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

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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

2 In recent years, plant-based nutritional supplements 3 and plant-based functional foods have gained signifi-4 cant popularity among individuals seeking to increase ⁵ their health and well-being^{1,2}. Consequently, inter-6 est in incorporating pigments from extracts into food 7 matrices to provide safety benefits and nutritional ⁸ benefits is increasing³⁻⁶. Extracts can enhance the 9 nutritional value and health benefits of food prod-10 ucts in various forms, including as a food colorant ¹¹ supplement⁴. Anthocyanins, a subgroup of polyphe-12 nols, are among the most well-known natural substrates. Anthocyanins contribute to the vibrant colors 13 observed in various fruits, vegetables, cereals, flow-¹⁵ ers, and other plants ^{7,8}. These compounds are found 16 in the flowers, fruits, foliage, roots, tubers, stems, 17 and bulbils of most angiosperms, as well as in some 18 gymnosperms, ferns, and bryophytes. The presence

of anthocyanins can protect against certain cancers⁹, ¹⁹ cardiovascular diseases¹⁰, and other oxidative stress- 20 related disorders¹¹. Notably, anthocyanins exhibit ²¹ significantly greater activity than do common antiox- 22 idants such as ascorbate¹². Previous studies have 23 shown that factors such as pH, temperature, and the 24 presence of light, oxygen, water, or metallic ions (e.g., 25 Fe^{2+} , Fe^{3+} , Ca^{2+} , K^+) significantly influence the stability of anthocyanins¹³. In addition to their role in 27 attracting pollinators and assisting in seed dispersal, dietary anthocyanins have been linked to being nat-29 ural food colorants. However, integrating extracts into food products poses challenges, depending on 31 the type of food product and requiring precise con-32 trol over the extraction and cooking process, which 33 can degrade the quality and applicability of the plant 34 extract in food products. 35

There is considerable interest in incorporating encapsulated anthocyanin powders into food products 37

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³⁸ because of their potential benefits^{14,15}. Encapsu-³⁹ lation powders are produced through encapsulation and spray-drying techniques, which transform the properties of extract solutions into powder forms¹⁶. 41 These techniques protect plant extract components, such as polyphenols, anthocyanins, and flavonoids, 43 from degradation, retain their vibrant colors, allow controlled release for improved bioavailability, mask 45 undesirable flavors, and enable their use in a wide range of food applications¹⁷. Various studies have 48 explored the use of encapsulated extracts in products such as jelly candy¹⁸, yogurt¹⁹, beverages³, and 49 jam²⁰. Encapsulated products not only preserve the 50 51 core nutritional value of the extract but also offer a solution for quality control, as the powder form al-52 lows for better measurement and consistency. How-53 ever, the effectiveness of extraction varies across dif-54 ferent food models with varying water activities^{21,22}. 55 For high-water activity products such as beverages, 56 57 juices, or desserts, microencapsulated powders focus on achieving uniform dissolution, maintaining nutritional content, and altering the color or flavor of the product. 60 In this study, the encapsulated product from roselle 61 extract was evaluated as a potential food colorant in a high-water-activity food model system. The mi-63 croencapsulated roselle powder was combined with nata de coco, a traditional Vietnamese dessert, to 65 produce a red nata de coco. Roselle (Hibiscus sabdariffa) is known for its high anthocyanin content 67 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 sta-70 ble quality. The microencapsulated powder produced 71 through spray drying offers enhanced stability, allowing better control over its properties and increasing 73 the potential of the roselle used in food products. 74 Roselles, which are rich in antioxidants, can be in-75 corporated into various foods. Recent research has 76 indicated that spray-dried roselle powder can significantly preserve its antioxidant properties when added 78 to gummies²⁴ and jam products²⁵ and can even pro-79 long the shelf-life of refrigerated chicken nuggets²⁶. 80 Despite its promising potential, further research is needed to explore its specific application in foods 82 such as natta de coco. By incorporating encapsulated 83 roselle extract into nata de coco, its potential to im-84 85 prove bioactive properties, health benefits, and sensory attributes could be altered. This study evaluated 86 the dyeing ability and physicochemical properties of 87 red nata de coco, which was created by incorporating microencapsulated hibiscus calyx extract into tradi-89

⁹⁰ tional nata de coco. The preservation of roselle prop-⁹¹ erties 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.

