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Optimization of methanol-water solvent extraction of anthocyanins from Roselle (*Hibiscus sabdariffa* L.) petals using response surface methodology

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ABSTRACT

Anthocyanins, a prominent group of water-soluble plant pigments, exhibit noteworthy health-promoting properties and are natural colorants in food applications. This study aimed to optimize the solvent extraction of anthocyanins from Roselle petals using methanol-water solvent, with three independent variables: extraction time (2 to 6 h), extraction temperature (20 to 40ºC), and Roselle petal to solvent weight ratio (2 to 6 g). Response Surface Methodology (RSM) based on Box-Behnken design (BBD) was employed for experimental design. The anthocyanin content extracted from Roselle petals ranged from 132.85 to 270.49 mg/L. Elevated extraction temperature, extended extraction time, and higher petal-to-solvent ratio significantly enhanced anthocyanin extraction efficiency ($p \leq 0.05$). The optimal conditions for maximum anthocyanin extraction were predicted as follows: extraction temperature of 40ºC, extraction time of 5.71 h, and petal-to-solvent ratio of 6. Under these conditions, 266.19 mg/l of anthocyanin was extracted, validating the model prediction (R^2=0.96).

1. Introduction

Color has perpetually captivated consumers, pivotal in fulfilling their psychological desires and aesthetic preferences. The application of pigments in food product preparation for industrial purposes is well-established (1). The burgeoning demand for natural colors in the food industry is attributable to the growing awareness of the detrimental impacts associated with artificial colorants. Anthocyanins offer a virtuous alternative to synthetic colors, possessing vibrant and appealing hues and demonstrating validated antioxidant, anticancer, and antiviral properties (2). These natural pigments, falling within the extensive class of flavonoids, are synthesized during the phenylpropanoid cycle in plants (3). Anthocyanins, however, exhibit susceptibility to degradation post-separation from the plant matrix, with factors such as chemical reactions, pH, storage conditions, temperature, humidity, concentration, light, moisture, extraction solvents, and the presence of enzymes, flavonoids, proteins, and metal ions influencing their stability (4). Extraction of anthocyanins typically employs aqueous-alcoholic solvents like ethanol, methanol, butanol, etc., preferably at temperatures below 30ºC and often under vacuum conditions to minimize degradation (5). Recent efforts have focused on enhancing anthocyanin extraction techniques from various plant sources, such as saffron petals, black carrot, blackberry, grape pulp, and blueberry (6-11). The medicinal plant Roselle, scientifically named Hibiscus sabdariffa L., belongs to the *Malvaceae* family. Multiple components of Roselle, including its flowers, leaves, and seeds, find utility in the food and pharmaceutical sectors. Indigenous to Asia (India to Malaysia) and Africa, Roselle thrives in regions like Central America, India, Africa, Brazil, Australia, Hawaii, Florida, and the Philippines, serving as a staple in home gardens and a pivotal export commodity in Sudan (12, 13). Endowed with abundant anthocyanins, flavonoids, ascorbic acid, and other valuable compounds, Roselle exhibits remarkable antioxidant and antibacterial efficacy. Noteworthy compounds such as ascorbic acid, aldehydes, beta-carotene, β-sitosterol, citric acid, cyanidin 3-rutinoside, delphinidin galactose, gossypetin, and

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phylloquinone are found in the petioles of this plant (13). The copious anthocyanin content contributes significantly to Roselle's attributes, with documented effects encompassing robust antioxidant and antitumor activities. Roselle finds application in managing hypertension, inflammatory conditions, and cancer (14). Roselle, also known as the traditional Chinese rose, has effectively addressed various health conditions such as high blood pressure, inflammatory diseases, and cancer (15). Abundant phenolic compounds, antioxidants, and anthocyanins in fruits like pomegranates, strawberries, blackberries, cherries, and charcoal inhibit low-density lipoprotein (LDL) cholesterol oxidation in the bloodstream, thereby mitigating cardiovascular risks (16). The vital role of antioxidants across industries has spurred research endeavors focused on refining antioxidant extraction methodologies to augment extraction efficiency and quality. However, scarce investigations exist concerning optimal anthocyanin extraction from Roselle. Consequently, this study endeavors to optimize Roselle solvent extraction conditions (water/methanol) for anthocyanins, employing response surface methodology (RSM) and examining variables such as extraction time, temperature, and petal-to-solvent ratio, to yield an anthocyanin-enriched extract suitable for food and nutraceutical applications.

