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Proximate Characteristics and Antioxidant Activity of Polyherbal Extract in Yogurt

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ABSTRACT

Fermented dairy products, such as yogurt, are well known for their health advantages. Enhancing their functional value using polyherbal mixtures constitutes a big step forward in functional food development. This study looks at the proximate composition (fat, protein, moisture, and ash content) and antioxidant activity of polyherbal yogurt enriched with green tea, cinnamon, and fenugreek seed extracts. Formulations were created with varied amounts of polyherbal extract at 0% (P0), 1% (P1), 2% (P2), and 3% (P3), with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as starter cultures (Lactina Ltd). A completely randomized design with three replications was used, and the data were analyzed using ANOVA at a 95% confidence level. The results showed substantial variations in fat (2.84−3.62%) and moisture (88.17−8.83%) content between treatments. Protein (3.92−5.03%) and ash level (0.76−0.77%) met the Indonesian National Standard (SNI) for yogurt. Polyherbal yogurt demonstrated considerably higher antioxidant activity and capacity than the control. The control yogurt (P0) had a poor antioxidant capacity of 60.81±10.89%, while polyherbal yogurt (P1, P2, and P3) had a high antioxidant capacity ranging from 252.40±11.20% to 267.44±1.44%. These findings indicate that including polyherbal extracts into yogurt not only ensures compliance with national nutritional standards (SNI), but also considerably increases antioxidant potential. Thus, polyherbal yogurt is an appealing prospect for development as a beneficial dairy product.

Keywords: antioxidant, polyherbal, proximate, yogurt

INTRODUCTION

Fermented dairy foods, such as yogurt, have been linked to numerous health advantages (Manzanares *et al*. 2021). Yogurt is a dairy product manufactured by inducing fermentation with lactic acid bacteria like *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Gharibzahedi and Chronakis 2018). Fermented foods can provide nutritional and physiological benefits (Wihansah *et al*. 2022). Yogurt has several health benefits, including maintaining intestinal health, increasing lipid and protein bioaccessibility, reducing food allergens, and producing B and K group vitamins, short-chain fatty acids, polyamines, and ω-3 unsaturated fatty acids, including conjugated linoleic acid and bioactive metabolites (Stanton *et al*. 2005).

The increased production of fermented foods has sparked a strong interest in the use of beneficial ingredients (Adler *et al*. 2013). Herbal plants are called functional components because they include a variety of active substances that add value to physiological systems, providing additional advantages without causing harm to the body. Green tea (*Camellia sinensis*), which is high in polyphenols and has a variety of biological activities such as anticarcinogenic, antiviral, antiallergic, and anti-inflammatory properties, as well as a stimulating effect on immunity (Scalbert *et al*. 2005), can enhance the functional properties of food products. Cinnamon (*Cinnamomum burmannii*) is commonly used as a spice and natural preservative in cuisine. Cinnamon possesses antioxidant, antibacterial, antipyretic, and antiallergenic properties. These favorable qualities can improve food safety and shelf life, particularly for fat-rich processed foods (Pazra *et al*. 2023). Fenugreek seeds (*Trigonella foenum-graecum*) is a perennial plant native to India and North Africa that has been used for centuries to treat a variety of ailments, including diabetes and hyperlipidemia. The plant is well known for its high fiber content and phytochemical composition (Dhawi *et al.* 2020).

The growing public interest in herbal medicine goods as an alternative treatment has prompted additional study into improvements in the production of fortified herbal products in food products (Mohamed *et al*. 2021). According to Cheigh *et al*. (1994), during the herbal fermentation process by *Lactobacillus* bacteria, organic acids, hydrolyzed proteins, and antioxidant ferulic acid are produced from dissolving plant cell walls. This demonstrates that the fermentation process

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in herbal plants can cause the breakdown of plant cell wall structures, which results in the release or synthesis of numerous antioxidant chemicals (Hussain *et al*. 2016). Proximate analysis and antioxidant activity are required as a foundation for the development of polyherbal yogurt products as functional foods that are expected to meet community needs while also raising public awareness of the importance of a healthy diet. They are also useful for adding value to processed herbal-based diary products.

