# **Evaluation of Microencapsulated Roselle Extract as a Food Colorant in the Red Nata de Coco Dessert Model System**

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#### History

Received: 2024-09-25
Revised: 2024-12-17
Accepted: 2024-12-19
Published Online: 2024-12-31

#### DOI:

https://doi.org/10.32508/stdj.v27i4.4365



#### ABSTRACT

Introduction: The incorporation of natural extracts into food products offers significant health benefits and promotes sustainable production methods. This study evaluated the incorporation of a microencapsulated roselle (Hibiscus sabdariffa) extract into nata de coco, a traditional Vietnamese dessert. Anthocyanins, a subgroup of polyphenols, are notable for their vibrant colors and health benefits. By transforming extract solutions into powder forms with maltodextrin and xanthan gum through spray-drying, their color is retained, and their bioavailability is improved, making them effective natural food colorants. **Methods:** The color dyeing ability of the microencapsulated roselle extract was determined on the Natta de coco by direct and indirect dyeing. The investigations on pH, total titratable acidity, anthocyanins, and DPPH were performed on components of the Natta de coco, which is the sugar syrup, and the Natta de coco. Changes in the chemical profiles of the dessert model during storage were also reported in this study. Discussion: This study demonstrated that the roselle-enhanced nata de coco exhibited high antioxidant activity and maintained anthocyanin stability. The optimal dyeing method produced samples with a pH ranging from 2.6 to 3.2, a titratable acidity ranging from 0.45% to 0.49%, and a total anthocyanin content ranging from 1.1 to 1.9 mg/L. The degree of antioxidant inhibition was approximately  $49.26 \pm 3.0\%$ . Stability studies of Roselle Nata de Coco (RDC) over four weeks revealed a consistent pH, titratable acidity, and antioxidant properties with minimal anthocyanin degradation. Conclusion: The encapsulated roselle powder enhances the color, nutritional value, and health benefits of nata de coco, demonstrating its potential as a stable natural food colorant. These findings suggest that the encapsulated roselle powder is a stable functional food ingredient with significant potential when combined with Natta de coco. These results highlight the stability and bioactive properties of the encapsulated extract in the food industry, especially for naturally derived and antioxidantsupported products.

**Key words:** Microencapsulated roselle, anthocyanins, antioxidant, food colorant, nata de coco

#### INTRODUCTION

In recent years, plant-based nutritional supplements and plant-based functional foods have gained significant popularity among individuals seeking to increase their health and well-being 1,2. Consequently, interest in incorporating pigments from extracts into food matrices to provide safety benefits and nutritional benefits is increasing 3-6. Extracts can enhance the nutritional value and health benefits of food products in various forms, including as a food colorant supplement 4. Anthocyanins, a subgroup of polyphenols, are among the most well-known natural substrates. Anthocyanins contribute to the vibrant colors observed in various fruits, vegetables, cereals, flowers, and other plants <sup>7,8</sup>. These compounds are found in the flowers, fruits, foliage, roots, tubers, stems, and bulbils of most angiosperms, as well as in some

gymnosperms, ferns, and bryophytes. The presence of anthocyanins can protect against certain cancers<sup>9</sup>, cardiovascular diseases 10, and other oxidative stressrelated disorders 11. Notably, anthocyanins exhibit significantly greater activity than do common antioxidants such as ascorbate 12. Previous studies have shown that factors such as pH, temperature, and the presence of light, oxygen, water, or metallic ions (e.g.,  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Ca^{2+}$ ,  $K^+$ ) significantly influence the stability of anthocyanins 13. In addition to their role in attracting pollinators and assisting in seed dispersal, dietary anthocyanins have been linked to being natural food colorants. However, integrating extracts into food products poses challenges, depending on the type of food product and requiring precise control over the extraction and cooking process, which can degrade the quality and applicability of the plant

Cite this article: Ngo M, Quan V M, Le C K, Ngoc H V, Vo T, Nguyen K T, Cao L T T, Tu T N, Doan H N, Nguyen T. Evaluation of Microencapsulated Roselle Extract as a Food Colorant in the Red Nata de Coco Dessert Model System. Sci. Tech. Dev. J. 2024; 27(4):3643-3653.

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extract in food products.

There is considerable interest in incorporating encapsulated anthocyanin powders into food products because of their potential benefits 14,15. Encapsulation powders are produced through encapsulation and spray-drying techniques, which transform the properties of extract solutions into powder forms <sup>16</sup>. These techniques protect plant extract components, such as polyphenols, anthocyanins, and flavonoids, from degradation, retain their vibrant colors, allow controlled release for improved bioavailability, mask undesirable flavors, and enable their use in a wide range of food applications 17. Various studies have explored the use of encapsulated extracts in products such as jelly candy 18, yogurt 19, beverages 3, and jam<sup>20</sup>. Encapsulated products not only preserve the core nutritional value of the extract but also offer a solution for quality control, as the powder form allows for better measurement and consistency. However, the effectiveness of extraction varies across different food models with varying water activities <sup>21,22</sup>. For high-water activity products such as beverages, juices, or desserts, microencapsulated powders focus on achieving uniform dissolution, maintaining nutritional content, and altering the color or flavor of the product.