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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 114 with a particle size of approximately 0.5 cm. An acetic 115 acid 2% extraction solvent was prepared for the extraction process. Roselle powder was immersed in the 117 solvent at a ratio of 4 g powder to 100 mL extraction 118 solvent, and the mixture was incubated at 4 °C. After 119 24 ± 2 h, the extraction mixture was collected, and the 120 remaining solid residue was subjected to a second extraction under the same conditions. The final roselle 122 extract was stored at 4 °C for subsequent analysis. 123

Preparation of spray-dried roselle powder 124

Before microencapsulation, each encapsulating agent 125 was dispersed into the roselle extracted solution and 126 mixed until fully homogenized. The encapsulation 127 concentration was 10% w/v. The components of the 128 wall materials used were maltodextrin and xanthan 129 gum at a 99:1 w/w ratio. The SD-1000 Eyela (EYELA, 130 Japan) was used to spray the encapsulating mixture. ¹³¹ The homogenized mixture was fed into the spray 132 dryer, with the inlet temperature set at 160 °C and the outlet temperature at 80 °C. The solution was fed into 134 the dryer with a spray air flow rate of $0.7 \text{ m}^3/\text{min}$ and a 135 pressure drop of 12×10 kPa. To maintain the homogeneity of the solution, gentle mixing was performed 137 during the pumping process. After spray drying, the 138 powders were weighed, stored in airtight plastic bags, 139 and stored at -20 °C until further evaluation. 140

141 Scanning electron microscopy of the spray-

142 dried 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.

148 Nata de coco preparation

Dry-pressed raw coconut nata de coco, which was 1.5 149 $_{150}$ 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 90-151 95 °C to remove contaminants and residual bacteria. 152 After 40-60 min of boiling, the nata de coco was im-153 mersed in water for more than 2 h, and the water was 154 155 changed to remove the acetic acid. After 4-5 cycles of immersion in deionized water, the nata de coco was 156 drained and squeezed to prepare for cooking red nata 157 de coco. 158

159 Preparation of Roselle Nata de Coco (RDC)

Microencapsulated roselle powder was used to color 160 nata de coco via two distinct methods: staining via 161 sugar syrup (Method 1 - RDC-1) and staining via 162 direct contact with the nata de coco (Method 2 -163 164 RDC-2). The sugar mixture used in both methods contained 26% white sugar (Table 1). Following the 165 staining process, RDC underwent hot packaging and 166 was kept in a refrigerator until further characteriza-167 tion. The processing scheme of the two staining meth-168 169 ods is presented in Figure 1. White nata de coco (nonstaining sample) and commercial colorant (ama-170 ranth) samples were prepared under the same condi-171 tions for comparison. 172

173 Physicochemical profile of RDC

174 pH determination and titratable acidity

The titratable acidity was determined following the instructions of Tyl and Sadler, 2009, in the Food Analysis book²⁷. 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:

 $_{183}$ % titratable acidity (wt/wt) = N×V×Eq.wt. $_{184}$ W×1000×100

- 185 where: N = normality of the titrant, usually NaOH
 186 (mEq/mL)
- $_{187}$ V = volume of titrant (mL)
- 188 Eq.wt. = equivalent weight of predominant acid189 (mg/mEq)

W = mass of sample (g) 190 1000 = factor relating mg to grams (mg/g) (1/10 = 191 100/1000) 192

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Total anthocyanin content

The anthocyanin content was determined according194to the procedure of Lee et al. (2005), with slight mod-195ifications 28. Two different buffers were prepared, in-196cluding a pH 1.0 buffer (potassium chloride, 0.025 M)197and a pH 4.5 buffer (sodium acetate, 0.4 M). The sam-198ple was mixed with each buffer at a ratio of 1:4. The199absorbance of each of these solutions was measured200at 520 nm and 700 nm via a microplate reader and a201UV spectrophotometer. The absorption difference of202the attenuated sample was computed according to the203following equation:204

