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Use of Roselle Calyx Wastes for the Enrichment of Biscuits: An Approach to Improve Their Functionality

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Abstract: The objective of the present study was to evaluate the use of powder made out of Roselle Calyx Wastes (RCP) in developing a biscuit formulation with acceptable sensory value. Roselle calyxes were infused in water in a 1:10 ratio. The residual infused calyxes were dried at 50 °C for 16 h, grounded, sieved through a 50 mesh, and stored in plastic bags until used. The biscuit formulations were enriched with RCP at 0% (BC), 5% (BRCP5), 10% (BRCP10), and 15% (BRCP15). The amount of RCP added to the biscuit formulation did not change the protein content. However, the addition of RCP significantly affected the biscuit's color; the lightness parameter (L*) decreased as the RP content increased from 69.66 to 49.04. The sensory evaluation showed that the control biscuit and the biscuit enriched with 5% of RP were the best accepted. As for the antiradical activity, the formulation with the highest activity was presented by the BRCP15 (587.43 µmol Trolox/100 g dwb). On the other hand, BRCP5 presented 189.96 µmol Trolox/100 g dwb. Therefore, the biscuit formulation with RCP at a 15% enrichment could be used to commercialize a functional product.

Keywords: biscuit; *hibiscus sabdariffa*; DPPH; digestibility

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1. Introduction

Many studies have been conducted to research the use of new nontraditional raw materials in food technologies to increase their nutritional value. The companies develop and create confectionery products for specialized, dietary, therapeutic, and protective diet purposes [1–3]. In addition to being a food widely consumed in the world by people of all ages, biscuits are a low-moisture food, which means that the shelf life of these products is long. Moreover, biscuits can be easily added with different unconventional ingredients to improve sensory characteristics or nutritional content, ingredients such as fruits and fruit byproducts [4], vegetables such as eggplant [5], edible flowers such as roses [6], and even some edible insects [7,8].

Roselle (*Hibiscus sabdariffa* L.) is a member of the Malvaceae family. It is widely distributed and cultivated in tropical and subtropical regions worldwide. Many parts of Roselle, including seeds, leaves, and roots, are utilized in different foods. Calyces are the most popular [9]. Calyces are consumed as a beneficial drink by infusing them and served cold or hot. Roselle has also been integrated into diverse applications, including foods, cosmetics, and pharmaceuticals. [10]. Roselle calyces are rich in vitamin C and other antioxidants such as anthocyanin, flavonoids, and phenols, which are hypothesized to be beneficial to human health, for example, preventing cancer and reducing chronic illnesses such as diabetes, dyslipidemia, high blood pressure, and coronary heart diseases [9].

Recently, Roselle has been used to prepare functional foods. Saleh et al. (2018) evaluated several biscuit models with a mixture of two or three natural supplements: pumpkin, yellow corn germ, white kidney bean, rice bran, and Roselle calyces; Roselle powder was added at a concentration of 2, 5, and 6%. The mixture formulae were analyzed by sensory analysis, texture analysis, chemical composition, and color [11]. El-Sayed et al. (2019) evaluated the effect of replacing wheat flour with roselle powder, either from roselle seed cake (0, 10, 20, and 30%) or roselle leaves (0, 2.5, 5, and 7.5%), on the nutritional and quality properties of biscuits. The obtained results proved that incorporating roselle cake or leaf powder in the biscuit formula improved the biscuits' nutritional profile and physical characteristics. Sensory evaluation revealed that the best replacement ratio was 10% of roselle cake and 2.5% for roselle leaves [12]. Marak et al. (2022) developed a Roselle muffin and assessed its acceptability, nutrition, and shelf life. According to the findings, the combination of roselle calyx extract volume of 45.37 mL, citric acid 1.11 g, and sodium bicarbonate 1.67 g produces the best muffin, with the panelist's sensory scores reaching up to 84% [13].

The infused calyces of roselle are considered agro-industrial waste. However, they still possess beneficial properties such as antioxidant activity, similar to the non-infused calyces. Typically, these calyces are thrown away despite the multiple therapeutic effects and could be used as an unconventional ingredient. For this reason, this work aimed to use roselle's infused calyces as a source of bioactive compounds to incorporate them into a bakery product. A different percentage of roselle calyx powder was substituted in a biscuit formulation to evaluate the biscuits' physical parameters, chemical composition, antioxidant activity, and in vitro digestibility.

2. Materials and Methods

2.1. Raw Material

Calyces of Roselle (*Hibiscus sabdariffa* L.) from the Tecoanapa variety were employed to prepare the infused Roselle Calyx Powder (RCP). First, 100 g of roselle calyces were mixed with distilled water in a ratio of (1:10) and infused by heating to boiling for 30 min. Next, the roselle extract was removed by filtration. The roselle calyces were subjected to a dehydration process in an oven at 55 °C for 24 h; once dehydrated, the dried calyces were pulverized in a coffee grinder and sieved using a mesh with an opening of 0.02 mm. It was kept in a hermetically sealed plastic bag to avoid any contamination at ambient temperature (30 °C).

