

Yoghurt Synbiotic with Fortification of Bawang Dayak (*Eleutherine palmifolia*) Extract

ABSTRACT

Aims: The purpose of this research was to study the quality of yoghurt symbiotic fortified with Bawang Dayak (*Eleutherine palmifolia*) extract.

Study design: This research used Completely Randomized Design (CRD) experimental method and consist of 4 treatments.

Place and Duration of Study: Sample: Animal Product Technology Laboratory Faculty of Animal Science Universitas Brawijaya, Food Quality and Safety Testing Laboratory Department of Agricultural Product Technology, Faculty of Agricultural Technology Universitas Brawijaya, between June 2022 – December 2022.

Methodology: The material used are skim milk, fresh milk, yoghurt starter bacteria (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*), and bawang dayak extract. Bawang Dayak were extracted using Microwave-Assisted Extraction (MAE) with temperature 60-70°C. This research which consist of 4 treatments and 3 replications with various concentration of Bawang Dayak extract of T0 (0%), T1 (1%), T2 (4%), and T3 (6%), respectively. The samples of yoghurt synbiotic with fortification of Bawang Dayak extract were analyzed for their total lactic acid bacteria, antioxidant activity, viscosity and color analysis.

Results: Fortification of Bawang dayak extract in synbiotic yogurt results in highly significant different effects on the quality parameters of antioxidant and color (lightness, redness and yellowness) ($P < 0.01$), but had no significant effect ($P > 0.05$) on the total quality of lactic acid bacteria. Fortification of 6% Bawang dayak extract (T3) in yogurt produced the best quality in terms of antioxidant content and color (lightness, redness, and yellowness).

Conclusion: The best treatment of yoghurt synbiotic was achieved in T3 with the 6% of Bawang Dayak extract.

Keywords: {yoghurt, synbiotic, bawang Dayak, extract}

1. INTRODUCTION

Yogurt is one of the milk fermentation products produced by lactose fermentation to lactic acid by the starter culture of yogurt. Yogurt starters usually consist of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* will interact with the protein in milk and then improve the texture and sensory quality of the product [1]. Yogurt is widely known as a healthy food because of the presence of protein, vitamins, minerals and probiotics that are good for health. More precisely, the probiotics that are friendly intestinal, omega fatty acids

3, antioxidants, vitamins, minerals, and plant extracts are functional foods [2]. Probiotics provide many beneficial effects such as anti cholesterol, antihypercholesterolemia, antidiabetic, antiulcerogenic, anti-inflammatory, antiobesity, improve the digestive tract and intestinal problems, inhibit the growth of pathogenic bacteria, and help essential micronutrient synthesis [3][4][5]. Increasing the effectiveness of probiotics through synbiotic products is carried out through prebiotic fortification, an undigested substance that can stimulate the growth of certain bacterial species in the intestine [3]. Inulin, is a prebiotic most commonly found in root roots such as chicory (*Cichorium intybus*), garlic (*Allium sativum*), wheat (*Triticum spp.*), Oat (*Avena sativa*) and dalia. The addition of inulin and oligofructose in food is proven to increase the beneficial effects mainly because it is able to spur the growth of lactic acid bacteria in the intestine, increase the bioavailability of minerals, increase antioxidant activity and immune systems [6]

To improve food quality with natural ingredients, the addition of antioxidants from natural ingredients such as plants has been proven efficiently able to protect from oxidation stress due to enzymes or non-enzymatic, because there are some nutritional substances that are only synthesized by plants and can only be accepted through food. In addition, food with the addition of bioactive components can improve the immune system [1]. Many biological activities can be found in plant extracts, such as antioxidants, immunostimulants, antimicrobials. One of the herbs that contain antioxidant components is Bawang Dayak (*Eleutherine palmifolia*), which grows on the island of Kalimantan and is included in the genus Iridaceae. This plant contains phenol and flavonoids which act as an antioxidant and antibacterial components. In addition to the antioxidant content, extracts from this plant also contain FOS (Fructo-Oligo-Saccharide) and Inulin as Prebiotics [7]. The main part of the Bawang dayak plant is the tubers that are often used either in dry, pickled or powder forms [8]. The tubers from Bawang dayaks have antioxidant activity with IC_{50} of 1.48 $\mu\text{g/ml}$ compared to ascorbic acid in DPPH absorption. Strong antioxidant activity can affect the growth of probiotic bacteria, so the tubers from Bawang dayaks have the potential as prebiotics and antioxidant agents [9].

