) test for yogurt samples began by diluting 5 g of the sample with 50 mL of distilled water. A total of 25 mL of this diluted yogurt sample was placed into an Erlenmeyer flask, and three drops of 1% phenolphthalein (PP) indicator were added. The sample was then titrated with 0.1 N NaOH, which had been standardized using oxalic acid. The titration was stopped when the mixture turned pink.

To generate a gallic acid standard curve, various concentrations (0.4, 0.8, 1.2, 1.6, and 2.4 mg/L) were prepared and their absorbance was measured at a wavelength of 765 nm. For testing total phenolic content, the Folin–Ciocalteu method was employed. In this procedure, 500  $\mu\text{L}$  of the sample was mixed with 500  $\mu\text{L}$  of 0.1 M Folin–Ciocalteu reagent and shaken at  $45^{\circ}\text{C}$  for 10 min. Subsequently, 500  $\mu\text{L}$  of 0.1 M  $\text{Na}_2\text{CO}_3$  was added, and the mixture was allowed to stand at room temperature for 30 min. Finally, the absorbance of the solution was read at a wavelength of

765 nm using a spectrophotometer (Camones *et al.* 2022).

### Total Flavonoid Test

In a 500  $\mu\text{L}$  sample, 500  $\mu\text{L}$  of 1.5%  $\text{NaNO}_2$  was added, and the mixture was shaken using a vortex for 10 min. Following this, 1000  $\mu\text{L}$  of 3%  $\text{AlCl}_3$  was added, and the solution was shaken again using a vortex for 2 min. Then, 1000  $\mu\text{L}$  of 1 N NaOH was added, and the mixture was shaken for an additional 2 to 8 minutes. The sample was subsequently analysed for absorbance at a wavelength of 490 nm using a spectrophotometer (Camones *et al.* 2022).

### Data Analysis

The design used in this study was a completely randomized design (CRD) with four levels of polyherbal addition treatment, each repeated three times. The treatments consisted of P0 (control): 0% polyherbal addition, P1: 1% polyherbal addition, P2: 2% polyherbal addition, and P3: 3% polyherbal addition. The statistical model used refers to Steel and Torrie (1997).

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where:

$Y_{ij}$  = Response to the  $i$ -th polyherbal addition treatment (0%, 1%, 2%, and 3%) and to the  $j$ -th replication (1, 2, and 3).

$\mu$  = General mean of the study response

$\alpha_i$  = Effect of  $i$ -th level of polyherbal addition (0%, 1%, 2%, 3%)

$\varepsilon_{ij}$  = Error effect of adding the  $i$ -th polyherbal (0%, 1%, 2%, and 3%) and the  $j$ -th replication (1, 2, and 3)

Data on physical and chemical characteristics were analyzed using the Analysis of Variance (ANOVA) to determine differences with a significant level of 95%.

## RESULTS AND DISCUSSION

### Physical Characteristics

There were no significant differences between the viscosity of the control group (P0) and the treatments (P1, P2, and P3), nor among the treatment groups themselves (P1, P2, and P3) ( $p > 0.05$ ) (Table 1). The viscosity value obtained in this study was lower than that reported by Dahlan and Sani (2017), which recorded a viscosity of 1.59 Pa.s. The lower viscosity in this study is likely due to a shorter incubation time of 8 h, compared to the 24 h used in Dahlan and Sani's work (2027). Similarly, Adiputra *et al.* (2022) found that an incubation time of 9 h resulted in a higher viscosity compared to an incubation time of 3 h. They concluded that incubation time significantly affects yogurt

viscosity; the longer the incubation, the more LAB grow, leading to increased viscosity.

### Chemical Characteristics

The average pH derived from the analysis of variance (Table 2) indicates that the addition of polyherbal significantly affects the pH of yogurt, especially at the highest treatment level of P3 (3%) with a significance level of  $p < 0.05$ . The polyherbal extract used in the treatment resulted in a slightly higher pH compared to yogurt without any treatment (P0), which had a pH of approximately 5.20. Therefore, a minimum treatment level of 3% is necessary to observe the effects of polyherbal extracts on the pH. It is noted that green tea and cinnamon extracts have acidic pH levels. According to Handayani *et al.* (2013), green tea extract has a pH of 5.5, while Nurmalasari *et al.* (2018) reported that cinnamon extract has an acidic pH ranging from 4 to 6.5 due to the presence of cinnamic acid. Additionally, chlabet seeds possess a pH that is close to neutral, around 6.8 to 6.9 (Dilshad 2017). The pH of the four samples examined in this study meet the quality requirements established by SNI (2009) for yogurt, which specifies a pH range of 3.8 to 4.5.

