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Effect of Roselle calyces extract on the chemical and sensory properties of functional cupcakes

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Abstract

Roselle calyces (RC) are a major crop for export and used to make a common drink in Egypt. Dried RC are commercially available and appreciated to obtain concentrated extracts which might be used in the food and pharmaceutical industries for color and heath benefits. The objective of this research was to determine the chemical and the sensory properties of cupcakes formulated with Roselle calyces extract (RCE). Proximate analysis, anthocyanins, ascorbic acid, titrable acidity, % retaining of anthocyanins, color and sensory evaluations were done. RC cupcakes had high sensory scores ($P < 0.05$) compared to control cupcakes. The parameter a^* was significantly red in the RC cupcakes compared to control cupcakes along with 77% retaining of anthocyanins. The consumption of 100 g of the RC cupcakes would provide 465 mg/100 g dry matter anthocyanin that is more than 2 folds of the minimum average of the daily intake of anthocyanins for Americans, along ~1/3 of the daily dietary fiber intake to achieve fiber adequacy according to the Scientific Advisory Committee on Nutrition. RC cupcake can be a functional food and would have a "clean" label with cost effective advantage.

Keywords: Roselle calyces; *Hibiscus sabdariffa* L.; Anthocyanins; Cupcakes; Fiber; Cost effective product

1. Introduction

To date, ~30,000 phytochemicals have been identified, of which 5000–10,000 are present in the food consumed in the human diet [1]. There is evidence that diets rich in fruit and vegetables could have a protective effect against a number of cancers [2] and other chronic health conditions such as cardiovascular disease [3].

Phytochemicals have many uses in the therapeutic, pharmaceutical, and food industries. Roselle calyces (RC) (*Hibiscus sabdariffa* L.) are a tropical plant in the Malvaceae family and is known in Egypt as *Karkadah*. It is probably a native of West Africa and is now widely cultivated throughout the tropics and subtropics, e.g., Sudan, China, Thailand, Egypt, Mexico, and the western part of India [4]. RC are one of the major Egyptian crops and is used in food, drinks, and cosmetics. Dried RC are commercially available and appreciated to obtain concentrated extracts which might be used in the food and pharmaceutical industries for color and heath benefits. It has been shown that ingestion of infusions of RC may help to reduce chronic diseases such as diabetes mellitus, dyslipidemia, and hypertension. This could be due to the activity of some compounds, mainly flavonoids and anthocyanins, found as natural antioxidants in Roselle calyces extract (RCE) [5].

RC anthocyanins might be used as a natural food colorant [6], as it is safer than most synthetic dyes that contain azo functional groups and aromatic rings, that may have negative effects on health including allergic and asthmatic reactions [7], DNA damage [8], and hyperactivity [9]. Some synthetic dyes are even considered to be potentially carcinogenic and mutagenic to humans [10].

Numerous researchers have pointed out that RC and its extracts possess functional properties from where advantages can be taken for developing new products with additional nutritious characteristics that may provide health benefits to

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consumers. In this sense, one of the main challenges that companies face today is the development of new value-added products to meet the consumer's demands [5]. Using RC into food products would also provide a cost effective products with "clean" label.

Cupcakes are a convenient bakery product for enrichment, is universal and easy to make. Therefore, the current study aimed to use Roselle calyx extracts as a natural source of color with its health benefits to enrich cupcakes.

2. Materials and methods

2.1. Cupcake ingredients

Cupcake ingredients included all purpose wheat flour (72% extraction), sun-dried loose dark red RC, caster sugar, unsalted butter, liquid skimmed milk, baking powder and iodized salt, eggs, pure white vanilla powder, were all purchased from local markets in Cairo, Egypt.

2.2. Chemicals

Cyanidin-3-glucoside, delphinidin-3-sambubioside, cyanindin-3-sambubioside, and delphinidin-3-glucoside were purchased from Sigma–Aldrich (Steinheim, Germany). Heat stable α-amylase (*Bacillus licheniformis*, solution, A3306), protease (*B. licheniformis*, lyophilized powder, P3910), and amyloglucosidase (*Aspergillus niger*, solution, A9913) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and solvents were Analar grade.

2.3. Preparation of Roselle calyces extract (RCE)

A 100 g of dried loose RC were cleaned by removing visually observed non-calyces matter, then dried in a vacuum dryer (Remplissage evacuation, Arthermo Gessate MI and Density Guide, Rome, Italy) at 28 °C for 3 h, cooled at room temperature (23 °C), weighed and ground to fine powder (0.55 mm) according to the optimized particle size measurement to produce more anthocyanins as described by Ref. [11], using a stainless steel Spray Veyco MPV mill model 100 (Shanghai, China). The fine powder was soaked overnight in 200 mL distilled water (DW). In the morning, the suspension was heated at 80 °C for 1 h after adding 450 mL (DW) in a 2 L Erlenmeyer flask. The suspension was strained, and added to the RC cupcake ingredients.