2.2. Biscuit Preparation

Biscuit formulations were prepared with butter (Gloria, Cremería Americana S.A. de C.V., Ciudad de México, México), sucrose (Zulka, Zucarmex, S.A. de C.V., Sinaloa, México), eggs (San Juan, Proteina Animal S.A. de C.V., Jalisco, México), powdered milk, and wheat flour (Selecta, Molinera de México S.A. de C.V., Estado de México, México). In addition, different concentrations of RCP were added to the biscuit formulation, according to Table 1. The procedure included the following actions. First, the butter was beaten at room temperature until a creamy consistency was achieved. Next, the sugar was incorporated with butter, followed by the egg. Additionally, the powdered milk, wheat flour, and RCP were mixed until a paste was formed. This paste was laminated and refrigerated for 30 min at −14 °C to maintain its consistency. It was later cut into 4 cm diameter circles and baked for 10 min at 180 °C. Finally, the biscuits (Figure 1) were stored in plastic bags until further use.

Table 1. Formulation of biscuits enriched with roselle calyx powder.

Sample	Wheat Flour (g)	Roselle Calyx Powder (g)	Egg (piece)	Butter (g)	Powdered Milk (g)	Sucrose (g)
BC	100	0	1	60	5	60
BRCP5	95	5	1	60	5	60
BRCP10	90	10	1	60	5	60
BRCP15	85	15	1	60	5	60

BC: Control Biscuit; BRCP5: Biscuit enriched with 5% of roselle calyx powder; BRCP10: Biscuit enriched with 10% of roselle calyx powder; BRP15: Biscuit enriched with 15% of roselle calyx powder.

BC BRCP5 BRCP10 BRCP15

Figure 1. Biscuit enriched with infused roselle calyx powder.

2.3. Physical Parameters of Biscuits

The physical parameters of the biscuits were determined using a digital vernier. The length (diameter) and thickness (width) were expressed as the mean and standard deviation of 10 measurements. The results were described in cm.

The color evaluation was investigated with tristimulus colorimetry. For this purpose, a colorimeter X-Rite Ci62 (X-Rite Inc., Grandville, MI, USA) was used. The colorimeter was calibrated at L = +78.4, a = −1.8, and b = +25 at 10° from the observer. The parameters recorded were L* (black–white), a* (green–red), and b* (blue–yellow). The Chroma parameter (C*) represents the degree of saturation or fullness of color. The Hue (H°) parameter is an angular position around a point in a color space co-ordinate diagram. The L*, a*, and b* values were used to calculate the C* and H°, using the following formulae:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$h^\circ = \tan h^{-1}\left\{\frac{b^*}{a^*}\right\}$$

2.4. Chemical Composition of Biscuits

The proximal analysis of the different biscuits was carried out. It included the following studies: Moisture was determined gravimetrically, through the official method 44-16 of the AACC (2000) [14]. 2 g of sample were weighed and dried in an oven at 130 ± 3 °C for 1 h. Protein content was determined by the official method 46-13 [14]. It was indirectly determined by quantifying total nitrogen using the Kjeldahl method, with a conversion factor of 5.85. Ash content was quantified by the official method 08-01 [14] by calculating the weight loss of 3 g of the sample after incineration at 600 °C in a muffle. Lipid content was determined by the official method 30-25, AOAC (2012) [15]. Finally, total starch analysis was determined using an enzymatic kit (Megazyme International, Co., Wicklow, Ireland), following the AOAC official method 76-13 [15].

2.5. Total Phenolic Content and Antioxidant Capacity of Biscuits

An aqueous extract was prepared, consisting of biscuit weighing 0.5 g, homogenized with 10 mL of methanol (MeOH 70%). The mixture was put under constant stirring for 1 h. Then, the solution was centrifuged at 11,000 rpm for 5 min at 25 °C, and the supernatant was stored. Next, the sediment was resuspended with 10 mL of MeOH (MeOH 70%) and put under constant stirring for another hour, then centrifuged again. This step was repeated one more time, completing a final volume of 30 mL. The supernatant was used for the following analysis.

2.5.1. Total Phenolic Content of Biscuits

The Total Phenolic Content (TPC) in the methanolic extract was determined via the Folin–Ciocalteu method [16], using gallic acid as the standard. The reaction was carried out with 0.1 mL of the extract, 0.1 mL of Folin–Ciocalteu reagent diluted (1:2) in distilled water, 2 mL of a 7.5% solution of Na_2CO_3, and 2.8 mL of distilled water. The absorbance was measured at 750 nm. The TPC was expressed as the gallic acid equivalent (GAE) per mass of dry biscuit (mg of gallic acid equivalent (GAE)/g of Biscuit).

2.5.2. Trolox Equivalent Antioxidant Capacity of Biscuits

The Trolox Equivalent Antioxidant Capacity (TEAC) analysis was evaluated following the methodology described by Brand-Williams et al. [17]. The extract samples were analyzed in terms of the hydrogen-donating or radical-scavenging ability of the stable DPPH radical. Briefly, the reaction mixture contained 0.1 mL of the extract and 3.9 mL of DPPH. The absorbance of the reaction mixture was measured at 515 nm against a blank sample containing only methanol. The results were expressed in terms of the mass of Trolox per mass of dried biscuit (mg of Trolox/g biscuit). The total equivalent values were calculated using the standard curve of Trolox.

2.5.3. Ferric Reducing Antioxidant Power of Biscuits

The Ferric Reducing Antioxidant Power (FRAP) of extracts was analyzed according to the methodology [18]. The method is based on reducing the Fe3+-TPTZ (2,4,6-tripyridyl-s-triazine) complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 593 nm. Briefly, 3 mL of working FRAP reagent, prepared daily, was mixed with 100 µL of biscuit extract; the absorbance at 593 nm was recorded after a 15 min incubation at 37 °C. FRAP values were obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Trolox and expressed as mmol of Trolox equivalents per 100 dwb biscuit.

