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Effect of Adding Chlorophyll Extract of Suji Leaves (*Dracaena angustifolia*) and Fermentation Time on Yogurt Characteristics

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ABSTRACT

Suji leaves contain a green pigment, namely chlorophyll. Suji leaf chlorophyll and its derivative compounds provide health benefits due to their anti-inflammatory and antioxidant activity. This research aims to produce suji leaf chlorophyll extract yogurt with antioxidant activity, soluble protein levels, and high numbers of lactic acid bacteria. This research used a completely randomized factorial design (CRD) consisting of 2 factors, namely the concentration of added chlorophyll extract (1%, 2%, 3%) and variations in fermentation time (6 hours, 8 hours, 10 hours). The research results showed that suji leaf chlorophyll extract yogurt with antioxidant activity, high levels of soluble protein, and a high number of lactic acid bacteria was found in the treatment with the addition of 3 percent chlorophyll and a fermentation time of 10 hours. In this treatment, suji leaf chlorophyll yogurt was obtained with antioxidant activity of 59.20 percent, dissolved protein content of 128.80 mg BSA/100 ml, total sugar content of 71.92 g glucose/ 100 ml, fat content of 5.91 percent, total chlorophyll of 2 .39 mg/L, viscosity 10.00 cP; color L (brightness) 77.09, color a* (reddish) -5.80, color b* (yellowish) 9.35 and total Lactic Acid Bacteria (LAB) around 8.08 Log CFU/mL. Yogurt, with the addition of suji leaf chlorophyll extract, has the potential to be an excellent alternative to functional food for consumption.*

INTRODUCTION

Yogurt is a dairy product that has been known since ancient times. Yogurt is loved for its distinctive aroma, freshness, and texture. The formation of lactic acid, acetone, carbonyl, acetone, diacetyl, and others influences the characteristic taste of yogurt (Astawan, 2008). Yogurt is produced through fermentation with the help of the bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. As time progresses and changes in consumption tastes have resulted in the demand for yogurt increasing, so there is a need for diversification of yogurt products that are more varied, both in terms of type, aroma, taste, and texture (Hidayat et al., 2006). A new strategy in diversifying yogurt is to use vegetables as additional ingredients to increase the functional value content of yogurt. One way is by using suji leaf chlorophyll extract to make yogurt.

Several factors can influence Lactic Acid Bacteria (LAB) growth in the yogurt fermentation process. One factor that influences the development of lactic acid bacteria is the fermentation time. Fermentation time must be considered when making yogurt because it will affect the quality of the yogurt produced. Research conducted by Syainah (2014) shows that different incubation temperatures affect the lactic acid content, protein content, viscosity, taste, and aroma of the resulting yogurt product.

Suji leaves are a plant known as a source of chlorophyll. Generally, suji leaves are only used as a natural food coloring agent. Suji leaves contain natural pigments such as chlorophyll, alkanoids, flavonoids, and saponins, which help improve color, increase nutrition, and preserve products (Aryanti, 2016). The antioxidant activity in suji leaves shows an IC50 value of 48.96 ppm, which means that the antioxidant activity is in the "strong" category (Putriyana, 2023). Suji leaf chlorophyll and its derivative compounds provide health benefits due to their anti-inflammatory and antioxidant activity and play a role in preventing chronic diseases, such as cancer and diabetes (Indrasti et al., 2019; McQuistan et al., 2012). Some green leaves, such as suji and pandan leaves, contain natural pigments such as chlorophyll, tannins, carotenoids, and flavonoids, which can be used as natural colorings in food products (Marta, 2020). Prangdimurti et al. (2006) reported that the administration of suji leaf extract could also improve the anti-oxidative status of mice, which was shown, among other things, by a decrease in liver MDA levels and an increase in liver SOD activity caused by the antioxidant activity of chlorophyll contained in suji leaf extract.

Much research has been done on making yogurt with green leaves. Still, yogurt with suji leaf chlorophyll has never been done. One of the research studies on yogurt with green leaves was carried out by Amalia

(2021); namely, the use of green grass jelly leaf extract in making soybean sprout milk yogurt has an effect on the physical properties (viscosity and pH), chemical properties (fiber content) and antioxidant activity of the yogurt. Still, it does not affect the sensory properties (color, texture, aroma, and taste). Apart from that, Fatmawati et al. (2021)also researched the effect of adding Moringa leaf extract on the quality of the yogurt produced. The research results showed that the addition of Moringa leaf extract had an impact on protein levels, increased calcium levels, and increased pH.

It is hoped that adding suji leaf chlorophyll extract to yogurt products can produce more nutritious yogurt with a delicious taste that people will like. Besides that, suji leaf chlorophyll extract is also used as a natural coloring in suji leaf chlorophyll yogurt. The different fermentation times used in this research are hoped to allow for the best yogurt treatment.

METHOD

The research was conducted at the Physicochemical Laboratory of the National Research and Innovation Agency (BRIN) Tawangmangu and the Microbiology Laboratory of the Faculty of Food Technology and Industry, Slamet Riyadi University, Surakarta. The method used was a Factorial Completely Randomized Design (CRD) method, which consisted of two factors, namely the addition of suji leaf chlorophyll extract to water (1, 2, 3%) and fermentation time (6 hours, 8 hours, 10 hours). This method refers to Suhartatik et al. (2020) with several modifications, namely, using milk, starter, and other ingredients (suji leaf chlorophyll).

Tool

The tools used include Kern ABS-2220-04 analytical balance, measuring cup, thermometer, Erlenmeyer, Memmert type digital incubator, vortex, and Thermo Scientific G10S UV-VIS spectrophotometer.