2.4. Batter preparation

The basic formulation, modified from Ref. [12], was used for the preparation of 250 g of each cupcake batter and is shown in Table 1. The dry ingredients were sieved together and then the melted butter, eggs, vanilla, and skimmed milk or RCE (replacing the milk) were mixed together for 5 min. Cupcake papers were fitted into each of 12 wells cupcake tray (34 × 26 cm). Four cupcake papers were filled with the batter on a balance and 60 g batter added, and then baked in a preheated gas oven at 175 °C for ∼20 min. After baking, cupcakes were left to cool and then packed in polyethylene bags and stored for 12 h (overnight) in a dry place before sensory testing began. Both moisture determination and sensory testing were begun that morning.

Table 1
The ingredients used in the cupcake batter formulations.

Ingredient (g)	Cupcake batter formulation	
	Control cupcake	Roselle cupcake
Egg	40.0	40.0
Pure vanilla powder	1.0	1.0
Caster sugar	55.0	55.0
Wheat flour	80.0	80.0
Salt	0.25	0.25
Milk	50.0	0.0
Butter	21.0	21.0
Baking powder	2.75	2.75
Roselle extract	0.0	50.0 (20%)
Total	250.0	250.0

2.5. Analytical methods

2.5.1. Proximate analysis

Proximate analysis of the samples was done in triplicate for protein, lipid, ash, moisture, and fiber contents. The crude protein was determined according to the Kjeldahl, official method 991.20 [13]. Hydrolysis was done using a Tecator Digestion System 20, 1015 Digestor (Tecator, Höganäs, Sweden) with modification by using a nitrogen factor of 5.70 as recommended by Ref. [14].

Total lipids were extracted by using the [15] method (Soxhlet extraction apparatus, extraction tube id 40 mm, Cat. No. 09-551 B, Fisher, Pittsburgh, PA, USA). Ash was determined gravimetrically in a muffle furnace (Nabertherm, D2804, Lilenthial-Bremen, Germany) by heating at 550 °C until constant weight [16] official method 930.30 for 6 h [17]. The carbohydrate was estimated by difference according to Ref. [18].

Moisture was determined using an Infrared Moisture Determination Balance (FD-610-Kett Electric Laboratory, Tokyo, Japan) by weighing 5 g of each cupcake crumb and measured at 80 °C for 60 min.

The water loss was calculated according to the following equation:

$$\%\ \text{ML (Moisture loss)} = W_1 - W_2 / W_1 \times 100$$

where W_1 is the weight of cupcake batter actually transferred into each cupcake paper (∼60 g) and W_2 is the weight of the baked cupcake 12 h after baking [19].

Total dietary fiber of cupcake samples was determined according to Ref. [20] method 960.52. Cupcakes samples were lyophilized (Snijders Scientific, Tilburg, Holland, capacity 3 kg ice). After lyophilization cupcakes were weighed again, ground, sieved through a 40-mesh sieve, and stored at −20 °C for up to 15 days until analysis. Total dietary fiber was the weight of the residue less the weight of the protein and ash.

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Calculation of total dietary fiber (TDF) was done according to the following equation:

$$\%\ \mathrm{TDF} = [(R_{Sample} - P_{Sample} - A_{Sample} - B)/SW] \times 100$$

where TDF = total dietary fiber, R = average residue weight (mg), P = average protein weight (mg), A = average ash weight (mg), SW = average sample weight (mg). The residue weight = $W_2 - W_1$, ash weight = $W_3 - W_1$, $B = R_{Blank} - P_{Blank} - A_{Blank}$, W_1 = celite + crucible weight, W_2 = residue + celite + crucible weight, W_3 = ash + celite + crucible weight.

2.5.2. Measurement of pH

The pH of RCE and cupcake samples were measured according to the method of Ref. [21] with slight modification. A 0.5 g sample of ground cupcake was mixed with 20 mL of deionized water (DIW) and vortexed for 3 min. The mixture was held at room temperature for 1 h to separate the solids and liquid. After centrifugation of the liquid for 3 min at 3050 × *g*, the pH of supernatants was measured.