In this study, the encapsulated product from roselle extract was evaluated as a potential food colorant in a high-water-activity food model system. The microencapsulated roselle powder was combined with nata de coco, a traditional Vietnamese dessert, to produce a red nata de coco. Roselle (Hibiscus sabdariffa) is known for its high anthocyanin content and is consumed in various forms, including herbal tea and juice 14,23. However, the use of roselle extract in solution poses challenges in maintaining stable quality. The microencapsulated powder produced through spray drying offers enhanced stability, allowing better control over its properties and increasing the potential of the roselle used in food products. Roselles, which are rich in antioxidants, can be incorporated into various foods. Recent research has indicated that spray-dried roselle powder can significantly preserve its antioxidant properties when added to gummies 24 and jam products 25 and can even prolong the shelf-life of refrigerated chicken nuggets <sup>26</sup>. Despite its promising potential, further research is needed to explore its specific application in foods such as natta de coco. By incorporating encapsulated roselle extract into nata de coco, its potential to improve bioactive properties, health benefits, and sensory attributes could be altered. This study evaluated the dyeing ability and physicochemical properties of red nata de coco, which was created by incorporating microencapsulated hibiscus calyx extract into traditional nata de coco. The preservation of roselle properties such as color stability and bioactive compounds in high-water-activity products such as nata de coco was thoroughly analyzed. Combining bioactive compounds from hibiscus with nata de coco is expected to produce a new food product with enhanced color, nutritional value, and health-promoting properties, potentially replacing commercial food colorants. This research could lead to the development of promising functional food products that promote healthier diets and lifestyles.

#### **MATERIALS & METHODS**

# **Materials**

Dry roselle (hibiscus) was purchased from Hichagol Co., Ltd., Hue, Vietnam. Acetic acid, citric acid, and sodium hydroxide were purchased from Xilong Chemical Co. Ltd. Potassium chloride and sodium acetate anhydrous were purchased from Guangdong Guanghua Sei-Tech Co. Ltd. Maltodextrin (DE: 10–12%) was received from Qinhuangdao Lihua Starch Co. Ltd. DPPH (2,2-diphenyl-1-picrylhydrazyl) and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich.

#### Roselle extraction

The dry roselle flower was ground into a fine powder with a particle size of approximately 0.5 cm. An acetic acid 2% extraction solvent was prepared for the extraction process. Roselle powder was immersed in the solvent at a ratio of 4 g powder to 100 mL extraction solvent, and the mixture was incubated at 4  $^{\circ}$ C. After 24  $\pm$  2 h, the extraction mixture was collected, and the remaining solid residue was subjected to a second extraction under the same conditions. The final roselle extract was stored at 4  $^{\circ}$ C for subsequent analysis.

#### Preparation of spray-dried roselle powder

Before microencapsulation, each encapsulating agent was dispersed into the roselle extracted solution and mixed until fully homogenized. The encapsulation concentration was 10% w/v. The components of the wall materials used were maltodextrin and xanthan gum at a 99:1 w/w ratio. The SD-1000 Eyela (EYELA, Japan) was used to spray the encapsulating mixture. The homogenized mixture was fed into the spray dryer, with the inlet temperature set at 160  $^{\circ}$ C and the outlet temperature at 80  $^{\circ}$ C. The solution was fed into the dryer with a spray air flow rate of 0.7 m<sup>3</sup>/min and a

pressure drop of  $12 \times 10$  kPa. To maintain the homogeneity of the solution, gentle mixing was performed during the pumping process. After spray drying, the powders were weighed, stored in airtight plastic bags, and stored at -20 °C until further evaluation.

# Scanning electron microscopy of the spraydried roselle powder

The microstructure of the powders was examined by scanning electron microscopy (SEM). The size and shape of the encapsulated roselle powder particles were determined through image analysis with ImageJ software.

#### Nata de coco preparation

Dry-pressed raw coconut nata de coco, which was 1.5 cm  $\times$  1.5 cm in size, was boiled in water containing 2% w/v acetic acid and 0.5% w/v iodized salt at 90–95 °C to remove contaminants and residual bacteria. After 40–60 min of boiling, the nata de coco was immersed in water for more than 2 h, and the water was changed to remove the acetic acid. After 4–5 cycles of immersion in deionized water, the nata de coco was drained and squeezed to prepare for cooking red nata de coco.

#### Preparation of Roselle Nata de Coco (RDC)

Microencapsulated roselle powder was used to color nata de coco via two distinct methods: staining via sugar syrup (Method 1 – RDC-1) and staining via direct contact with the nata de coco (Method 2 – RDC-2). The sugar mixture used in both methods contained 26% white sugar (Table 1). Following the staining process, RDC underwent hot packaging and was kept in a refrigerator until further characterization. The processing scheme of the two staining methods is presented in Figure 1. White nata de coco (nonstaining sample) and commercial colorant (amaranth) samples were prepared under the same conditions for comparison.

