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Utilization of Rosella as a Natural Color and Source of Antioxidants for Soybean Hull Nata

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Abstract— Nata is a fiber-rich cellulose formed through the fermentation of sugar by *Acetobacter xylinum* bacteria. Soybean hull waste has a nutritional content of 14.45% protein, 3.04% fat, 3.15% ash, 47.01% crude fiber, and 32% total iron minerals, making it suitable as raw material in making nata sheets. Rosella flowers contain anthocyanins that can be used as a natural red colorant in food products. The addition of rosella flower crude extract to nata will increase the visual and functional values of nata. This study aims to determine the best concentration of rosella flower crude extract (0%, 2%, 4%, 6%) based on the characteristics and functional value of nata made from soybean hulls. The best concentration of rosella flower crude extract in this study is 6%, producing nata with 0.776 ± 0.020 mM antioxidant, 2.230 ± 0.276 mg/L anthocyanin, 67.40 ± 0.063 L*, 48.84 ± 0.740 °hue (red color), 13.96 ± 0.770 C*. The scoring test results show that nata with 6% rosella flower crude extract has 'slightly no foreign taste' (4.55 ± 1.197), 'slightly no foreign aroma' (4.80 ± 0.966), and 'slightly red' color (4.20 ± 0.687). The hedonic test results show that panelists 'slightly like' the taste (5.12 ± 1.22), aroma (5.15 ± 1.17), and color (5.57 ± 1.11) of the nata.

Keywords— *Acetobacter xylinum*; nata; rosella flower; soybean hull

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I. INTRODUCTION

Nata is an extracellular polysaccharide (cellulose) layer rich in fiber, formed from the fermentation of sugar into cellulose by *Acetobacter xylinum* bacteria [1]. In the production of tempeh, there is a process of separating the skin (hull) of soybeans that will not be used in the subsequent tempeh-making process, resulting in waste. The soybean hull waste generated in the manufacture of tempeh amounts to 15% of the raw soybean materials used. This shows that the average soybean hull produced is 420 thousand tons/year, generating a considerable amount of soybean hull waste [2].

Soybean hull waste has an energy of 3,060.48 kcal/kg with nutritional content of 14.45% protein, 3.04% fat, 3.15% ash, carbon compounds in the form of 47.01% crude fiber, and 32% total mineral iron [3]. The nutritional content such as protein, fiber, and minerals that are still available in soybean hull waste

makes it suitable as raw material in the making of nata. Therefore, the production of nata made from soybean hull waste is conducted.

The addition of color to food products has a significant influence on the level of consumer acceptance a product [4]. Nata is a white and slightly transparent color. According to Handarini [5], the addition of dragon fruit extract affects the level of panelists' preference for the Nata de Soya product. The preference level of panelists increased as the concentration of dragon fruit extract increased. The higher the concentration of dragon fruit extract that was used, the redder the color of the nata produced.

Rosella flower (*Hibiscus sabdariffa* L.) contains anthocyanins that can be used as a natural red colorant in food products. The red color in anthocyanin pigments consists of delphinidin-3-silo glucoside, delphinidin-3-glucoside, and cyanidin-3-silo glucoside. The utilization of rosella flower crude extract as a

natural colorant has been investigated in several food products such as yogurt, Nata de Aloe, and jelly drinks [5]. The addition of rosella flower crude extract to nata is expected to enhance its visual characteristics in terms of color.

According to Santosa *et al.* [6] the highest nutritional content in nata only comes in the form of 2.5% crude fiber, indicating that its functional properties for health are suboptimal and need improvement. Rosella flower crude extract can also act as a source of antioxidants. According to Djaeni *et al.* [7] rosella flower crude extract has strong antioxidant activity, with an IC_{50} value of 50-100 ppm.

This research aims to determine the best concentration of rosella flower crude extract based on the characteristics and functional value of nata made from soybean hulls.