2. Materials and methods

2.1. Materials

Roselle leaves (containing 12.2% carbohydrate, 1.1% protein, 0.7% fat, and 86% moisture) were procured from a local market in Tehran, Iran. Methanol, chloric acid, sodium acetate, hydrochloric acid, and other chemicals were acquired from Merck Company (Berlin, Germany).

Extraction of pigment from Roselle leaves

Roselle leaves were soaked in tap water at 50°C for 2 hours to extract the pigment, followed by a solvent extraction approach. The focus was on anthocyanin extraction from Roselle leaves. Methanol (96%) and water were used as the solvent systems at ratios of 0.5 (1:2) to 1.5 (3:2) at ambient temperature.

Table 1. Independent variables and their levels used in the Box–Behnken design.

Factors	Coded symbols	Levels		
		+1	0	-1
Time (h)	A	6	4	2
Weight ratio (g)	B	6	4	2
Temperature (˚C)	C	40	30	20

Specifically, 50 mL of solvent was added to 250 mL Erlenmeyer flasks containing a predetermined amount of leaves. The Erlenmeyer flasks were sealed with a polyethylene coating to prevent solvent evaporation and placed on an orbital oscillator with adjustable temperature and time. The extract was subsequently separated from undesired substances using Whatman No. 1 filter paper and concentrated using a rotary evaporator (BUCHI Rotavapor, Model R-114, Lausanne, Switzerland) at 60°C to reach Brix 60 (17). Independent variables for optimizing Roselle anthocyanin extraction are presented in Table 1.

2.2. pH and acidity measurement

pH was determined using a pH meter (Istek, South Korea), and acidity was measured through titration using 0.1 N NaOH to achieve a pH of 1.8 for both treated and control samples (18). Acidity was calculated using the following equation:

$$Acidity=(N\times 0.9)/V \quad (1)$$

where, N represents normality, and V is the volume of NaOH used.

2.3. Determination of anthocyanins

Anthocyanin content was determined using the pH differential method and spectrophotometric testing, measuring the absorbance of extracts at pH 1.0 and 4.5 (19). The absorbance of Roselle extracts was recorded at 510 and 700 nm, and results were reported as mg cyanidin-3-O-glucoside equivalents per gram of dry matter (mg/l) using the following equations:

$$Total\ anthocyanin\ content\ \left(\frac{mg}{100g}\right)=\frac{A*MW*DF*1000}{\varepsilon*C} \quad (2)$$

where, A is the extract absorbance and calculated as follow:

$$A=(A510-A700)_{pH\ 1.0}-(A510-A700)_{pH\ 4.5} \quad (3)$$

MW is the molecular weight of cyanidin-3-O-glucoside, DF is the extract dilution factor, ε is the molar absorptivity of cyanidin-3-O-glucoside, and C is the buffer concentration in mg/mL.

2.4. Statistical analysis

The experimental design and data analysis were conducted using response surface methodology (RSM) based on Box-Behnken in Minitab v.16. Three independent variables – time (2 to 6 h), temperature (20 to 40°C), and Roselle leaf-to-solvent ratio weight (2 to 6 g) – were examined at three levels.

3. Results and discussion

3.1. pH and acidity

The role of pH in anthocyanin stability and color preservation is well established, as alterations in pH can trigger anthocyanin degradation and subsequent color instability. However, the inherent structure of anthocyanins lends them a notable degree of reversibility in response to changes in pH. The outcomes pertaining to pH, acidity, and anthocyanin content under various treatment conditions are documented in Table 2. It is evident that diverse extraction conditions exert