Material

The ingredients used are suji leaves that have fully bloomed, namely ± the fifth leaf from the tip to the bottom, obtained in the Ngawi area, East Java, fresh cow's milk from a cow's milk agent in the Surakarta area, granulated sugar (Gulaku), skim milk (Indoprima), and yogurt starter (Biokul) obtained from Superindo in Solo City. Chemical analysis materials include glucose, 0.5 mM DPPH solution, 45 percent NaOH, 30 percent HCl, arsenomolybdate, Nelson-Somogyi reagent, methanol, 10 percent Na2CO3, CuSO4, K-tartrate, Follin-Ciocalteu reagent, MRS (de Man Rogosa Sharpe, Oxoid), and CaCO3 obtained from the Food Chemistry and Biochemistry Laboratory, Faculty of Food Technology and Industry, Slamet Riyadi University, Surakarta, Indonesia with pro analysis (pa) quality.

Making Suji Leaf Chlorophyll Extract

Making suji leaf chlorophyll extract based on the method used by Jokopriyambodo et al. (2014), which has been modified, is washed using clean water and cut into pieces measuring 2-5 cm. Then 100 grams of suji leaves were added to 800 mL of water, blended using a commercial blender for 2 minutes, and filtered. The filtrate obtained is boiled until coagulation (a water-insoluble chlorophyll extract of suji leaves) forms at the top of the water. After that, the coagulant was taken, and 10 percent powdered sugar (w/w) was added, then dried in an oven at 40^0C for 24 hours. The dried extract is then blended and filtered through a 60-mesh size.

Making Yogurt

Making yogurt based on the method of Suhartatik et al. (2020), which has been modified, fresh cow's milk is homogenized with 10 percent (w/v) skim milk and 3 percent (w/v) glucose. Then, the homogeneous mixture was heated at 85ºC for 15 minutes and cooled to 40^0C. After that, divided into 9 containers and added to each 10 ml of suji leaf chlorophyll extract with a concentration according to the treatment (1%, 2%, 3%), then inoculated with 3 percent (v/v) plain yogurt starter and incubated at 39ºC for 6, 8, and 10 hours.

Research Parameters

The analysis carried out in this study included fat content using the Mojonnier method (Association of Official Analytical Chemists [AOAC], 2005), total chlorophyll analysis (Jokopriyambodo et al., 2014), dissolved protein content using the Lowry-Folin method (Rahmawati et al., 2016), analysis of total sugars using the Nelson-Somogyi method (Sudarmadji et al., 1997), analysis of antioxidant activity using the DPPH method (Widodo et al., 2019), viscosity analysis (Kartikasari & Nisa, 2014), color analysis (Kartikasari & Nisa, 2014) and microbiological analysis, namely determining total Lactic Acid Bacteria (LAB) using the Total Plate Count method (Kartikasari & Nisa, 2014).

Data analysis

The data obtained is entered into the data tabulation table. Then, the data was analyzed using Analysis of Variance (ANOVA), followed by the Tukey Test, which showed a significance level of 5 percent using IBM SPSS version 20.

RESULTS AND DISCUSSION

The results of the chemical analysis of suji leaf chlorophyll yogurt with the addition of suji leaf chlorophyll extract and fermentation time can be seen in Table 1.

Antioxidant Activity of Suji Leaf Chlorophyll Yogurt

The antioxidant activity of suji leaf chlorophyll yogurt in the treatment with the addition of suji leaf chlorophyll extract and fermentation time showed no significant difference. The highest antioxidant activity resulted from the treatment with the addition of 2 persen and 3 persen chlorophyll extract with a

Table 1. Chemical characteristics of suji leaf chlorophyll yogurt with the addition of suji leaf chlorophyll extract and variations in fermentation time

Suji Leaf Chlorophyll Extract	Fermentation Time	Antioxidant Activity (%)	Protein Content (mg BSA/100 ml)	Total Sugar (g glucose/100 ml)	Fat Content (%)
1% (K1)	6 Hours (T1)	57.94±7.71a	122.94±9.84a	71.96±6.24a	6.63±0.01de
	8 Hours (T2)	53.51±3.23a	125.92 ± 10.48a	71.64±7.25a	6.36±0.05cd
	10 Hours (T3)	58.02 ± 2.04a	125.14±1.41a	70.94 ± 3.82a	7.05±0.06e
2% (K2)	6 Hours (T1)	54.51±4.90a	119.50±12.95a	73.37±9.21a	6.16±0.11bc
	8 Hours (T2)	55.83 ± 0.26a	126.62±7.87a	72.05±8.32a	6 .30±0.04cd
	10 Hours (T3)	59.20±8.37a	138.49 ± 4.04a	71.62±6.73a	6.26±0.29cd
3% (K3)	6 Hours (T1)	55.71±1.44a	110.72±14.10a	72.34±6.27a	5.69 ± 0.13a
	8 Hours (T2)	55.83 ± 0.83a	121.27±5.69a	72.62±8.63a	5.77±0.06ab
	10 Hours (T3)	59.20 ± 3.63a	128.80±14.55a	71.92 ± 5.21a	5.91±0.04abc

Note: Numbers followed by different letters on the same line are significantly different (p>0.05)

fermentation time of 10 hours, amounting to 59.20 persen, while the lowest antioxidant activity was obtained from the treatment with the addition of 1 persen chlorophyll extract and a fermentation time of 8 hours amounting to 53.51 persen. The addition of suji leaf chlorophyll extract did not significantly increase or decrease antioxidant activity. However, the longer the fermentation time, the higher the antioxidant activity value obtained. This is due to the sugar content present; with the hydrolysis of sugar carried out by lactic acid bacteria causing more phenolic compounds to be liberated, the antioxidant activity will increase. Sugar that undergoes breakdown will produce lactic acid with the help of Lactic Acid Bacteria (LAB), which is synergistic by providing H+ ions to free radicals to increase the primary antioxidant activity (Primurdia & Kusnadi, 2014). According to research by Af'idah & Trimulyono (2019), the yogurt fermentation process by lactic acid bacteria can increase antioxidant activity by increasing lactic acid levels during fermentation.

In research conducted by Angelina (2022), adding 9 percent pandan leaf extract can increase the antioxidant activity of the yogurt produced. Adding green tea leaf powder to durian seed jackfruit yogurt products can also increase antioxidant activity (Princess, 2022). This proves that adding green leaf extract to yogurt can increase its antioxidant activity. Still, the concentration of the added extract must be appropriate.