METHODS

Preparation of Polyherbal Extract

The maceration method was used to extract the dry polyherbal powder, which contained green tea, cinnamon, and fenugreek seeds (16:5:4). Maceration was performed by mixing 500 g of polyherbal preparations with 1000 mL of water (1:10) (Emilia *et al*. 2023). After that, the mixture was brought to a boil, cooled to 45°C for 15 min, and filtered. The filtered polyherbal macerate was then evaporated using a double boiler or a bain-marie technique at temperatures ranging from 45 to 50°C until the extract thickened and yielded 20%. Furthermore, as a natural preservative, 10% honey was added to the polyherbal extract, which was then refrigerated at a maximum temperature of 4°C.

Polyherbal Yoghurt Preparation

Cow's milk was pasteurized at 90°C for 3 min before cooling to 40−45°C (Ayar and Gürlin 2014). The milk (1L) was then inoculated by 1 g Bulgarian yogurt starter (Lactina Ltd.) contains of *S. thermophilus* and *L. bulgaricus*) followed by varying amounts of polyherbal. The inclusion of polyherbal before incubation is intended to cause polyherbal to undergo lactic acid fermentation (Zain and Mirdhayati 2021). The milk was then incubated at 37°C for 8 h to produce yogurt. Following that, polyherbal yogurt was served with 1.5% stevia as a natural sweetener.

Proximate Characteristics Analysis

The proximate properties of polyherbal yogurt, such as fat, protein, moisture, and ash content, were examined using AOAC (2005) standard techniques. The fat content was assessed using the Soxhlet extraction method, with hexane as the solvent. The protein content was determined using the Kjeldahl method, and the resultant nitrogen content was converted to protein using a conversion factor of 6.38. The moisture content was evaluated gravimetrically by drying the sample in a porcelain crucible at 105°C for 24 h. The weight loss was then utilized to quantify the moisture level. The ash content was determined by estimating the inorganic residue left after incineration in a muffle furnace at 600°C for 7 h. The mass of the residual inorganic residue was utilized to calculate the overall ash content.

Antioxidant Analysis

Antioxidant activity was assessed using DPPH inhibitor (% scavenging activity). A vial was filled with 0.15 mL of each test solution, followed by 0.9 mL of 0.1 mM DPPH solution (Tangkanakul *et al.* 2009). The samples were then incubated in a dark room for 20 min. The absorbance was measured using a spectrophotometer at a wavelength of 517 nm. The %SA value was converted based on the standard absorbance curve of ascorbic acid at concentrations of 0; 0.5; 1.0; 1.5; 2.0; and 2.5 mg/100 mL. A linear regression was created based on the linear curve with the equation $y = ax + b$. The antioxidant capacity value is expressed as the vitamin C equivalent value (mgEVC/100g).

Data Analysis

The data analysis used in the study of yogurt with polyherbal combination is a completely randomized design (CRD) with the inclusion of polyherbal formulations (green tea, cinnamon, and fenugreek seeds) at concentrations of 0% (control), 1%, 2%, and 3%, repeated three times. Data on proximate features and antioxidant activity will be examined using analysis of variance (ANOVA) to determine each treatment's effect with a 95% confidence interval.

RESULTS AND DISCUSSION

Proximate Characteristics

The examination of fat content (Table 1) revealed a substantial difference in yogurt with varying degrees of polyherbal inclusion. Adding 3% polyherbal extract yielded the lowest fat content (2.84 ± 0.15%). The decrease in fat content associated withthe dilution effect of the initial composition of the extract. The higher the concentration of polyherbal extract, the greater the volume of fat-free components contained in the yogurt, thereby causing a dilution effect on the total fat composition in percentage terms. According to SNI (2009), the minimal yoghurt fat level is 3%, while 0.6−2.9% is considered low-fat yogurt. Fat in yogurt plays an important effect in decreasing the physicochemical quality of yogurt. Low fat levels can result in poor textural quality due to whey formation or susceptibility to syneresis (Domagala *et al.* 2013). Nonetheless, the present market demand for low-fat yoghurt products is increasing due to consumer perception of health benefits (Zhao *et al*. 2023).