However, there is no study that explains the evaluation of the provision of Bawang dayak extract to yogurt in terms of quality. Therefore, evaluation is needed for the quality of Synbiotic yogurt with total parameters of lactic acid bacteria, viscosity, antioxidants and colors of Synbiotic yogurt products. The purpose of this study is to evaluate the quality parameters of the yogurt Synbiotic with the fortification of bawang Dayak extract.

2. MATERIAL AND METHODS

The study was conducted using Completely Randomized Design (CRD). The factors used are the concentration of Bawang Dayak extract for yogurt, namely 0% (T0), 1% (T1), 4% (T2) and 6% (T3) with 3 repetitions. The data obtained are tabulated and calculated on average and standard deviations. Data were analyzed using Analysis of Variance (ANOVA), if there was a difference in the influence it would be followed by the Duncan Multiple Test (UJBD)

The material used in this research is synbiotic yogurt made from fresh milk, skim milk, yogurt starter bacteria, and Bawang Dayak extract. The material used to conduct analysis includes MRSA, Pepton Water, 70% alcohol, DPPH, and 97% ethanol. The equipment used includes color readers, erlenmeyer, measuring cups, beaker glass, stirrer, scales, filter cloth, microwaves, latex gloves, spoons, bunsen, stove, and thermometer.

2.1 Bawang Dayak Extraction

Bawang Dayak was extracted using Microwave-Assisted Extraction (MAE). Weighed as much as 50 grams of Bawang Dayak and then added 500 ml of distilled water (ratio of 1:10). Extracted for 12 minutes with temperature 70°C. Extraction results are removed from the microwave and filtered using a filter cloth. The filtrate was then evaporated with temperature 80°C. After evaporation a thick extract will be obtained. The extract then tested for total phenol and phytochemicals. Bawang dayak extract is ready to be fortified into yoghurt.

2.2 Phytochemical Screening on Bawang Dayak Extract.

Phytochemical screening is carried out with qualitative flavonoids, alkaloids, tannins, phenols, steroids and triterpenoids which are carried out following Ministry of Health of The Republic of Indonesia in *Materia Medika Indonesia* [10].

2.3 Total Phenol Analysis of Bawang Dayak Extract .

Total phenol was analyzed according to Sassi, et al [11] by preparing 100 µL of gallic acid solution mixed with 500 µL of water and 100 µL of Folin-Ciocalteu reagent (FCR) and then left for 6 minutes. Furthermore, 500 µL of distilled water and 7% sodium carbonate were added to the previously prepared mixture and stored in a dark room for 90 minutes. The absorbance was measured using a spectrophotometer at 760 nm. The procedure was repeated for each extract. The total phenol in the sample was calculated as the gallic acid equivalent using the gallic acid standard curve. Repeat the procedure 3 times repetition

2.4 Preparation of Yoghurt Synbiotic

Measured as much as 250 ml of milk and poured on Erlenmeyer, then pasteurized with a temperature of 70°C for 15 seconds. Then cooled to a temperature of 40°C. Added bawang dayak extract according to the treatment (0%, 1%, 4% and 6%) and then homogenized. Inoculation of yogurt starter bacteria as much as 3% and incubated in room temperature for 24 hours.

2.5 Determination of Lactic Acid Bacteria

Testing Total Lactic Acid Bacteria According to Pangestu et al. [12] begins with taking a sample of 10 ml with a micropipette into a test tube containing 90 ml of sterile peptone water as the first dilution (10^1), then homogenized with vortex. Take 1 ml from the first dilution (10^1) insert it into the next test tube, and do it until 7 dilutions (10^7) are obtained. Take 1 ml of the last 3 dilutions (10^5 , 10^6 , 10^7) and inserted each in a petri dish. Pour the sterile MRSA media as much as 10 ml, homogenized with the movement of number 8. Waited until it solidified, then incubated at 37°C for 48 hours with an inverted position. Calculate the petri dish with growth of as much as 30-300 colonies.