Soluble Protein Content of Suji Leaf Chlorophyll Yogurt

The results of the ANOVA test that was carried out (Table 1) showed that the soluble protein levels of suji leaf chlorophyll yogurt were not significantly different. The highest dissolved protein content of suji leaf chlorophyll yogurt was 138.49 mg BSA/100 ml, which was produced from the treatment of adding 2 percent chlorophyll extract and a fermentation time of 10 hours, while the lowest soluble protein content was 110.72 mg BSA/100 ml which produced from treatment with the addition of 3 percent chlorophyll

extract and a fermentation time of 6 hours. The addition of chlorophyll extract did not affect the dissolved protein content in suji leaf chlorophyll yogurt. However, the longer the fermentation took to make yogurt, the higher the dissolved protein content in suji leaf chlorophyll yogurt, and there was no significant difference. This is different from the research conducted by Diana(2012); the results of fermentation for 48 hours showed an increase in protein levels in red bean milk yogurt products. Changes in dissolved protein levels during the fermentation process are due to the bacteria Lactobacillus bulgaricus and Streptococcus thermophilus in yogurt continuing to grow so that they require a source of energy/food substances for growth and reproduction (Nurhayati et al., 2014). In addition, during the fermentation process, lactic acid is formed by lactic acid bacteria, which triggers a decrease in the pH of the product. When milk's pH changes, the protein's solubility in yogurt will be affected (Haque, Timilsena & Adikari, 2016). A pH decrease also causes the yogurt proteins to aggregate, forming a thick yogurt texture (Hoxha, Evstatieva & Nikolova, 2023).

Total Sugar Content of Suji Leaf Chlorophyll Yogurt

The results of research on the total sugar content of suji leaf chlorophyll yogurt in the treatment with the addition of suji leaf chlorophyll extract and fermentation time showed no significant difference. The highest total sugar content was 73.37 g glucose/100 ml from the treatment with the addition of 2 percent chlorophyll extract and fermentation time of 6 hours, while the lowest total sugar content result was 70.94 g glucose/100 ml in the treatment with the addition of 1 percent chlorophyll extract. The longest fermentation time is 10 hours. The greater the concentration of chlorophyll extract added does not affect the total sugar content. However, the longer the fermentation takes, the lower the total sugar content. The longer the fermentation process results in the breakdown of glucose by lactic acid bacteria, the more it is used for metabolism so that the total sugar content in yogurt decreases. Treatment variations in fermentation time affect the total sugar content produced during fermentation (Salminen et al., 2004). The length of fermentation will influence how much sugar can be converted into lactic acid due to metabolites from lactic acid bacteria. Lactic Acid Bacteria (LAB) use sugar as a carbon source, grow, and can produce metabolites in the form of lactic acid during fermentation. The more lactic acid bacteria cells are formed, the more glucose will be used for cell metabolism.

Fat Content of Suji Leaf Chlorophyll Yogurt

The ANOVA test analysis (Table 1) results for fat content show a significant difference. The highest fat content was 7.05 percent, obtained from the treatment with the addition of 1 percent suji leaf chlorophyll

extract. The fermentation time was 10 hours, while the lowest fat content was obtained from the treatment with the addition of suji leaf chlorophyll extract at 5.69 percent after the treatment with 3 percent chlorophyll extract and a fermentation time of 6 hours. This research is in line with Diyah et al. (2015), who researched the effect of ratios and variations in fermentation time on the characteristics of sweet corn yogurt, which shows that the longer the fermentation, the more the fat content will increase. Research by Manurung et al. (2014) explains that the fat content is influenced by the total lactic acid bacteria and fatty acids contained in the additional ingredients. One of the ingredients for making yogurt, which has quite a high-fat content, is fresh milk of 3.28-3.51percent (Suhendra et. al. 2017). Milk is used as a medium for bacterial growth. Thus, the fat content in yogurt is greatly influenced by the condition of the medium, the initial fat content, and the development of yogurt bacteria. The high-fat content in milk as a medium for yogurt will affect the final fat content produced. In addition, according to Nguyen & Hwang (2016), fat content can be influenced by the breakdown of fat in milk into free fatty acids due to the LAB fermentation process. The resulting fat content in the suji leaf chlorophyll yogurt sample calculation meets the Indonesian National Standard (2009): a minimum of 3.0.

Total Chlorophyll of Suji Leaf Yogurt Chlorophyll

The total chlorophyll analysis of suji leaves showed significant differences. The total chlorophyll value of suji leaf chlorophyll yogurt can be seen in Table 2. The total chlorophyll value of suji leaf chlorophyll yogurt with the addition of chlorophyll extract and fermentation time ranged from 2.17-2.39 mg/L. The highest total chlorophyll was obtained from the treatment with the

Table 2. Total Chlorophyll and Viscosity of Suji Leaf Chlorophyll Yogurt with the Addition of Suji Leaf Chlorophyll Extract and Variations in Fermentation Time

Suji Leaf Chlorophyll Extract	Fermentation Time	Total Chlorophyll (mg/L)	Viscosity (cP)
1% (K1)	6 Hours (T1)	2.19±0.08ab	8.75±0.35a
	8 Hours (T2)	2.25±0.02abc	8.75±0.35a
	10 Hours (T3)	2.17 ± 0.01[a]	10.25±1.06a
2% (K2)	6 Hours (T1)	2.28±0.04abcd	8.75±0.35a
	8 Hours (T2)	2.32±0.00bcd	9.00 ± 0.71a
	10 Hours (T3)	2.28±0.01abcd	9.50 ± 0.71a
3% (K3)	6 Hours (T1)	2.36±0.00cd	8.75±0.35a
	8 Hours (T2)	2.39±0.06d	9.75±0.35a
	10 Hours (T3)	2.37±0.01cd	10.00±0.00a

Note: Numbers followed by different letters are significantly different (P=0.05)

addition of 3 percent chlorophyll extract and a fermentation time of 8 hours, while the lowest total chlorophyll was obtained from the treatment with the addition of 1 percent chlorophyll extract and a fermentation time of 10 hours. The total amount of chlorophyll increases with each concentration of chlorophyll extract addition because the chlorophyll of yogurt is influenced by the chlorophyll content in the yogurt product. Meanwhile, the fermentation time did not show a significant increase or decrease in the total chlorophyll in yogurt.