# Physicochemical profile of RDC pH determination and titratable acidity

The titratable acidity was determined following the instructions of Tyl and Sadler, 2009, in the Food Analysis book <sup>27</sup>. Five milliliters of RDC was added to 45 mL of distilled water. The pH of the solution was measured. The samples were titrated by adding 0.1 N NaOH to the mixture in the presence of phenolphthalein until the pH reached 7.0. The titratable acidity of RDC was calculated via the following equation:

% titratable acidity (wt/wt) =  $N \times V \times Eq.wt$ .  $W \times 1000 \times 100$ 

where: N = normality of the titrant, usually NaOH (mEq/mL)

V = volume of titrant (mL)

Eq.wt. = equivalent weight of predominant acid (mg/mEq)

W = mass of sample (g)

1000 = factor relating mg to grams (mg/g) (1/10 = 100/1000)

#### Total anthocyanin content

The anthocyanin content was determined according to the procedure of Lee et al. (2005), with slight modifications  $^{28}$ . Two different buffers were prepared, including a pH 1.0 buffer (potassium chloride, 0.025 M) and a pH 4.5 buffer (sodium acetate, 0.4 M). The sample was mixed with each buffer at a ratio of 1:4. The absorbance of each of these solutions was measured at 520 nm and 700 nm via a microplate reader and a UV spectrophotometer. The absorption difference of the attenuated sample was computed according to the following equation:

 $A = (A_{520} - A_{700})pH 1.0 - (A_{520} - A_{700})pH 4.5$ 

The total anthocyanin concentration was expressed as cyanidin-3-glucoside equivalents (% w/w) via the following formula:

Anthocyanin [mg/L] =  $A \times MW \times DF \times 1000\varepsilon \times 1$ 

where A = absorbance MW = molecular weight (449.2 g/mol)

DF = dilution factor

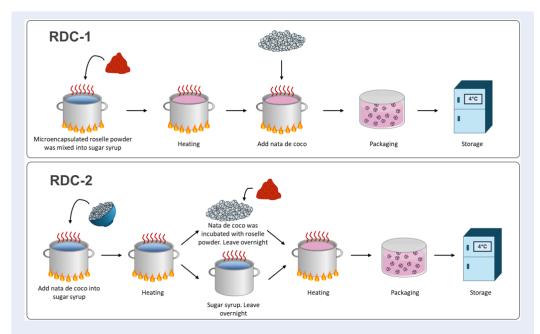
 $\varepsilon$  = extinction molar coefficient (26,900 L/cm.mol)

# Antioxidant assay – DPPH assay

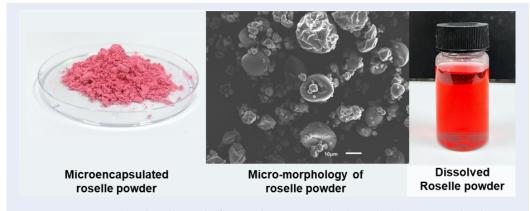
DPPH was prepared at 0.1 mM in a DMSO solution. The RDC in each cooking method, nata de coco without spray-dried roselle extract as a control, spraydried roselle extract in distilled water, and DMSO as a blank were sampled. Different concentrations of samples were diluted in DMSO. A total of 180  $\mu$ L of 0.1 mM DPPH solution was incubated with a 20  $\mu$ L sample for 30 minutes in the dark. A series of ascorbic acid (VitC) dilutions in DMSO at concentrations ranging from 0–200  $\mu$ g/mL were also prepared and measured at the same time. The decrease in the absorbance of DPPH was measured at 517 nm via a microplate reader. The proportion of the scavenging effect of DPPH was calculated via the following equation:

DPPH scavenging effect (%) =  $[(A_0-A_1)/A_0] \times 100$ where A0 = the absorbance of the control.

A1 =the absorbance of the sample



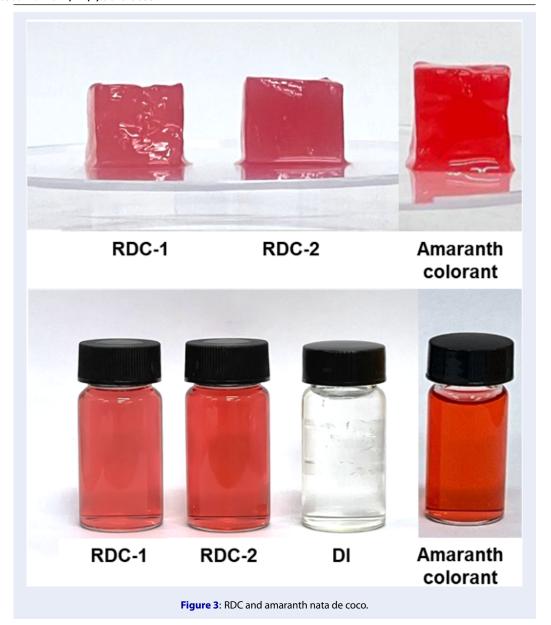
**Figure 1**: Scheme process of RDC preparation with microencapsulated roselle powder via two different dyeing approaches.



**Figure 2**: Microencapsulated roselle powder from xanthan gum mixed with a maltodextrin-encapsulated carrier.

Table 1: Ingredients of RDC

INGREDIENTS	RDC	White nata de coco	Amaranth nata de coco
Sugar concentration (w/w):	26%	26%	26%
Sugar (°Brix):	21 °Brix	21 °Brix	21 °Brix
Nata de coco: syrup ratio:	60:40	60:40	60:40
Roselle concentration (w/w):	3%	-	-
Commercial colorant (w/w)	-	-	0.5



# Storage stability

RDC was prepared and packaged in 150 mL plastic sealed bags. For storage ability analysis, RDC samples were stored for 4 weeks in a refrigerator (4  $^{\circ}$ C) without light. For quantitative analysis, the pH, titratable acidity, total anthocyanin content, and antioxidant activity of the samples were determined via the DPPH method to evaluate the changes in the degradation of the sample characteristics each week.