2.6 Antioxidant Activity

Antioxidant activity was analyzed according to Sutakwa et al. [13] using the DPPH antioxidant level method (2,2-diphenyl-1-picrylhydrazyl). DPPH radical absorption is tested with 3.9 ml of DPPH ethanol solution (DPPH concentration 0.004 g/ml of ethanol) mixed with 0.1 ml of samples. Incubated the mixture for 30 minutes in a dark room. The absorbance is measured with a wavelength of 515 nm in the spectrophotometer. Antioxidant activity is calculated using a linear equation standard

2.7 Viscosity .

Measurement of viscosity is carried out according to Setyawardhani, et al. [14] using a viscometer. Yogurt samples are prepared in a container and stirred first. spindle cylinder (number 3) is used in the viscometer, with a speed of 30 rpm with a duration of 60 seconds.

2.8 Color Analysis .

Product color analysis according to Wibawanti and Rinawidiastuti [15] was carried out using a color reader with the indicator $l^*a^*b^*$. Yogurt samples are taken as much as 10 ml and placed on transparent plastic packaging. Attached color reader on the surface of the plastic. Arranged the reading button to $l^* a^* b^*$ then pressed the target button. Read and record the results

3. RESULTS AND DISCUSSION

3.1 Total Phenols an Antioxidant Levels on Bawang dayak Extract

The total phenol test aims to determine the levels of phenolic compounds in the Bawang dayak extract. Based on the test results, it was known that the total phenol in the Bawang dayak extract with water solvent was $4.85 \pm 0.01\%$. Polyphenolic substances in plants are known as reducing agents which donate hydrogen atoms as antioxidants and reduce the formation of singlet oxygen. Phenol has high antioxidant activity by providing hydrogen or electrons and is able to stabilize and transfer unpaired electrons through the chain breaking function [16].

The free radical inhibition ability of Bawang dayak extract is expressed as IC_{50} in ppm units. Based on the test results it is known that the antioxidant content in the extract is 171.607 ± 0.1 ppm. The antioxidant activity of Bawang dayak extract correlates with the presence of flavonoids and total phenols. The greater the total phenol and flavonoid content, the stronger the antioxidant activity in a material [17].

3.2 Qualitative Phytochemical Screening on Bawang dayak Extract

The results of the phytochemical screening indicated the presence of flavonoids, alkaloids, tannins, phenols and steroids. These compounds are also known as secondary metabolites of plants that have a pharmacological activity that can provide protection from disease. Previous studies have suggested that different phytochemical compounds have various important roles as antimicrobials and cures for pathogenic bacterial infections. Flavonoids and phenols have been known for their radical inhibitory activity, which makes them have potential as antioxidants, anti-inflammatories, and others. Tannins are known as anti-fungal and anti-microbial which can inhibit the growth of microorganisms [8]. Alkaloids as phytochemical substances are known to be able to prevent oxidation stress and inflammatory diseases [18]. Triterpenoids are also known to be able to inhibit the activity of pathogenic bacteria [19].

Table 1. Qualitative phytochemical screening on bawang dayak extract

Parameters	Result on Bawang dayak Extract
Flavonoid	+

Alkaloid	+
Tannin	+
Phenol	+
Steroid	+
Triterpenoid	-

+ - Mean presence parameters in the bawang dayak extract

The various compounds above are known to have a role in antioxidant activity and the quality of Bawang dayak extract on health.

3.3 Determination of Lactic Acid Bacteria

Yogurt is a functional drink that involves the fermentation of lactose by lactic acid bacteria. Lactobacillus strains used in making yogurts such as L. Acidophilus, L. Rhamnosus, L. Casei, and L. Plantarum ferment using milk sugar (lactose) which produces lactic acid, carbon dioxide, acetic acid, dactyls, acetaldehyde and other components that form structure, taste and smell of yogurt. The number of these bacteria must be viable and sufficient for the final product of the yogurt [20].

The result show that total Lactic Acid Bacteria not significantly different ($P > 0.05$) in all treatments. Average value of total Lactic Acid Bacteria fortification bawang dayak in the ranged 7.57 – 8.95 (LOG CFU/mL). It can be seen that the amount still meet with the Standar Nasional Indonesia 2981:2009 that require minimum as much as 10^7 probiotic bacteria cells. *Streptococcus thermophilus* that stimulate the growth of other bacteria by synthesizing formic acid so it can be increasing total population of microbes and the activity metabolism will be increase [21].