 $A = (A_{520} - A_{700})pH 1.0 - (A_{520} - A_{700})pH 4.5$ 205 The total anthocyanin concentration was expressed as 206 cyanidin-3-glucoside equivalents (% w/w) via the fol- 207 lowing formula: 208 Anthocyanin $[mg/L] = A \times MW \times DF \times 1000\varepsilon \times 1$ 200 where A = absorbance 210 MW = molecular weight (449.2 g/mol) 211 DF = dilution factor 212 ε = extinction molar coefficient (26,900 L/cm.mol) 213

Antioxidant assay – DPPH assay

DPPH was prepared at 0.1 mM in a DMSO solu- 215 tion. The RDC in each cooking method, nata de coco 216 without spray-dried roselle extract as a control, spray- 217 dried roselle extract in distilled water, and DMSO as 218 a blank were sampled. Different concentrations of 219 samples were diluted in DMSO. A total of 180 μ L of 220 0.1 mM DPPH solution was incubated with a 20 μ L 221 sample for 30 minutes in the dark. A series of ascor- 222 bic acid (VitC) dilutions in DMSO at concentrations 223 ranging from 0–200 μ g/mL were also prepared and 224 measured at the same time. The decrease in the ab- 225 sorbance of DPPH was measured at 517 nm via a microplate reader. The proportion of the scavenging ef- 227 fect of DPPH was calculated via the following equation: 229

DPPH scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$	23
where $A0 =$ the absorbance of the control.	23
A1 = the absorbance of the sample	23

Storage stability

RDC was prepared and packaged in 150 mL plastic ²³⁴ sealed bags. For storage ability analysis, RDC sam- ²³⁵ ples were stored for 4 weeks in a refrigerator (4 °C) ²³⁶ without light. For quantitative analysis, the pH, titrat- ²³⁷ able acidity, total anthocyanin content, and antioxi- ²³⁸ dant activity of the samples were determined via the ²³⁹







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



240 DPPH method to evaluate the changes in the degra-241 dation of the sample characteristics each week.

242 Statistical analysis

²⁴³ All the results are presented as the means \pm standard ²⁴⁴ deviations. Statistical analysis was conducted via one-²⁴⁵ way ANOVA for all tests, followed by Tukey's post hoc ²⁴⁶ analysis to determine significant differences between ²⁴⁷ groups. A p value of less than 0.05 was considered ²⁴⁸ statistically significant.

249 **RESULTS**

Microencapsulated roselle powder

The spray-dried hibiscus extract powder exhibited ²⁵¹ light pink coloration, combining the characteristic red ²⁵² 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 ²⁵⁶ microencapsulated powder exhibited 70% solubility. ²⁵⁷ The anthocyanin content and antioxidant properties ²⁵⁸ determined via DPPH were 25.07 mg/mL. Accord-²⁵⁹ ing to the SEM results, the microencapsulated roselle ²⁶⁰ powder had a circular morphology with rough and ²⁶¹ wrinkled surfaces. The microparticles were approxi-²⁶²





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

	рН	TTA (%)	Anthocyanin content (mg/L)	DPPH scaveng- ing (%)	µgVitC/mL
Sugar syrup					
RDC-1	2.6 ± 0.0	0.49 ± 0.00	1.17 ± 0.08^c	41.00 ± 2.37^{b}	115.67 ± 6.78^b
RDC-2	2.7 ± 0.0	0.47 ± 0.00	1.70 ± 0.11^b	44.57 ± 0.97^b	126.01 ± 2.78^b
White nata de coco	3.8 ± 0.0	0.04 ± 0.00	-	-	-
Roselle powder in DI	2.9 ± 0.1	0.47 ± 0.00	2.26 ± 0.00^a	57.45 ± 1.80^a	162.86 ± 5.16^a
Amaranth nata de coco	4.56 ± 0.0	0.01 ± 0.00	-	-	-
Nata de coco					
RDC-1	3.2 ± 0.5	0.45 ± 0.02	1.15 ± 0.09^{c}	42.85 ± 4.8^{c}	115.49 ± 9.62^c
RDC-2	2.7 ± 0.0	0.47 ± 0.01	1.92 ± 0.07^b	$49.26\pm3.0b^{c}$	143.27 ± 4.88^b
White nata de coco	3.9 ± 0.0		-	-	-
Roselle powder in DI	2.9 ± 0.1	0.47 ± 0.00	2.26 ± 0.00^a	57.45 ± 1.80^a	162.86 ± 5.16^a
Amaranth nata de coco	4.3 ± 0.1	0.01 ± 0.00	-	-	-