II. MATERIAL AND METHODS

A. Material

The research material consists of soybean hulls obtained from Rumah Tempe Indonesia, fresh rosella flower purchased from the online retail market "Rosella Borobudur", sugar brand of Gulaku, *Acetobacter xylinum* starter obtained from Nata Pelita, Indonesia, and 99% food grade acetic acid. The chemical ingredients used were HCl (Smart Lab, Indonesia), 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma, United States), $FeCl_3$ (Merck, Germany), CH_3COOH (Merck, Germany), ethanol (Merck, Germany), and KCl (Merck, Germany).

B. Methods

The experimental design in this research is a completely randomized design with 1 factor and 4 levels of rosella crude extract concentration, which are 0%, 2%, 4%, and 6%, with 2 replications. The stages in this research include the preparation of soybean hull powder, the making of nata, the making of rosella soaking media, and chemical analysis. The observed parameters are antioxidant activity, anthocyanin content, color characteristics, and organoleptic tests. The data obtained are analyzed using ANOVA statistical analysis.

Soybean Hull Drying

Nata was made using soybean hull powder. The soybean hulls were washed under running water until clean. Then, they were steamed for 25 minutes. Next, the soybean hulls were dried using an oven at 125°C for 2 hours and 30 minutes. The dried soybean hulls were reduced in size using a herb grinder, and then sieved with a 60-mesh sieve, resulting in soybean hull powder.

The Making of Nata

The nata fermentation medium was prepared by mixing 15% soybean hull powder (w/v) and 400 mL of water. Glacial acetic acid (99%) was gradually added until the pH of the solution reached 3.5. The pH was measured using a pH meter. The solution was then filtered using a tea strainer and the filtrate was

obtained. A total of 300 mL of filtrate was heated to boiling. Subsequently, 0.5% (w/v) ZA and 10% (w/v) sugar were added and stirred until homogeneous, resulting in the fermentation medium. The fermentation medium was transferred into a sterilized jar and stored for 3 hours covered with sterilized paper. Next, a 12% nata starter (v/v) was added to the sterilized fermentation medium and stirred until homogeneous. The fermentation medium was stored for 7 days at room temperature ($\pm 25^\circ C$). After 7 days of fermentation, the formed nata was removed. Then, the nata was rinsed with clean water and soaked for 1 day to eliminate the sour taste and aroma. Afterward, the nata was boiled in water until the boiling point ($100^\circ C$) was reached.

The Preparation of Rosella Soaking Media

The incorporation of rosella crude extract in the nata was done using the soaking method. Soaking media was prepared by combining rosella flower crude extract and sugar solution. The formula of rosella soaking media can be seen in **Table 1**.

TABLE I
ROSELLA SOAKING MEDIA FORMULATION

Material	Formulation*			
	I (0%)	II (2%)	III (4%)	IV (6%)
Sugar Solution	100	100	100	100
Rosella Crude extract	0	2	4	6

*The rosella crude extract amount is based on the sugar solution percentage

Rosella flower crude extract was made using fresh, non-wilted, and mold-free rosella flowers. The flowers were washed with running water until clean. Then, the rosella flowers were mixed with water in a ratio of 1:2 (rosella flowers: water) and mixed until homogeneous with a blender, then filtered to obtain a filtrate free from sediments or impurities, resulting in rosella flower crude extract. The sugar solution was made with a sugar-to-water ratio of 1:2.5. Then, the sugar solution was added with rosella flower crude extract in concentrations of 0% (control), 2%, 4%, and 6%, creating the rosella soaking media. Nata was soaked in rosella soaking media for 2 days at chiller temperature ($\pm 8^\circ C$).

Analysis Parameters

Antioxidant activity was measured using the Ferric Reducing Antioxidant Power (FRAP) method as described by Maesaroh *et al.* [8]. Antioxidant analysis was performed on the nata. To obtain a sample, the nata was crushed using a blender and filtrated using a vacuum pump. A total of 0.125 mL of sample was mixed with 3 mL of FRAP reagent and incubated for 30 minutes in a water bath ($37^\circ C$). The absorbance was measured with a visible spectrophotometer at 593 nm. The antioxidant activity in the sample was calculated using the regression equation.