2.6. Free Glucose and Starch Fractions of Biscuits

The digestion properties of biscuits were analyzed using the Englyst test with some modifications [19]. First, 200 mg of biscuit was mixed with 2 mL of deionized water, then 4 mL of pepsin/guar gum/hydrochloric acid solution was added and incubated for 30 min at 37 °C. Subsequently, 2 mL of sodium acetate buffer was added and placed in a water bath at 37 °C, stirring at 160 rpm for 20 min. Finally, 2 mL of an enzymatic mixture of pancreatin, amyloglucosidase, and invertase were added at intervals of 1 min per sample. Next, at 0, 20, and 120 min, 50 µL of digestion solution was removed and placed in microcentrifuge tubes containing 950 µL of 80% ethanol. Next, the microtubes were centrifuged at 11,000 rpm for 5 min. After that, 50 µL of the supernatant was analyzed for glucose content by the D-Glucose (GOPOD Format) Assay Kit (Megazyme International Ltd., Bray, Ireland), and the percentage of digested starch was calculated as follows:

$$\mathrm{FG}\ (\%) = \left(\frac{\text{weight of released glucose (mg) at 0 min}}{\text{weight of biscuit (mg)}}\right) \times (0.9) \times (100)$$

$$\text{RDS } (\%) = \left(\frac{\text{weight of released glucose (mg)at 20 min}}{\text{weight of biscuit (mg)}} \right) \times (0.9) \times (100) - \text{FG}$$

$$\text{SDS } (\%) = \left(\frac{\text{weight of released glucose (mg)at 120 min}}{\text{weight of biscuit (mg)}} \right) \times (0.9) \times (100) - \text{RDS} - \text{FG}$$

where 0.9 is the ratio of the molecular weight of the anhydroglucose monomer unit in starch to that of glucose. FG is the amount of free glucose; RDS is the rapidly digestible starch fraction; and SDS is the slowly digestible starch fraction. Each sample was analyzed in triplicate.

2.7. Sensory Evaluation of Biscuits

For the sensory evaluation, 70 voluntary untrained subjects supported the analysis. Each individual was given a plate with a random biscuit (BC, BRCP5, BRCP10, and BRCP15). Every sample was coded with a five-digit code; among samples, crackers and plain water was used to avoid mixing the flavors in the mouth. The panelists answered anonymously a questionnaire where they evaluated the overall acceptance of biscuit samples using a 5-point hedonic scale, where 1 = dislike very much, 3 = neither like nor dislike, and 5 = like very much.

2.8. Statistic Analysis

For the statistical analysis of the results, the Minitab17 package was used. A one-way analysis of variance (ANOVA) was performed. Where differences between the means were found, a Tukey's test was conducted to compare means with a significance level of $p = 0.05$ and establish differences between the samples.

3. Results and Discussion

3.1. Physical Parameters of Biscuits

Table 2 shows the physical parameters and color of biscuits added with infused roselle calyx powder. Diameter and thickness decreased significantly with the addition of RCP. This effect could be related to the dilution of gluten and the interaction of gluten with RCP fiber resulting in less gas retention ability during baking [20]. This result is in line with a report on biscuits enriched with other ingredients such as fruit waste and insect powder, where the diameter and thickness decreased [21,22].

Table 2. Physical parameters and color of biscuits.

Sample	Diameter (cm)	Thickness (cm)	L*	a*	b*	C*	h°
BC	4.76 ± 0.02^{a}	0.79 ± 0.01^{a}	69.66 ± 0.66^{a}	4.16 ± 0.36^{a}	27.02 ± 0.53^{a}	27.34 ± 0.57^{a}	1.42 ± 0.01^{a}
BRCP5	4.55 ± 0.69^{b}	0.69 ± 0.02^{b}	53.75 ± 1.33^{b}	3.71 ± 0.20^{b}	16.07 ± 0.24^{b}	16.50 ± 0.27^{b}	1.34 ± 0.01^{b}
BRCP10	$4.52 \pm 0.03^{b,c}$	0.62 ± 0.02^{c}	$51.13 \pm 1.45^{b,c}$	5.46 ± 0.22^{c}	$15.41 \pm 0.90^{b,c}$	16.35 ± 0.84^{b}	1.23 ± 0.03^{c}
BRCP15	4.37 ± 0.04^{c}	0.51 ± 0.01^{c}	49.04 ± 0.55^{c}	6.29 ± 0.22^{c}	14.23 ± 0.24^{c}	15.56 ± 0.21^{b}	1.15 ± 0.02^{d}

Results are presented as means ± SD ($n = 3$) on dry weight basis. Values followed by the different letters in the same line are significantly difference in the Tukey's test ($p < 0.05$). BC = Biscuit Control; BRCP5 = Biscuit added with 5% of roselle calyx powder; BRCP10 = Biscuit added with 10% of roselle calyx powder; BRCP15 = Biscuit added with 15% of roselle calyx powder. L*: lightness from black (0) to white (100); a*:green-red colour (a* > 0 indicates redness, a* < 0 indicates greenness); b*: blue-yellow colour (b* > 0 indicates yellowness, b* < 0 indicates blueness).