*Different letters represent significantly different data

- $_{\rm 263}\,$ mately 15 $\mu{\rm m}$ in size. This morphology indicated suc-
- 264 cessful microencapsulation using maltodextrin, xan-

²⁶⁵ than gum, and hibiscus extract. Figure 2 presents the

²⁶⁶ characteristics of the roselle encapsulated powder.

267 Red RDC

To produce red-colored nata de coco, two staining ap-268 proaches were tested to evaluate the effectiveness of 269 microencapsulated roselle powder as a food coloring 270 agent. The powder was stained indirectly by mixing 271 with syrup via one method and directly with nata de 272 coco via the other method. Both techniques resulted 273 in noticeable color heterogeneity in the nata de coco. 274 Method 2, where the powder was added to the nata de 275 coco, produced a slightly darker hue than did Method 276 1, where the powder was added to the syrup. This 277 color difference is attributed to the timing of the ad-278 dition of the spray-dried roselle extract powder dur-279 ing the cooking process. The morphology of the red-280 colored nata de coco (RDC) produced by both meth-281 ods is shown in Figure 3. 282

The physiochemical properties of the syrup and nata 283 de coco are presented in Table 2. To assess the staining 284 efficiency of the encapsulated roselle powder in RDC, 285 the physio-chemical profiles of RDC, microencapsu-286 287 lated roselle powder in deionized water (DI), and a blank sample without a roselle were analyzed. Ta-288 ble 2 presents the differences in properties under var-289 ious treatments. In the sugar mixture, the pH of the 290 RDC produced via both methods was approximately 291 2.6. However, in nata de coco, the pH of Method 2 292 (2.7) was lower than that of Method 1 (3.2). This may 293 be due to the complexity of the cellulose matrix of the nata de coco, which does not easily enable the extract 295 to be absorbed. This finding indicated that directly 296 introducing roselle powder into drained nata de coco 297 enhances the absorption of the extract. Conversely, 298 the titratable acidity between Method 1 and Method 299 2 (0.45-0.49%) showed minimal differences. 300

The anthocyanin content and antioxidant activity are 301 crucial parameters for evaluating the functionality of 302 roselle powder in nata de coco. Both metrics were 303 higher in the samples from Method 2 (RDC-2) than in 304 those from Method 1 (RDC-1). Specifically, RDC-2 had anthocyanin concentrations of 1.7 mg/L in syrup 306 and 1.93 mg/L in nata de coco, whereas the RDC-1 307 samples contained approximately 1.15 to 1.17 mg/L. 308 This trend was also observed for the antioxidant activ-309 ity, suggesting that adding roselle to drained nata de 310 coco and incubating overnight enhances the incorpo-311 312 ration and diffusion into both nata de coco and sugar 313 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 320 properties of RDC stored at 6 \pm 2 °C for four weeks ₃₂₁ were evaluated to monitor potential changes over 322 time. The pH of the products did not change over 323 four weeks, with the pH ranging from 2.5 to 4.0 un- 324 der both storage conditions. Similarly, the titratable 325 acidity (TTA) exhibited minimal variation. However, 326 as shown in Figure 4, the anthocyanin content in each 327 sample decreased considerably by approximately 10- 328 20% compared with that in the previous week. De- 329 spite the decrease in anthocyanin content, Figure 4 330 shows that the antioxidant properties of each sam- 331 ple remained approximately the same. These findings 332 suggest that antioxidant activity is not solely depen- 333 dent on anthocyanin levels. 334