Analysis of anthocyanin content was carried out by a differential pH method based on Anggraeni *et al.* [9] with

modifications. The anthocyanin analysis was conducted on the nata. To obtain a sample, the nata was crushed using a blender and filtrated using a vacuum pump. A total of 1 mL sample, each was added to 5 mL KCl 0.025 M pH 1 and 5 mL sodium acetate 0.4 M pH 4.5 separately. The solution was then stored for 30 minutes. The absorbance of each solution was measured at 510 nm and 700 nm. The anthocyanin content in nata was calculated using the formula:

$$A = (A_{vis-max} - A_{700})_{1.0} - (A_{vis-max} - A_{700})_{4.5} \dots \text{eq.(1)}$$

$$KA = \frac{A \times MW \times DF \times 1000}{\epsilon \times L} \dots \text{eq.(2)}$$

*A as sample absorbance; KA as anthocyanin concentration in mg/100 g; MW as molecular weight count as cyanidine-3-glucoside (MW=449.2); DF as dilute factor; L as cuvette width (1 cm); ϵ as absorptivity of cyanidine-3-glucoside (26.900).

The color characteristics of nata were measured according to the International Commission on Illumination (CIE) system [10]. Color analysis was performed using a chromameter. The surface of the chromameter was placed on the sample until the color results appeared on the device's display. The sample was measured based on the values of lightness (L*), redness (a*), and yellowness (b*), which were then converted into °hue and chroma value (C*). The °hue (h*) and chroma value (C*) were calculated using the formula:

$$h^* = \tan^{-1} \left(\frac{b^*}{a^*} \right) \dots \text{eq.(3)}$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \dots \text{eq.(4)}$$

Sensory evaluation was conducted through scoring and hedonic tests. The evaluation parameters consist of three aspects, which are foreign taste, foreign aroma, and color. The organoleptic test was carried out by 40 panelists on samples with randomly assigned codes [11].

III. RESULT AND DISCUSSION

Antioxidant Activity (Ferric Reducing Antioxidant Power)

Antioxidant activity is a parameter that indicates the amount of antioxidants entering the cellulose tissue of nata during the soaking process. Antioxidant activity is measured based on its ability to reduce Fe³⁺ ions to Fe²⁺. **Table 2** presents the results of the antioxidant activity of the nata using the FRAP assay.

TABLE 2
ANTIOXIDANT ACTIVITY

Rosella Crude extract Concentration (%)	Antioxidant Activity (mM)*
0	0.26±0.01 ^a
2	0.28±0.01 ^b
4	0.52±0.01 ^c
6	0.78±0.02 ^d

*Data shown is average value ± deviation standard

*The difference in letter notation indicates a significant difference (p<0.05)

The highest antioxidant activity is found in the nata with the addition of 6% rosella flower crude extract, which is 0.776±0.020 mM against the FeSO₄ compound. The analysis results show that the antioxidant activity of the nata increases with the increasing concentration of added rosella flower crude extract. According to **Table 3**, it is evident that the anthocyanin content increases with the rising concentration of rosella flower crude extract, confirming the consistency of the results obtained in this study. According to Santosa *et al.* [6], antioxidant activity will increase with the increase in anthocyanin content.

In **Table 2**, it is observed that even without the addition of rosella flower crude extract (0%), nata still exhibits antioxidant activity against the FeSO₄ compound. According to Rahayu and Erna [12], soybeans contain antioxidants in the form of anthocyanins in its hull. Without the addition of rosella flower crude extract, nata made from soybean hulls contain antioxidants, but the addition of rosella flower crude extract will enhance its antioxidant content.