The CIE-LAB color coordinates L*, a*, and b*, and the calculated parameters C* and h° shows that the lightness of biscuits decreased significantly ($p = 0.05$) with the addition of RCP in the formulations. Lightness was found in the interval value of 69.66 for the control biscuit (BC) and 49.04 for the biscuit added with 15% (BRCP15). Generally, the luminosity is negatively affected when there is an incorporation of alternative ingredients to the traditional formulation of bakery products. Authors reported this behavior using alternative ingredients to prepare cookies, such as apple fiber, wheat bran, and tagose [20,23,24].

BRCP15 presented a higher a* value (6.29) and a lower b* value (14.23) than all the biscuit formulations, demonstrating that with a higher concentration of RCP in the biscuit formulations, the final product will present red tones and a tendency towards blue tones. The chroma parameter that indicated the color saturation decreased significantly with the increasing RCP concentration. On the other hand, the hue angle 0° or 360° represents red color, 90° represents yellow, 180° green, and 270° the color blue. All the biscuits had hue values less than 90°, indicating that they had red color in the CIE-LAB color space. The flavonoid and anthocyanins are pigments in the roselle calyx [25]; these pigments might be responsible for those values.

3.2. Chemical Composition of Biscuits

The proximate composition of biscuits formulated with infused roselle calyx powder as a partial substitute for wheat flour at three different levels is presented in Table 3. RCP had a significant ($p < 0.05$) effect on the moisture content of the biscuits compared to the control, with a decrease in moisture content from 6.45 to 3.85 g/100 g of biscuit. Similar results were reported for biscuits added with flaxseed flour [26]. These authors found that as the flaxseed flour concentration increased in the formulation, the moisture content of cookies decreased; those results were attributed to the low moisture of flaxseed flour. Biscuits are foods that are characterized by low moisture levels. During baking, water evaporates from the surface of the dough. In contrast, in the center of the dough, moisture condenses, causing changes in the moisture content. Moisture found in the BC and those added with RCP varied significantly due to the addition of a low-moisture ingredient. Reduced moisture content is valuable to the shelf life of biscuits because most unwanted microbes might struggle to survive [27]. Table 3 also showed a significant ($p < 0.05$) reduction in starch content from 39.65 to 37.67 g/100 g. Total starch content is essential because it is a source of glucose released during digestion. In foods where the starch is highly gelatinized, and its structure is very porous, rapid degradation is generated in the small intestine, producing a fast glycemic response.

Table 3. Proximal composition of biscuits.

Sample	Moisture (g/100 g)	Ash (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Total Starch (g/100 g)	Carbohydrates (g/100 g)
BC	6.45 ± 0.18^{a}	0.68 ± 0.02^{a}	23.7 ± 0.51^{a}	10.33 ± 0.16^{a}	39.65 ± 0.16^{a}	65.29 ± 0.00
BRCP5	6.01 ± 0.08^{b}	0.73 ± 0.01^{a}	23.3 ± 0.62^{a}	10.48 ± 0.95^{a}	39.47 ± 0.27^{a}	65.49 ± 0.00
BRCP10	4.67 ± 0.17^{c}	0.73 ± 0.02^{a}	23.9 ± 0.48^{a}	10.50 ± 0.13^{a}	38.99 ± 0.14^{b}	64.87 ± 0.00
BRCP15	3.85 ± 0.15^{d}	0.70 ± 0.05^{a}	23.5 ± 0.49^{a}	10.55 ± 0.16^{a}	37.67 ± 0.25^{c}	65.29 ± 0.00

Results are presented as means ± SD (n = 3) on dry weight basis. Values followed by the different letters in the same line are significantly difference in the Tukey´s test ($p < 0.05$). BC = Biscuit control; BRCP5 = Biscuit added with 5% of roselle calyx powder; BRCP10 = Biscuit added with 10% of roselle calyx powder; BRCP15 = Biscuit added with 15% of roselle calyx powder.

In contrast, the protein, ash, fat, and carbohydrate content did not present a significant effect due to the addition of RCP. The protein content in biscuits added with RCP was higher than those reported by [26] for biscuits added with flaxseed flour but remains similar to those reported by [6] for biscuits added with rose petals. In our investigation, we used eggs and powdered milk, accounting for the higher protein content. It is no surprise that most proximal analyses remained the same since the roselle calyx powder has been reported to have similar values [28] to wheat flour.

3.3. Total Phenolic Content and Antioxidant Capacity of Biscuits

The total phenolic compound (TPC) of biscuits enriched with roselle calyx powder was measured; the contents are listed in Table 4. All the biscuit formulations showed higher TPC levels above the biscuit control (BC). TPC contents of the biscuits significantly increased with the addition of the infused roselle calyx powder, from 3.6 mg GAE/100 g dwb to 76.2 mg GAE/100 g dwb in the BRCP15 sample. For instance, the TPC of infused roselle calyx powder was around 672.9 mg GAE/100 g dwb. This value represents ninefold the value of the BRCP15. Najjar et al. [29] studied the enrichment of cookies with seeds of six varieties of Emirati dates, using different substitution percentages of 2.5, 5.0, and 7.5; two types of wheat flour; and two cooking temperatures of 180 °C and 200 °C. They reported a maximum TPC content of 96.2 mg/100 g for cookies substituted at 7.5%. This value was higher than that found in BRCP15 75.2 mg GAE/100 g of biscuit. On the other hand, Soares et al. [5] studied the enrichment of cookies with eggplant flour at 2.5, 5.0, and 7.5% substitution. They reported a maximum TPC of 32.2 mg GAE/100 g cookie with 7.5% substitution. This value was lower than the maximum value found in our investigation. As can be observed, many strategies have been explored in searching for archiving high TPC in cookies and biscuit formulations. The antioxidant activity of biscuits enriched with RCP was determined by two different methods, the DPPH and FRAP assays (Table 4). In both analyses, as the amount of RCP increased in the biscuit formulation, the μmol Trolox Equivalent increased. Most of the beneficial effects of roselle calyces are mainly attributed to anthocyanins, phenolic acids, and flavonoids, which have shown antioxidant, hypocholesterolemic, antihypertensive, antimicrobial, anti-inflammatory, anti-diabetic, and anti-cancer potential [30]. For this reason, the antioxidant activities found in the biscuit may be related to beneficial effects.