Table 2. Effect of fortification bawang dayak extract on Lactic Acid Bacteria (LAB)

Treatment	LAB (log CFU/ml)
P0	8,26 ± 0,09 ^{ab}
P1	7,85 ± 1,21 ^{ab}
P2	8,95 ± 0,14 ^b
P3	7,57 ± 0,45 ^a

^{abcd} Mean values in the same row without common superscript differ at $p > .05$

The decrease of total lactic acid bacteria in treatment P1 and P3 suspected because of the addition of extract bawang dayak that contained nutrition such as carbohydrates and protein as substrate of the growth, but if its existence is greater than critical value it can inhibiting factor while in other hand the excess nitrogen can reduce substrate conversion into polysaccharides [22].

3.4 Antioxidant Capacity

The results of the analysis of antioxidant activists showed that the results of antioxidant activity were highly significant ($p < 0.01$) due to the fortification of Bawang dayak extract in yogurt. The antioxidant activity value is expressed in the IC_{50} value with PPM units, which is the concentration of the solution needed to inhibit 50% of DPPH free radicals. Where the higher the antioxidant activity, the smaller the IC_{50} value [23]. Based on the results of the analysis, the smallest IC_{50} value is 728.89 ± 9.83 in the control treatment (P0). While the largest IC_{50} value is 379.84 ± 8.23 ppm, namely in the treatment of the 6% Bawang dayak extract. The value of IC_{50} is reduced proportionally to an increase in the concentration of extracts that are defined into yogurt. This is in accordance with the results of the research Cho, et al. [24] shows the result that an increase in the concentration of olive leaf extract

results in an increase in antioxidant activity. It is also known that there is a high correlation between antioxidant activity and phenolic components in a plant. The presence of hydrolysis in milk protein or organic acid production can also be the reason for the activity of antioxidants in yogurt, as well as the presence of metabolic activity from microbes during fermentation and product storage.

Table 3. Effect of fortification bawang dayak extract on yoghurt antioxidant levels

Treatment	Antioxidant (ppm)
P0	728,89 ± 9,83 ^d
P1	579,46 ± 13,15 ^c
P2	464,86 ± 10,01 ^b
P3	379,84 ± 8,23 ^a

^{abcd} Mean values in the same row without common superscript differ at $p > .05$

The high levels of antioxidants are suspected as the effect of phytochemical content in plants such as xanthenes, isoquinolines, naphthalenes and phenols [25]. This is an advantage of the presence of phytochemical content in herbal plants, one of which is the compiler of antioxidant activity. In previous studies, yogurt samples with herbal fortification showed that DPPH radical capture activities were higher than yogurt plain (shori, 2020). Increased phenolic content in yogurt can cause increased radical absorption activities [26]. Antioxidants of phenolic substances provide hydrogen atoms that are able to stabilize free radicals and become relatively stable phenoxy radical intermediate so that the continued initiation of free radical chains can be stopped. Flavonoids are also effective in inhibiting lipid peroxidation because of its reduction potential which is relative to radical peroxy. [20]. Molecular changes during the fermentation process can also produce differences in ingredients with antioxidant activity such as peptides, free amino acids and fatty acids. The presence of probiotics also produces antioxidant activity in yogurt [13].

3.5 Viscosity

Viscosity defined with a measure thickness of yoghurt or the total amount of resistance for the flow of liquid [27]. Viscosity of yoghurt affected by pH, homogenization, processing parameters can be stirred or set and also heat treatment [28]. The presence of phenolic compounds in bawang dayak that interacted with milk protein such as casein in the yoghurt, resulting higher viscosity. Yoghurt contain casein micelle with entrapped serum, and viscosity is related to the gel structure of yoghurt [29].

According to the statistical analysis, viscosity of yoghurt synbiotic no significantly different ($P > 0.05$) in all treatments, with the ranged between $30,60 \pm 0,53^a$ for lowest average with the addition bawang dayak 4% and $37,67 \pm 1,00^a$ for highest average with the addition 1% concentration. Viscosity influenced by the strength and number of bonds between casein micelles in yoghurt such as the structure and spatial distribution [30]. Viscosity can be affected by the pH which can decrease casein solubility so that hydrophobic interaction between casein micelles form the structure and consistency of yoghurt get thicker and increase viscosity [31].

