

ANTHOCYANIN EXTRACTION FROM RED SPINACH UTILIZING MICROWAVE IRRADIATION AND ITS APPLICATION IN FOOD PRODUCTS

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Abstract

In contemporary times, using food coloring agents is necessary to capture attention. The extensive use of synthetic food coloring agents has raised significant health concerns. Red spinach, a plentiful vegetable in Indonesia, contains anthocyanins that give it a reddish-purple color. The presence of anthocyanins makes red spinach a promising natural coloring agent. This research aims to identify the optimal operational conditions for extracting anthocyanins from red spinach, with the ultimate goal of applying these findings in developing innovative food products. The fixed variables included 20 grams of dried red spinach, a 10-minute extraction duration, and distilled water as the solvent. The independent variables were microwave power levels (100, 300, 500 W) and the material-to-solvent (M/S) ratio (1:10, 1:15 g/mL). The results revealed a moisture content of 7.6% in red spinach. The highest yield was achieved at 100 W with an M/S ratio of 1:15 g/mL, yielding 80%. The best anthocyanin extract was determined based on the highest anthocyanin content, which was obtained at 300 W with an M/S ratio of 1:15 g/mL, resulting in 56.93 mg/L. Anthocyanins were stable at low temperatures (4°C) but highly unstable at high temperatures. Sensory testing to determine consumer acceptability showed no significant taste difference between beverages with added anthocyanin extract and those without. The resulting taste was mild, and the extract's color was appealing. Therefore, the anthocyanin extract from red spinach can be used as a natural coloring agent, replacing hazardous synthetic food colorants.

Keywords: Anthocyanin; Extraction; Food coloring; Microwave irradiation; Red spinach

1. INTRODUCTION

The present era is witnessing rapid advancements in the food industry, driven by the increasing demand for food supplies. However, this rapid growth in the food industry sector has not been entirely matched by improved product quality and safety standards of the materials used. Numerous incidents of food poisoning have been closely linked to the use of synthetic additives in food products. In response to this situation, an alternative approach involving using natural ingredients in food products has been proposed (Jumiati & Mardhiana, 2017).

Natural food coloring agents are derived from plants, animals, and minerals through extraction processes and have traditionally been widely recognized as safe for consumption. On the other hand, synthetic food coloring agents are produced from chemical compounds such as iron oxide, titanium

dioxide, and others (Sulistiawati et al., 2017). Approved coloring agents in food products are called "certified color" or "permitted color." However, synthetic food coloring agents are still common in certain food products in Indonesia, such as crackers, shrimp paste, candy, syrup, biscuits, sausages, fried noodles, soft drinks, and more. This phenomenon poses serious health risks (Surati, 2015).

In Indonesia, some examples of commonly used synthetic food coloring agents include tartrazine and quinoline yellow (yellow), brilliant blue and indigo carmine (blue), violet GB (purple), fast green FCF (green), sunset yellow (orange), and rhodamine B (red) (WHO, 2023). While the use of synthetic food coloring agents provides significant visual benefits to manufacturers to enhance the appeal of their products, it has negative consequences for consumers, including health problems such as the risk of

poisoning, lung, eye, throat, and intestinal irritations, and even potential cancer risks (Hevira, 2020).

As an alternative, red spinach extract can be considered an option to reduce dependence on synthesis food colorants. Red spinach contains anthocyanin compounds that have the potential to serve as a natural food coloring source while offering health benefits due to their ability to combat the effects of free radicals (Murningsih, 2019). Anthocyanins are polar compounds, making polar solvents suitable for extraction. Besides considering solvent polarity, the criteria for solvent selection also include safety, availability, affordability, and neutrality (Baciang & Inda, 2020). According to data from the Central Statistics Agency (BPS) in 2021, the production of red spinach in Indonesia and North Sumatra Province indicates abundant availability, as shown in Table 1. This opens up opportunities to optimize the potential of red spinach as a raw material for natural food coloring.