Table 4. Total phenolic content and antioxidant capacity of biscuits.

Sample	TPC (mg GAE/100 g dwb)	$TEAC_{DPPH}$ (μmol TE/100 g dwb)	FRAP (μmol TE/100 g dwb)
BC	3.6 ± 0^{e}	48.49 ± 29.12^{e}	64 ± 1.30^{e}
BRCP5	17.3 ± 1.9^{d}	189.96 ± 21.99^{d}	100.63 ± 1.91^{d}
BRCP10	28.2 ± 3.1^{c}	414.41 ± 18.00^{c}	140.35 ± 1.30^{c}
BRCP15	76.2 ± 2.0^{b}	587.43 ± 46.81^{b}	218.15 ± 1.87^{b}
RCP	672.9 ± 13.4^{a}	3940.46 ± 25.19^{a}	2031.61 ± 36.60^{a}

Results are presented as means ± SD (n = 3) on dry weight basis. Values followed by the different letters in the same line are significantly difference in the Tukey's test ($p < 0.05$). BC: Biscuit control; BRCP5: Biscuit added with 5% of roselle calyx powder; BRCP10: Biscuit added with 10% of roselle calyx powder; BRCP15: Biscuit added with 15% of roselle calyx powder.

3.4. Free Glucose and Starch Fractions of Biscuits

The four biscuit formulations in the current investigation only varied in the amount of RCP. The rest of the ingredients, especially sugar, in the biscuits were maintained at the same levels. Therefore, any difference observed in the in vitro starch digestibilities was mainly due to the RCP. Figure 2 shows the amount of free glucose (FG), rapidly digestible starch (RDS), and slowly digestible starch (SDS) of biscuits enriched with RCP. FG and RDS were the higher fractions found in BC. With the increment of RCP, a significant statistical decrease was observed in these fractions. RDS encountered in BC and biscuits added with RCP were in the interval of 36.13 g/100 g–16.87 g/100 g. However, the values found in this study were lower than those reported by Agama-Acevedo et al. [31] in cookies made with green plantain flour (61.8–52.8). Starch can be classified into different fractions using the in vitro Englyst assay: the starch fraction digested within 20 min of incubation is classified as RDS. RDS induces a rapid increase in blood glucose and insulin levels, which can induce health complications such as diabetes and cardiovascular diseases. This fraction is especially important for active people [32]. Slowly digestible starch (SDS) is gradually absorbed in the small intestine, and it causes a gradual increase in postprandial glucose and insulin levels [33]. The SDS fraction increased significantly as the proportion of RCP in the biscuit formulation (4.77 g/100 g–22.73 g/100 g) increased. The SDS values in the biscuits added with a higher concentration of RCP were higher than those reported by Agama-Acevedo and collaborators in green plantain flour cookies (10.9 g/100 g). The slow starch digestibility of biscuits enriched with RCP is likely due to its non-starch components, such as phenolic compounds, that can inhibit starch digestion. The inhibitory effect is caused by binding interactions between polyphenols and enzymes that largely depend on polyphenol molecular structure [34].