significant influence on pH modulation and the augmentation of acidity levels ($p \leq 0.05$), leading to a range of pH values within Roselle extracts, spanning from 2.68 to 2.86. Notably, heightened extraction temperature (20 to 40ºC), prolonged extraction time (2 to 6 h), and an increased sample weight to solvent ratio (2 to 6 g) all contribute to substantial pH reduction ($p \leq 0.05$). The most elevated pH value (2.86) was attained under conditions involving an extraction temperature of 20ºC, 4-hour extraction time, and a sample-to-solvent weight ratio of 2 g. Conversely, the lowest pH level within Roselle extracts (2.68) was achieved at an extraction temperature of 40ºC, a 4-hour extraction time, and a sample-to-solvent weight ratio of 6 g. This decline in pH can likely be attributed to the disruption of the delicate co-pigmentation bonds inherent in anthocyanins (20). Anthocyanins exhibit distinct chemical forms at different pH ranges, manifesting as cationic flavylium structures (associated with red color in water-soluble form) at low acidic pH (pH<2) and transitioning to pinkish blue forms within an intermediate acidic pH range (pH =4-7) (6). The perturbation of pH equilibrium can induce anthocyanin instability, leading to color alteration (3). Furthermore, pH serves not only to modulate anthocyanin color but also to reinforce its structural integrity. Anthocyanins in acidic solutions demonstrate heightened resilience in comparison to neutral or alkaline conditions. Oxygen, within specific pH ranges, emerges as a significant factor impacting anthocyanin stability (3, 6). Chumsri et al. (21) noted the presence of natural organic acid compounds, including citric, malic, and 3-indole acetic acids, within fresh rose petals, which play a pivotal role in enhancing the vivid red hue of juice samples. In environments with an acidic pH, anthocyanins exist in equilibrium across four distinct structures: flavilium cation, quinonoidol base, carbinol pseudobase, and chalcone (20). Concomitantly, the range of acidity within the Roselle extracts spanned from 4.67 to 4.97%. The experimental outcomes underscore the significant impact of elevated extraction temperature (20 to 40ºC), extended extraction time (2 to 6 h), and augmented sample-to-solvent weight ratio (2 to 6 g) on amplifying acidity values ($P \leq 0.05$). As indicated in Table 2, the pinnacle of acidity within Roselle extracts (4.97) was achieved under conditions involving a 40ºC extraction temperature, 4-hour extraction time, and a weight of 6 g; conversely, the lowest acidity (4.65) was attained with an extraction temperature of 20ºC, a 4-hour extraction time, and a sample-to-solvent weight ratio of 2 g. Notably, an escalation in acidity corresponds to heightened anthocyanin content. For instance, Sadeghi et al. (22) delved into the stability of strawberry color during concentrate production, revealing that a pH value of 3 emerged as the most efficacious in preserving anthocyanins and ascorbic acid, yielding a more aromatic outcome. Similarly, Farhadi Chitgar et al. (23) investigated the stability of anthocyanins within three barberry species across a pH spectrum of 1 to 7, establishing the applicability of a first-order kinetic equation for anthocyanin degradation. Their findings indicated a direct relationship between increasing pH and a higher constant reaction rate across all three barberry varieties, with the highest stability observed at pH=1. Khalili et al. (24) explored anthocyanin extraction from red cabbage, demonstrating that an initial pH of 2.5 was pivotal, as subsequent pH elevation followed by reversion to the original pH led to restoring the original red cabbage color. Consequently, a lower pH contributes to heightened anthocyanin content, thus restoring the initial properties of the substance.

Table 2. Experimental and predicted values for the pH, acidity, and anthocyanins of Roselle extract according to Box-Behnken design.

Treatment	Weight (g)	Time (h)	Temperature (˚C)	pH		Acidity (% malic acid)		Anthocyanin (mg/L)	
				Measured responses	Predicted responses	Measured responses	Predicted responses	Measured responses	Predicted responses
1	2	4	40	2.75	2.74	4.83	4.84	210.140	211.406
2	4	2	40	2.74	2.72	4.89	4.89	220.490	221.993
3	4	2	20	2.84	2.83	4.67	4.67	139.87	135.690
4	4	6	20	2.83	2.83	4.68	4.68	142.750	141.247
5	6	4	40	2.68	2.69	4.97	4.96	270.490	263.541
6	4	6	40	2.71	2.72	4.94	4.93	230.93	235.110
7	4	4	30	2.78	2.77	4.75	4.75	180.630	180.507
8	6	2	30	2.77	2.75	4.77	4.77	189.180	194.626
9	2	2	30	2.80	2.80	4.72	4.70	163.06	160.291
10	4	4	30	2.78	2.77	4.75	4.75	180.52	180.520
11	2	4	20	2.86	2.85	4.65	4.65	132.85	132.850
12	6	6	30	2.76	2.75	4.79	4.80	200.52	200.520
13	2	6	30	2.79	2.80	4.73	4.72	175.75	175.750
14	6	4	20	2.82	2.81	4.70	4.68	156.250	156.250
15	4	4	20	2.78	2.77	4.75	4.75	180.37	180.370

3.2. Anthocyanins

Anthocyanins are the largest group of water-soluble pigments in plants and belong to a group of molecules called flavonoids (25). Anthocyanin test results are reported in Table 2. As can be seen, the amount of anthocyanins in Roselle petal extracts is in the range of 132.85 to 270.490 mg/l. The results showed that with increasing temperature (from 20 to 40ºC), extraction time (from 2 to 6 h), and the weight ratio of Roselle petal to solvent (from 2 to 6 g), the anthocyanin content of the extracts developed significantly ($p \leq 0.05$). The highest amount of anthocyanin extracted from the Roselle petal (270.490 mg/l) was obtained at 40ºC, extraction time of 4 h and Roselle petal weight ratio of 6 g, while the lowest amount of anthocyanin