DISCUSSION

Plant pigments have long been used as natural food 336 colorants in the culinary industry. When applied to 337 nata de coco, microencapsulated roselle powder can 338 increase the demand for roselle, potentially increas- 339 ing production and benefiting the economies of the 340 roselle- and nata de coco-producing regions. Pro- 341 ducers can achieve greater profits by incorporating 342 spray-dried roselle into their products. The bene- 343 ficial use of roselle in nata de coco reduces the re- 344 liance on synthetic food colorants. Consumers ben- 345 efit from the added nutritional value and reduced 346 risk of allergic reactions or problems caused by com- 347 mercial colorants. This natural alternative to syn- 348 thetic colorants also provides antioxidants and anti- 349 inflammatory compounds, enhancing the health ben- 350 efits of the product. While it may not serve as a treatment for specific conditions, it offers a healthful alter- 352 native to synthetic food colorants, positively impact- 353 ing consumer health. 354

Encapsulation involves surrounding a central material with a wall material to prevent chemical and environmental interactions²⁹. This method addresses the instability of substances by inhibiting volatilization and protecting against adverse conditions³⁰. 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

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³⁶⁵ Figure 2. The combination of plant extracts with mal-366 todextrin and xanthan gum effectively provided color and antioxidant properties. Thus, microencapsulated granules under these conditions are a viable option for 368 developing functional foods. 369 In combining and developing nata de coco products by using roselle encapsulated powder, understanding 371 the characteristics of nata de coco is essential. Cel-372 lulose, a natural polymer composed of carbohydrate polysaccharides, is obtained via fermentation and ad-374 heres to the cellulose fibers produced by Acetobacter 375 xylinum. Physical characteristics can affect food qual-376 ity and consumer acceptability. Nata de coco has high 377 aesthetic value because of its pure white hue, dense consistency, and fresh scent³¹. To produce bacte-379 rial cellulose for nata de coco, the product is thor-380 oughly rinsed to remove the sour taste and then boiled 381 to eliminate the acidic flavor and eliminate any mi-382 croorganisms. After purification, the bacterial cellu-383 lose is cooked with sugar and other ingredients to cre-384 ate a dessert known as nata de coco. In this study, 385 microencapsulated roselle powder was used as a colorant to introduce a safe, aesthetically appealing, and 387 antioxidant-rich nata de coco. Given that water activ-388

ity 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 col-393 rant for nata de coco was assessed via two different methods. In Method 1, roselle powder was added 395 during the initial cooking phase when the nata de 396 coco remained porous and could absorb liquid. The structural matrix of the gel likely absorbed some phy-398 tochemicals, resulting in less color in the residual 399 syrup. In contrast, in Method 2, roselle was added 400 after cooking the nata de coco, leaving more extract 401 particles suspended in the sugar syrup rather than ab-402 sorbed into the nata de coco fibers. The dispersed pigments in method 2 imparted a more intense red 404 coloration to the solution. The results indicated that 405 encapsulated roselle powders exhibited their primary 406 ability to stain the subject directly or indirectly after 407 being dissolved in a solution. 408

⁴⁰⁹ Titratable acidity and pH offer complementary in-⁴¹⁰ sights into food quality, with titratable acidity more ⁴¹¹ accurately reflecting the impact of organic acids on ⁴¹² flavor. Food acids only partially ionize, affecting some ⁴¹³ properties on the basis of the ionized fraction and ⁴¹⁴ others on the total acid content. pH determination ⁴¹⁵ is crucial for developing high-quality products with ⁴¹⁶ optimal phytochemical characteristics³². According ⁴¹⁷ to Paramee Chumsri et al., the optimal pH of dried roselle calyxes in beverages ranges from 2.8 to 2.9³³. ⁴¹⁸ Similarly, the pH of RDC in this study was approx-⁴¹⁹ imately 2.6–3.2, making the product sour due to the⁴²⁰ acidity of the encapsulated roselle powder. The acid-⁴²¹ ity level of spray-dried RDC is approximately 0.4%. ⁴²² The antioxidant properties were approximately 50% ⁴²³ in both samples. These findings indicate that spraydried roselle powder is appropriate for use in food and ⁴²⁵ beverages. Using spray-dried roselle in nata de coco can reduce the need for synthetic food colorants, offering a natural and healthy alternative. ⁴²⁸