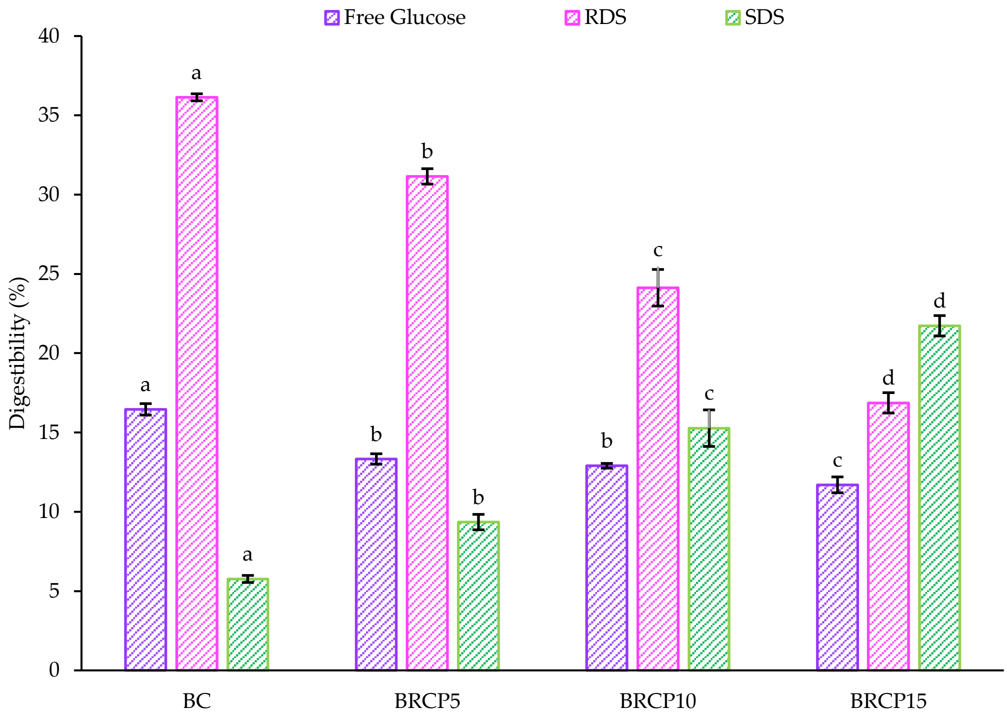

Figure 2. Free Glucose (FG), Rapidly Digestible Starch (RDS), and Slowly Digestible Starch (SDS) of biscuits enriched with RCP. Results are expressed as means ± SD (n = 3) on a dry weight basis. Values followed by different letters in the same line are statistically different with Tukey's test ($p < 0.05$). BC = Biscuit control; BRCP5 = Biscuit enriched with 5% roselle calyx powder; BRCP10 = Biscuit enriched with 10% roselle calyx powder; BRCP15 = Biscuit enriched with 15% of roselle calyx powder.

3.5. Sensory Evaluation of Biscuits

The sensory analysis of biscuits enriched with RCP are shown in Figure 3. The results indicated that the control biscuit and the one added with 5% RCP did not have statistically significant differences ($p < 0.05$) and presented good acceptability among the panelists. Although the sensory acceptance of the biscuits significantly decreased as the RCP content in the formulations increased, they still showed good acceptance by the judges. Although the overall acceptance values of the biscuit added with 10 and 15% of RCP was less accepted by the panelist, they had a good evaluation level. The addition of the RCP produced dark biscuits. The antioxidant content increased and improved the darkness in the biscuits; this could be considered an acceptable or rejected factor because the first sensory perception is the view [35].

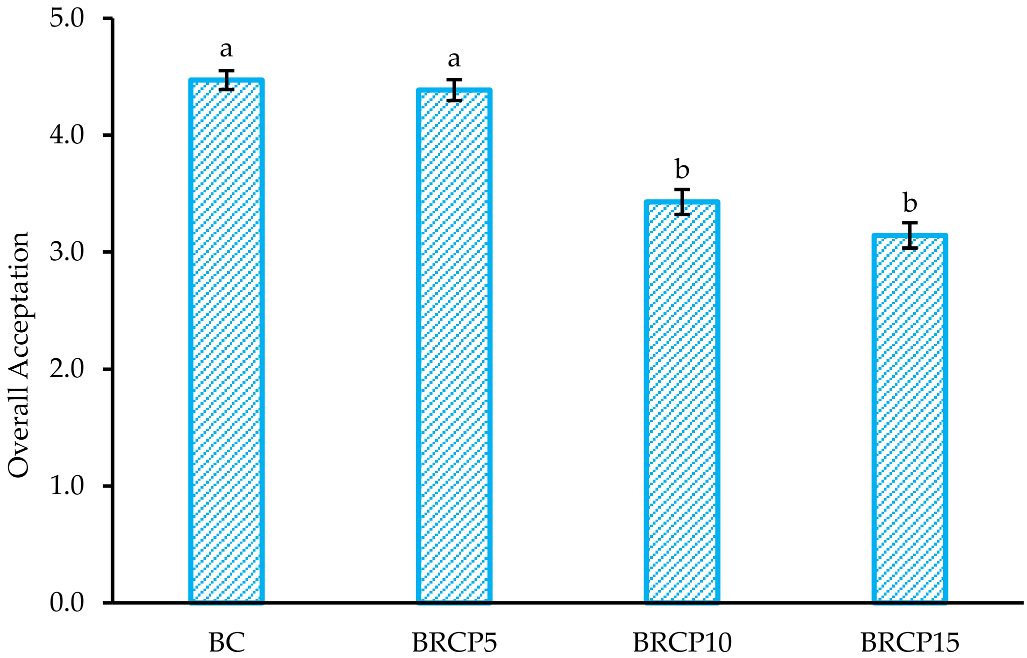

Figure 3. Sensory acceptance of biscuits enriched with roselle calyx powder. Results are presented as means ± SE (n = 70) on a dry weight basis. Values followed by the different letters in the same line are significantly different in Tukey's test ($p < 0.05$). BC = biscuit control; BRCP5 = Biscuit enriched with 5% roselle calyx powder; BRCP10 = Biscuit enriched with 10% roselle calyx powder; BRCP15 = Biscuit enriched with 15% roselle calyx powder.

4. Conclusions

Adding infused roselle calyx powder into the formulations of biscuits did not cause the loss of the amount of protein and minerals in the final product. However, the humidity and starch content decreased according to the percentage of RCP added. On the other hand, the content of starch rapidly decreased in the biscuits added with different proportions of RCP. At the same time, the samples with 10 and 15% of RCP showed higher values of slowly digestible carbohydrates. Regarding the color, it was perceived that the biscuit with 10% and 15% roselle calyx powder presented a reddish tone and less luminosity. The control biscuit and the biscuit enriched with 5% RCP showed the highest sensory acceptability of the evaluated biscuits. This research showed a strategy to use an agro-industrial byproduct as a functional ingredient that will retard the digestibility of carbohydrates and possesses an antioxidant capacity. Extending the use of the Roselle flower to other types of products can promote its sowing and commercialization and can be a key factor in economic and social development, improving the living conditions of producers and inhabitants of rural areas. Innovation on food products also opens up other areas of opportunity for the development of agro-industrial machinery and other aspects related to science, technology, engineering, and mathematics areas.