(132.85 mg/l) obtained at 20ºC, extraction time of 4 h and Roselle petal weight ratio of 2 g. The increase in temperature increases yield efficiency by affecting extracts' viscosity, solubility, and surface tension (26). Mahdavi Khazaei et al. (6) optimized the extraction of anthocyanins from saffron petals using RSM. The results showed that in the optimal conditions of the 20 ml solvent/g sample ratio, ethanol percent 25.02%, extraction temperature 25.8ºC, and extraction time 24 h, the maximum anthocyanin content of 1609 mg/L was obtained. Kirca et al. (7) also stated that the amount of anthocyanin in black carrots is 1750 mg /kg in the optimum condition. In another study, Lee et al. (10) reported that the amount of anthocyanin in blackberry extract was 13.6 mg, strawberry extract to be 63.6 mg, raspberry extract to be 36.7 mg, and blackberry extract to be 3,800.6 mg, representing the presence of considerable amounts of health-promoting anthocyanins. The experimental and predicted values for the pH, acidity, and anthocyanin content of Roselle extract are summarized in Table 2. Anthocyanins, the largest group of water-soluble pigments in plants, belong to the flavonoid family (25). The anthocyanin content of Roselle petal extracts was found to range from 132.85 to 270.490 mg/l. This variation was influenced by changes in extraction conditions, such as temperature (20 to 40ºC), extraction time (2 to 6 h), and the weight ratio of Roselle petals to solvent (2 to 6 g), which had a significant impact on anthocyanin content ($p \leq 0.05$). The highest anthocyanin content (270.490 mg/l) was obtained under conditions of 40ºC extraction temperature, 4-hour extraction time, and a petal-to-solvent weight ratio of 6 g. Conversely, the lowest anthocyanin content (132.85 mg/l) was observed at 20ºC extraction temperature, 4-hour extraction time, and a weight ratio of 2 g. Elevated temperature was shown to enhance extraction efficiency through its influence on factors such as viscosity, solubility, and surface tension (26). The optimization of anthocyanin extraction from various plant sources has been explored in prior studies. For instance, Mahdavi Khazaei et al. (6) optimized anthocyanin extraction from saffron petals using Response Surface Methodology (RSM), achieving a maximum anthocyanin content of 1609 mg/L under optimal conditions. Similarly, Kirca et al. (7) determined that the optimal condition for anthocyanin extraction from black carrots was 1750 mg/kg. Lee et al. (10) reported substantial anthocyanin in various fruit extracts, such as 13.6 mg in blackberry, 63.6 mg in strawberry, 36.7 mg in raspberry, and a notable 3800.6 mg in blackberry.

3.3. Regression coefficients and model of pH, acidity, and anthocyanin

The analysis of variance was carried out on the second-order polynomial model, as presented in Table 3. The model exhibited a good fit to the experimental data, with a correlation coefficient (R^2) of 98.54% for pH and 99.21% for acidity, along with their respective adjusted correlation coefficients (R^2-adj) of 95.91% and 97.79%. Linear effects of temperature and sample-to-solvent weight ratio, secondary effects, and certain interaction effects were found to significantly influence the changes in acidity ($p \leq 0.05$). However, the linear effects of time, second-degree effects, and certain interaction effects were not significant ($p > 0.05$) in the context of acidity changes. Similarly, the analysis of variance for anthocyanin content indicated suitable fitness of the model to the experimental data, with a correlation coefficient (R^2) of 97.79% and an adjusted correlation coefficient (R^2-adj) of 99.21%. Linear effects of temperature and sample-to-solvent weight ratio, along with the interaction effect of weight ratio × temperature, were found to significantly influence changes in anthocyanin content ($p \leq 0.05$).

Table 3. Response surface model of pH, Acidity, and anthocyanin of Roselle extract.

Response	Model	R^2	R^2-adj
pH	2.78–0.0075 A–0.02125 B–0.05875C+0.00125 A^2–0.00125 B^2-0.00125 C^2–0.00500 AC–0.00750BC	98.54	95.91
Acidity	4.75000+0.01125A+0.03750 B+0.11625 C+0.00500 A^2 –0.00250 B^2+0.04000C^2-0.00250 AB+0.01000AC+0.02250 BC	99.21	97.79
Anthocyanin	180.507+4.669 A+16.830 B+45.041C–3.651 A^2+5.272 B^2+6.654 C^2–0.337 AB+1.890 AC+9.238 BC	97.79	99.21

A: temperature; B: time and C: sample-to-solvent weight ratio

Table 4. Regression coefficients results of anthocyanin, acidity and pH Roselle extract.

