

Evaluation of functional characteristics of roselle seed and its use as a partial replacement of wheat flour in soft bread making

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ARTICLE INFO

Article history:

Received on: July 20, 2022

Accepted on: October 24, 2022

Available online: January 22, 2023

Key words:

Antioxidant activity,
Functional properties,
Nutritional properties,
Roselle seeds,
Soft bread.

ABSTRACT

Roselle seeds (*Hibiscus sabdariffa* L.) are often discarded during processing, while they can be used as a source of nutritional and functional compounds, especially bioactive compounds and dietary fiber. The research was carried out to take advantage of this unused raw material and apply it in soft bread making. Seeds after collection were dried and analyzed for functional characteristics. Roselle seeds flour was then used as a partial replacement of wheat flour in amounts varying from 4.4% to 26.4% (interval 4.4%) in soft bread processing with samples coded from B1 to B6 and the control sample B0 (without the addition of roselle seed flour). The influence of roselle seeds flour on the functional and nutritional properties of soft bread was evaluated. Research results showed that roselle seeds were high in fiber (25.42%) and phenolic compounds (1.8 mg gallic acid equivalent/g). Besides, this powder showed high antioxidant activity, with a value of 38.36% inhibition of 2,2-diphenyl-1-picrylhydrazyl radical. Among the seven bread recipes, recipe B2 (8.8% roselle seed flour) was rated the highest for its sensory properties and was the most preferred by consumers. Besides that, sample B2 showed a higher total fiber content (3.2 g/100 g) than the control sample (0.29 g/100 g). The addition of roselle seed powder in soft bread products resulted in an upward trend in the total phenolic content, which was 1.8 times higher than that of the control sample.

1. INTRODUCTION

The fresh red calyx between the parts of roselle (*Hibiscus sabdariffa* L.) is used mainly in food processing, such as syrup, wine, juicy drinks, and jam, while the dried calyx is used for making tea and spices. Roselle seeds are a by-product of the roselle processing industry; however, which can be recycled as a value-added food supplement providing beneficial bioactive compounds, fats, proteins, and carotenoids [1-4]. Al-Wandawi [5] found that the oxalic acid content in the seed coat was lower than the calyx (44.6%), healthy people can safely consume moderate amounts of oxalic acid. Roselle seeds contain high dietary fiber, with soluble and insoluble fiber ratios ranging from 1.2 to 3.3 [6]; therefore, it may serve as a potential source of functional ingredients. According to the American Dietetic Association, the recommended fiber intake for adults is 25–30 g/day, with a ratio of insoluble to the soluble fiber of 3:1 [7].

Among convenience foods, bread is a popular food all over the world. However, bread is a food with a high glycemic index, so adding a

source of fiber can improve product functionality while maintaining good quality. Instead of being discarded, roselle seeds can be a good supplement in this case. Seeds can be heated after collection [8], ground to a fine powder, and used in value-added products [9]. Substituting roselle seed flour in recipes may reduce gluten content [10]. Several studies have been done to determine how to substitute gluten proteins in baked goods [11] and look at other flours that partially replace wheat flour. It has also been found that combining specific flours with quality has allowed gluten-reduced breads with good properties to be created.

At present, in Vietnam, seeds are only considered waste products and are discarded; there has not been any research to exploit the biological nutritional potential of rose seeds to process value-added products. Adding nutrients from various sources, like roselle seeds in this case, can create new food sources (such as soft bread) with more diverse and complete nutrients. Taking advantage of the nutritional value of wheat and the functional characteristics of roselle seeds incorporated in bread recipes can improve the quality of food products. Recently, roselle seeds have also been applied to cookies, which exhibited improved antioxidant properties [12]. However, there is still limited knowledge on the application of roselle seeds as the source of functional ingredient for soft bread preparation. Therefore, the objective of this study was to determine the physicochemical properties of roselle seeds and propose the application of their flour to the preparation of soft bread.