# Statistical analysis

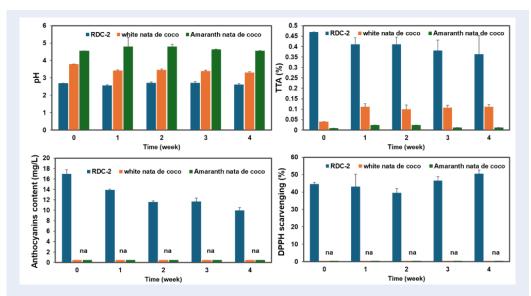
All the results are presented as the means  $\pm$  standard deviations. Statistical analysis was conducted via one-

way ANOVA for all tests, followed by Tukey's post hoc analysis to determine significant differences between groups. A p value of less than 0.05 was considered statistically significant.

# **RESULTS**

# Microencapsulated roselle powder

The spray-dried hibiscus extract powder exhibited light pink coloration, combining the characteristic red hue from the hibiscus with the white tone of the encapsulated carrier. The powder was a fine, particulate powder comprising small, granular particles. Initially, upon testing the solubility of the powder, the



**Figure 4**: Physiochemical properties of RDC, white nata de coco, and amaranth nata de coco during four weeks of storage in a refrigerator.

Table 2: Physiochemical profile of nata de coco produced under different treatments

		•			
	рН	TTA (%)	Anthocyanin content (mg/L)	DPPH scavenging (%)	μgVitC/mL
Sugar syrup					
RDC-1	$2.6 \pm 0.0$	$0.49 \pm 0.00$	$1.17\pm0.08^{\it c}$	$41.00\pm2.37^b$	$115.67 \pm 6.78^b$
RDC-2	$2.7 \pm 0.0$	$\textbf{0.47} \pm \textbf{0.00}$	$1.70\pm0.11^b$	$44.57\pm0.97^b$	$126.01 \pm 2.78^b$
White nata de coco	$3.8 \pm 0.0$	$\textbf{0.04} \pm \textbf{0.00}$	-	-	-
Roselle powder in DI	$2.9 \pm 0.1$	$\textbf{0.47} \pm \textbf{0.00}$	$2.26\pm0.00^a$	$57.45 \pm 1.80^a$	$162.86 \pm 5.16^a$
Amaranth nata de coco	$4.56 \pm 0.0$	$\pm0.00$	-	-	-
Nata de coco					
RDC-1	$3.2 \pm 0.5$	$0.45 \pm 0.02$	$1.15\pm0.09^{c}$	$42.85 \pm 4.8^{\textit{c}}$	$115.49 \pm 9.62^{c}$
RDC-2	$2.7 \pm 0.0$	$\textbf{0.47} \pm \textbf{0.01}$	$1.92\pm0.07^b$	$49.26\pm3.0b^{\it c}$	$143.27 \pm 4.88^b$
White nata de coco	$3.9 \pm 0.0$		-	-	-
Roselle powder in DI	$2.9 \pm 0.1$	$\textbf{0.47} \pm \textbf{0.00}$	$2.26\pm0.00^a$	$57.45 \pm 1.80^a$	$162.86 \pm 5.16^a$
Amaranth nata de coco	$4.3 \pm 0.1$	$0.01 \pm 0.00$	-	-	-

<sup>\*</sup>Different letters represent significantly different data

microencapsulated powder exhibited 70% solubility. The anthocyanin content and antioxidant properties determined via DPPH were 25.07 mg/mL. According to the SEM results, the microencapsulated roselle powder had a circular morphology with rough and wrinkled surfaces. The microparticles were approximately 15  $\mu \rm m$  in size. This morphology indicated successful microencapsulation using maltodextrin, xanthan gum, and hibiscus extract. Figure 2 presents the characteristics of the roselle encapsulated powder.

#### **Red RDC**

To produce red-colored nata de coco, two staining approaches were tested to evaluate the effectiveness of microencapsulated roselle powder as a food coloring agent. The powder was stained indirectly by mixing with syrup via one method and directly with nata de coco via the other method. Both techniques resulted in noticeable color heterogeneity in the nata de coco. Method 2, where the powder was added to the nata de coco, produced a slightly darker hue than did Method 1, where the powder was added to the syrup. This color difference is attributed to the timing of the addition of the spray-dried roselle extract powder during the cooking process. The morphology of the red-colored nata de coco (RDC) produced by both methods is shown in Figure 3.

The physiochemical properties of the syrup and nata de coco are presented in Table 2. To assess the staining efficiency of the encapsulated roselle powder in RDC, the physio-chemical profiles of RDC, microencapsulated roselle powder in deionized water (DI), and a blank sample without a roselle were analyzed. Table 2 presents the differences in properties under various treatments. In the sugar mixture, the pH of the RDC produced via both methods was approximately 2.6. However, in nata de coco, the pH of Method 2 (2.7) was lower than that of Method 1 (3.2). This may be due to the complexity of the cellulose matrix of the nata de coco, which does not easily enable the extract to be absorbed. This finding indicated that directly introducing roselle powder into drained nata de coco enhances the absorption of the extract. Conversely, the titratable acidity between Method 1 and Method 2 (0.45-0.49%) showed minimal differences.