Table 1. Red spinach production data

Year of Production	Quantity (Tons)	
	Indonesia	North Sumatra Province
2021	169,715	17,980
2020	157,024	12,786
2019	160,305	16,610
2018	162,263	20,244
2017	148,289	20,435

Research on anthocyanin extraction from red spinach has been conducted using conventional methods, such as maceration and vacuum drying, with polar solvents, including a combination of distilled water with acid and a mixture of ethanol with acid. Previous research showed anthocyanins were more stable at acidic pH, with an anthocyanin content reaching 43.08 mg/L (Adam, 2017). However, this study investigated a non-conventional method, microwave-assisted extraction (MAE), utilizing distilled water as a solvent, with microwave power and M/S ratio as independent variables. This research aims to identify the optimal operational conditions for extracting anthocyanins from red spinach, with the ultimate goal of applying these findings in developing innovative food products.

2. MATERIALS AND METHOD

Red spinach was obtained from local farmers in North Sumatra. Distilled water (H₂O), ACS reagent grade of potassium chloride (HCl), sodium acetate (CH₃COONa.3H₂O), and ethanol (C₂H₅OH) were purchased from a local chemical store. Tonic water and fermented milk were obtained from the supermarket, while clear milk tea was prepared using a clarified method.

2.1 Preparation of Raw Materials

The red spinach was thoroughly cleaned with running water to remove impurities. It was then

dehydrated using a food dehydrator at a constant temperature of 35°C until it reached a consistent weight. After dehydration, the red spinach was finely pulverized into a powder using a blender and sieved through a 20-mesh sieve. The resulting red spinach powder was stored in a dry container.

2.2 Extraction and Evaporation

A quantity of 20 grams of red spinach powder was weighed. Subsequently, the red spinach powder (M) was mixed with distilled water (S) at two M/S ratios of 1:10 and 1:15 g/mL. After mixing, the mixture was placed in a microwave for extraction, utilizing 100, 300, and 500 W power settings, each for 10 minutes. Following the extraction process, filtration was carried out to separate the filtrate from the residue. The filtrate was then evaporated using a rotary vacuum evaporator at 45°C for 2 hours. The resulting concentrated anthocyanin extract was stored in opaque glass bottles.

2.3 Characteristics of Red Spinach

2.3.1 Determination of moisture content of red spinach powder

Porcelain dishes are dried at 105°C for 30 minutes and cooled in a desiccator to a constant weight. Next, 2 grams of red amaranth powder are added and dried for 1 hour, then cooled in a desiccator. Next, the porcelain dish and sample powder are weighed. The moisture content of the material is calculated using Equation 1.

$$\text{Water Content} = \frac{C_1 - C_2}{C_2} \times 100\% \quad (1)$$

C₁ is the weight of the porcelain dish and sample, grams; C₂ is the weight of a constant porcelain dish, grams.

2.3.2 Yield of red spinach anthocyanin extract

Anthocyanin extract yield is calculated using Equation 2.

$$\% \text{yield} = \frac{\text{Volume of concentrated extract (ml)}}{\text{Initial Volume (ml)}} \times 100\% \quad (2)$$

2.3.3 Anthocyanin content

Anthocyanin content absorbance was measured using a UV-Vis Spectrophotometer by dissolving 0.1 grams of concentrated extract with 10 mL aquadest. Then, as much as 0.2 mL was inserted into the cuvette, and buffer solution KCl pH 1 and CH₃COONa.3H₂O pH 4.5 were added. Absorbance is carried out at a maximum wave of 700 nm. The final absorbance is calculated using Equation 3.

$$A = [(A_{\lambda_{\text{vis-max}}} - A_{700})_{\text{pH } 1,0} - (A_{\lambda_{\text{vis-max}}} - A_{700})_{\text{pH } 4,5}] \quad (3)$$

where A is the final absorbance value; A_{λ_{vis-max}} is the value of the absorbance length at the maximum wave, nm; A₇₀₀ is the value of the absorbance length in the 700 wave, nm.

Anthocyanin levels are calculated using Equation 4.

$$\text{Anthocyanin (mg/L)} = \frac{A \times \text{BM} \times \text{FP} \times 1000 \text{ mg}}{\epsilon \times L} \quad (4)$$

where A is the final absorbance value; BM is the molecular weight of cyanidin-3-glucoside, g/mol; FP is

the dilution factor; ϵ is the molar cyanidin-3-glucoside absorptivity, L/mol.cm; L is the cuvette width, cm.

2.3.4 Anthocyanin stability

A 5 mL mixture of each extract is put into a test tube. Then, it is heated at 40, 50, and 60°C in a water bath for 30 minutes. Then, the color change is observed for 60 minutes (influence of temperature). In addition, samples are also stored in the refrigerator (-4°C) and rooms and placed in dark bottles for 7 days, with observation every 24 hours (influence of storage conditions).

