



# Synbiotic Yogurt Fortified with Bawang Dayak (*Eleutherine palmifolia*) Extract

R. D. Andriani <sup>a\*</sup>, M. E. Sawitri <sup>a</sup>, V. T. Widayanti <sup>b</sup>,  
A. Manab <sup>a</sup>, P. P. Rahayu <sup>a</sup> and R. N. Fadilah <sup>c\*\*</sup>

<sup>a</sup> Department of Animal Product Technology, Faculty of Animal Science, Universitas Brawijaya, JL. Veteran, Malang, East Java 65145, Indonesia.

<sup>b</sup> Department of Agro-Industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, JL. Veteran, Malang, East Java 65145, Indonesia.

<sup>c</sup> Faculty of Animal Science, Universitas Brawijaya, JL. Veteran, Malang, East Java 65145, Indonesia.

## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** The purpose of this research was to study the quality of yogurt 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, yogurt starter bacteria (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*), and bawang dayak extract. Bawang Dayak were extracted using Microwave-Assisted Extraction (MAE) with temperature 60-

\*\*Student;

\*Corresponding author: Email: riaandriani@ub.ac.id;

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 synbiotic yogurt 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 synbiotic yogurt was achieved in T3 with the 6% of Bawang Dayak extract.

**Keywords:** Yogurt; symbiotic; 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, antherosclerosis, antidiabetic, antiulcerogenic, anti-inflammatory, antiobesity, improve the digestive tract and intestinal problems, inhibit the growth of pathogenic bacteria, and help essential micronutrition 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 sources such as plants has been proven to be an efficient method 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, anantimicrobials. One of the herbs that contain antioxidant components is Bawang Dayak (*Eleutherine palmifolia*), which grows on the island of Kalimantan and belongs to the genus Iridaceae. This plant contains phenol and flavonoids which act as an antioxidant and antibacterial agents. In addition to its 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].

Many studies related to synbiotic yogurt with the addition of prebiotics rich in antioxidants have been carried out. the use of various natural additives in yogurt has a significant effect on the antioxidant capacity and quality of synbiotic yogurt [10,11,12]. 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 Sinbiotic yogurt with total parameters of lactic acid bacteria, viscosity, antioxidants and colors is needed. The purpose of this study is to evaluate the quality parameters of the synbiotic yogurt with the fortification of Bawang dayak extract.

## 2. MATERIALS 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 were 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 Range Test (DMRT).

The material used in this research were synbiotic yogurt made from fresh cow milk that obtained from fresh milk shop "lloe", skim milk, lactic acid bacteria culture in liquid form, 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). 50 grams of Bawang Dayak was weighed and then added 500 ml of distilled water (ratio of 1:10). The extraction was conducted for 12 minutes with temperature 70°C. Extraction results were 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 was ready to be fortified into yogurt.

### 2.2 Phytochemical Screening on Bawang Dayak Extract

Phytochemical screening was carried out to identify the presence of flavonoids, alkaloids, tannins, phenols, steroids and triterpenoids using qualitative method in accordance with the Ministry of Health of The Republic of Indonesia in *Materia Medika Indonesia* [13].

#### 2.2.1 Flavonoid Identification

Weighed 10 grams of sample then add 10 ml of hot water. heated for 5 minutes, then filtered in hot conditions. in 5 ml of the filtrate, 0.1 gram of magnesium powder, 1 ml of concentrated hydrochloric acid, and 2 ml of amyl alcohol were

added, then shaken and waited for it to separate. if red, yellow, or orange appears then the sample is positive for flavonoids.

#### 2.2.2 Alkaloid Identification

As much 0.5 grams of sample was added with 1 ml of 2 N hydrochloric acid and 9 ml of distilled water. heated for 2 minutes, then cooled and filtered to obtain the filtrate. Prepare 3 test tubes. Put 0.5 ml of the filtrate in each tube. 3 different reagents were used in each tube, namely Mayer, Bouchard and dragendorff, used 2 drops of each reagent. the sample is tested positive for alkaloids if sediment or turbidity appears in at least two of the three tubes.