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2. MATERIALS AND METHODS

2.1. Preparation of Roselle Seeds

The fresh roselle fruits [Figure 1a] were harvested from the farm. The calyx was split to get the seeds [Figure 1b]. The red calyx was kept and used for various processing purposes. Fresh seeds were collected and washed under running water four times. The rinsed seeds were then air-dried by drier (SIBATA, SD-600, Nakane Soka, Japan) at 70°C for 4 h until the moisture content was 7% [13]. The dried seeds [Figure 1c] were ground to a particle size of 0.045 mm using a laboratory mill (Shanghai, China). The finely ground flour was packaged in vacuum-sealed bags and stored in the dark at room temperature (25°C) for further use.

2.2. Preparation of Bread

Wheat flour (Bakers' Choice flour, Interflour company, Vung Tau, Vietnam), salt, eggs, margarine Tuong An (Ho Chi Minh, Vietnam), TH true milk (Vietnam), and Lesaffre - Red Saf-instant Dry Yeast (Marquette-lez-Lille, France) were used in this study.

The volume/mass of some ingredients was constant as 42 g of rapeseed oil, 20 g of sugar, 1 g of salt, 130 g of fresh milk, 5 g of yeast, and 25 g of egg. The total weight of formulation was 437 g. The ingredients were prepared and weighed according to Table 1. The control sample (B0) was made without roselle seed flour, while in the other recipes, a part of wheat flour was replaced with roselle seeds flour from 4.4% to 26.4% (formula B1 to B6). A bread maker (Unold BackMeister Edel 68456, German) with baking temperature of 165°C was applied. Total time of mixing, incubation, and baking was about 3 h and 40 min. The spread factor, moisture content, hardness, color, and sensory properties of each product were analyzed.

2.3. Analysis of Physicochemical Properties, Bioactive Compounds and Antioxidant Activities

All chemicals used for analysis in the present study were of analytical grade.



Figure 1: Fresh fruit (a), roselle calyces and capsule (b) and dried roselle seeds (c).

Table 1: Formulations of soft bread making.

Ingredients (g)	B0	B1	B2	B3	B4	B5	B6
Wheat flour	250	239	228	217	206	195	184
Roselle seeds flour	0	11	22	33	44	55	66

The content of moisture, protein, lipid, carbohydrate, fiber, ash of roselle seeds flour was analyzed by the standard procedure of Association of Official Analytical Chemists [14]. The total phenolics content of the sample was determined according to Waterhouse [15] and Thuy *et al.* [16] and expressed in terms of gallic acid equivalent (mg GAE)/100 g of sample.

Water holding capacity (WHC) was determined according to the method from Rosell *et al.* [17]. Oil holding capacity (OHC) was determined using the modified method of Vazquez- Vazquez-Ovando *et al.* [18]. Water retention capacity (WRC) was determined according to the method from Garau *et al.* [19]. 2,2-diphenyl-1 picrylhydrazyl (DPPH) radical scavenging capacity was determined according to the method of Liu and Yao [20]. Bread firmness was measured using Rheotex, bread loaf was sliced into 2 cm thick slices and placed on the texture analyzer platform. The sliced bread was compressed with a cylindrical probe using 50% strain. The spread factor was determined by the thickness and diameter of the bread [21]. The color of the bread surface was measured using UltraScan Pro HunterLab Color measuring system (HunterLab, Virginia, US).

2.4. Sensory Analysis

The setting of sensory evaluation room and procedure was followed by described of Pasqualone *et al.* [22]. Fifty consumers that were not affected by food allergies or intolerances and were regular consumers of bread samples were involved. They were given information about study aims, and individual written informed consent was obtained from each participant. The consumers were asked to state if they liked or disliked each samples (coded with three-digit random numbers), rating their disliking or liking levels on a structured scale from 1 to 9 (1 = dislike extremely; and 9 = like extremely) for each attributes (appearance, odor, structure, taste, and consumer preference).