	Anthocyanin		Acidity		pH	
Variation source	P-value	F-value	P-value	F-value	P-value	F-value
Regression	0.000*	50.25	0.000*	69.85	0.000*	37.45
Linear effects	0.000*	111.14	0.000*	195.73	0.000*	111.14
Temperature (A)	0.000*	52.96	0.000*	527.38	0.000*	290.66
Time (B)	0.100	145.46	0.077	4.94	0.081	4.47
Weight ratio (C)	0.001*	4.08	0.001*	54.88	0.002*	38.09
Second-order effects	0.172	0.06	0.015*	9.83	0.976	0.006
Temperature×Temperature (A^2)	0.108	2.77	0.003*	28.82	0.815	0.006
Time×Time (B^2)	0.3133	379.33	0.532	0.045	0.815	0.006
Weight ratio (C^2)	0.182	2.52	0.751	0.011	0.815	0.006
Interactions	0.150	1.14	0.085	3.98	0.418	1.14
Temperature×Time (A×B)	0.558	0.33	0.221	1.95	0.352	0.05
Temperature×Weight ratio (A×C)	0.037*	7.98	0.026*	9.88	0.185	2.37
Time×Weight ratio (B×C)	0.922	0.01	0.741	0.12	1.000	0.000
Lack of fit	-	-	-	-	-	-

Conversely, the second-degree effects of independent variables, linear effects of time, and certain interaction effects were not significant ($p>0.05$) in the context of anthocyanin changes. The presented data and analysis underscore the intricate interplay of extraction conditions and their effects on pH, acidity, and anthocyanin content in Roselle extracts. These findings contribute to a deeper understanding of the factors influencing the extraction of valuable bioactive compounds from botanical sources.

3.4. Interaction effects

3.4.1.Interaction effects of different extraction conditions on the pH value of the Roselle extract

In Figure 1, the interaction effects of various extraction conditions on the pH value of the Roselle extract are presented in three subplots. Subplot (a) illustrates the Roselle extract's pH changes based on the interaction between the weight ratio of Roselle petals to solvent and the extraction temperature. It is evident that as both the temperature and weight ratio increase while maintaining a constant extraction time of 4 hours, the pH level of the extract decreases. Subplot (b) depicts the pH changes of the Roselle petal extract in relation to the interaction between the extraction temperature and extraction time. It can be observed that increasing both the temperature and extraction time, with the weight ratio of Roselle petals to solvent kept constant at 4 g, leads to a decrease in the pH level of the extract. Subplot (c) demonstrates the pH variations of the Roselle petal extract based on the interaction between the extraction temperature and the weight ratio of Roselle petals. The results indicate that increasing both the extraction time and weight ratio while maintaining a constant temperature of 30°C results in a decrease in the pH level of the extract. These interactions provide valuable insights into how different extraction conditions, such as temperature, extraction time, and the weight ratio of Roselle petals to solvent, collectively influence the pH level of the Roselle extract. The results highlight the complex relationships between these variables and their impact on the pH of the extracted solution.