2.4 Application in Food Products (Beverages)

The anthocyanin extract was applied to tonic water, clear milk tea, and fermented milk. The process commenced with measuring the pH level in each beverage. Subsequently, 5 mL of anthocyanin extract was added to 100 mL of each beverage. Observations were then conducted for 3 minutes to assess solubility.

2.5 Organoleptic Testing

Five panelists performed organoleptic testing by randomly trying each drink that had been added extract; panelists gave a scale of 1-5 depending on their preference for each drink's taste. Concluding organoleptic testing using statistical calculations: the hedonic method.

3. RESULTS AND DISCUSSION

3.1 Solvent Selection

In microwave-assisted extraction (MAE), solvent selection should consider the dielectric constant value. The dielectric constant value indicates how much a solvent can interact with microwave radiation. The higher the dielectric constant value of a solvent, the better it can absorb microwave energy. As a result, both energy and mass transfer are enhanced. The dielectric constant values for various polar solvents are shown in Table 2.

Table 2. The dielectric constant value of polar solvents (Triesty & Mahfud, 2017)

Solvent	Dielectric Constant
Distilled water	80,4
Methanol	32,6
Chloroform	24,3
Toluene	4,8
Hexane	2,4

From Table 2, it can be observed that distilled water has the highest dielectric constant value, thus facilitating a more effective transfer of energy and mass during the extraction process. In brief, the energy transfer phase involves the absorption of electromagnetic energy by molecules within the sample and the solvent, which elevates their temperature. Subsequently, the mass transfer phase occurs, where the desired substances diffuse out of the sample and dissolve in the heated solvent.

3.2 Moisture Content of Red Spinach Raw Material

The method employed to determine the moisture content of red spinach adheres to the standard procedure test (2021), which involves the drying or evaporation of water from a substance. The analysis yielded a moisture content of 7.6% in the red spinach, which complies with the moisture content standard for organic material powder, which is below 10% (Rosyidah et al., 2021).

3.3 Anthocyanin Yield

Figure 1 shows the influence of the M/S ratio at various microwave power levels on the anthocyanins yield.

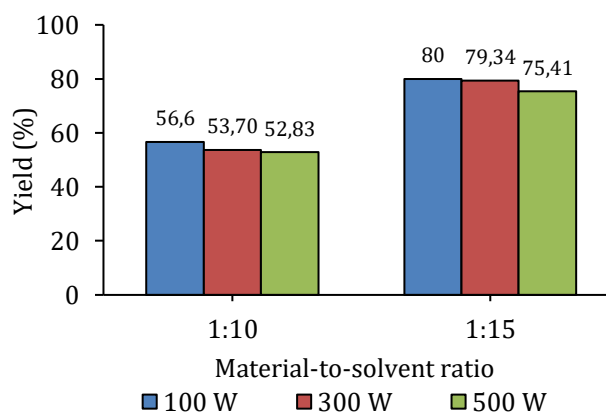


Figure 1. Influence of the M/S ratio at various microwave power levels on the anthocyanin yield

Figure 1 shows that the highest yield was obtained at 100 W with an M/S ratio of 1:15. Under these conditions, the yield reached 80%. Meanwhile, the lowest yield was achieved at 500 W with an M/S ratio of 1:10, resulting in 52.83%.

This phenomenon can be attributed to the correlation between power and temperature during extraction. Higher temperatures increase the solubility of phytochemical compounds in the solvent and the equilibrium constant (Nicoue et al., 2007). Microwave power can elevate the temperature to increase pressure within plant cell walls. This pressure causes the cell walls to rupture, releasing components into the extraction solution to facilitate the diffusion of components from the source material (Xue et al., 2018). However, using higher power can also lead to lower extraction yields due to excessive evaporation in the sample. Excessive evaporation can reduce the solvent dissolving anthocyanins (Hasdar, 2021).

In the extraction process, an increase in solvent usage can also result in higher yields (Rifai et al., 2018). This phenomenon occurs because an increased quantity of solvent enhances the efficacy of extracting the target compounds into the solvent while preventing solvent saturation. However, an increase in extraction yield does not always correspond to an increase in anthocyanin content. This is due to the presence of impurity compounds that contribute to the yield results. These impurity compounds can

include sugars, organic acids, phenolic compounds, and substances other than anthocyanins (Wahyuni, 2015). Nevertheless, yield analysis is still essential because it does not require specialized instrumentation and can provide initial information about extraction efficiency.