#### 2.2.3 Tannin Identification

In 1 grams of sample was added with 100 ml of distilled water and then cooled for 3 minutes, then cooled and filtered. prepared 2 ml of solution and then add 1 to 2 drops of 1% FeCl<sub>3</sub> reagent. The sample is contain tannins if a blue-black or black-green color occurs.

#### 2.2.4 Phenol Identification

The sample was put in a test tube and added a little ether and then shaken. The ether layer is dried on the drip plate, then added with the FeCl<sub>3</sub> solution. The form of bluish-purple color indicated the presence of phenolic compounds in the sample.

#### 2.2.5 Steroid and Triterpenoid Identification

Samples were taken then dripped on the spot test board and dried. Add 3 drops of acetic anhydride and 1 drop of concentrated sulfuric acid. The presence of terpenoid compounds will cause a red color, while the presence of steroid compounds will cause a blue color.

### 2.3 Total Phenol Analysis of Bawang Dayak Extract

Total phenol content was analyzed according to Sassi, et al. [14] 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. Then, 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 Synbiotic Yogurt

Measured 250 ml of milk and pour it into on Erlenmeyer, then pasteurize it by heating it to 70°C for 15 seconds. Then cool it down to 40°C. Added bawang dayak extract according to the treatment (0%, 1%, 4% and 6%) and then homogenized. Inoculate 3% of yogurt starter bacteria and incubated at room temperature for 24 hours.

## 2.5 Determination of Lactic Acid Bacteria

Testing Total Lactic Acid Bacteria According to Pangestu et al. [15] begins by taking a sample of 10 ml with a micropipette into a test tube containing 90 ml of sterile peptone water for the first dilution ( $10^1$ ), then homogenized using 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. [16] by using the DPPH antioxidant level method (2,2-diphenyl-1-picrylhydrazyl). The DPPH radical absorption was tested by mixing 3.9 ml of DPPH ethanol solution (DPPH concentration 0.004 g/ml of ethanol) with 0.1 ml of samples. The mixture was then incubated for 30 minutes in a dark room. The absorbance was measured at a wavelength of 515 nm in the spectrophotometer. Antioxidant activity is calculated using a linear equation standard.

## 2.7 Viscosity .

Measurement of viscosity was carried out according to Setyawardhani, et al. [17] using a viscometer. Yogurt samples were 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 [18] was carried out using a color reader with the indicator  $L^*$ ,  $a^*$  and  $b^*$ . Yogurt samples were 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 purpose of total phenol test is to determine the levels of phenolic compounds in the Bawang dayak extract. Result showed that the total phenol content in the Bawang dayak extract with water as a 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 [19].

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  $171.607 \pm 0.1$  ppm. The antioxidant activity of Bawang dayak extract related to the presence of flavonoids and total phenols. The higher the total phenol and flavonoid content, the stronger the antioxidant activity in a material [20].

### 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 known as secondary metabolites of plants that possess pharmacological activity that can provide protection against disease. Previous studies have shown that various phytochemical compounds have important roles as antimicrobials and cures for pathogenic bacterial infections. Flavonoids and phenols are known for their radical inhibitory activity, which makes them have potential as antioxidants, anti-inflammatory, and others. Tannins are known

as anti-fungal and anti-microbial properties and have the ability to inhibit the growth of microorganisms [8]. Alkaloids as phytochemical substances, are known to be able to prevent oxidation stress and inflammatory diseases [21]. Triterpenoids are also known to be able to inhibit the activity of pathogenic bacteria [22].

**Table 1. Qualitative phytochemical screening on bawang dayak extract**

Parameters	Result on Bawang dayak Extract
Flavonoid	+
Alkaloid	+
Tannin	+
Phenol	+
Steroid	+
Triterpenoid	-

Source: phytochemical screening on bawang dayak extract in *Materia Medica Batu, Malang. (August, 2022)*

+ - 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. Casei*, and *L. plantarum* ferment using milk sugar (lactose) which produces lactic acid, carbon dioxide, acetic acid, dactyls, acetaldehyde and other components that form the structure, taste and smell of yogurt. The number of these bacteria must be viable and sufficient for the final product of the yogurt [23].