The anthocyanin content and antioxidant activity are crucial parameters for evaluating the functionality of roselle powder in nata de coco. Both metrics were higher in the samples from Method 2 (RDC-2) than in those from Method 1 (RDC-1). Specifically, RDC-2 had anthocyanin concentrations of 1.7 mg/L in syrup and 1.93 mg/L in nata de coco, whereas the RDC-1

samples contained approximately 1.15 to 1.17 mg/L. This trend was also observed for the antioxidant activity, suggesting that adding roselle to drained nata de coco and incubating overnight enhances the incorporation and diffusion into both nata de coco and sugar syrup. Compared with the sample of roselle powder dissolved in DI water, the RDC samples had minimal effects on the cooking process. Moreover, no anthocyanins or antioxidant activities were detected in samples colored with a commercial amaranth colorant, further highlighting the functional benefits of encapsulated roselle powder.

In the stability investigation, the physicochemical properties of RDC stored at  $6\pm2\,^{\circ}\mathrm{C}$  for four weeks were evaluated to monitor potential changes over time. The pH of the products did not change over four weeks, with the pH ranging from 2.5 to 4.0 under both storage conditions. Similarly, the titratable acidity (TTA) exhibited minimal variation. However, as shown in Figure 4, the anthocyanin content in each sample decreased considerably by approximately 10–20% compared with that in the previous week. Despite the decrease in anthocyanin content, Figure 4 shows that the antioxidant properties of each sample remained approximately the same. These findings suggest that antioxidant activity is not solely dependent on anthocyanin levels.

# **DISCUSSION**

Plant pigments have long been used as natural food colorants in the culinary industry. When applied to nata de coco, microencapsulated roselle powder can increase the demand for roselle, potentially increasing production and benefiting the economies of the roselle- and nata de coco-producing regions. Producers can achieve greater profits by incorporating spray-dried roselle into their products. The beneficial use of roselle in nata de coco reduces the reliance on synthetic food colorants. Consumers benefit from the added nutritional value and reduced risk of allergic reactions or problems caused by commercial colorants. This natural alternative to synthetic colorants also provides antioxidants and antiinflammatory compounds, enhancing the health benefits of the product. While it may not serve as a treatment for specific conditions, it offers a healthful alternative to synthetic food colorants, positively impacting consumer health.

Encapsulation involves surrounding a central material with a wall material to prevent chemical and environmental interactions<sup>29</sup>. This method addresses the instability of substances by inhibiting volatilization and protecting against adverse conditions<sup>30</sup>. In

this study, roselle powder was encapsulated with maltodextrin and xanthan gum. The concentration of xanthan gum is crucial for maintaining the antioxidant and color properties of roselle extracts. The characteristics of the encapsulated powder are shown in Figure 2. The combination of plant extracts with maltodextrin and xanthan gum effectively provided color and antioxidant properties. Thus, microencapsulated granules under these conditions are a viable option for developing functional foods.

In combining and developing nata de coco products by using roselle encapsulated powder, understanding the characteristics of nata de coco is essential. Cellulose, a natural polymer composed of carbohydrate polysaccharides, is obtained via fermentation and adheres to the cellulose fibers produced by Acetobacter xylinum. Physical characteristics can affect food quality and consumer acceptability. Nata de coco has high aesthetic value because of its pure white hue, dense consistency, and fresh scent 31. To produce bacterial cellulose for nata de coco, the product is thoroughly rinsed to remove the sour taste and then boiled to eliminate the acidic flavor and eliminate any microorganisms. After purification, the bacterial cellulose is cooked with sugar and other ingredients to create a dessert known as nata de coco. In this study, microencapsulated roselle powder was used as a colorant to introduce a safe, aesthetically appealing, and antioxidant-rich nata de coco. Given that water activity is crucial in regulating pigment degradation during storage, this study aims to understand the protective effect of encapsulation on roselle extract in a highwater-activity food model such as nata de coco.

The ability of the microencapsulated powder as a colorant for nata de coco was assessed via two different methods. In Method 1, roselle powder was added during the initial cooking phase when the nata de coco remained porous and could absorb liquid. The structural matrix of the gel likely absorbed some phytochemicals, resulting in less color in the residual syrup. In contrast, in Method 2, roselle was added after cooking the nata de coco, leaving more extract particles suspended in the sugar syrup rather than absorbed into the nata de coco fibers. The dispersed pigments in method 2 imparted a more intense red coloration to the solution. The results indicated that encapsulated roselle powders exhibited their primary ability to stain the subject directly or indirectly after being dissolved in a solution.

Titratable acidity and pH offer complementary insights into food quality, with titratable acidity more accurately reflecting the impact of organic acids on flavor. Food acids only partially ionize, affecting some

properties on the basis of the ionized fraction and others on the total acid content. pH determination is crucial for developing high-quality products with optimal phytochemical characteristics<sup>32</sup>. According to Paramee Chumsri et al., the optimal pH of dried roselle calyxes in beverages ranges from 2.8 to  $2.9^{33}$ . Similarly, the pH of RDC in this study was approximately 2.6-3.2, making the product sour due to the acidity of the encapsulated roselle powder. The acidity level of spray-dried RDC is approximately 0.4%. The antioxidant properties were approximately 50% in both samples. These findings indicate that spraydried roselle powder is appropriate for use in food and beverages. Using spray-dried roselle in nata de coco can reduce the need for synthetic food colorants, offering a natural and healthy alternative.