3.4 Anthocyanin Content

Figure 2 shows the influence of the M/S ratio at various microwave power levels on the anthocyanin content in red spinach.

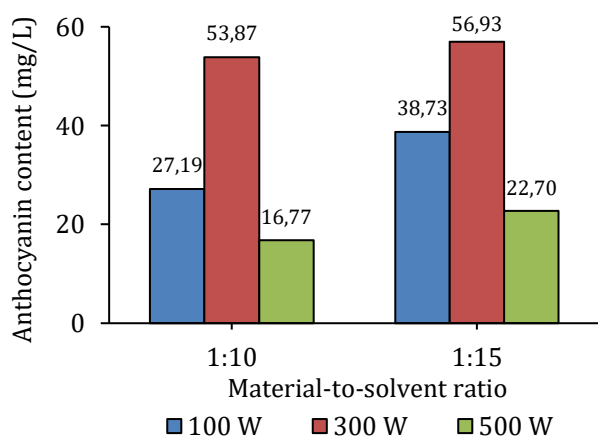


Figure 2. Influence of the M/S ratio at various microwave power levels on the anthocyanins content in red spinach

Figure 2 shows that the highest anthocyanin content is achieved at 300 W with an M/S ratio of 1:15. Under these conditions, the anthocyanin content reaches 56.93 mg/L. Meanwhile, the lowest anthocyanin content is obtained at 500 W with an M/S ratio of 1:10, resulting in 27.19 mg/L. Figure 2 also shows that the anthocyanin content increases from 100 to 300 W but then decreases at 500 W. Additionally, increasing the M/S ratio from 1:10 to 1:15 enhances the anthocyanin content.

This phenomenon occurs because higher microwave power levels transfer more electromagnetic energy to molecules through ionic conduction and dipole rotation. This interaction leads to rapid heating of the extraction system (Kaderides et al., 2019). The relation between microwave power and temperature has been studied, where increased power causes higher microwave irradiation intensity, ultimately converting more electromagnetic energy into heat, as indicated by temperature elevation (Gala et al., 2016). At 500 W, the resulting temperature increases, causing anthocyanin compounds' degradation in red spinach. These results imply a direct proportional relationship between temperature and microwave power, and anthocyanin is susceptible to degradation, especially at high temperatures.

Apart from microwave power, the M/S ratio also plays a significant role in the MAE process, and the two are closely interrelated. This is reinforced by the fact that the extracted raw material's properties and the solution's equilibrium can affect the extraction outcome (Dinira et al., 2021). The research results

indicate that an M/S ratio of 1:15, in combination with an appropriate power level (300 W), produces the highest anthocyanin content compared to a 1:10 ratio with the same power. This is because, in the 1:15 ratio, the solvent has not reached saturation, allowing for a greater capacity to extract anthocyanin compounds efficiently.

These results align with Farida and Nisa's (2015) research, which conducted anthocyanin extraction from mangosteen peel using the MAE method. Their study indicated that an M/S ratio of 1:20 resulted in a higher anthocyanin content compared to ratios of 1:10 and 1:30. This is because, at a 1:20 ratio, the appropriate proportion between the amount of raw material and the solvent is achieved, allowing the solvent to dissolve effectively into the raw material and reach its optimal condition, resulting in the highest anthocyanin content. Therefore, selecting power and M/S ratio is a crucial factor to consider in MAE.

3.5 Application in Food Products (Beverages)

3.5.1 Solubility of anthocyanin extract

The anthocyanin extract from red spinach was applied in three beverages: tonic water, clear milk tea, and fermented milk, as shown in Figure 3.

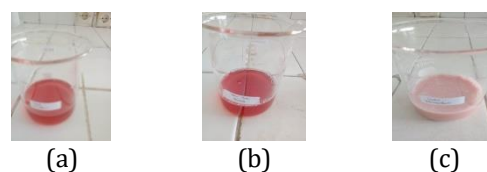


Figure 3. Application of anthocyanin extract in beverages (a) tonic water, (b) clear milk tea, and (c) fermented milk

Before applying anthocyanin extract to the beverages, the pH of the extract and the three types of beverages were measured. pH analysis was conducted initially because the chemical properties of anthocyanin are greatly influenced by pH. When anthocyanin extract is mixed with alkaline compounds, its color changes to greenish-yellow. However, its color remains unchanged if anthocyanin extract is mixed with acidic compounds. Lower pH values result in a darker color because anthocyanin is in the form of flavilium cations under such conditions (Adam, 2017).