2.5. Data Analysis

Data analyses were carried out using STATGRAPHICS Centurion XV (U.S.A.). Values were expressed as mean \pm standard deviation.

3. RESULTS AND DISCUSSION

3.1. Physicochemical and Functional Properties of Roselle Seed Powder

Roselle seeds were dried at 70°C for 4 h, ground, and the physicochemical and functional properties of obtained powder were determined [Table 2]. The moisture content of dried roselle seed powder is about 7%, indicating that the seeds/flour could be stored

Table 2: Physicochemical and functional characteristics of roselle seeds powder.

Parameters, %	Content	Parameters	Content
Moisture content	7.01 \pm 0.135	Total phenolics content (mg GAE/g)	1.80 \pm 0.05
Ash	2.39 \pm 0.125	Antioxidant activity (%)	38.36 \pm 0.007
Carbohydrate	27.38 \pm 0.047	Water holding capacity (g/g)	1.33 \pm 0.04
Protein	18.33 \pm 0.157	Water retention capacity (g/g)	1.51 \pm 0.02
Lipid	19.47 \pm 0.08	Oil holding capacity (g/g)	10.37 \pm 0.15
Fiber	25.42 \pm 0.24		

The value was expressed as mean \pm STD. mg GAE: mg Gallic acid equivalent

for a long period and microbial activity will be prevented, as reported by Ellis *et al.* [23]. The results obtained were slightly higher than in the earlier studies [9-10], which used dry roselle seeds with moisture content in the range of 5.66–6.66%.

Roselle seed powder had a high content of carbohydrates ($27.38 \pm 0.047\%$) and lipids ($19.47 \pm 0.08\%$). It was observed that the major constituent in roselle seeds is represented by carbohydrates; however the analytical data from this experiment is lower than the contents previously published (35.6% and 37.3%) [9,24]. Besides, our obtained results on lipids content are also lower than the studies of Rimamcwe and Chavan [10], who reported the lipid content of 23.80% for Indian roselle seeds.

The average crude fiber in our study was found to be $25.42 \pm 0.24\%$ which was more than the finding of several authors. Mabrouk *et al.* [25] reported that the roselle seeds had crude fiber $18.95 \pm 0.5\%$. Rimamcwe and Chavan [10] found that Indian roselle seeds had fiber source of 19.87%. Roselle seeds have a balanced amount of soluble and insoluble fiber [6], between 1.2 and 3.3, which have physiological effects such as reducing the risk of cardiovascular disease, gastrointestinal disease, colon cancer, glycemic response, and obesity [26].

The results of protein and total ash content of roselle seeds in our study were observed as $18.33 \pm 0.16\%$ (higher than protein of wheat flour, about 13–14.5%) and $2.39 \pm 0.13\%$, respectively, which are lower than protein and total ash content determined by Mabrouk *et al.* [25], $25.87 \pm 0.36\%$ and $5.66 \pm 0.01\%$, respectively. Karma and Chavan [27] also found Indian roselle seeds had good source of proteins (38.06%). The rich source of proteins with high biological values, albumin, and globulins was found in roselle seeds [28]. There is convincing evidence that higher protein intake increases thermogenesis and induces satiety compared with low protein intake [29]. Therefore, a high-protein diet is recommended for weight loss [30]. The different results obtained in the analysis may be due to genetics, variety, environment and ecological conditions. It also depends on the harvesting time of roselle fruit and seed collection.

The total polyphenol content was 1.80 ± 0.05 mg GAE/g with the DPPH radical scavenging activity being $38.36 \pm 0.007\%$. Polyphenols play an extremely important role in the composition of plants because they contribute positively to the antioxidant activities. Dietary polyphenols have also been reported to reduce the rise in postprandial blood glucose levels, suggesting an inhibition of starch digestion in the body [31].