Table 4. Effect of fortification bawang dayak extract on yoghurt viscosity

Treatment	Viscosity (cPs)
P0	37,6 ± 5,39 ^a

P1	37,67 ± 1,00 ^a
P2	30,60 ± 0,53 ^a
P3	37,07 ± 5,92 ^a

^{abcd} Mean values in the same row without common superscript differ at $p > .05$

Increasing viscosity of yoghurt due to fermentation process of lactic acid bacteria that synthesize short chain fatty acid vitamins, and exopolysaccharides as natural bio thickening agent [32]. Lactic acid bacteria will form lactic acid thus forming a gel by casein coagulation and resulting in texture changes and viscosity of yoghurt (Wibawanti and Rina, 2018). Viscosity describes the consistency of a foodstuff, the higher total solid and fat content in milk the higher viscosity in the yoghurt [33].

3.6 Color Analysis

Changes in yogurt color by fortification of Bawang dayak extract are measured using Color Reader with L (Lightness), a* (Redness) and b* (Yellowness) parameters. The analysis results show that the addition of Bawang dayak extract in yogurt decreases the value of L highly significant ($p < 0.01$) from the highest value of $88.26 \pm 1,04$ in P0 (control treatment) and the lowest value is $76.21 \pm 1,78$ in P3 (the addition of extract as much as 6%).

The analysis results show that the addition of Bawang dayak extract in yogurt decreases the value of L highly significant ($p < 0.01$) from the highest value of $88.26 \pm 1,04$ in P0 (control treatment) and the lowest value is $76.21 \pm 1,78$ in P3 (the addition of extract as much as 6%). Light dispersion from the casein micelles and fat globules influences the appearance of white and brightness on dairy products [34]. the result is in accordance with research by Wahyuni, et al. [35] which shows the result that the highest L value is in the addition of the lowest anthocyanin extract, while the lowest L value is in the highest extract addition.

Table 5. Color analysis in yoghurt fortified bawang dayak extract results

Treatment	Parameters		
	L (Lightness)	a* (Redness)	b* (Yellowness)
P0	$88,26 \pm 1,04^d$	$-2,45 \pm 0,66^a$	$6,62 \pm 0,89^a$
P1	$84,59 \pm 0,71^c$	$0,91 \pm 0,06^b$	$9,21 \pm 0,27^b$
P2	$78,46 \pm 0,17^b$	$6,28 \pm 0,42^c$	$12,62 \pm 0,63^c$
P3	$76,21 \pm 1,78^a$	$7,94 \pm 0,31^d$	$14,66 \pm 0,98^d$

^{abcd} Mean values in the same row without common superscript differ at $p < 0.01$

Color parameters a* (redness) show the results of a very significant difference ($p < 0.01$) with the lowest result being of -2.45 ± 0.66 in the control treatment (p0) and the highest result being 7.94 ± 0.31 in the addition extract as much as 6% (P3). The increase in the value of L is followed by a decrease in the value of a*. As explained by Khalifa and Gomaa [36] that the highest a* value is found in the addition of extracts with the highest anthocyanin pigment, with the lowest a* in the least extract addition. Color changes in the product are caused by the anthocyanin levels in the product [37]. Yogurt with control treatment shows the results of a* negative because there are no additional natural dyes. The decrease in the L value is greater with the increasing concentration of extracts with natural dyes. Acid conditions during the yogurt fermentation process also explain the causes of changes in the value of a*. Acid conditions allow changes in the structure of the tissue in yogurt so that the anthocyanin pigment is absorbed by yogurt [38]

The value of b* (yellowness) on the analysis results shows the lowest value of P0 with a value of 6.62 ± 0.89 and the highest value is in P3 with a value of 14.66 ± 0.98 . These results indicate that there are very significant differences ($p < 0.01$) in the fortification of Bawang dayak extract in yogurt against the value of b*. The results show that the value of b*

in all treatments shows a positive number that indicates the appearance of color tends to be yellow in yogurt. In accordance with Arifin, et al. [39] that the value of B* indicates the chromatic color level of blue-yellow with 0 to +70 being yellow and 0 to -70 being blue. The difference in the color value of the identified and unproductive yogurt is caused by a variation in the concentration of phenol substances that are known to interact with anthocyanin and play a role in the color intensity that appears [40].

4. CONCLUSION

Fortification of Bawang dayak extract in synbiotic yogurt results in highly significant different effects on the quality parameters of antioxidant and color (lightness, redness and yellowness), but had no significant effect on the total quality of lactic acid bacteria. Fortification of 6% Bawang dayak extract (P3) in yogurt produced the best quality in terms of antioxidant content and color (lightness, redness, and yellowness).

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