The pH analysis results showed that the pH of anthocyanin extract from red spinach, tonic water, clear milk tea, and fermented milk were 5.3, 2.8, 4.5, and 4.0, respectively. Subsequently, 5 mL of concentrated extract was added to 100 mL of each beverage. Observations were made for 3 minutes to assess its solubility, as shown in Figure 3. The observations revealed that the anthocyanin extract dissolved completely in tonic water and clear milk tea, while it caused coagulation in the fermented milk. These results align with the research of Sanchez-Bravo et al. (2015), which focuses on the interaction between anthocyanin extract and proteins in fermented milk,

inducing changes in the colloid state and forming clumps. Consequently, fermented milk experienced coagulation due to alterations in protein structure influenced by pH changes resulting from the addition of acidic anthocyanin extract.

3.5.2 Stability of anthocyanin extract

The anthocyanin extract, added to various beverages, was subsequently collected in 5 mL for stability analysis. Table 3 shows the influence of heating temperature on the color stability of anthocyanins in various beverages. In contrast, Table 4 shows the influence of storage conditions on the color stability of anthocyanins in various beverages.

Table 3. Test results for the influence of heating temperature on the color stability of anthocyanins in various beverages

Treatment	Heating Time (30 Minutes)		
	40°C	50°C	60°C
Tonic water	-	+	+
Clear milk tea	-	+	+
Fermented milk	-	+	+

Notes:

(-) = no color changed

(+) = color changed

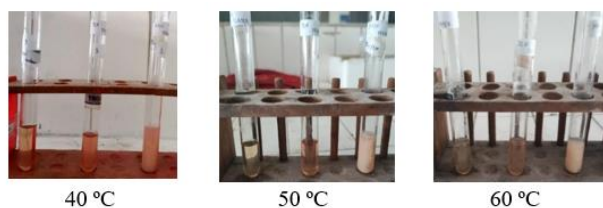


Figure 4. Anthocyanin stability in various beverages under different heating temperatures (from left to right: tonic water-clear milk tea-fermentation milk)

The heating temperature is a crucial factor in anthocyanin extraction and processing. Anthocyanins are highly sensitive to high temperatures, so assessing their ability to withstand a specific temperature without degradation or with minimal degradation is essential. The optimal temperature for anthocyanin processing can be identified through stability analysis at various heating temperatures.

Table 3 shows that after heating at 40°C for 30 minutes, the color of the beverages still exhibits relatively good stability. This indicates that at this temperature and time, the structure of the anthocyanin mixed into the beverages has not been significantly damaged. However, heating at 50 and 60°C for 30 minutes results in color fading in all beverages, as seen in Figure 4. This occurs because heat can lead to the degradation of anthocyanin pigments into chalcone compounds (brown color) (Gala, 2016). Therefore, these results illustrate that temperatures of 50 and 60°C have caused damage to anthocyanin compounds.

Table 4. Test results for the influence of storage conditions on the color stability of anthocyanins in various beverages

Treatment	Variable	Storage Condition	
		Room Temp.	Refrigerator (4°C)
Tonic water	Day 1	-	-
	Day 2	-	-
	Day 3	+	-
	Day 4	+	-
	Day 5	+	-
	Day 6	+	-
	Day 7	+	-
Clear milk tea	Day 1	-	-
	Day 2	-	-
	Day 3	+	-
	Day 4	+	-
	Day 5	+	-
	Day 6	+	-
	Day 7	+	-
Fermented milk	Day 1	-	-
	Day 2	-	-
	Day 3	+	-
	Day 4	+	-
	Day 5	+	-
	Day 6	+	-
	Day 7	+	-

Notes:

(-) = no color changed

(+) = color changed

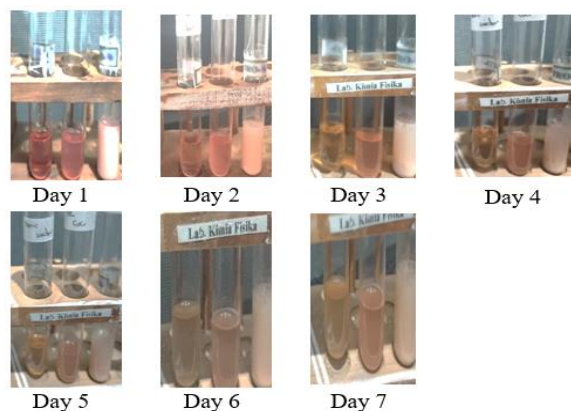


Figure 5. Anthocyanin stability in various beverages at room temperature for 7 days (from left to right: tonic water-clear milk tea-fermentation milk)

Analyzing anthocyanin stability under various storage conditions is also a crucial factor. Through stability analysis in different storage conditions, it can be determined how to store the applied anthocyanin extract to maintain its quality for an extended period.

Table 4 shows that all beverages to which anthocyanin extract was added began to change color on the third day at room temperature, as observed in Figure 5. This color change indicates anthocyanin degradation influenced by oxidation and exposure to light (heat). In addition to these factors, a high sugar concentration also accelerates anthocyanin degradation (Nasrullah et al., 2020). Therefore, if the

goal of storage is to maintain the stability and quality of anthocyanin over a long period, it is advisable not to store the anthocyanin extract at room temperature.

On the other hand, at refrigerator temperature (4°C), as seen in Table 4 and Figure 6, it is evident that the three beverages with added anthocyanin extract did not change color during the 7 days. This is because the low temperature in the refrigerator can slow down chemical reactions and enzymatic activities that could potentially degrade anthocyanins (Mossfika & Aziz, 2022). Therefore, refrigerator temperature provides a more stable storage environment, protecting anthocyanin extract from degradation.



Figure 6. Anthocyanin stability in various beverages at refrigerator temperature for 7 days (from left to right: tonic water-clear milk tea-fermentation milk)

3.5.3 Organoleptic testing

Organoleptic testing is a method used to evaluate the consumer acceptance of a product based on sensory characteristics (Gusnadi et al., 2021). In this study, organoleptic testing was conducted on three types of beverages: tonic water, clear milk tea, and fermented milk, to which anthocyanin extracts from red spinach had been added.

The organoleptic evaluation involved five teenage panelists, aged between 19 and 22 years, tasked with assessing and comparing taste parameters for each beverage. These beverages were evaluated both without the addition of anthocyanin extract and with the addition of various anthocyanin extract variations: a (M/S ratio of 1:10, 100 W), b (M/S ratio of 1:10, 300 W), c (M/S ratio of 1:10, 500 W), d (M/S ratio of 1:15, 100 W), e (M/S ratio of 1:15, 300 W), and f (M/S ratio of 1:15, 500 W). The panelists provided ratings using a scoring sheet with a scale ranging from 1 to 5, as shown in Table 5-Table 7. This scale was used to measure the panelists' level of preference for the taste of the beverages, with the following interpretations:

- 1 = total dissatisfaction (strong taste of red spinach);
- 2 = dissatisfaction (taste of red spinach was noticeable);
- 3 = neutral impression (the taste of red spinach was still slightly noticeable);
- 4 = satisfaction (the original taste of the beverage was still predominant);

- 5 = high satisfaction (the original taste of the beverage was very dominant).

Table 5. Results of organoleptic testing for tonic water

No.	Name	Extract Variations					Total	
		a	b	c	d	e		f
1	Hendy	5	4	4	4	3	3	23
2	Fortuna	4	3	3	3	3	4	20
3	Wan	5	4	4	3	4	4	24
4	Jhon	4	4	4	4	3	3	22
5	Ricky	3	3	3	3	3	3	18
Total		21	18	18	17	16	17	Y=107

Table 6. Results of organoleptic testing for clear milk tea

No.	Name	Extract Variations					Total	
		a	b	c	d	e		f
1	Hendy	3	3	4	3	4	3	20
2	Fortuna	3	4	4	3	4	4	22
3	Wan	4	3	3	4	3	3	20
4	Jhon	3	4	4	3	3	4	21
5	Ricky	4	4	4	4	4	4	24
Total		17	18	19	17	18	18	Y=107

Table 7. Results of organoleptic testing for fermented milk

No.	Name	Extract Variations					Total	
		a	b	c	d	e		f
1	Hendy	4	3	3	4	3	3	20
2	Fortuna	4	5	5	4	3	4	25
3	Wan	3	3	3	3	3	3	18
4	Jhon	3	4	4	3	3	3	20
5	Ricky	3	3	3	4	3	3	18
Total		17	18	18	17	15	16	Y=101

The conclusions drawn from the organoleptic testing were based on statistical analysis using the hedonic method, which was processed using Analysis of Variance (ANOVA). The results of the ANOVA analysis are presented in Tables 8, 9, and 10.