Viscosity of Suji Leaf Chlorophyll Yogurt

Data from ANOVA analysis of suji leaf chlorophyll yogurt with the addition of suji leaf chlorophyll extract and fermentation time showed no significant difference. The average viscosity value of suji leaf chlorophyll yogurt with the addition of suji leaf chlorophyll extract and fermentation time ranged from 8.75-10.25 cP. Viscosity is a measure of the viscosity of a fluid, which states the amount of friction in the fluid. The greater the viscosity value, the more difficult it is for the liquid to flow and move within the fluid. The highest viscosity value was obtained from the treatment with the addition of 1 percent chlorophyll extract and a fermentation time of 10 hours of 10.25 cP, while the lowest viscosity value was obtained from several treatments, namely the addition of 1percent chlorophyll extract and a fermentation time of 6 hours and 8 hours, the addition of 2 chlorophyll extracts. % and fermentation time of 6 hours, as well as the addition of 3 percent chlorophyll extract and fermentation time of 6 hours, each of which has a viscosity value of 8.75 cP. The effect of treatment on the concentration of suji leaf chlorophyll extract and fermentation time can be seen in Table 2.

Table 2 shows the results of the average viscosity value of each treatment, showing that the longer the fermentation, the higher the viscosity value of the yogurt product. This is thought to be influenced by lactic acid produced by Lactic Acid Bacteria (LAB). The acidic conditions caused by lactic acid can affect the viscosity of the yogurt product. When the acidity level in the yogurt product reaches the isoelectric point, the proteins in the yogurt will coagulate. The isoelectric point occurs because the number of cations and anions in the yogurt product is balanced, resulting in the attraction of these ions.(Widagdha & Nisa, 2015).

Color Analysis L* (Brightness)

The color of suji leaf chlorophyll yogurt was measured using a coloimeter. The results of the ANOVA analysis of L* color analysis (brightness) stated that there was no significant difference. The average lightness value (L*) of suji leaf chlorophyll yogurt can be seen in Table 3. The lightness value indicates the darkness (brightness) of a color. The notation L* states the brightness parameter from 0 (black) to 100 (white). The

data from this research shows that the brightness value (L*) in suji leaf chlorophyll yogurt ranges from 73.00-77.09. The highest L* value was obtained from the treatment with the addition of 3 percent chlorophyll extract and a fermentation time of 10 hours, while the lowest value was obtained from the treatment with the addition of 3 percent chlorophyll extract and a fermentation time of 6 hours.

Table 3 shows that the brightness level of suji leaf chlorophyll yogurt decreases as the concentration of chlorophyll extract is added because the green pigment chlorophyll affects the brightness level of the yogurt. Meanwhile, the fermentation time did not show a significant increase or decrease in the brightness level of the yogurt.

Color Analysis a* (Redness)

Analysis results from ANOVA color a* (reddish) show that the addition of suji leaf chlorophyll extract and fermentation time significantly differed from the reddish color (a*) of suji leaf chlorophyll yogurt. The average value of the reddish color of suji leaf chlorophyll yogurt can be seen in Table 3. The notation a* is a reddish to greenish color coordinate with a* value of +60 (reddish) and -60 (greenish), so minus the value of a*, the color of suji leaf chlorophyll yogurt will get greener. From the research data results, the average redness value (a*) in suji leaf chlorophyll yogurt ranged from -5.80 to -7.89. The highest value was obtained from suji leaf chlorophyll yogurt with 3 percent chlorophyll extract and a fermentation time of 10 hours, while the lowest a* value was obtained from adding 3 percent chlorophyll extract and a fermentation time of 8 hours.

Table 3 shows that the more concentration of chlorophyll extract is added, the redness value of suji leaf chlorophyll yogurt decreases

Table 3. Color (L*, a*, b*) of Suji Leaf Chlorophyll Yogurt with the Addition of Suji Leaf Chlorophyll Extract and Variations in Fermentation Time

Treatment	L* value	a* value	b* value
K1T1	76.56 ± 0.09a	-6.17±0.13cd	9.58±0.24bc
K1T2	75.41 ± 0.61a	-6.58±0.13bc	6.88 ± 0.24a
K1T3	76.01 ± 0.6a	-6.12±0.13cd	8.84±0.24b
K2T1	73.69 ± 0.06a	-6.43±0.13bcd	9.70±0.24bc
K2T2	74.78 ± 0.56a	-6.26±0.13cd	10.17±0.24bc
K2T3	73.31±3.47a	-7.01±0.13b	10.41±0.24c
K3T1	73.00 ± 0.24a	-8.26±0.13a	13.99±0.24d
K3T2	74.24 ± 0.4a	-7.89±0.13a	14.06±0.24d
K3T3	77.09 ± 0.86a	-5.80±0.13d	9.35±0.24bc

Note: Numbers followed by different letters on the same line are not significantly different (p>0.05)
L* = brightness parameter (L value=0 (black), L=100 (white))
a* = red to green color coordinates (Value a =+60 (red), a=-60 (green))
b* = yellow to blue coordinates (value b=+60 (yellow), b=-60 (blue)

because the amount of green chlorophyll pigment increases. This is because the color of the chlorophyll pigment is composed of a combination of red and blue (Joko priyambodo et al., 2014). Long fermentation treatment did not affect the redness value of suji leaf chlorophyll yogurt.

Color Analysis b* (Yellowish)

Analysis results from ANOVA color b* (yellow) show that adding chlorophyll extract and fermentation time are significantly different. The average b* color value of suji leaf chlorophyll yogurt can be seen in Table 3. The notation b* indicates a yellowish to bluish color where +60 (yellowish) and -60 (bluish), the more positive the b* value will make the color of the chlorophyll yogurt yellower. From the analysis results, the average yellowness value (b*) in yogurt with adding chlorophyll extract and fermentation time ranged from 6.88 to 14.06. The highest b* value was obtained from the treatment with 3 percent chlorophyll extract and a fermentation time of 8 hours, while the lowest b* value was obtained from the treatment with adding 1percent chlorophyll extract and a fermentation time of 8 hours. The yellowish color of yogurt is thought to be due to the presence of carotene dissolved in the fat in the milk ingredients (Shodiq et al., 2023).