Anthocyanin Content

Anthocyanin content is a parameter indicating the amount of anthocyanin pigment that enters the cellulose tissue of nata during the soaking process. **Table 3** displays the results of the anthocyanin content in nata.

TABLE 3
ANTHOCYANIN CONTENT

Rosella Flower Crude extract Concentration (%)	Anthocyanin Content (mg/L)*
0	0.15±0.24 ^a
2	0.60±0.30 ^a
4	1.28±0.41 ^b
6	2.23±0.28 ^c

*Data shown is average value ± deviation standard

*The difference in letter notation indicates a significant difference (p<0.05)

According to **Table 3**, it is evident that the anthocyanin content in the nata is significantly different between the concentrations of 0%; 2% with 4%, and 6%. The highest anthocyanin content is found in nata with the addition of 6% rosella flower crude extract. This aligns with the study conducted by Santosa *et al.* [6], where a higher concentration of dragon fruit extract leads to increased anthocyanin content. The higher anthocyanin content in the nata results in a more intense red color. This is consistent with the C* value in Table 4, where the color intensity of nata increases as the concentration of rosella flower crude extract increases.

Color Characteristics

Color is one of the factors that influence consumer acceptance of a product. **Figure 1** illustrates that soaking nata with rosella crude extract produces nata with a reddish color.

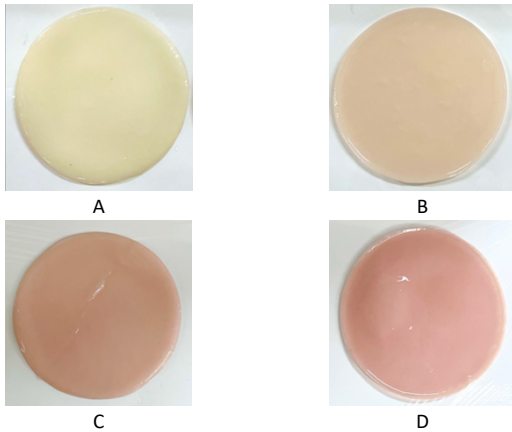


Fig. 1 Nata made from Soybean Hull with Concentration Variation of Rosella Crude extract

*A is nata with 0% rosella flower crude extract treatment and represents control; B is nata with 2% rosella flower crude extract treatment; C is nata with 4% rosella flower crude extract treatment; D is nata with 6% rosella flower crude extract treatment.

In the color analysis, a chromameter with the CIElab system measuring L, a*, and b* values, was used. Subsequently, the °hue value (h*), and chroma value (C*) of the nata were obtained using the equations. **Table 4** presents the color characteristics of the nata.

TABLE 4
 COLOR CHARACTERISTICS

	Rosella Flower Crude extract Concentration (%)			
	0	2	4	6
Lightness*	74.52±0.11 ^c	72.50±0.26 ^b	67.12±0.33 ^a	67.40±0.06 ^a
(h*)*	90.38±0.20 ^d	73.78±0.18 ^c	51.09±0.89 ^b	48.84±0.74 ^a
(C*) *	7.54±0.17 ^a	10.00±0.39 ^b	11.87±0.16 ^c	13.96±0.77 ^d
Color	Yellow	Yellow-red	Red	Red

*Data shown is average value ± deviation standard
 *The difference in letter notation indicates a significant difference (p<0.05)
 (C) as chroma value; (h*) as °hue

The statistical analysis results indicate significant differences in lightness (p<0.05) between concentrations of 4 and 6% and the other concentrations. The higher the lightness value, the brighter the color of the nata. Nata without the addition of rosella flower crude extract (0%) has the highest lightness value of 74.52±0.107 followed by nata with rosella flower crude extract concentrations of 2%, 6%, and 4%. This indicates that as the concentration of rosella flower crude extract increases, the lightness of the nata product decreases. Lightness represents the brightness of the color in a product, the closer the lightness value is to 100%, the whiter the color [13]. The addition of rosella flower crude extract changes the color of the nata to red. Higher concentrations of rosella flower crude extract result in increased color intensity, causing the lightness of the nata to decrease.