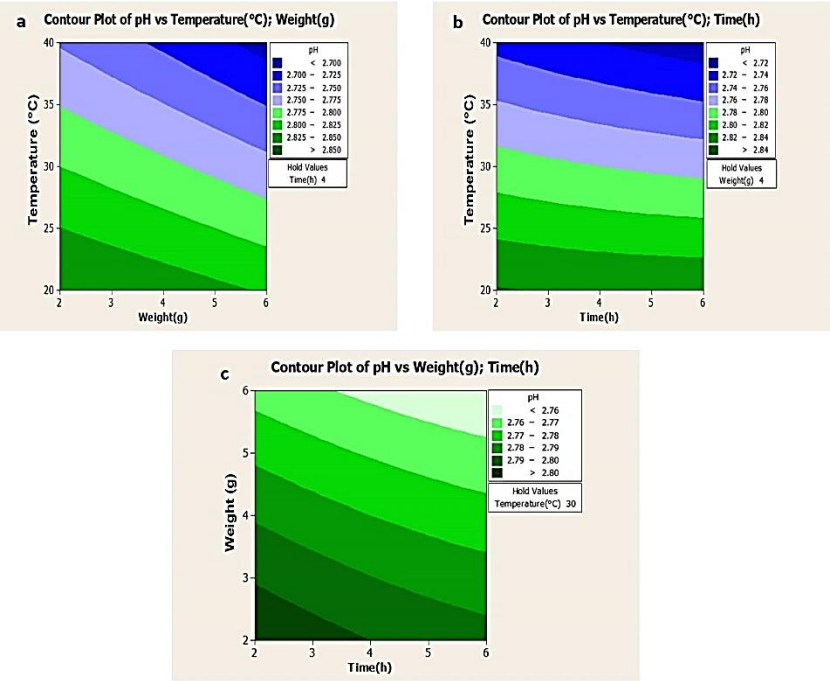

Fig. 1. Interactions of independent variables of different extraction conditions on the pH of Roselle petals. a) The interaction of the weight ratio of the petals to solvent and the extraction temperature, b) The interaction of the extraction temperature and the extraction time, c) The interaction of the weight ratio of the petals to solvent and the extraction time.

3.4.2. Interaction effects of different extraction conditions on the Acidity value of the Roselle extract

The results of the interaction effects of various extraction conditions on the acidity of the Roselle petal extracts are presented in Figure 2. Fig. 2 (b) presents the variations in the acidity of the Roselle petal extract under the interaction effect between the extraction temperature and extraction time. The results indicate that with an increase in both temperature and extraction time, while keeping the weight ratio of Roselle petal to solvent constant at 4 g, the acidity level of the extract increases. In Fig. 2 (c), the changes in the acidity of the Roselle petal extracts are depicted under the extraction conditions of the interaction effect between the weight ratio of the Roselle petal to solvent and the extraction temperature. The findings reveal that as the extraction time and the weight ratio of Roselle petal to solvent increase, the acidity level of the extract also increases, while the extraction temperature is maintained at the central point of 30°C.

3.4.3. Interaction effects of different extraction conditions on the anthocyanin value of the Roselle extract

The results of the surface and interaction effects of different extraction conditions on the anthocyanin content of Roselle extract are presented in Figure 3. In Fig. 3 (a), the changes in anthocyanin content in Roselle petal extracts are shown under the conditions of the interaction effect between the weight ratio of petals to solvent and the extraction temperature. It is observed that with an increase in temperature and weight ratio while maintaining a constant extraction time of 4 hours, there is a significant increase ($p \leq 0.05$) in the amount of anthocyanins. Specifically, an anthocyanin content of 250 mg/l was obtained at 38°C and an extraction time between 5-6 hours. Fig. 3 (b) illustrates the variations in anthocyanin content of Roselle extract under the conditions of the interaction effect between the extraction temperature and extraction time. The results indicate that with an increase in temperature and extraction time while keeping the weight ratio

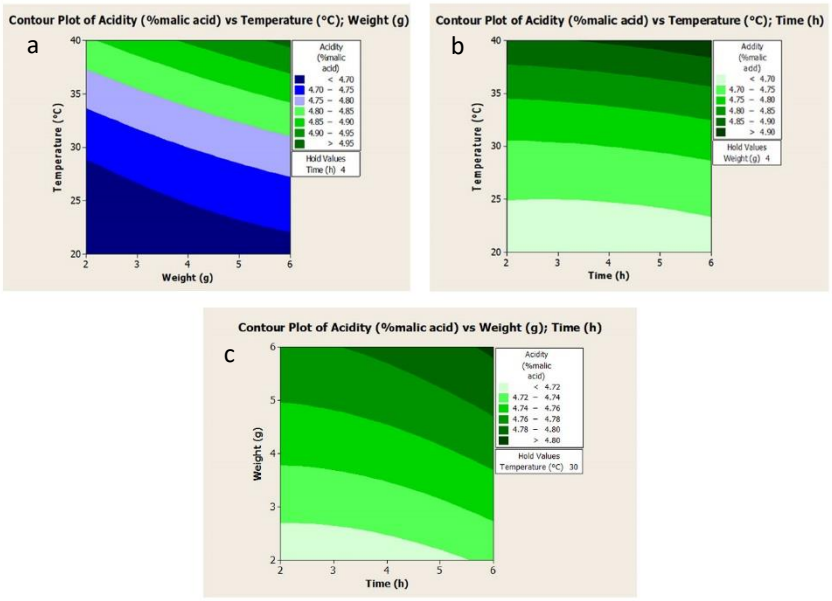

Fig. 2. Interactions of independent variables of different extraction conditions on the acidity of Roselle petals. a) The interaction of the extraction temperature and the weight ratio of the petals to solvent, b) The interaction of the extraction temperature and the extraction time, c) The interaction of the weight ratio of the petals to solvent and the extraction time.