The total anthocyanin content serves as an indicator of both the color and potential health benefits provided by the anthocyanins in roselle extract. The results revealed that Method 2 had a significantly greater anthocyanin content (1.7-1.93 mg/L) than Method 1 did (1.15-1.17 mg/L). In Method 1 (RDC-1), adding the spray-dried extract while cooking with sugar syrup allowed more roselle to remain suspended in the residual syrup rather than being absorbed into the nata de coco. Conversely, in Method 2 (RDC-2), the roselle powder was added during the cooking of nata de coco, which has significant water-holding capacity, enabling a portion of the anthocyanins to be absorbed into the cellulose matrix of the nata de coco. This is visually evident, as the color of the sugar syrup in Method 2 is slightly less red than that in Method 1, but the nata de coco in Method 2 is redder. The two methods assess a sample's antioxidant activities to determine the anthocyanin antioxidant outcome following nata de coco production. This disparity likely arises from differences in processing conditions. In RDC-1, the nata de coco exposed the extract to potentially longer temperature effects, decreasing anthocyanin stability and activity. However, in RDC-2, the addition of roselle extract after cooling prevented exposure to sudden temperature changes during prolonged cooking. As described in the section on total anthocyanin content, the heating procedure affects the stability of anthocyanins 22,34,35, leading to a decrease in their antioxidant activity in nata de coco. Furthermore, anthocyanins degrade in response to various factors, including pH, temperature, intermolecular pigmentation, ascorbic acid, oxygen, etc. 36. The cleavage of the intramolecular bonds of anthocyanins, resulting in colorless, smaller molecules, is more active in the

extracts than in the cells because of the high temperature. For example, coumarin glycoside has been identified as a common byproduct of the thermal degradation of cyanidin 3,5-diclycosides over time <sup>37</sup>. Specifically, at 37 °C, delphinidin 3-sambubioside and cyanidin 3-sambubioside in anthocyanins lead to the formation of colorless molecules such as gallic acid and protocatechuic acid <sup>38</sup>. This explains why the anthocyanin content in RDC-1 was lower than that in RDC-2, and the color was also brighter red in RDC-1 than in RDC-2. The effects of temperature on anthocyanin properties were also observed when the anthocyanin contents produced in treatments 1 and 2 were compared with those of the same amount of roselle powder mixed with DI. After processing, the medicinal properties were reduced by approximately 30% compared with those of the original product.

In the stability study, the physicochemical properties of the RDC samples remained stable over four weeks, except for the anthocyanin content. The stability of anthocyanins in roselle powder is influenced by various factors, such as heat and pH, particularly in lowacid environments where anthocyanins degrade more rapidly. However, the study revealed that the anthocyanins in the roselle powder presented high color stability and heat resistance under acidic conditions. These findings suggest that the quality and appearance of roselle powder can be preserved by adjusting the pH and temperature during processing. Furthermore, the roselle extract used in this study was rich in anthocyanins and exhibited strong antioxidant properties. Despite a decrease in anthocyanin levels over time, the antioxidant activity remained stable, further supporting the potential of roselle powder as a food ingredient with health benefits. Some studies have indicated that roselle anthocyanins are relatively stable at high temperatures, particularly when the heating duration is short  $(30-45 \text{ min})^{39,40}$ . The preservation of roselle properties in a high-water-activity food model such as nata de coco demonstrates the potential of roselleencapsulated powder as a natural colorant. Therefore, further research is warranted to determine the optimal conditions for encapsulating roselle anthocyanins during the heating process. Importantly, this study assessed the stability of anthocyanins in roselle powder under specific conditions, and the results may not be applicable to other anthocyanin-containing products or processing methods. Additional research is needed to explore the stability of anthocyanins in various food and beverage products and their potential applications.

#### **CONCLUSIONS**

In conclusion, this study successfully developed a nata de coco product using encapsulated roselle powder as a natural food colorant. The results demonstrated high antioxidant activity and maintained the stability of the anthocyanins in the RDC product. The optimal method involved incubating roselle in drained nata de coco, producing samples with pH values ranging from 2.6-3.2, titratable acidities between 0.45% and 0.49%, and total anthocyanin contents varying from 1.1-1.9 mg/L. The degree of antioxidant inhibition ranged from 40-50%. Stability studies confirmed that the pH, titratable acidity, and antioxidant properties of the samples remained consistent over four weeks, despite some anthocyanin degradation. This research explored the potential of a natural food colorant to create an innovative product, offering valuable insights for future research and development. By providing a low-cost and effective natural alternative to synthetic colorants, this approach may positively impact consumer health. These findings suggest that the use of microencapsulated RDC powder can lead to the production of stable functional foods with significant potential. Future research should prioritize the assessment of RDC stability under various storage conditions and conduct broader sensory evaluations of consumer preferences. Although roselle powder provides antioxidants and anti-inflammatory compounds, potentially improving health benefits for traditional nata de coco, further research is needed to fully understand the health benefits of spray-dried roselle. Once the functional food's suitability and health benefits are confirmed, further in vivo and in vitro studies will be conducted to evaluate its efficacy. These investigations provide crucial insights into the potential culinary and health applications of RDC.

# **LIST OF ABBREVIATIONS USED**

DPPH: 2,2-diphenyl-1-picrylhydrazyl DMSO: Dimethyl sulfoxide RDC: Roselle Nata de Coco TTA: titratable acidity

# COMPETING INTERESTS

The authors declare that they have no competing interests.

# **ACKNOWLEDGMENT**

The authors gratefully acknowledge the financial support from the Department of Science & Technology and the People's Committee of Ben Tre Province, Vietnam, under contract 271/HD-SKHCN. We also thank all the members of the TERM laboratory for useful discussions and support.