The total anthocyanin content serves as an indicator 429 of both the color and potential health benefits pro- 430 vided by the anthocyanins in roselle extract. The 431 results revealed that Method 2 had a significantly 432 greater anthocyanin content (1.7-1.93 mg/L) than 433 Method 1 did (1.15-1.17 mg/L). In Method 1 (RDC- 434 1), adding the spray-dried extract while cooking with 435 sugar syrup allowed more roselle to remain suspended 436 in the residual syrup rather than being absorbed into 437 the nata de coco. Conversely, in Method 2 (RDC- 438 2), the roselle powder was added during the cooking 439 of nata de coco, which has significant water-holding 440 capacity, enabling a portion of the anthocyanins to 441 be absorbed into the cellulose matrix of the nata de 442 coco. This is visually evident, as the color of the 443 sugar syrup in Method 2 is slightly less red than that 444 in Method 1, but the nata de coco in Method 2 is 445 redder. The two methods assess a sample's antioxi- 446 dant activities to determine the anthocyanin antioxidant outcome following nata de coco production. 448 This disparity likely arises from differences in pro- 449 cessing conditions. In RDC-1, the nata de coco exposed the extract to potentially longer temperature 451 effects, decreasing anthocyanin stability and activ- 452 ity. However, in RDC-2, the addition of roselle extract after cooling prevented exposure to sudden tem- 454 perature changes during prolonged cooking. As de- 455 scribed in the section on total anthocyanin content, 456 the heating procedure affects the stability of antho- 457 cyanins^{22,34,35}, leading to a decrease in their antiox- 458 idant activity in nata de coco. Furthermore, antho- 459 cyanins degrade in response to various factors, in- 460 cluding pH, temperature, intermolecular pigmenta- 461 tion, ascorbic acid, oxygen, etc. 36. The cleavage of 462 the intramolecular bonds of anthocyanins, resulting 463 in colorless, smaller molecules, is more active in the 464 extracts than in the cells because of the high tem- 465 perature. For example, coumarin glycoside has been 466 identified as a common byproduct of the thermal 467 degradation of cvanidin 3,5-diclycosides over time³⁷. 468 Specifically, at 37 °C, delphinidin 3-sambubioside 469 and cyanidin 3-sambubioside in anthocyanins lead 470 471 to the formation of colorless molecules such as gallic ⁴⁷² acid and protocatechuic acid ³⁸. 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-1 474 than in RDC-2. The effects of temperature on antho-475 cyanin properties were also observed when the anthocvanin contents produced in treatments 1 and 2 were 477 compared with those of the same amount of roselle 478 powder mixed with DI. After processing, the medicinal properties were reduced by approximately 30% 480 compared with those of the original product. 481

In the stability study, the physicochemical properties 482 of the RDC samples remained stable over four weeks, 483 except for the anthocyanin content. The stability of 484 anthocyanins in roselle powder is influenced by vari-485 ous factors, such as heat and pH, particularly in low-486 acid environments where anthocyanins degrade more rapidly. However, the study revealed that the antho-488 cyanins in the roselle powder presented high color 489 stability and heat resistance under acidic conditions. 491 These findings suggest that the quality and appearance of roselle powder can be preserved by adjusting the 492 pH and temperature during processing. Furthermore, 493 the roselle extract used in this study was rich in antho-494 495 cyanins and exhibited strong antioxidant properties. Despite a decrease in anthocyanin levels over time, the 496 antioxidant activity remained stable, further support-497 ing the potential of roselle powder as a food ingredient with health benefits. Some studies have indicated 499 that roselle anthocyanins are relatively stable at high 500 temperatures, particularly when the heating duration 501 is short $(30-45 \text{ min})^{39,40}$. The preservation of roselle 502 properties in a high-water-activity food model such 503 as nata de coco demonstrates the potential of roselle-504 encapsulated powder as a natural colorant. Therefore, 505 further research is warranted to determine the opti-506 mal conditions for encapsulating roselle anthocyanins 507 during the heating process. Importantly, this study 508 assessed the stability of anthocyanins in roselle pow-509 der under specific conditions, and the results may not 510 be applicable to other anthocyanin-containing prod-511 ucts or processing methods. Additional research is 512 513 needed to explore the stability of anthocyanins in various food and beverage products and their potential applications. 515