The WHC of roselle seed measured (1.334 ± 0.042 g/g) is lower than that wheat flour (1.58 ± 0.08 g/g). Differences in protein structure and hydrophilic carbohydrates contribute to the variation in the WHC of flour. The fiber in roselle seeds affects WRC (1.511 ± 0.019 g/g) and WHC (1.334 ± 0.042 g/g), both indexes of roselle seeds/flour are relatively low due to the lower number of free hydroxyl groups [32].

The OHC of food materials may be related to the overall charge density and hydrophobic character of the grain. Alterations in the presence of nonpolar side chains in powders, which are linked to the hydrocarbon side chains of oils, altered the OHC of the powders [33]. In this study, OHC of roselle seeds (10.37 ± 0.15 g/g) was significantly different between wheat flour ($0.7 \pm 0.03\%$) [34]. Food ingredients with high WHC and OHC can act as functional ingredients. In addition, high-OHC food materials can play an important role in stabilizing high-fat food systems and can act as emulsifiers [35]. In soft bread making,

emulsifiers are used mainly to enhance gluten-gluten interactions, promote the formation of protein-starch complexes and improve aeration. The high OHC of hibiscus seed powder suggests that it can be used as a fiber-rich ingredient in foods to help reduce the amount of fat and cholesterol absorbed by the body. Therefore, high OHC roselle seed flour can be an alternative emulsifier for soft bread in all designed recipes.

3.2. Processing Soft Bread With Roselle Seed Flour Adding

Bread is among the oldest and most consumed foods in the world [36]. However, food companies pursue the search for new ingredients for preparing new products with improved nutritional and health properties. As mentioned, partial replacement of wheat flour with roselle seeds flour in different proportions has also been carried out.

3.3. Physical Properties of Soft Bread with Roselle Seed Flour Adding

Figure 2 shows the color of the missed dough (during incubation) according to the formulas as shown in Table 1 (in experimental design). The physical properties of breads are shown in Table 3. The moisture content of samples had significantly difference, the lowest moisture content was found in sample of formula B6 ($17.1 \pm 0.03\%$) and the highest was obtained in sample of formula B0 ($44.7 \pm 1.3\%$).

The storage stability of food products is based on the water activity in the finished product. Low water activity can prevent microbial activity, lipid oxidation, and enzymatic reactions during food product preservation. The analysis results showed that the water activity showed a significant difference ($P \leq 0.05$) between the samples. The control sample (using all wheat flour) showed the highest water activity and gradually decreased as the percentage of hibiscus seed powder gradually increased in the different formulations investigated. From sample B2 onwards showed lower water activity value than control sample. Probably due to the presence of the seed coat that acts as a humectant in the samples that were substituted with roselle seeds flour in different proportions. Beuchat *et al.* [37] reported that microorganisms can grow at the minimum a_w of 0.60. The lowest a_w for various food borne bacteria growth is about 0.87, except several halophilic bacteria can grow at lower a_w of 0.75.

The spread factor of soft bread decreased from B0 sample (42.6 ± 0.9) to B6 (29.6 ± 0.5), the data for other samples ranges between B0 and B6. The lowest spread factor is shown in two samples B5 and B6. Increasing the amount of roselle seed flour in the preparation of soft bread was reduced the spread factor of the sample. The hardness of bread supplemented with 4.4% to 26.4% roselle seed meal was shown

Table 3: Physical characteristics of bread products prepared by different recipes.