Table 4 shows significant differences in °Hue values between the concentrations of 0%, 2%, 4%, and 6%. Nata without the addition of rosella flower crude extract falls within the yellow color range. Meanwhile, nata with the addition of rosella flower crude extract is in the yellow red, and red color ranges. This corresponds to the conversion of °hue value, where 90°-126° represents the yellow color range, 54°-90° represents the yellow-red color range, and 18°-54° represents the red color range. Rosella flowers contain anthocyanin pigments that contribute to providing red color [7]. The higher the concentration of rosella flower crude extract used, the more the nata color enters the red color range.

Table 4 shows significant differences in C* values for all concentration treatments. Nata with 6% rosella flower crude extract has the highest C* value. The higher the concentration of rosella flower crude extract added, the higher the C* value. According to Fadhlurrohman *et al.* [13], the higher the C* value, the higher the saturation of the product, resulting in a more intense color. This indicates that with the increase in the added concentration of rosella flower crude extract, nata was produced with a more intense color.

Sensory Evaluation

Sensory evaluation was conducted through scoring and hedonic tests. The scoring test represents the intensity of each treatment based on three parameters, which are unfamiliar flavor, unfamiliar aroma, and color. The hedonic test represents the preference of panelists on each treatment based on three parameters, namely unfamiliar flavor, unfamiliar aroma, and color. **Table 5** shows the sensory characteristics based on the scoring test and **Table 6** shows the sensory characteristics based on the hedonic test.

TABLE 5
 SENSORY CHARACTERISTICS (SCORING TEST)

Rosella Flower Crude extract Concentration (%)	Foreign Taste*	Foreign Aroma*	Color*
0	5.00±0.88 ^a	5.02±0.80 ^a	1.15±0.43 ^a
2	4.93±0.97 ^a	4.73±1.01 ^a	1.95±0.78 ^b
4	4.75±0.98 ^a	4.83±0.98 ^a	3.55±0.78 ^c
6	4.55±1.20 ^a	4.80±0.97 ^a	4.20±0.69 ^d

*Data shown is average value ± deviation standard
 *The difference in letter notation indicates a significant difference (p<0.05)
 *Scale 1 (Extremely foreign taste, extremely foreign aroma, extremely not red)- 6(Extremely no foreign taste, extremely no foreign aroma, extremely red).

The variation of rosella flower crude extract concentration did not have a significant effect (p>0.05) on foreign taste and foreign aroma. The scores given by the panelists ranged between 'slightly not foreign; slightly not aromatic' and 'not foreign; not aromatic'. In the scoring test, the results indicated no foreign taste and aroma in the nata with the addition of rosella flower crude extract. This suggests that the addition of

rosella flower crude extract does not introduce undesirable foreign taste and aroma to the nata. According to Yazid and Muchammad [14], rosella flower petals contain betalain compounds. Betalain is a compound that produces an earthy taste and aromas due to the presence of pyrazine and geosmin compounds [15]. These betalain compounds can cause the emergence of foreign tastes and aromas in nata.

Table 5 shows significant differences ($p < 0.05$) in the color scoring test. The results of the scoring test without the addition of rosella flower crude extract (1.15 ± 0.43) and with the addition of 2% rosella flower crude extract (1.95 ± 0.78) fall between the ranges of 'very not red' and 'not red' with scores 1 and 2. The results of the nata scoring test with the addition of 4% rosella flower crude extract (3.55 ± 0.78) fall between the ranges of 'slightly not red' and 'slightly red' with scores 3 and 4. Meanwhile, the results of the scoring test of the addition of 6% rosella crude extract (4.20 ± 0.69) fall between the ranges of 'slightly red' and 'red' with scores 4 and 5. The higher the concentration of rosella flower crude extract added, the higher the color scoring value. This indicates that the color of the nata visually becomes increasingly red with the increase in concentration. This aligns with the °hue value in **Table 4**. According to **Table 4**, nata without the addition of rosella flower crude extract has a yellow color, and as the concentration increases, the nata color turns red. Rosella flowers contain anthocyanin pigments that contribute to providing a red color [7]. Increasing the concentration of rosella flower crude extract will enhance the anthocyanin content entering the nata tissue during soaking [6], resulting in nata with increasingly intense red color.