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 rang-523 ing from 2.6-3.2, titratable acidities between 0.45% 524 and 0.49%, and total anthocyanin contents varying 525 from 1.1-1.9 mg/L. The degree of antioxidant inhibi- 526 tion ranged from 40-50%. Stability studies confirmed 527 that the pH, titratable acidity, and antioxidant properties of the samples remained consistent over four 529 weeks, despite some anthocyanin degradation. This 530 research explored the potential of a natural food col- 531 orant to create an innovative product, offering valu- 532 able insights for future research and development. By 533 providing a low-cost and effective natural alterna- 534 tive to synthetic colorants, this approach may posi- 535 tively impact consumer health. These findings sug- 536 gest that the use of microencapsulated RDC powder 537 can lead to the production of stable functional foods 538 with significant potential. Future research should pri- 539 oritize the assessment of RDC stability under various 540 storage conditions and conduct broader sensory eval- 541 uations of consumer preferences. Although roselle 542 powder provides antioxidants and anti-inflammatory 543 compounds, potentially improving health benefits for 544 traditional nata de coco, further research is needed 545 to fully understand the health benefits of spray-dried 546 roselle. Once the functional food's suitability and 547 health benefits are confirmed, further in vivo and in 548 vitro studies will be conducted to evaluate its efficacy. 549 These investigations provide crucial insights into the 550 potential culinary and health applications of RDC. 551

LIST OF ABBREVIATIONS USED

DPPH: 2,2-diphenyl-1-picrylhydrazyl	553
DMSO: Dimethyl sulfoxide	554
RDC: Roselle Nata de Coco	555
TTA: titratable acidity	556