Total Lactic Acid Bacteria (LAB)

The data from this research showed that the total Lactic Acid Bacteria (LAB) in suji leaf chlorophyll yogurt ranged from 7.76-8.08 CFU/ml. The results of the analysis stated that the highest log value of total lactic acid bacteria was 8.08 CFU/ml from the treatment with the addition of 3 percent chlorophyll extract and fermentation time of 10 hours, while the lowest log value was 7.76 CFU/ml from the treatment with the addition of the extract. 2 percent chlorophyll and fermentation time 8 hours.

The results of the total BAL test tend to increase with the length of fermentation. This is because the Lactic Acid Bacteria (LAB) found in yogurt take longer to use the nutrients in the yogurt medium to produce more lactic acid. Lactic acid bacteria will hydrolyze lactose (milk sugar) and sucrose by the enzyme lactate dehydrogenase produced by Lactic Acid Bacteria (LAB) (Ramadzanti, 2006). The total LAB produced by calculating suji leaf chlorophyll yogurt samples meets the Indonesian National Standards(2009): a minimum of 1 x 107 CFU/ml (7.00 log CFU/ml).

CONCLUSION

The combination of treatments that had the best effect on yogurt products was the addition of suji leaf chlorophyll extract and fermentation time, which was 3% suji leaf chlorophyll extract and a fermentation time of 10 hours. The best treatment produced antioxidant activity of 59.20 percent, dissolved protein content of 128.80 mg BSA/100 ml, total sugar content of 71.92 g

glucose/100 ml, fat content of 5.91percent, total chlorophyll of 2.39%, viscosity 10.00 cP, color L* (brightness) -77.09, color a* (reddish) -5.80, color b* (yellowish) 9.35, and total BAL 8.08 log CFU/ml.

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REFERENCES

Af'idah, F., & Trimulyono, G. 2019. *Test the antioxidant activity and lactic acid levels of soybean tempeh yogurt (Glycine max) and green bean tempeh yogurt (Vigna radiata)*. LenteraBio: Biological Scientific Periodical, 8(1), 17–24. https://doi.org/10.3934/agrfood.2022035

Amalia, NRP, & Aminah, S. 2021. *Fiber content, antioxidant activity, physical and sensory characteristics of soybean sprout milk yogurt with the addition of green grass jelly extract*. Journal of Food and Nutrition, 11(01), 50–59. https://doi.org/10.26714/jpg.11.1.2021.50-59

Angelina, R. 2022. *The effect of different concentrations of green tea leaves (Camellia sinensis) on the physical and sensory properties of durian seed pickled yogurt*. (Bachelor Thesis, Widya Mandala University Surabaya). http://repository.ukwms.ac.id/id/eprint/28866

AOAC. 2005. *Official Methods of Analysis (17th ed.). The Association of Official Analytical Chemists, Gaithersburg, MD, USA*. Methods 925.10, 974.24, 992.16

Aryanti, N. 2016. *Extraction and characterization of chlorophyll from suji (Pleomele angustifolia) leaves as a natural food coloring*. Journal of Food Technology Applications, 5(4), 129–135. https://doi.org/10.17728/jatp.196

Astawan, M. 2008. *Healthy with animal dishes*. Jakarta: Penebar Swadaya

National Standardization Agency. 2009. SNI 2981:2009 Yogurt. Indonesian National Standards. National Standardization Agency. http://sispk.bsn.go.id/SNI/DetailSNI/8111#:~:text=Detail%20SNI%20Nomor%20SNI%20SNI%202981:2009

Diana, N. 2012. *Making vegetable yogurt by fermenting red bean milk (Phaseolus vulgaris) using backslop culture*. (Bachelor Thesis, University of Indonesia).

Haque, M.A., Timilsena, Y.P., & Adhikari, B. 2016. *Food proteins, structure, and*

function. Ref. Module. Food Sci., 1-8. https://doi.org/10.1016/b978-0-08-100596-5.03057-2

Hesti Wardhani, D., Catur Maharani, D., & Agus Prasetyo, E. 2015. *Study of the influence of the method of making corn milk, ratio and fermentation time on the characteristics of sweet corn yogurt*. Momentum, 11(1), 7–12. http://dx.doi.org/10.36499/jim.v11i1.1075

Hidayat, N., Suhartini, S., & Padaga, MC 2006. *Industrial microbiology (I)*. Yogyakarta: CV. Andi Offset.

Hoxha, R., Evstatieva, Y. & Nikolova, D. 2023. *Physicochemical, rheological, and sensory characteristics of yogurt fermented by lactic acid bacteria with probiotic potential and bioprotective properties*. Foods, 12(13), 2552. https://doi.org/10.3390/foods12132552

Indrasti, D., Andarwulan, N., Hari Purnomo, E., & Wulandari, N. 2019. *Suji leaf chlorophyll: potential and challenges as natural colorant*. Indonesian Journal of Agricultural Sciences, 24(2), 109–116. https://doi.org/10.18343/jipi.24.2.109

Jokopriyambodo, W., Sudarsono, & Rohman, A. 2014. *The antiradical activity of insoluble water suji (Pleomele angustifolia N. E. Brown) leaf extract and its application as natural colorant in bred products*. Journal of Food and Pharmaceutical Sciences, 2, 52–56. https://doi.org/10.14499/jfps

Kartikasari, DI, & Nisa, FC. 2014. *The effect of adding soursop juice and fermentation time on the physical and chemical characteristics of yogurt*. Journal of Food and Agroindustry, 2(4), 239–248. https://jpa.ub.ac.id/index.php/jpa/article/view/96