2.5.3. Colorimetric determination of anthocyanins

Anthocyanins were determined according to the method of Ref. [22]. The method is based on the monomeric anthocyanin pigments reversibly changing color with a change in pH; the colored oxonium form exists at pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance at 520 nm at these two pH is proportional to the pigment concentration.

The absorption of anthocyanins was measured at both pH at 520 and 700 nm using an E-Chrom Tech Spectrophotometer (CT-2200, Taipei, Taiwan) with a DIW blank. The absorbances were measured within 20–50 min of preparation. Calibration curves were prepared using Cyd-3-Glu concentrations of 0, 150, 300, 600, and 1200 mg/L in DIW based on powder weight.

The concentration of anthocyanins were calculated in the samples and expressed as Cyd-3-Glu equivalents (mg/100 g), as follows:

$$\frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

where $A = (A_{520\,nm} - A_{700\,nm})$ pH 1.0 $- (A_{520\,nm} - A_{700\,nm})$ pH 4.5; MW (molecular weight) = 449.2 g/mol for Cyd-3-Glu; DF = dilution factor = 1.5; 1 = path length in cm; $\in$ = 26,900 molar extinction coefficient of Cyd-3-Glu [22].

Known amount of Cyd-3-Glu was also added to the samples before testing. The recovery ranged from 88 to 106% for the samples and 95% for the controls. This recovery was within the acceptable range.

2.5.4. HPLC determination of anthocyanins

To refine the colorimetric method for anthocyanins, HPLC was used to identify anthocyanin compounds in RC powder, extracts and cupcake samples. Anthocyanins extraction, purification, and identification were done according to Refs. [23,24], respectively with slight modifications. The HPLC system employed consisted of a Waters 600 controller gradient pump, a model 717 plus Waters autosampler with an autoinjector 20 μL fixed loop, and a Waters 996 photodiode array detector set at 520 nm, all controlled with a Waters Millennium 32 Software System (Waters LTD, Mertsfordshire, UK). A 25 × 0.4 cm reverse-phase C18 column (LiCrospher 100 RP-18, Darmstadt, Germany), with a particle size of 5 mm in diameter, was used. Purified Roselle extract (20 μL) was injected and eluted in an isocratic phase using acetonitrile:formic acid (4.5%) with a flow rate of 1.5 mL/min at proportions of 1:90, 13:87, and 100:0 during 0, 11, and 21 min, respectively. Mixtures of anthocyanin compounds were quantified from HPLC data using the external standard (ESTD) (0.5 mg/mL), dissolved in ethanol: water (50:50). The concentration of each anthocyanin detected and confirmed in a sample was determined by measuring its peak area and comparing that with the average peak area of the corresponding anthocyanin in the ESTD. The anthocyanins were calculated according to the following equation:

$$\text{Anthocyanin (mg/100 mL)} = AUC_{Sample} \times 0.5 \times (\text{mg/mL}) \times DF \times 100/AUC_{Standard}$$

where AUC_{Sample} is the area under the curve of the samples, $AUC_{Standard}$ is the area under the curve of the standard prepared at a 0.5 mg of anthocyanin/mL, and *DF* is the dilution factor.

All the anthocyanin compounds gave a linear calibration response with $r^2 > 0.9995$ over the range of concentrations. The procedure was tested each time by carrying out a reagent recovery using only reagents and also a sample spiked at the 0.5 mg/mL level with each of the 3 anthocyanin standards. Percent recovery of spiked reagent and samples were 92 and 105%, respectively.

2.5.5. Determination of vitamin C

Ascorbic acid content of the RC powder, extracts and cupcakes were determined using 2,6-dichlorophenolindophenol (DCIP) dye-titrimetric method [25]. Samples were extracted with 15 g of trichloroacetic acid and dissolved in 40 mL acetic acid and 200 mL DW. Afterwards, diluted to 500 mL with DW and filtered with Whatman No. 2 filter paper. Standard solution of ascorbic acid was prepared by weighing 0.05 g of standard ascorbic acid then dissolved in 60 mL of the extractant and made up to 250 mL with DW. The DCIP standard solution was prepared from 0.05 g of 2,6-dichlorophenolindophenol, weighed into a beaker and an equal amount of Na_2CO_3 was added to facilitate stability of the dye solution. The mixture was dissolved in 100 mL of distilled water and filtered. The DCIP was thereafter standardized by titrating against 10 mL of ascorbic acid stock solution until a faint pink color was obtained. The amount (mg) of ascorbic acid equivalent to 1 mL of dye solution was then calculated.