The result show that total Lactic Acid Bacteria not significantly different ( $P > 0.05$ ) in all treatments. The average value of total Lactic Acid Bacteria fortification bawang dayak ranges from 7.57 – 8.95 (LOG CFU/mL). It can be seen that the amount still meets 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 boosting metabolic activity [24,25].

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

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

<sup>abcd</sup> 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 [26].

### 3.4 Antioxidant Capacity

The results of the analysis of antioxidant activity showed that the fortification of bawang Dayak extract in yogurt were highly significant ( $p < 0.01$ ) on the antioxidant activity. 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 [27]. Based on the results of the analysis, the smallest  $IC_{50}$  value is  $728.89 \pm 9.83$  in the control treatment (P0). While the largest  $IC_{50}$  value is  $379.84 \pm 8.23$  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. [11] who observed 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 yogurt antioxidant levels**

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

<sup>abcd</sup> Mean values in the same row without common superscript differ at  $p > .05$

The high levels of antioxidants are believed to be the result of phytochemical content in plants such as xanthonenes, isoquinolines, naphthalenes and phenols [14]. This is an advantage of the presence of phytochemical content in herbal plants, one of which contributes to 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 [28]. 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 [17].

### 3.5 Viscosity

Viscosity defined with a measure thickness of yogurt or the total amount of resistance for the flow of liquid [12]. Viscosity of yogurt affected by several factors such as pH, homogenization, processing parameters can be stirred or set and heat treatment [29]. The presence of phenolic compounds in bawang dayak that interacted with milk protein such as casein in the yogurt, resulting higher viscosity. Yogurt contains casein micelle entrapped with serum, and viscosity is related to the gel structure of yogurt [30].

According to the statistical analysis, viscosity of synbiotic yogurt was not significantly different ( $P > 0.05$ ) in all treatments, ranging from  $30,60 \pm 0,53^a$  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 yogurt such as the structure and spatial distribution [31]. 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 yogurt get thicker and increase viscosity [32].

**Table 4. Effect of fortification bawang dayak extract on yogurt viscosity**

Treatment	Viscosity (cPs)
P0	37,6 ± 5,39 <sup>a</sup>
P1	37,67 ± 1,00 <sup>a</sup>
P2	30,60 ± 0,53 <sup>a</sup>
P3	37,07 ± 5,92 <sup>a</sup>

<sup>abcd</sup> Mean values in the same row without common superscript differ at  $p > .05$

Increasing viscosity of yogurt due to fermentation process of lactic acid bacteria that synthesize short chain fatty acid vitamins, and exopolysaccharides as natural bio thickening agent [33]. Lactic acid bacteria will form lactic acid, which leads to casein coagulation and resulting in texture changes and viscosity of yogurt [18]. Viscosity describes the consistency of a foodstuff, the higher total solid and fat content in milk the higher viscosity in the yogurt [34].

### 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 [35]. the result is in accordance with research by Wahyuni, et al. [36] 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 yogurt fortified bawang dayak extract results**

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

<sup>abcd</sup> Mean values in the same row without common superscript differ at  $p < 0.01$

Color parameters a\* (redness) showed very significant difference ( $p < 0.01$ ) with the lowest result being of  $-2.45 \pm 0.66$  in the control treatment (p0) and the highest result being  $7.94 \pm 0.31$  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 [37] 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 [38]. 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 [39].

The value of b\* (yellowness) on the analysis results reveals the lowest value of P0 with a value of  $6.62 \pm 0.89$  and the highest value is in P3 with a value of  $14.66 \pm 0.98$ . 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. [40] 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 [41].

#### 4. CONCLUSION

It can be conclude that the fortification of bawang dayak can improve the quality of synbiotic yogurt

in terms of antioxidant activity and yogurt color on the L\*a\*b\* indicator. Fortification of 6% Bawang dayak extract (P3) in yogurt produced the best quality in terms of antioxidant content and color (lightness, redness, and yellowness). It is recommended to conduct further research on the sensory quality of yogurt fortified with bawang dayak extract to determine consumer acceptance.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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