Manurung, D. ., Fahrunisa, D., & Ridwansyah. 2014. *The effect of the ratio of jackfruit seed juice to red dragon fruit juice and the ratio of stabilizers on the quality of dragon fruit yogurt*. Journal of Food and Agricultural Engineering, 2, 9–19. https://repository.usu.ac.id/handle/123456789/52252

Martha, NWVT. 2020. *The characteristics of the buns are based on the addition of liquid extract of Moringa oleifera leaves*. (Bachelor's Thesis, Health Polytechnic, Ministry of Health, Denpasar). http://repository.poltekkes-denpasar.ac.id/3167/

McQuistan, TJ, Simonich, MT, & Pratt, MM. 2012. *Cancer chemoprevention by dietary chlorophylls: A 12,000-animal*

Treatment	L* value	a* value	b* value
K1T1	76.56 ± 0.09a	-6.17±0.13cd	9.58±0.24bc
K1T2	75.41 ± 0.61a	-6.58±0.13b	6.88±0.24a
K1T3	76.01 ± 0.6a	-6.12±0.13cd	8.84±0.24b
K2T1	73.69 ± 0.06a	-6.43±0.13bcd	9.70±0.24bc
K2T2	74.78 ± 0.56a	-6.26±0.13cd	10.17±0.24bc
K2T3	73.31±3.47a	-7.01±0.13b	10.41±0.24c
K3T1	75.00 ± 0.24a	-8.26±0.13a	13.99±0.24d
K3T2	74.24 ± 0.44a	-7.89±0.13a	14.06±0.24d
K3T3	77.09 ± 0.86a	-5.80±0.13d	9.75±0.24bc

Note: Numbers followed by different letters on the same line are not significantly different (p>0.05)
L* = brightness parameter (L value=0 (black), L=100 (white))
a* = red to green color coordinates (Value a =+60 (red), a=-60 (green))
b* = yellow to blue coordinates (value b=+60 (yellow), b=-60 (blue)

and a fermentation time of 6 hours and 8 hours, the addition of 2 chlorophyll extracts. % and fermentation time of 6 hours, as well as the addition of 3 percent chlorophyll extract and fermentation time of 6 hours, each of which has a viscosity value of 8.75 cP. The effect of treatment on the concentration of suji leaf chlorophyll extract and fermentation time can be seen in Table 2.

Table 2 shows the results of the average viscosity value of each treatment, showing that the longer the fermentation, the higher the viscosity value of the yogurt product. This is thought to be influenced by lactic acid produced by Lactic Acid Bacteria (LAB). The acidic conditions caused by lactic acid can affect the viscosity of the yogurt product. When the acidity level in the yogurt product reaches the isoelectric point, the proteins in the yogurt will coagulate. The isoelectric point occurs because the number of cations and anions in the yogurt product is balanced, resulting in the attraction of these ions.(Widagdha & Nisa, 2015).

Color Analysis L* (Brightness)

The color of suji leaf chlorophyll yogurt was measured using a coloimeter. The results of the ANOVA analysis of L* color analysis (brightness) stated that there was no significant difference. The average lightness value (L*) of suji leaf chlorophyll yogurt can be seen in Table 3. The lightness value indicates the darkness (brightness) of a color. The notation L* states the brightness parameter from 0 (black) to 100 (white). The data from this research shows that the brightness value (L*) in suji leaf chlorophyll yogurt ranges from 73.00-77.09. The highest L* value was obtained from the treatment with the addition of 3 percent chlorophyll extract and a fermentation time of 10 hours, while the lowest value was obtained from the treatment with the addition of 3 percent chlorophyll extract and a fermentation time of 6 hours.

Table 3 shows that the brightness level of suji leaf chlorophyll yogurt decreases as the concentration of chlorophyll extract is added because the green pigment chlorophyll affects the brightness level of the yogurt. Meanwhile, the fermentation time did not show a significant increase or decrease in the brightness level of the yogurt.

Color Analysis a* (Redness)

Analysis results from ANOVA color a* (reddish) show that the addition of suji leaf chlorophyll extract and fermentation time significantly differed from the reddish color (a*) of suji leaf chlorophyll yogurt. The average value of the reddish color of suji leaf chlorophyll yogurt can be seen in Table 3. The notation a* is a reddish to greenish color coordinate with a* value of +60 (reddish) and -60 (greenish), so minus the

value of a*, the color of suji leaf chlorophyll yogurt will get greener. From the research data results, the average redness value (a*) in suji leaf chlorophyll yogurt ranged from -5.80 to -7.89. The highest value was obtained from suji leaf chlorophyll yogurt with 3 percent chlorophyll extract and a fermentation time of 10 hours, while the lowest a* value was obtained from adding 3 percent chlorophyll extract and a fermentation time of 8 hours.

Analysis results from ANOVA color b* (yellow) show that adding chlorophyll extract and fermentation time are significantly different. The average b* color value of suji leaf chlorophyll yogurt can be seen in Table 3. The notation b* indicates a yellowish to bluish color where +60 (yellowish) and -60 (bluish), the more positive the b* value will make the color of the chlorophyll yogurt yellower. From the analysis results, the average yellowness value (b*) in yogurt with adding chlorophyll

Table 4. Total Lactic Acid Bacteria in Suji Leaf Chlorophyll Yogurt with the Addition of Suji Leaf Chlorophyll Extract and Variations in Fermentation Time

Suji Leaf Chlorophyll Extract	Fermentation Time	Total LAB (Log CFU/ml)
1% (K1)	6 Hours (T1)	7.83
	8 Hours (T2)	7.82
	10 Hours (T3)	8.00
2% (K2)	6 Hours (T1)	7.78
	8 Hours (T2)	7.76
	10 Hours (T3)	8.04
3% (K3)	6 Hours (T1)	7.85
	8 Hours (T2)	7.80
	10 Hours (T3)	8.08

Table 3 shows that the more concentration of chlorophyll extract is added, the redness value of suji leaf chlorophyll yogurt decreases because the amount of green chlorophyll pigment increases. This is because the color of the chlorophyll pigment is composed of a combination of red and blue (Jokopriyambodo et al., 2014). Long fermentation treatment did not affect the redness value of suji leaf chlorophyll yogurt.