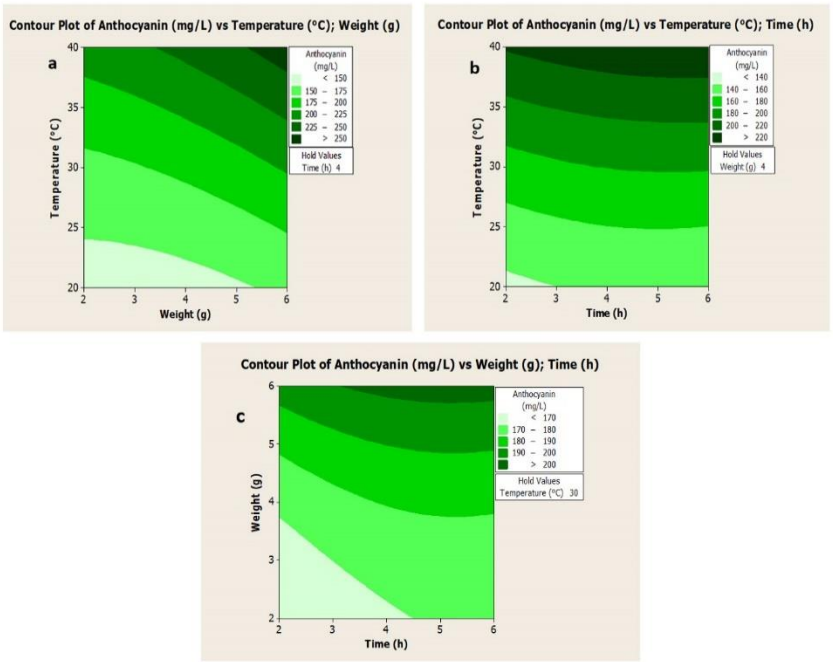

Fig. 3. Interactions of independent variables of different extraction conditions on the anthocyanin content of Roselle petal. a) The interaction of the weight ratio of the petals to solvent and the extraction temperature, b) The interaction of the extraction temperature and the extraction time, c) The interaction of the weight ratio of the petals to solvent and the extraction time

constant at the central point of 4 g, there is a significant increase in the amount of anthocyanins. For instance, an anthocyanin content of approximately 220 mg/l was achieved at 40°C during an extraction time range of 2-6 hours. In Fig. 3 (c), the changes in the anthocyanin content of Roselle extract are demonstrated under the interaction effect between the weight ratio of petals to solvent and the extraction temperature. The findings reveal that an increase in extraction time and weight ratio while maintaining a constant temperature of 30°C leads to a significant increase ($p \leq 0.05$) in anthocyanin content. Specifically, an anthocyanin content of 200 mg/l was obtained within an extraction time range of 3-6 hours and a weight ratio ranging from 5 to 6 g.

3.5. Single optimization

3.5.1.Single optimization of pH value of Roselle petal extracts in different extraction conditions

In order to achieve the minimum pH as optimal conditions for extracting anthocyanins from Roselle petal extract, the extraction time of 6 h, the extraction temperature of 40ºC, and the weight ratio of Roselle petal to solvent of 6 g was determined as optimal conditions. Under these conditions, the pH of Roselle petal extract was predicted to be 2.6787 with 100% desirability. The results are displayed in Figure 4.

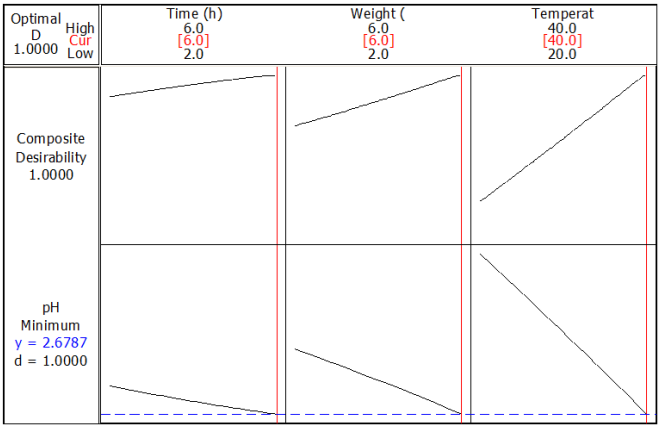

Fig. 4. pH optimization conditions of Roselle petal extract.