Parameters	Formulas						
	B0	B1	B2	B3	B4	B5	B6
Water activity	0.9 ^a	0.9 ^a	0.8 ^b	0.8 ^b	0.7 ^c	0.7 ^c	0.7 ^c
Moisture content (%)	44.7 ^a	39.1 ^b	30.0 ^c	25.5 ^d	23.8 ^e	19.4 ^f	17.1 ^g
Spread factor	42.6 ^a	40.6 ^b	39.5 ^c	38.1 ^d	31.6 ^e	30.4 ^f	29.6 ^f
Hardness (g force)	841 ^a	789 ^b	738 ^c	695 ^d	651.7 ^e	629 ^f	503.7 ^g
L*	66.3 ^a	62.0 ^b	58.5 ^c	56.8 ^d	54.4 ^e	52.3 ^e	49.8 ^f
a*	7.3 ^a	6.3 ^b	5.8 ^c	5.5 ^d	5.4 ^{de}	5.2 ^e	4.7 ^f
b*	21.5 ^a	20.4 ^b	18.1 ^c	17.8 ^c	16.1 ^d	14.7 ^e	13.8 ^f

Mean values with different superscripts in the same row differ significantly at $P \leq 0.05$.

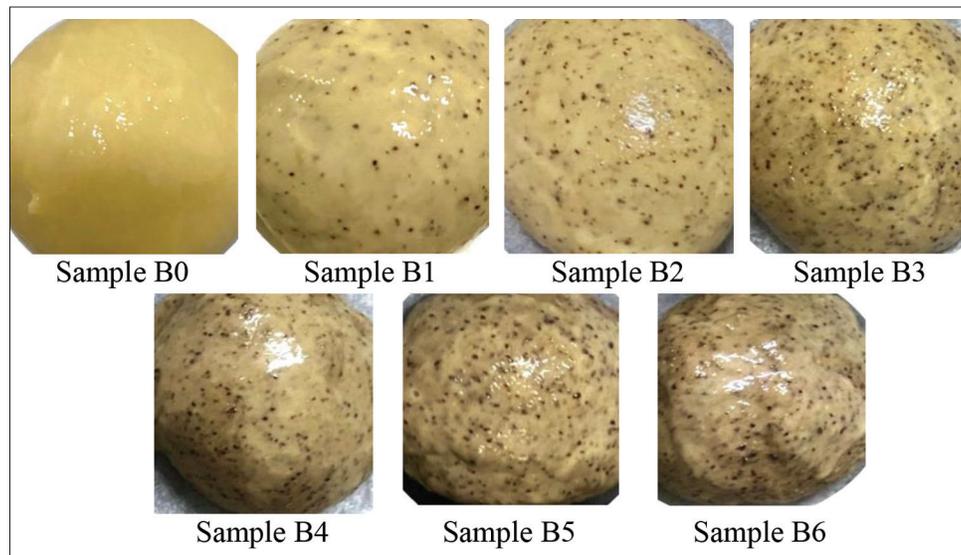


Figure 2: The dough is tempered before baking with different roselle seed flour replaced.

to be significantly different from that of the control sample (100% wheat flour dough).

The hardness of the bread is clearly affected by the amount of roselle seed flour substituted in bread recipes, perhaps because roselle's WHC significantly affects the firmness of soft bread. Roselle seed flour added to bread caused the dough to have a low water content, so it will not create an expanded gluten structure and results in even softer bread [38]. This yielded good results in soft bread making in this case of study.

The color measurement resulted in Table 3 was shown to be different significantly in lightness (L^*), redness (a^*), and yellowness (b^*) values for the different added roselle seed flours studied. In this study, wheat flour was found to be significantly different in L^* value (66.3 ± 0.4) as compared to the other samples, indicating that wheat flour has the lightest color [Figure 2]. Our results are higher than results obtained by several authors [34,39] who reported that the wheat flour utilized in their studies had an L^* value of 92.6 and 91.43, respectively. This is probably due to the different degree of milling of the flour used. Wheat flour is generally considered white due to all brans have been removed. Increasing the added roselle seeds flour, L^* values significantly lower ($P \leq 0.05$) than wheat flour, indicating an increased darkness in the color of the dough, this might be due to the nature of the roselle seeds flour with the seed coat does not separate completely during the flour making process.

3.4. Sensory Evaluation

Sensory value is also an important indicator that directly affects consumers' decision to choose products [40-43]. Sensory evaluation by hedonic test was performed with seven (07) samples (01 control sample and 06 samples were replaced with different percent of roselle seeds flour) to assess consumer acceptance of the attributes [Table 4].