TABLE 6
SENSORY CHARACTERISTICS (HEDONIC TEST)

Rosella Flower Crude extract Concentration (%)	Foreign Taste*	Foreign Aroma*	Color*
0	5.02±1.48 ^a	5.33±1.33 ^a	4.98±1.37 ^a
2	5.05±1.32 ^a	5.20±1.18 ^a	4.85±1.37 ^a
4	5.28±1.13 ^a	5.35±1.15 ^a	5.25±1.06 ^{ab}
6	5.12±1.22 ^a	5.15±1.17 ^a	5.57±1.11 ^b

*Data shown is average value ± deviation standard

*The difference in letter notation indicates a significant difference ($p < 0.05$)

*Scale 1 (Extremely dislike); scale 4 (Neutral); Scale 7 (Extremely like)

Based on **Table 6**, the concentration of rosella flower crude extract did not have a significant effect ($p > 0.05$) on the foreign taste and aroma. The scores given by the panelists range between 'slightly like' and 'like' with scores of 5 and 6. The results align with the results of the scoring test. No significant difference in taste and foreign aroma in the scoring test, made the panelists' preference also not significantly different between concentrations. In this study, the nata produced has the characteristics of a 'slightly not foreign' taste referring to the scoring test, and based on the hedonic test, panelists tend to 'slightly like' the taste and aroma of the nata. Betalain

compounds found in rosella flowers can impart earthy taste and aroma characteristics that might be perceived as foreign in nata [14].

Table 6 shows a significant difference ($p < 0.05$) in the color hedonic test. This indicates that the addition of color significantly influences the preference of panelists towards nata. The color hedonic test results for nata without the addition of rosella flower crude extract (4.98 ± 1.37) and the addition of 2% rosella crude extract (4.85 ± 1.37) fall between the ranges of 'neutral' and 'slightly like' with scores of 4 and 5. Meanwhile, the results of the color hedonic test for the addition of 4% rosella flower crude extract (5.25 ± 1.06) and 6% rosella flower crude extract (5.57 ± 1.06) fall between the ranges of 'neutral' and 'slightly like' with scores of 5 and 6. This indicates that the treatment most preferred by the panelists is the 6% treatment, which produces nata with the most intense red color compared to other concentrations. The intensity of the red color in the nata will increase with the increase of the concentration used, referring to the C* value in **Table 4**. Rosella flowers contain anthocyanin pigments that contribute to providing red color [7]. These results are consistent with the study conducted by Ratnasari *et al.* [16], where the higher the concentration of colorant used, the higher the preference level of panelists towards the nata.

IV. CONCLUSION

In this study, the results indicate that the addition of 6% rosella flower crude extract is the best concentration based on its characteristics (anthocyanin content, color characteristics, and sensory evaluations) and functional value (antioxidant activity). The addition of 6% rosella flower crude extract producing nata with 0.776 ± 0.020 mM antioxidant activity, 2.230 ± 0.276 mg/L anthocyanin, 67.40 ± 0.063 L*, 48.84 ± 0.740 °hue (red color), 13.96 ± 0.770 C*. The scoring test results show that nata with 6% rosella flower crude extract has 'slightly no foreign taste' (4.55 ± 1.197), 'slightly no foreign aroma' (4.80 ± 0.966), and 'slightly red' color (4.20 ± 0.687). The hedonic test results show that panelists 'slightly like' the taste (5.12 ± 1.22), aroma (5.15 ± 1.17), and color (5.57 ± 1.11) of the nata.

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CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

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