The average TTA values from the analysis of variance (Table 2) indicated a significant difference ( $p < 0.05$ ) between treatments P0 and P3. According to Aleman *et al.* (2020), higher pH in yogurt correspond to lower TTA. This is because a low pH indicates the presence of acidic substances; consequently, TTA, measured as a percentage of lactic acid or total acid resulting from the conversion of lactose by LAB, tends to be higher (Kamaluddin and Handayani 2018; Zakaria 2008). The TTA for each treatment were ranked in descending order as follows: P0, P1, P2, and P3. Conversely, the pH values for each treatment were

ranked from highest to lowest as follows: P3, P2, P1, and P0. Notably, the TTA values of all four samples in this study met the quality standards for yogurt established by SNI (2009), which stipulate a range of 0.5–2.0%.

The analysis of variance for total flavonoids (Table 2) indicates that adding polyherbal extracts at different treatment levels has a significant effect ( $p < 0.05$ ), even though the differences between the treatment levels were only about 0.5% to 1%. Additionally, the results of total flavonoids in this study were directly proportional to total phenolics, which aligns with findings by Camones *et al.* (2022). This correlation is likely due to flavonoid compounds being a major component of phenolics, as noted by Balasundram *et al.* (2006). Among the extracts used, green tea extract contributed the most flavonoids to the polyherbal yogurt, significantly more than fenugreek seed and cinnamon extracts. This is because green tea contains approximately 30% flavonoids, primarily derived from the catechin group (Ratnani and Malik 2022).

## CONCLUSIONS

The lactic acid fermentation process used in yogurt production is influenced by the addition of polyherbal extracts, which enhances several chemical characteristics of yogurt. These characteristics include pH, total titratable acidity (TTA), total phenolic content, and total flavonoid content. The combined polyherbal yogurt produced in this study meets the quality standards set by the SNI yogurt specifications (2009). The optimal treatment level identified in this research was the addition of 3% polyherbal extract (denoted as P3).

Table 1 Physical characteristics of polyherbal yogurt combination

Parameter	Treatments			
	P0	P1	P2	P3
Viscosity (Pa.s)	1.03±0.06	1.10±1.00	1.10±0.00	1.10±1.00

Description: P0: yogurt with 0% polyherbal addition; P1: yogurt with 1% polyherbal addition; P2: yogurt with 2% polyherbal addition; P3: yogurt with 3% polyherbal addition; Means having the different superscript in the same row are significantly different ( $P < 0.05$ ).

Table 2 Chemical characteristics of polyherbal yogurt combination

Parameters	Treatments				SNI
	P0	P1	P2	P3	
pH	4.11±0.02 <sup>b</sup>	4.13±0.01 <sup>b</sup>	4.15±0.02 <sup>ab</sup>	4.19±0.02 <sup>a</sup>	3.8–4.5
TTA (%)	1.12±0.11 <sup>a</sup>	1.05±0.06 <sup>ab</sup>	0.96±0.11 <sup>ab</sup>	0.88±0.04 <sup>b</sup>	0.5–2.0
Total phenolics (mg GAE/g)	0.06±0.02 <sup>c</sup>	0.14±0.01 <sup>b</sup>	0.26±0.03 <sup>a</sup>	0.32±0.04 <sup>a</sup>	–
Total flavonoids (mg QE/g)	0.026±0.03 <sup>d</sup>	0.033±0.00 <sup>c</sup>	0.037±2.18 <sup>b</sup>	0.045±0.00 <sup>a</sup>	–

Description: P0: yogurt with 0% polyherbal addition; P1: yogurt with 1% polyherbal addition; P2: yogurt with 2% polyherbal addition; P3: yogurt with 3% polyherbal addition; Means having the different superscripts in the same row are significantly different ( $P < 0.05$ ).



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