Color Analysis b* (Yellowish)

extract and fermentation time ranged from 6.88 to 14.06. The highest b* value was obtained from the treatment with 3 percent chlorophyll extract and a fermentation time of 8 hours, while the lowest b* value was obtained from the treatment with adding 1percent chlorophyll extract and a fermentation time of 8 hours. The yellowish color of yogurt is thought to be due to the presence of carotene dissolved in the fat in the milk ingredients(Shodiq et al., 2023).

Total Lactic Acid Bacteria (LAB)

The data from this research showed that the total Lactic Acid Bacteria (LAB) in suji leaf chlorophyll yogurt ranged from 7.76-8.08 CFU/ml. The results of the analysis stated that the highest log value of total lactic acid bacteria was 8.08 CFU/ml from the treatment with the addition of 3 percent chlorophyll extract and fermentation time of 10 hours, while the lowest log value was 7.76 CFU/ml from the treatment with the addition of the extract. 2 percent chlorophyll and fermentation time 8 hours.

The results of the total BAL test tend to increase with the length of fermentation. This is because the Lactic Acid Bacteria (LAB) found in yogurt take longer to use the nutrients in the yogurt medium to produce more lactic acid. Lactic acid bacteria will hydrolyze lactose (milk sugar) and sucrose by the enzyme lactate dehydrogenase produced by Lactic Acid Bacteria (LAB) (Ramadzanti, 2006). The total LAB produced by calculating suji leaf chlorophyll yogurt samples meets the Indonesian National Standards(2009): a minimum of 1 x 107 CFU/ml (7.00 log CFU/ml).

CONCLUSION

The combination of treatments that had the best effect on yogurt products was the addition of suji leaf chlorophyll extract and fermentation time, which was 3 percent suji leaf chlorophyll extract and a fermentation time of 10 hours. The best treatment produced antioxidant activity of 59.20 percent, dissolved protein content of 128.80 mg BSA/100 ml, total sugar content of 71.92g glucose/100 ml, fat content of 5.91percent, total chlorophyll of 2.39 percent, viscosity 10.00 cP, color L* (brightness) -77.09, color a* (reddish) -5.80, color b* (yellowish) 9.35, and total BAL 8.08 log CFU/ml.

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REFERENCES

Af'idah, F., & Trimulyono, G. 2019. Test the antioxidant activity and lactic acid levels of soybean tempeh yogurt (Glycine max) and green bean tempeh yogurt (Vigna radiata). *LenteraBio: Biological Scientific Periodical, 8(1)*, 17–24. https://doi.org/10.3934/agrfood.2022035

Amalia, NRP, & Aminah, S. 2021. Fiber content, antioxidant activity, physical and sensory characteristics of soybean sprout milk yogurt with the addition of green grass jelly extract. *Journal of Food and Nutrition, 11(01)*, 50–59. https://doi.org/10.26714/jpg.11.1.2021.50-59

Angelina, R. 2022. *The effect of different concentrations of green tea leaves (Camellia sinensis) on the physical and sensory properties of durian seed pickled yogurt*. (Bachelor Thesis, Widya Mandala University Surabaya). http://repository.ukwms.ac.id/id/eprint/28866

AOAC. 2005. *Official Methods of Analysis (17th ed.). The Association of Official Analytical Chemists, Gaithersburg, MD, USA*. Methods 925.10, 974.24, 992.16

Aryanti, N. 2016. *Extraction and characterization of chlorophyll from suji (Pleomele angustifolia) leaves as a natural food coloring*. Journal of Food Technology *Applications, 5(4)*, 129–135. https://doi.org/10.17728/jatp.196

Astawan, M. 2008. *Healthy with animal dishes*. Jakarta: Penebar Swadaya

National Standardization Agency. 2009. *SNI 2981:2009 Yogurt. Indonesian National Standards*. National Standardization Agency. http://sispk.bsn.go.id/SNI/DetailSNI/8111# :~:t ext= Detail% 2 0 S N I % 2 0 N o m o r % 2 0 S N I % 20SNI%202981:2009

Diana, N. 2012. *Making vegetable yogurt by fermenting red bean milk (Phaseolus vulgaris) using backslop culture*. (Bachelor Thesis, University of Indonesia).

Haque, M.A., Timilsena, Y.P., & Adhikari, B. 2016. Food proteins, structure, and function. *Ref. Module. Food Sci.*, 1-8. https://doi.org/10.1016/b978-0-08-100596-5.03057-2

Hesti Wardhani, D., Catur Maharani, D., & Agus Prasetyo, E. 2015. Study of the influence of the method of making corn milk, ratio and fermentation time on the characteristics of sweet corn yogurt. *Momentum, 11(1)*, 7–12. http://dx.doi.org/10.36499/jim.v11i1.1075

Hidayat, N., Suhartini, S., & Padaga, MC 2006. *Industrial microbiology (I)*. Yogyakarta: CV. Andi Offset.

Hoxha, R., Evstatieva, Y. & Nikolova, D. 2023. Physicochemical, rheological, and sensory characteristics of yogurt fermented by lactic acid bacteria with probiotic potential and bioprotective properties. *Foods, 12(13)*, 2552. https//doi.org/10.3390/foods12132552

Indrasti, D., Andarwulan, N., Hari Purnomo, E., & Wulandari, N. 2019. *Suji leaf chlorophyll: potential and challenges as natural colorant*. Indonesian Journal of Agricultural Sciences, 24(2), 109–116. https://doi.org/10.18343/jipi. 24.2.109

Jokopriyambodo, W., Sudarsono, & Rohman, A. 2014. *The antiradical activity of insoluble water suji (Pleomele angustifolia N. E. Brown) leaf extract and its application as natural colorant in bred products*. Journal of Food and Pharmaceutical Sciences, 2, 52–56. https://doi.org/10.14499/jfps