In ANOVA analysis, if the calculated F value is lower than the critical F value at a specific significance level (typically 5% or 1%), it can be concluded that there is no significant difference between the groups. The analysis of variance (ANOVA) results in this study indicate that the addition of six variations of anthocyanin extract to three types of beverages did not result in a significant difference in taste between the beverages with added anthocyanin extract and those without. Furthermore, the test results also show that adding anthocyanin extract from red spinach to the beverages imparts a light flavor and an attractive color. This conclusion suggests that the use of anthocyanin extract from red spinach can be a safe alternative to replace synthetic food colorants, which may be visually appealing but pose health risks.

Table 8. Analysis of variance for tonic water

VS	DF	SS	MS	Calc F	F Table		N S D
					5%	1%	
S	5	2,97	0,59	2,6	2,7	4,1	
P	4	3,87	0,97				
E	20	4,53	0,23				
Total	29	11,37					

Table 9. Analysis of variance for clear milk tea

VS	DF	SS	MS	Calc F	F Table		N S D
					5%	1%	
S	5	0,57	0,11	0,46	2,7	4,1	
P	4	1,87	0,47				
E	20	4,93	0,25				
Total	29	7,37					

Table 10. Analysis of variance for fermented milk

VS	DF	SS	MS	Calc F	F Table		N S D
					5%	1%	
S	5	1,37	0,27	1,32	2,7	4,1	
P	4	5,47	1,37				
E	20	4,13	0,21				
Total	29	10,97					

Notes:

S = sample

P = panelist

E = error

VS = variability source

DF = degree of freedom

SS = sum of squares

MS = mean square

Calc F = calculated F

NSD = not significantly different

4. CONCLUSION

Research findings reveal that the moisture content of the red spinach powder used in this study aligns with the standard moisture content of 7.6%. The highest yield was achieved at a microwave power of 100 W and an M/S ratio of 1:15, resulting 80% extraction yield. Additionally, the anthocyanin extract of superior quality, characterized by the highest anthocyanin content, was obtained at a microwave power of 300 W and M/S ratio of 1:15, yielding an anthocyanin content of 56.93 mg/L. Remarkably, anthocyanin added to various beverages exhibits resilience to heating at 40°C for up to 30 minutes and maintains stability during storage at a refrigerator temperature of 4°C for 7 days. Furthermore, applying anthocyanin from red spinach to beverages successfully provides a delicate flavor and is visually appealing.

5. REFERENCES

Adam, D. H. (2017). Determination of anthocyanins from red amaranth leaves (*Alternanthera amoena* Voss.) as well as their alkanine as a drink coloring. *Journal of Nuclear Learning and Biology*, 3(1), 10-16.

Baciang, J. N., & Inda, N. I. (2020). Extraction and stability test of natural dyes from red spinach

(*Alternanthera amoena* Voss). *Covalent: Journal of Chemical Research*, 6(3), 212-217.

Central Bureau of Statistics and Directorate General of Horticulture. (2021). *Vegetable production in Indonesia*. BPS North Sumatra. Terrain

Dinira, L., Rosyida, N., & Wulandari, E. R. N. (2021). Ultrasonic wave-assisted maceration method for the extraction of red-purple pigments from six varieties of red amaranth. *Journal of Natural Materials Engineering and Sustainable Energy*, 5(2), 10-15.

Farida, R., & Nisa, F. C. (2015). Anthocyanin extraction of mangosteen peel waste microwave assisted extraction method (extraction duration and ratio of material: solvent). *Journal of Food and Agroindustry*, 3(2), 362-373.

Gala, S., Kusuma, H. S., Sudrajat, R. G. M., Susanto, D. F., & Mahfud, M. (2016). Extraction of natural dyes from mahogany wood (*swietenia mahagoni*) using the microwave assisted extraction method. *Journal of Chemical Engineering*, 11(1), 7-13.