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# **REFERENCES**

- Day L, Seymour RB, Pitts KF, Konczak I, Lundin L. Incorporation of functional ingredients into foods. Trends Food Sci Technol. 2009 Sep 1;20(9):388–95;Available from: www.doi.org/10. 1016/j.tifs.2009.04.003.
- Oliveira A, Amaro A, Pintado M. Impact of food matrix components on nutritional and functional properties of fruit-based products. Curr Opin Food Sci. 2018 Apr 1;22;Available from: www.doi.org/10.1016/j.cofs.2018.01.007.
- 3. Duangmal K, Saicheua B, Sueeprasan S. Roselle anthocyanins as a natural food colorant and improvement of its color stability. Proceedings. 2004 Jan 1;.
- Fernández-López JA, Fernández-Lledó V, Angosto JM. New insights into red plant pigments: more than just natural colorants. RSC Adv. 10(41):24669–82; Available from: www.doi.org/10.1039/C9RA10911H.
- Leong H, Show PL, Lim M, Chien Wei O, Ling T. Natural red pigments from plants and their health benefits – A review. Food Rev Int. 2017 May 10;34;Available from: www.doi.org/10.1080/ 87559129.2016.1225824.
- Wiczkowski W, Szawara-Nowak D, Topolska J. Red cabbage anthocyanins: Profile, isolation, identification, and antioxidant activity. Food Res Int. 2013 Apr 1;51:303–9;Available from: www.doi.org/10.1016/j.foodres.2013.01.020.
- Abou-Arab A, Abu-Salem F, Abou-Arab EA. Physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (Hibiscus sabdariffa). Proceedings. 2011;Available from: www.semanticscholar.org/paper/Physico-chemicalproperties-of-natural-pigments-Abou-Arab-Abu-Salem/ 640f12530704acb17e43beaf27c17235114ab392.
- Rodriguez-Amaya DB. Update on natural food pigments -A mini-review on carotenoids, anthocyanins, and betalains. Food Res Int. 2019 Oct;124:200–5;Available from: www.doi. org/10.1016/j.foodres.2019.01.010.
- Liu Y, Fernandes I, Mateus N, Oliveira H, Han F. The role of anthocyanins in alleviating intestinal diseases: A mini-review.
   J Agric Food Chem. 2024 Mar 20;72(11):5491–502;Available from: www.doi.org/10.1021/acs.jafc.4c00000.
- Festa J, Da Boit M, Hussain A, Singh H. Potential benefits of berry anthocyanins on vascular function. Mol Nutr Food Res. 2021;65(19):2100170;Available from: www.doi.org/10.1002/ mnfr.202100170.

- Alam MdA, Islam P, Subhan N, Rahman MdM, Khan F, Burrows GE, et al. Potential health benefits of anthocyanins in oxidative stress-related disorders. Phytochem Rev. 2021 Aug 1;20(4):705–49;Available from: www.doi.org/10.1007/s11101-021-09739-2.
- Garcia C, Blesso CN. Antioxidant properties of anthocyanins and their mechanism of action in atherosclerosis. Free Radic Biol Med. 2021 Aug 20;172:152–66;Available from: www.doi. org/10.1016/j.freeradbiomed.2021.07.021.
- Oancea S. A review of the current knowledge of thermal stability of anthocyanins and approaches to their stabilization to heat. Antioxidants. 2021 Aug 24;10(9):1337;Available from: www.doi.org/10.3390/antiox10091337.
- Cid-Ortega S, Guerrero-Beltran J. Roselle calyces (Hibiscus sabdariffa), an alternative to the food and beverages industries: A review. J Food Sci Technol. 2015 Apr 21;52;Available from: www.doi.org/10.1007/s13197-013-1086-4.
- Ozdal T, Yolci Omeroglu P, Tamer C. Role of encapsulation in functional beverages. In 2020. p. 195–232;.
- Halahlah A, Piironen V, Mikkonen KS, Ho TM. Polysaccharides as wall materials in spray-dried microencapsulation of bioactive compounds: Physicochemical properties and characterization. Crit Rev Food Sci Nutr. 2023;63(24):6983