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References

1. Ivanova, N.G.; Nikitin, I.A.; Terentyev, S.E.; Ushakova, S.G.; Mitrokhin, M.A. Butter biscuit technology for specialized nutrition. *IOP Conf. Ser. Earth Environ. Sci.* **2022**, *949*, 012058. [CrossRef]
2. Mamat, H.; Abu Hardan, M.O.; Hill, S.E. Physicochemical properties of commercial semi-sweet biscuit. *Food Chem.* **2010**, *121*, 1029–1038. [CrossRef]
3. Yu, S.; Dong, K.; Pora, B.L.R.; Hasjim, J. The Roles of a Native Starch and a Resistant Dextrin in Texture Improvement and Low Glycemic Index of Biscuits. *Processes* **2022**, *10*, 2404. [CrossRef]
4. Laganà, V.; Giuffrè, A.M.; De Bruno, A.; Poiana, M. Formulation of Biscuits Fortified with a Flour Obtained from Bergamot By-Products (*Citrus bergamia*, Risso). *Foods* **2022**, *11*, 1137. [CrossRef]
5. Soares, J.M.; Teixeira, F.; Oliveira, M.L.d.; Amaral, L.A.d.; Almeida, T.d.S.F.d.; Souza, G.H.O.d.; Hokama, L.M.; Menegassi, B.; Santos, E.F.d.; Novello, D. Eggplant Flour Addition in Cookie: Nutritional Enrichment Alternative for Children. *Foods* **2022**, *11*, 1667. [CrossRef]
6. Šťastná, K.; Sumczynski, D.; Yalcin, E. Nutritional Composition, In Vitro Antioxidant Activity and Phenolic Profile of Shortcrust Cookies Supplemented by Edible Flowers. *Foods* **2021**, *10*, 2531. [CrossRef]
7. Awobusuyi, T.D.; Pillay, K.; Siwela, M. Consumer Acceptance of Biscuits Supplemented with a Sorghum–Insect Meal. *Nutrients* **2020**, *12*, 895. [CrossRef]
8. Bas, A.; El, S.N. Nutritional Evaluation of Biscuits Enriched with Cricket Flour (*Acheta domesticus*). *Int. J. Gastron. Food Sci.* **2022**, *29*, 100583. [CrossRef]
9. Juhari, N.H.; Martens, H.J.; Petersen, M.A. Changes in Physicochemical Properties and Volatile Compounds of Roselle (*Hibiscus sabdariffa* L.) Calyx during Different Drying Methods. *Molecules* **2021**, *26*, 6260. [CrossRef]
10. Hapsari, B.W.; Manikharda; Setyaningsih, W. Methodologies in the Analysis of Phenolic Compounds in Roselle (*Hibiscus sabdariffa* L.): Composition, Biological Activity, and Beneficial Effects on Human Health. *Horticulturae* **2021**, *7*, 35. [CrossRef]
11. Saleh, S.A.A.; Abbas, M.S.; Doweidar, M.M.M.; Soliman, A.S. Preparation and Evaluation of Biscuits Supplemented with some Natural Additives for Children and Adolescents Feeding. *Suez Canal Univ. J. Food Sci.* **2018**, *5*, 69–90. [CrossRef]
12. El-Sayed, W.M.; El-Kalyoubi, M.H.; Mostafa, M.M.; Farghal, S.A. Nutritional evaluation of roselle (*Hibiscus sabdariffa* L.) and its application in biscuit supplementation. *Arab Univ. J. Agric. Sci.* **2019**, *27*, 2563–2572. [CrossRef]
13. Marak, S.; Kaushik, N.; Dikiy, A.; Shumilina, E.; Falch, E. Nutritionally Enriched Muffins from Roselle Calyx Extract Using Response Surface Methodology. *Foods* **2022**, *11*, 3982. [CrossRef]
14. AACC, *Approved Methods of the American Association of Cereal Chemists*, 11th ed.; American Association of Cereal Chemists: St. Paul, MN, USA, 2002.
15. AOAC, *Official Methods of Analysis of Association of Official Analytical Chemists International*, 19th ed.; AOAC International: Washington, DC, USA, 2012.
16. Saavedra-Leos, M.Z.; Leyva-Porras, C.; Toxqui-Terán, A.; Espinosa-Solis, V. Physicochemical Properties and Antioxidant Activity of Spray-Dry Broccoli (*Brassica oleracea var Italica*) Stalk and Floret Juice Powders. *Molecules* **2021**, *26*, 1973. [CrossRef]
17. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT* **1995**, *28*, 25–30. [CrossRef]
18. Xiao, F.; Xu, T.; Lu, B.; Liu, R. Guidelines for Antioxidant Assays for Food Components. *Food Front.* **2020**, *1*, 60–69. [CrossRef]
19. Espinosa-Solís, V.; García-Tejeda, Y.V.; Portilla-Rivera, O.M.; Barrera-Figueroa, V. Tailoring Olive Oil Microcapsules via Microfluidization of Pickering o/w Emulsions. *Food Bioprocess. Technol.* **2021**, *14*, 1835–1843. [CrossRef]
20. Kohajdová, Z.; Karovicova, J.; Jurasová, M.; Kukurová, K. Effect of the Addition of Commercial Apple Fibre Powder on the Baking and Sensory Properties of Cookies. *Acta Chim. Slovaca* **2011**, *4*, 88–97.
21. Larrea, M.A.; Chang, Y.K.; Martinez-Bustos, F. Some functional properties of Extruded Orange Pulp and its Effect on the Quality of Cookies. *LWT-Food Sci. Technol.* **2005**, *38*, 213–220. [CrossRef]
22. Xie, X.; Yuan, Z.; Fu, K.; An, J.; Deng, L. Effect of Partial Substitution of Flour with Mealworm (*Tenebrio molitor* L.) Powder on Dough and Biscuit Properties. *Foods* **2022**, *11*, 2156. [CrossRef]
23. Reyes-Pérez, F.; Salazar-García, M.G.; Romero-Baranzini, A.L.; Islas-Rubio, A.R.; Ramírez-Wong, B. Estimated Glycemic Index and Dietary Fiber Content of Cookies Elaborated with Extruded Wheat Bran. *Plant Foods Hum Nutr.* **2013**, *68*, 52–56. [CrossRef]
24. Taylor, T.; Fasina, O.; Bell, L. Physical Properties and Consumer Liking of Cookies Prepared by Replacing Sucrose with Tagatose. *J. Food Sci.* **2008**, *7*, S145–S151. [CrossRef]
25. Borrás-Linares, I.; Fernández-Arroyo, S.; Arráez-Roman, D.; Palmeros-Suárez, P.A.; Del Val-Díaz, R.; Andrade-Gonzáles, I.; Fernández-Gutiérrez, A.; Gómez-Leyva, J.F.; Segura-Carretero, A. Characterization of phenolic compounds anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*). *Ind. Crop. Prod.* **2015**, *69*, 385–394. [CrossRef]
26. Man, S.M.; Stan, L.; Păucean, A.; Chiş, M.S.; Mureşan, V.; Socaci, S.A.; Pop, A.; Muste, S. Nutritional, Sensory, Texture Properties and Volatile Compounds Profile of Biscuits with Roasted Flaxseed Flour Partially Substituting for Wheat Flour. *Appl. Sci.* **2021**, *11*, 4791. [CrossRef]
27. Ramashia, S.E.; Mamadisa, F.M.; Mashau, M.E. Effect of Parinari curatellifolia Peel Flour on the Nutritional, Physical and Antioxidant Properties of Biscuits. *Processes* **2021**, *9*, 1262. [CrossRef]