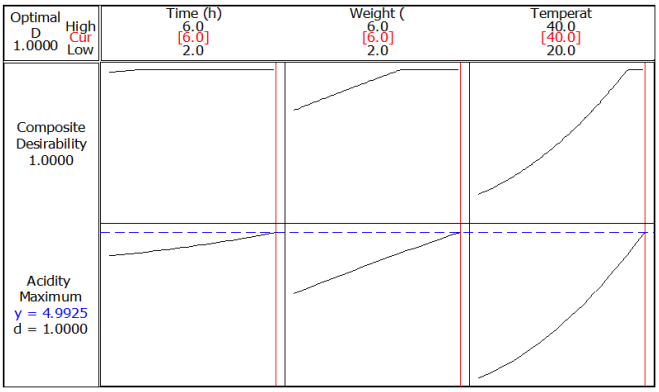

Fig. 5. Acidity optimization conditions of Roselle petal extract.

3.5.2.Single optimization of acidity of Roselle petal extracts in different extraction conditions

To achieve the maximum acidity value as the optimal conditions for extracting anthocyanins from Roselle petal extract, the extraction time of 6 hours, the extraction temperature of 40°C, and the weight ratio of Roselle petal to solvent of 6 g were once again determined as the optimal conditions. Under these conditions, the acidity of the Roselle petal extract was predicted to be 4.9925% with desirability of 100%. The results of the Optimization are presented in Figure 5.

3.5.3.Single optimization of extracted anthocyanin from Roselle petal in different extraction conditions

To achieve the maximum amount of anthocyanins as the optimal conditions for solvent extraction of Roselle petals, an extraction time of 5.71 hours, an extraction temperature of 40°C, and a weight ratio of Roselle petals to solvent of 6 g were determined. Under these conditions, the amount of anthocyanins in the extract was predicted to be 266.19 mg/l with a prediction desirability of 96%. The results of the Optimization are displayed in Figure 6.

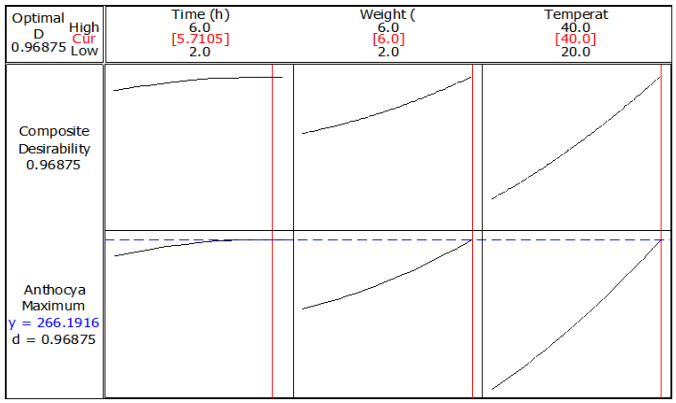

Fig. 6. Anthocyanin optimization conditions of Roselle petal extract.

3.5.3.Simultaneous optimization of pH, acidity, and anthocyanin of Roselle extracts in different extraction conditions

To achieve the minimum pH, maximum acidity, and maximum anthocyanin content as optimal conditions for extraction from Roselle petals, the following parameters were identified: an extraction time of 6 hours, an extraction temperature of 40°C, and a petal-to-solvent weight ratio of 6 g. Under these optimal conditions, the pH of the Roselle petal extract was predicted to be 3.4179, indicating a lower pH value. The acidity of the extract was predicted to be 4.9925, representing a higher acidity level. Additionally, the anthocyanin content was estimated to be 256.0288 mg/l, indicating a higher anthocyanin concentration in the extract. The overall desirability of these optimized conditions was determined to be 94%, suggesting a high level of desirability

for achieving the desired outcomes regarding pH, acidity, and anthocyanin content.

4. Conclusion

Based on the obtained results, it can be concluded that increasing the extraction temperature, extraction time, and petal-to-solvent weight ratio led to an increase in the amount of anthocyanins extracted from Roselle petals. The range of anthocyanin content extracted from Roselle petals varied from 123.85 to 270.49 mg/l. The highest amount of anthocyanins (270.49 mg/l) was obtained under the extraction conditions of 40°C extraction temperature, 4 hours extraction time, and a Roselle petal-to-solvent ratio of 6 g. On the other hand, the lowest anthocyanin yield (132.85 mg/l) was observed at 20°C extraction temperature, 4 hours extraction time, and a Roselle petal-to-solvent ratio of 2 g. These findings highlight that Roselle petals have a high content of anthocyanins, making them a valuable natural source for the food, nutraceutical, and pharmaceutical industries. The potential health-promoting attributes of Roselle petals and their rich anthocyanin content make them a promising alternative to artificial colors.

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