The appearance of B2 sample (8.8% roselle seeds flour replaced) was the most preferred by the panelists, followed by B1, B0, and B3. The color of B6 is the worst because it is very dark after baking because of the high percentage of roselle seeds flour used in the recipe. The aroma of samples B2 and B3 was the most preferred by the panelists. It was found that the flavor of soft bread was improved with the

incorporation of roselle seed powder, since the flavor of roselle seeds closely resembled that of cranberries [9]; therefore, the flavor of soft bread has been enhanced, more fragrant after incorporating the amount of roselle seeds flour. In addition, bread with a moderately soft texture have also received a high sensory rating and are preferred over softer or harder samples, samples B2 and B3 have been highly evaluated according to this criterion. Overall organoleptic evaluation showed that the B2 sample [Figure 3] scored the highest of all recipes, showing 8.8% of the combined roselle seed flour/wheat flour replacement as the preferred level in soft bread making.

The preference mapping also showed similar results [Figure 4]. Bread samples were evaluated according to preference scores. The results from the preference mapping again confirmed that formula B1, B2 is the most preferred by consumers (80–100%). Sample B3 also received a relatively high preference (60–80%), while sample B0 received only moderate preference (40–60%). Samples with the remaining formulations (B4, B5, and B6) received the lowest acceptance from consumer.

3.5. Comparison of Physicochemical and Functional Characteristics of Two Bread Samples B2 and B0

Two product samples, the control sample (B0) and the most favored bread sample (B2) in the Hedonic test were selected for analysis of physicochemical and functional components, along with antioxidant activity. The results are presented in Table 5. Partial replacement of wheat flour with hibiscus seed meal significantly affected the quality of soft bread and resulted in products with outstanding characteristics. The bread sample with the recipe using 8.8% roselle seeds flour (B2) showed a significant difference ($P \leq 0.05$) compared with the control sample (B0). In particular, the total fiber content was 11 times higher than that of the control sample. Other nutritional and functional components increased from 1.04, 1.65, and 1.66 times, respectively, with DPPH%, protein, and polyphenols.

Besides, the carbohydrate content of sample B2 (selected sample) was lower than the control sample, while the lipid content was higher. Mohamed *et al.* [44] announced roselle seed oil belongs to the linoleic/oleic category, its most abundant fatty acids being C18:2 (40.1%) and C18:1 (28%). Therefore, with this increasing and decreasing trend, the B2 soft bread product has significantly improved its quality and is good for human health when consumed.

Table 4: Sensory evaluation scores of bread samples with different percentages of roselle seed flour.

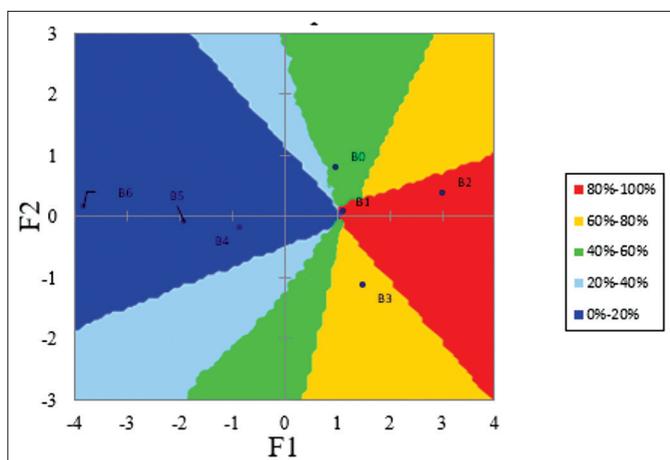
Sensory characteristics	B0	B1	B2	B3	B4	B5	B6
Appearance	6.9 ^a ±0.5	7.7 ^b ±0.5	8.5 ^a ±0.5	6.7 ^c ±0.5	5.6 ^d ±0.5	4.6 ^e ±0.3	3.1 ^f ±1.2
Odor	6.8 ^b ±0.7	7.4 ^c ±0.4	8.2 ^a ±0.7	7.8 ^d ±0.5	6.1 ^e ±0.4	5.1 ^e ±0.4	3.9 ^f ±0.2
Structure	7.3 ^a ±0.6	6.0 ^b ±0.4	8.1 ^a ±0.6	5.0 ^d ±0.2	4.5 ^e ±0.4	4.0 ^e ±0.1	3.0 ^f ±0.1
Taste	7.0 ^b ±0.7	7.1 ^b ±0.5	8.3 ^a ±0.7	8.3 ^a ±0.7	6.2 ^c ±0.4	5.7 ^c ±1.1	4.6 ^d ±0.3
Consumer preference	7.0 ^b ±0.1	7.1 ^b ±0.1	8.2 ^a ±0.2	8 ^a ±0.3	6.4 ^c ±0.2	5.9 ^c ±0.3	4.6 ^d ±0.2