28. Mayo-Mayo, G.; Navarrete-García, A.; Maldonado-Astudillo, Y.I.; Jiménez-Hernández, J.; Santiago-Ramos, D.; Arámbula-Villa, G.; Álvarez-Fitz, P.; Ramirez, M.; Salazar, R. Addition of Roselle and Mango Peel Powder in Tortilla Chips: A Strategy for Increasing their Functionality. *Food Meas.* **2020**, *14*, 1511–1519. [CrossRef]
29. Najjar, Z.; Kizhakkayil, J.; Shakoor, H.; Platat, C.; Stathopoulos, C.; Ranasinghe, M. Antioxidant Potential of Cookies Formulated with Date Seed Powder. *Foods* **2022**, *11*, 448. [CrossRef]
30. Izquierdo-Vega, J.A.; Arteaga-Badillo, D.A.; Sánchez-Gutiérrez, M.; Morales-González, J.A.; Vargas-Mendoza, N.; Gómez-Aldapa, C.A.; Castro-Rosas, J.; Delgado-Olivares, L.; Madrigal-Bujaidar, E.; Madrigal-Santillán, E. Organic Acids from Roselle (*Hibiscus sabdariffa* L.)—A Brief Review of Its Pharmacological Effects. *Biomedicines* **2020**, *8*, 100. [CrossRef]
31. Agama-Acevedo, E.; Islas-Hernández, J.J.; Pacheco-Vargas, G.; Osorio-Díaz, P.; Bello-Pérez, L.A. Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour. *LWT-Food Sci. Technol.* **2012**, *1*, 177–182. [CrossRef]
32. Miao, M.; Jiang, B.; Cui, S.W.; Zhang, T.; Jin, Z. Slowly Digestible Starch—A Review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1642–1657. [CrossRef]
33. Jenkins, D.J.; Wolever, T.M.; Leeds, A.R.; Gassull, M.A.; Haisman, P.; Dilawari, J.; Goff, D.V.; Metz, G.L.; Alberti, K.G. Dietary Fibres, Fibre Analogues, and Glucose Tolerance: Importance of Viscosity. *Br. Med. J.* **1978**, *1*, 1392–1394. [CrossRef] [PubMed]
34. Sun, L.; Miao, M. Dietary polyphenols modulate starch digestion and glycaemic level: A review. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 541–555. [CrossRef] [PubMed]
35. Chung, L.M.Y.; Fong, S.S.M.; Chung, J.W.Y. Identification on Sensory Attributes and Children's Ratings of Fruits and Vegetables with and without Appearance Modification: A Pilot Study. *J. Child Adolesc. Behav.* **2016**, *4*, 324–332.