Kartikasari, DI, & Nisa, FC. 2014. *The effect of adding soursop juice and fermentation time on the physical and chemical characteristics of yogurt*. Journal of

Food and Agroindustry, 2(4), 239–248. https://jpa.ub.ac.id/index.php/jpa/article/view/96

Manurung, D. ., Fahrunisa, D., & Ridwansyah. 2014. *The effect of the ratio of jackfruit seed juice to red dragon fruit juice and the ratio of stabilizers on the quality of dragon fruit yogurt*. Journal of Food and Agricultural Engineering, 2, 9–19. https://repository.usu.ac.id/handle/123456789/52252

Martha, NWVT. 2020. *The characteristics of the buns are based on the addition of liquid extract of Moringa oleifera leaves*. (Bachelor's Thesis, Health Polytechnic, Ministry of Health, Denpasar). http://repository.poltekkes-denpasar.ac.id/3167/

McQuistan, TJ, Simonich, MT, & Pratt, MM. 2012. *Cancer chemoprevention by dietary chlorophylls: A 12,000-animal dose–dose matrix biomarker and tumor study*. Food and Chemical Toxicology, 50(2), 341–352. https://doi.org/https://doi.org/10.1016/j.fct.2011.10.065.

Nguyen, L. & Hwang, E. S. 2016. *Quality characteristics and antioxidant activity of yogurt supplemented with Aronia (Aronia melanocarpa) Juice*. Prev Nutr Food Sci., 21(4), 330-337. https://doi.org/10.3746/pnf. 2016.21.4.330.

Nurhayati, Nelwida, & Berliana. 2014. *Changes in the protein and crude fiber content of pineapple skin fermented with plain yogurt*. Scientific Journal of Animal Science, 17(1), 31-38. https://doi.orh/10.22437/jiiip.v17i1.2044

Prangdimurti, E., Muchtadi, D., Astawan, M., & Zakaria, FR 2006. *Antioxidant activity of suji leaf extract (Pleomele angustifolia NE Brown)*. Journal of Food Technology and Industry, 17(2), 79–86. https://journal.ipb.ac.id/index.php/jtip/article/view/420

Primurdia, EK, & Kusnadi, J. 2014. *Antioxidant activity of probiotic drink from date palm juice (Phoenix dactilyfera L.) with L. Plantarum and L. casei isolates*. Journal of Food and Agroindustry, 2(3), 98–109. https://jpa.ub.ac.id/index.php/jpa/article/view/57

Putri, HRD. 2022. *Antioxidant activity of cow's milk yogurt with curd starter and the addition of pandan leaf extract (Pandanus amaryllifolius Roxb.)*. (Bachelor Thesis, Syarif Hidayatullah State Islamic University)

Putriyana. 2023. *Test the antioxidant activity of suji (Dracaena angustifolia) leaf extract using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method*. Journal of Health and Medical Research, 3(1), 86–97. http://repository.umnaw.ac.id/jspui/handle/123456789/2821

Rahmawati, Taurina, W., & Andrie, M. 2016. The effect of clove oil (Syzygium aromaticum L.) on the protein stability of water phase ointment preparations from snakehead fish (Channa striata) extract by determining protein content using the Lowry method. *Journal of Pharmacy*, 1–23. https://jurnal. untan.

ac.id/index.php/jmfarmasi/article/view/42846?__im-vfoPSxFn=11726738020732028760

Ramadzanti, A. 2006. *Protease activity and lactic acid content in yogurt modified with Bifidobacterium bifidum*. (Bachelor's Thesis, Bogor Agricultural Institute).

Salminen, S., S. Gorbach, Y. Lee and Y. Benno. 2004. *Human studies on probiotics: what is beneficially proven today? In Salminen, S., A. Von Wright and A. Ouwehand (eds)*. Lactic Acid Bacteria. Marcel Dekker, New York. Pg 515-530.

Shodiq, AN, Wanniatie, V., Qisthone, A., & Adianto, K. 2023. Physical characteristics of dairy cow's milk: study of the status of smallholder dairy farming in Tenggamus Regency. *Journal of Animal Husbandry Research and Innovation, 7(1)*, 125-132. https://doi.org/10.23960/jrip.2023.7.1.125-132.

Simanihuruk, AVM, Lubis, LM, & Sinaga, H. 2019. The effect of the percentage of added egg yolk and starter on the physicochemical characteristics of purple sweet potato frozen yogurt. *Journal of Engineering and Agriculture, 7(4)*, 233-240. https://dupakdosen.usu. ac.id/handle/123456789/6527

Sudarmadji, S., Haryono, B., & Suhardi. 1997. *Food and agricultural material analysis procedures*. Yogyakarta: Liberty.

Suhartatik, N., Widanti, YA, Wulandari, YW, & Lestari, WN 2020. *Ketapang seed milk yogurt (Terminalia catappa L) with variations in starter type and fermentation time*. *Forest Products Industry Research Journal, 11(2)*, 77. https://doi.org/10.24111/jrihh.v11i2.5575.

Suhendra, D., Sudjatmiko, & Widiyanto. 2017. The addition of protected corn oil with various levels of protein in Holstein Friesian cow rations increases the level of unsaturated fatty acids in milk. *Veterinary Journal, 19(1)*, 100-108. https://doi.org/10.19087/jveteriner. 2018.19.1.100

Syainah, E., Novita, S., & Yanti, R. 2014. Study of making yogurt from various types of milk and different incubations on quality and acceptability. *Journal of Health Scales, 5(1*), 48–58. https://doi.org/10.31964/jsk.v5i1.10

Widagdha, S., & Nisa, CF. 2015. Effect of adding grape juice (Vitis vinifera L.) and fermentation time on the physico-chemical characteristics of yogurt. *Journal of Food and Agroindustry, 3(1)*, 248–258.https://jpa.ub.ac.id/index.php/jpa/article/view/129

Widodo, H., Sismindari, S., Asmara, W., & Rohman, A. 2019. *Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and their classification* with chemometrics. *Journal of Applied Pharmaceutical Science, 9(6)*, 99–105. https://doi.org/10.7324/JAPS.2019.90614.

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