Data are expressed as mean±standard deviation. Mean values with different superscripts in the same row differ significantly at $P \leq 0.05$.

Table 5: Nutritional value and antioxidant activity of soft bread produced from formula B0 (control sample) and B2 (8.8% roselle seeds flour).

Parameters	Formula B0	Formula B2
Protein (%)	6.72 ^a ±0.33	11.11 ^b ±0.15
Total fiber (%)	0.29 ^a ±0.01	3.2 ^b ±0.13
Ash (%)	0.65 ^a ±0.2	0.72 ^b ±0.4
Carbohydrate (%)	77.70 ^b ±0.16	66.8 ^a ±0.15
Lipid (%)	12.75 ^a ±0.34	15.64 ^b ±0.2
DPPH (%)	69.6 ^a ±0.39	72.1 ^b ±0.2
Total phenolic (mg GAE/g)	1.95 ^a ±0.58	3.53 ^b ±0.3

Data are expressed as mean±standard deviation. Mean values with different superscripts in the same column differ significantly at $P \leq 0.05$. DPPH: 2,2-diphenyl-1-picrylhydrazyl, mg GAE: mg Gallic acid equivalent

**Figure 3:** Bread has been replaced with 8.8% wheat flour with roselle seed flour (formula B2).**Figure 4:** Preference mapping of soft bread with different formulas.

4. CONCLUSION

Roselle seeds contain highly nutritious and functional components, which can be used and developed as a functional ingredient in food products that are useful for human consumption. With the selected B2 recipe (8.8% roselle seeds flour), the soft bread product produced has

shown its superiority in quality. Bread that has been partially replaced with roselle seed flour has improved fiber and antioxidant properties. It was observed that roselle seeds can be used as a value-added food ingredient with potential health-promoting properties, in addition to providing a solution to the environmental problems associated with roselle seed by-products processing.

5. ACKNOWLEDGMENTS

The authors acknowledge the financial support to Can Tho University, Vietnam, from the Research fund project number TSV2021-46.

6. AUTHORS' CONTRIBUTIONS

Conceptualization, methodology, validation, writing-review, and editing: NMT and NVT; software, VQT and NVT; formal analysis, NBT, DGC, HKD, and LTA; investigation, NBT, DGC, HKD, LTA, VQT, TNG and NVT; writing-original draft preparation, NMT, VQT, NVT; supervision, NMT. All authors have read and agreed to the published version of the manuscript.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

10. PUBLISHER'S NOTE

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How to cite this article:

Thuy NM, Tran NB, Cuong DG, Duy HK, An LT, Tien VQ, Giao TN, Tai NV. Evaluation of functional characteristics of roselle seed and its use as a partial replacement of wheat flour in soft bread making. J App Biol Biotech. 2023;11(2):238-243. DOI: 10.7324/JABB.2023.110226