  7015;Available from: www.doi.org/10.1080/10408398.2022.
- Grgić J, Šelo G, Planinić M, Tišma M, Bucić-Kojić A. Role of the encapsulation in bioavailability of phenolic compounds. Antioxidants. 2020 Sep 26;9(10):923;Available from: www.doi. org/10.3390/antiox9100923.
- Moura SCSR, Berling CL, Garcia AO, Queiroz MB, Alvim ID, Hubinger MD. Release of anthocyanins from the hibiscus extract encapsulated by ionic gelation and application of microparticles in jelly candy. Food Res Int. 2019 Jul;121:542–52;Available from: www.doi.org/10.1016/j.foodres.2018.11.035.
- Moura S, Schettini G, Garcia A, Gallina D, Alvim I, Hubinger M. Stability of hibiscus extract encapsulated by ionic gelation incorporated in yogurt. Food Bioprocess Technol. 2019 Sep 1;12;Available from: www.doi.org/10.1007/s11947-019-02321-3
- Ashaye O, Adeleke TO. Quality attributes of stored roselle jam. Int Food Res J. 2009 Jan 1;16:363–71;.
- Garzón G, Wrolstad R. The stability of pelargonidin-based anthocyanins at varying water activity. Food Chem. 2001 Nov 1;75:185–96;Available from: www.doi.org/10.1016/S0308-8146(01)00189-6.
- Jiménez N, Dornier M, Bonazzi C, Pérez A, Vaillant F. Effect of water activity on anthocyanin degradation and browning kinetics at high temperatures (100–140 °C). Food Res Int. 2012 Jun 1;47:106–15;Available from: www.doi.org/10.1016/j. foodres.2011.04.020.
- Selim K, Khalil KE, Abdel-Bary MS, Abdel-Azeim NA. Extraction, encapsulation, and utilization of red pigments from Roselle (Hibiscus sabdariffa L.) as natural food colorants. Alex J Food Sci Technol. 2008 Jan 1;7–20;.
- Younesi M, Peighambardoust SH, Sarabandi K, Akbarmehr A, Ahaninjan M, Soltanzadeh M. Application of structurally modified WPC in combination with maltodextrin for microencapsulation of Roselle (Hibiscus sabdariffa) extract as a natural colorant source for gummy candy. Int J Biol Macromol. 2023 Jul 1;242:124903;Available from: www.doi.org/10.1016/j. ijbiomac.2023.124903.
- Department of Food Technology, University of Ibadan, Ibadan, Nigeria, Arueya GL. Stability studies of microencapsulated anthocyanins of Roselle (Hibiscus sabdariffa L.) in native starch and its potential application in jam production. IOSR J Environ Sci Toxicol Food Technol. 2014;8(7):112–22;.
- Bahrami Feridoni S, Khademi Shurmasti D. Effect of the nanoencapsulated sour tea (Hibiscus sabdariffa L.) extract with carboxymethylcellulose on quality and shelf life of chicken nugget. Food Sci Nutr. 2020 Jul;8(7):3704–15;Available from: www.doi.org/10.1002/fsn3.1658.

- 27. Sadler G, Murphy P. PH and titratable acidity. In: Food Analysis. 2010. p. 219–38:.
- Lee J, Durst R, Wrolstad R. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. J AOAC Int. 2005 Feb 20;88:1269–78;.
- Marcillo V, Tupuna Yerovi D, González Z, Ruales J. Encapsulation of bioactive compounds from fruit and vegetable byproducts for food application – A review. Trends Food Sci Technol. 2021 Jul 1;116;Available from: www.doi.org/10.1016/ i.tifs.2021.06.004.
- Díaz-Torres R, Alonso-Castro A, Carrillo M, Carranza Alvarez C. Bioactive compounds obtained from plants, their pharmacological applications and encapsulation. In 2021. p. 181–205;
- Herawati H, Kamsiati E, Widyaputri S, Sutanto. Physic-chemical characteristic of nata de coco. IOP Conf Ser Earth Environ Sci. 2020 Apr 4;458:012014;Available from: www.doi.org/10.1088/ 1755-1315/458/1/012014.
- Andrés-Bello A, Barreto-Palacios V, García-Segovia P, Mir-Bel J, Martínez-Monzó J. Effect of pH on color and texture of food products. Food Eng Rev. 2013 Sep 1;5(3):158–70;Available from: www.doi.org/10.1007/s12393-013-9050-9.
- Chumsri P, Anchalee S, Itharat A. Studies on the optimum conditions for the extraction and concentration of roselle (Hibiscus sabdariffa Linn.) extract. Songklanakarin J Sci Technol. 2008 Apr 1;30:133–9;.
- Garofulić I, Zorić Z, Sandra P, Verica DU. Retention of polyphenols in encapsulated sour cherry juice in dependence of drying temperature and wall material. LWT - Food Sci Tech-

- nol. 2017 May 1;83;Available from: www.doi.org/10.1016/j.lwt. 2017.05.036.
- Wu HY, Yang KM, Chiang PY. Roselle anthocyanins: Antioxidant properties and stability to heat and pH. Mol Basel Switz.
   2018 Jun 5;23(6):1357;Available from: www.doi.org/10.3390/molecules23061357.
- Oancea S. A review of the current knowledge of thermal stability of anthocyanins and approaches to their stabilization to heat. Antioxidants. 2021 Aug 24;10:1337;Available from: www.doi.org/10.3390/antiox10091337.
- Hendry GAF, Houghton JD. Natural Food Colorants. G. A. F. Hendry (Auth.), G. A. F. Hendry, J. D. Houghton; Available from: https://www.scribd.com/document/522204562/Naturalfood-colorants-G-A-F-Hendry-auth-G-A-F-Hendry-J-D-Houghton.
- Sinela A, Rawat N, Mertz C, Achir N, Fulcrand H, Dornier M. Anthocyanins degradation during storage of Hibiscus sabdariffa extract and evolution of its degradation products. Food Chem. 2017 Jan 1;214:234–41;Available from: www.doi.org/10.1016/j.foodchem.2016.07.119.
- Dyrby M, Westergaard N, Stapelfeldt H. Light and heat sensitivity of red cabbage extract in soft drink model systems. Food Chem. 2001 Mar 1;72(4):431–7;Available from: www.doi.org/ 10.1016/S0308-8146(00)00319-4.
- Kırca A, Cemeroğlu B. Degradation kinetics of anthocyanins in blood orange juice and concentrate. Food Chem. 2003;4(81):583–7;Available from: www.doi.org/10.1016/S0308-8146(02)00469-6.