The tested polyherbal yogurt had an average ash level of 0.77% (Table 1). The ash content test findings in polyherbal yogurt met the SNI criteria (maximum 1%). The results of statistical analysis, which were not substantially different in the ash content test, revealed that varying degrees of polyherbal addition had no meaningful effect on the ash content of yogurt. A product's ashes can indicate the amount of minerals it contains (Gemede *et al*. 2015). The more minerals a product has, the higher its ash content. Minerals are considered important because of their function as body regulators. Green tea, as one of the polyherbal formulation ingredients used, has a mineral composition of 5–7% consisting of potassium (K), phosphorus (P), and magnesium (Mg), as well as small amounts of manganese (Mn), zinc (Zn), and copper (Cu) (Ratnani and Malik 2022).

According to the study results in Table 1, the percentage of moisture content varies greatly. The control has the greatest water content test findings (88.83 ± 0.15%), whereas yogurt with 3% polyherbal extract has the lowest (88.17 ± 0.11%). This value is similar to the findings of Soni *et al*. (2020), who found an 87.32% of yogurt moisture content when using *S. thermophilus* and *L. bulgaricus* starter cultures. Furthermore, when the level of polyherbal addition increased, the moisture content in yogurt dropped. Fiber and other water-retaining herbs can have an impact on this. According to Al-Shawi *et al*. (2020), the inclusion of herbs has a significant impact on yogurt moisture content. Herbs can reduce moisture content, which is also affected by the physicochemical properties of yogurt. Cinnamon in yogurt can boost water-holding capacity (WHC) during storage and interact positively with moisture content (Shori *et al*. 2013).

Antioxidant Activity

Table 2 reveals the antioxidant activity and capacity study of yoghurt with varying degrees of polyherbal extract addition. Control yoghurt exhibited a lower percentage of antioxidant activity (17.97%) than polyherbal yogurt. The antioxidant activity in polyherbal yogurt ranged from 91.19 to 96.94%. According to the findings, adding polyherbal to yogurt can boost the antioxidant content when compared to control yogurt. These findings suggest that antioxidants produced from polyherbal extract prevent intermolecular free radical oxidation reactions (Jeki *et al*. 2019). Antioxidants, as electron-providing atoms or molecules, have the potential to minimize the detrimental impact of free radicals by neutralizing radical chemicals that can disrupt the body's biological systems due to excessive oxidation reactions (Hidayah *et al*. 2024). Excessive oxidation processes, also known as oxidative stress, occur when the body's reactive oxygen species (ROS) levels surpass its antioxidants. Oxidative stress can damage cell membranes, proteins, unsaturated lipids, enzymes, and DNA, resulting in a variety of disorders (Aman 2017). This is created by unstable, harmful free radical molecules (Putri *et al*. 2021).

Furthermore, the antioxidant capacity study exposed a substantial difference between the treated yogurt and the control. P1 (1%), P2 (2%), and P3 (3%) represent the antioxidant capacity of the treated yogurt in sequence of decreasing to increasing. Antioxidant capacity measures a molecule's ability to trap DPPH free radicals. Tangkanakul *et al*. (2009) identified four types of antioxidant capacity: very high (>500 mg EVC/100 g DW), high (200−500 mg EVC/100 g DW), medium (100−200 mg EVC/100 g DW), and low (<100 mg EVC/100 g DW). According to the results, the antioxidant capacity value of control yogurt is low, whereas polyherbal yogurt has a high antioxidant capacity.