COMPETING INTERESTS

The authors declare that they have no competing in- 558 terests. 559

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AUTHORS' CONTRIBUTION

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569 **REFERENCES**

- 570 1. 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.
- 573 1016/i.tifs.2009.04.003.
- 574 2. Oliveira A, Amaro A, Pintado M. Impact of food matrix compo-
- nents 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.
- 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.
- 585 5. Leong H, Show PL, Lim M, Chien Wei O, Ling T. Natural red pigments from plants and their health benefits – A review. Food
 787 Rev Int. 2017 May 10;34;Available from: www.doi.org/10.1080/
 7588 87559129.2016.1225824.
- 589 6. Wiczkowski W, Szawara-Nowak D, Topolska J. Red cabbage
- anthocyanins: Profile, isolation, identification, and antiox idant activity. Food Res Int. 2013 Apr 1;51:303–9;Available
- 592 from: www.doi.org/10.1016/j.foodres.2013.01.020.
- 593 7. Abou-Arab A, Abu-Salem F, Abou-Arab EA. Physico-chemical
- 594 properties of natural pigments (anthocyanin) extracted from 595 Roselle calvces (Hibiscus sabdariffa). Proceedings. 2011;Avail-
- able from: www.semanticscholar.org/paper/Physico-chemical-
- 597 properties-of-natural-pigments-Abou-Arab-Abu-Salem/
- ⁵⁹⁸ 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/i.foodres.2019.01.010.
- 9. Liu Y, Fernandes I, Mateus N, Oliveira H, Han F. The role of an-
- thocyanins in alleviating intestinal diseases: A mini-review.
 J Agric Food Chem. 2024 Mar 20;72(11):5491–502;Available
- 606 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/
- 610 mnfr.202100170.
- 611 11. Alam MdA, Islam P, Subhan N, Rahman MdM, Khan F, Bur612 rows GE, et al. Potential health benefits of anthocyanins in
 613 oxidative stress-related disorders. Phytochem Rev. 2021 Aug
- 6141;20(4):705-49;Available from:www.doi.org/10.1007/s11101-615021-09739-2.
- 616 12. Garcia C, Blesso CN. Antioxidant properties of anthocyanins
- 617and their mechanism of action in atherosclerosis. Free Radic618Biol 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 the current kno
- Oancea S. A review of the current knowledge of thermal sta bility of anthocyanins and approaches to their stabilization
 to heat. Antioxidants. 2021 Aug 24;10(9):1337;Available from:
- 623 www.doi.org/10.3390/antiox10091337.
- 624 14. Cid-Ortega S, Guerrero-Beltran J. Roselle calyces (Hibiscus
- sabdariffa), an alternative to the food and beverages indus tries: A review. J Food Sci Technol. 2015 Apr 21;52;Available
 from: www.doi.org/10.1007/s13197-013-1086-4.
- 628 15. Ozdal T, Yolci Omeroglu P, Tamer C. Role of encapsulation in 629 functional beverages. In 2020, p. 195–232;
- functional beverages. In 2020, p. 195-202,
 for the transferring of the transferr
- characterization. Crit Rev Food Sci Nutr. 2023;63(24):6983–
 7015;Available from: www.doi.org/10.1080/10408398.2022.
 2130124.
- 636 17. Grgić J, Šelo G, Planinić M, Tišma M, Bucić-Kojić A. Role of
- the encapsulation in bioavailability of phenolic compounds.
- 638 Antioxidants. 2020 Sep 26;9(10):923;Available from: www.doi.
- 639 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 microen capsulation of Roselle (Hibiscus sabdariffa) extract as a nat ural colorant source for gummy candy. Int J Biol Macromol.
 2023 Jul 1;242:124903;Available from: www.doi.org/10.1016/j.
 ibiomac.2023.124903.
- Department of Food Technology, University of Ibadan, 672
 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 675
 Environ Sci Toxicol Food Technol. 2014;8(7):112–22;. 676
- 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.
- Sadler G, Murphy P. PH and titratable acidity. In: Food Analysis. 682 2010. p. 219–38;. 683
- 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/ j.tifs.2021.06.004.
- Díaz-Torres R, Alonso-Castro A, Carrillo M, Carranza Alvarez C.
 Bioactive compounds obtained from plants, their pharmaco logical applications and encapsulation. In 2021. p. 181–205;.
 695
- 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 700 J, Martínez-Monzó J. Effect of pH on color and texture of food 701 products. Food Eng Rev. 2013 Sep 1;5(3):158–70;Available 702 from: www.doi.org/10.1007/s12393-013-9050-9. 703
- Chumsri P, Anchalee S, Itharat A. Studies on the optimum 704 conditions for the extraction and concentration of roselle (Hi-705 biscus sabdariffa Linn.) extract. Songklanakarin J Sci Technol. 706 2008 Apr 1;30:133–9;. 707
- 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-710

- 711 nol. 2017 May 1;83;Available from: www.doi.org/10.1016/j.lwt. 712 2017.05.036.
- 713 35. Wu HY, Yang KM, Chiang PY. Roselle anthocyanins: Antioxi-
- 714 dant properties and stability to heat and pH. Mol Basel Switz.
- 2018 Jun 5;23(6):1357;Available from:
 www.doi.org/10.3390/

 molecules23061357.
 molecules23061357.
- 717 36. Oancea S. A review of the current knowledge of thermal sta-
- bility of anthocyanins and approaches to their stabilization
 to heat. Antioxidants. 2021 Aug 24;10:1337;Available from:
- 720 www.doi.org/10.3390/antiox10091337.
- 721 37. 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/Natural-
- food-colorants-G-A-F-Hendry-auth-G-A-F-Hendry-J-D-
- 725 Houghton.
- 726 38. Sinela A, Rawat N, Mertz C, Achir N, Fulcrand H, Dornier M.
- Anthocyanins degradation during storage of Hibiscus sabdar-iffa 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.
- 731 39. Dyrby M, Westergaard N, Stapelfeldt H. Light and heat sensi-
- tivity of red cabbage extract in soft drink model systems. Food
 Chem. 2001 Mar 1;72(4):431–7;Available from: www.doi.org/
- 733 Chem. 2001 Mar 1;72(4):431–7;Available
 734 10.1016/S0308-8146(00)00319-4.
- 735 40. Kirca A, Cemeroğlu B. Degradation kinetics of an-736 thocyanins in blood orange juice and concen-
- thocyanins in blood orange juice and concen trate. Food Chem. 2003;4(81):583-7;Available from:
- 738 www.doi.org/10.1016/S0308-8146(02)00469-6.