Gusnadi, D., Taufiq, R., & Baharta, E. (2021). Oranoleptic test and acceptability on cassava tapi-based mousse products as MSME commodities in Bandung Regency. *Journal of Research Innovation*, 1(12), 2883-2888.

Hasdar, M. (2021). Microwave assisted extraction (MAE) of sirampog black rice. *Journal of Food Processing*, 6(2), 49-53.

Hevira, L., Alwinda, D., & Hilaliyati, N. (2020). Analysis of rhodamine B dye on red crackers in Payakumbuh. *Chempublish Journal*, 5(1), 27-35.

Jumiati, E., & Mardhiana, I. M. A. (2017). Utilization of caramunting fruit as natural dyes foods. *Agrifor: Journal of Agricultural and Forestry Sciences*, 16(2):163-170.

Kaderides, K., Papaoikonomou, L., Serafim, M., & Goula, A. M. (2019). Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction. *Chemical Engineering and Processing Process Intensification*, 1(37), 1-11.

Mossfika, E., & Aziz, H. (2022). The process of coating red meranti wood with mangosteen peel dye on antifungal properties: the effect of temperature variations. *MSSB Journal: Medisains West Sumatra*, 3(1), 1-5.

Murningsih, K. (2019). The effect of adding red dragon fruit peel and citric acid on antioxidant activity, suspension stability and red dragon juice favorability. *Dissertation*. Mercu Buana University Yogyakarta.

Nasrullah, N., Husain, H., & Syahrir, M. (2020). The effect of temperature and heating time on the stability of anthocyanin pigments, citric acid extract, red dragon fruit peel (*Hylocereus polyrizus*) and application to foodstuffs. *Chemica*, 21(2), 150-162.

Nicoue, E. E., Savard, S., & Belkacemi, K. (2007). Anthocyanins in wild blueberries of quebec: extraction and identification. *Journal of*

Agricultural and Food Chemistry, 55(14), 5626-5635.

- Rifai, G., Widarta, I. W. R., & Nocianitri, K. A. (2018). The influence of the type of solvent and the ratio of ingredients to solvent on the content of phenolic compounds and antioxidant activity of avocado seed extract (*Persea Americana* Mill.). *ITEPA Journal*, 7(2), 22-32.
- Rosyidah, K. A., Rahmawati, R. P., & Fadel, M. N. (2021). The effect of red spinach (*Amaranthus Tricolor* L.) leaf ethanol extract concentration on the physical quality of face serum and antioxidant activity using 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method. In *The International Conference on Public Health Proceedings* (Vol. 6, No. 01, pp. 1132-1148).
- Sulistiawati, E., Swastika, P., & Qadaryah, L. (2017). Extraction of natural dyes from young teak leaves (*Tectona grandis*) and secang wood (*Caesalpinia sappan*) by ultrasound assisted extraction method for the application of textile products, muhail. Surabaya Institute of Technology, Surabaya.
- Sanchez-Bravo, P., Zapata, P. J., Martinez-Espla, A., Carbonell-Barrachina, A. A & Sendra, E. (2018). Antioxidant and anthocyanin content in fermented milks with sweet cherry is affected by the starter culture and the ripening stage of the cherry. *Beverages*, 4(3), 57.
- Surati, S. (2015). The danger of rhodamine B additives in food. *Biosel (Journal of Science and Education Research)*, 4(1), 22-28.
- Triesty, I., and Mahfud, M. (2017). Extraction of essential oil from agarwood using microwave hydrodistillation and soxhlet extraction methods. *ITS Engineering Journal*. 6(2):393-396.
- Wahyuni, D. T., & Widjanarko, S. B. (2015). Influence of solvent type and extraction duration on yellow pumpkin carotenoid extract by ultrasonic wave method. *Journal of Food and Agroindustry*, 3(2), 390-401.
- World Health Organization. (2023). Evaluation of certain contaminants in food: ninety-third report of the joint fao/who expert committee on food additives.
- Xue, H., Xu, H., Wang, X., Shen, L., Liu, H., Liu, C., & Li, Q. (2018). Effects of microwave power on kinetic extraction of anthocyanins from blueberry powder considering absorption of microwave energy. *Journal of Food Quality*, 2018, 1-13.