Green tea is an important functional food in medicine and health because of its polyphenolic components, which have strong antioxidant qualities. Catechins are the primary polyphenols in tea, accounting for 30−42% of the dry weight of green tea leaves (Muniandy *et al*. 2016) and serving as the primary component that contributes to tea's functional properties. The catechin complex present in tea consists of gallocatechin (GC), epigallocatechin (EGC),

Table 1 Proximate characteristics of polyherbal extracts in yogurt

Parameter	Treatment			
	P0	P1	P2	P3
Protein (%)	3.93±0.04	4.14±0.47	5.03±0.99	4.09±0.19
Fat (%)	3.20±0.07^{ab}	3.62±0.49^{a}	3.10±0.14^{ab}	2.84±0.15^{b}
Ash (%)	0.78±0.01	0.76±0.01	0.77±0.01	0.78±0.03
Moisture (%)	88.83±0.15^{a}	88.63±0.19^{ab}	88.31±0.03^{bc}	88.17±0.11^{c}

Remarks: P0 = 0% polyherbal extract, P1 = 1% polyherbal extract, P2 = 2% polyherbal extract, and P3 = 3% polyherbal extract. Different letters on the same line indicate significant differences (*p* < 0,05).

Table 2 Antioxidant properties of polyherbal yogurt

Treatment	Antioxidant activity (%)	Antioxidant capacity (mgECV/100g)
P0	17.97±4.16b	60.81±10.89b
P1	91.19±4.28a	252.40±11.20a
P2	93.62±1.35a	258.84±3.52a
P3	96.94±0.55a	267.44±1.44a

Remarks: P0 = 0% polyherbal extract, P1 = 1% polyherbal extract, P2 = 2% polyherbal extract, and P3 = 3% polyherbal extract. Different letters on the same line indicate significant differences (*p* < 0,05).

epigallocatechin-3-gallate (EGCG), epicatechin (EC), and epicatechin-3-gallate (ECG) (Anjarsari 2016). Cinnamon contains a high concentration of phenolic and volatile chemicals, which have been linked to improved human health (Rahadian *et al*. 2017). Furthermore, the seeds include polyphenolic compounds such as rhaponticin and isovitexin, flavonoids, alkaloids, amino acids, coumarins, vitamins, saponins, and other antioxidants that are regarded to be the primary bioactive ingredients (He *et al*. 2015).

In general, polyphenols, which are abundant in herbal plants, work by increasing the activity of antioxidant enzymes, inhibiting lipid peroxidation, capturing free radicals, and reducing oxidation through metal ion chelation (Yiannakopoulou 2013). According to Rong and Li (2012), the antioxidant mechanism of phenolic compounds can be summarized as the process of hydrogen atom transfer or single electron transfer via protons. Based on in vitro test results in Xing *et al.* (2019), EGCG in green tea was reported to reduce the number of reactive oxygen species (ROS), increase cell viability, and inhibit H_2O_2. The ability of polyphenols to scavenge ROS depends on the number of hydroxyl groups in the structure, the environment, and the stability of phenolic oxygen radicals. Polyphenols react with ROS to form relatively stable phenolic oxygen radicals, thereby eliminating free radicals.

Additionally, the electrons in the benzene ring of the polyphenol structure have a conjugating effect on the single electron of the oxygen atom in the phenolic hydroxyl group. These lone electrons tend to move to the benzene ring, thereby reducing the hydrogen-oxygen bond activity in the phenolic hydroxyl group. The hydrogen activity in the phenolic hydroxyl group increases, and free radicals compete for active oxygen, halting the auto-oxidation reaction of free radicals (Yan *et al.* 2020). With aging, natural antioxidant synthesis and absorption drop, necessitating the use of exogenous antioxidants. These can be generated from herbal plants such as green tea, cinnamon, and fenugreek seeds, and their bioactivities can be increased further through LAB fermentation, as observed in polyherbal yogurt.

CONCLUSION

The addition of polyherbal extracts to yogurt affects fat and moisture content but has no significant effect on protein or ash level. The observed increase in antioxidant activity and capacity after adding polyherbal extracts suggests that these extracts can improve the antioxidant performance of yogurt. According to the findings, polyherbal yogurt had favorable proximate features and antioxidant activity in line with the Indonesian National Standard (SNI) for yogurt.

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