The samples were prepared by weighing 0.5 g of each sample and homogenized with 20 mL of the extractant. The mixture was filtered into a 50 mL volumetric flask and made up to mark with DW. A 10 mL volume of the resulting solution was pipetted into a conical flask and titrated against the standard indophenol

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solution and the titre value recorded. This was done in triplicate for each sample. Ascorbic acid was calculated as:

$$\text{Ascorbic acid (mg)/100 g sample} = C \times V \times (DF/W) \times 100$$

where C = mg ascorbic acid/mL dye; V = volume of dye used for titration of 1 mL of diluted sample; DF = dilution factor and W = weight of sample (g).

2.5.6. Total titratable acidity

It was determined according to Ref. [26]. One gram of sample was weighed and placed in a 100 mL glass beaker. A 40-mL of DW was added, heated to 70 °C, and left for 1 h. The supernatant was filtered through Whatman paper No. 4. The Roselle residues were rinsed with two portions of 20 mL of hot DW. The filtrate and the washings were transferred to a 100 mL flask, cooled down to room temperature (24 °C), brought to volume and thoroughly mixed. An aliquot of 25 mL of the extract was titrated with 0.1 N NaOH until reaching pH 8.3 using an Orion 5-star model pH meter (Thermo Scientific, Beverly, MA, USA). Results are reported as g of citric acid per 100 g of sample.

2.5.7. Anthocyanin % retaining

Percentage retaining of RC anthocyanins in the extract and cupcakes samples were determined [27]. In this method, appropriate amount of RC anthocyanins extract was diluted with citric acid phosphate buffer at pH 2.0 and the total anthocyanins were determined before and after heating/baking. For % retention value of RC anthocyanins was calculated according to the following equation:

$$\text{Retention (\%)} = \text{Total anthocyanins after baking/ total anthocyanins before baking (extract)} \times 100.$$

2.5.8. Color measurement

Color measurements (CIE $L^*a^*b^*$) of baked cupcakes were done using an image analysis technique [28]. A color image was obtained using a digital camera (Canon, Power Shot A470, 3.4X optical zoom, 7.1 megapixels, Shanghai, China) under controlled and defined illumination conditions using 2 Philips Natural Daylight 18 W fluorescent lamps with a color temperature of 6500 K according to the manufacturer. The color of the surface and the crumb of the cupcakes were measured. Sample photos were taken 30.5 cm above the cupcakes at an angle of 45° using a tripod. The pictures were downloaded to a personal computer using a USB digital film reader. Once the color images of the cupcake samples were captured, the color was analyzed quantitatively using Photoshop [29]. The captured photos were viewed in the Adobe Photoshop window and from the Info Palette and Histogram Window (Figs. 2 and 3). The L^*, a^*, b^*, values were calculated (Fig. 3), using the following equations according to Refs. [30,28].

$$L^* = \frac{\text{Lightness}}{255} \times 100$$

$$a^* = \frac{240a}{255} - 120$$

$$b^* = \frac{240b}{255} - 120$$

2.5.9. Sensory evaluation

Sensory evaluation was used to assess the sensory acceptability of the cupcakes using an acceptance test. Cupcakes were subjected to sensory evaluation by untrained panelists. Prospective panelists were screened using the following criteria: (1) female/male >25 year ($n = 75$), (2) employees who have eaten cake or cupcakes at least once a week for the last three months, and (3) have no food allergies. They were not given prior information about any of the ingredients use. The room temperature cupcakes were presented to the panelists in random order according to Ref. [31]. The panelists were instructed to score their liking for each of the attributes being studied. A 5-point hedonic scale with 1 = dislike very much, 2 = dislike moderately, 3 = neutral, 4 = like moderately, and 5 = like very much was used to evaluate the color, appearance, texture (visual and eating), taste, volume, lightness, aroma and overall liking of the cupcakes.

2.5.10. Statistical analysis

Proximate analysis, pH, anthocyanins, color measurements, and sensory evaluations were done in triplicate, and mean values and standard deviations were calculated using Excel for Microsoft Windows Operating System. One-way ANOVA was done and the differences of the means were evaluated using Tukey's HSD test ($P < 0.05$) and SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

The results of nutritional characteristics of RC and cupcakes are summarized in Table 2.

3.1. Proximate analysis

The results of proximate analysis of RC and enriched cupcakes with Roselle calyces extract (RCE) are summarized in Table 2. Moisture content in the cupcakes found ~29%. Incorporation of RCE at 20% in the cupcakes did not affect on the moisture content compared to the control cupcakes. The added value of the RC is with the fiber and ash fraction to RC cupcakes. Adding RCE to cupcakes significantly ($P < 0.05$) increased the ash and crude fiber content in the samples. The ash and fiber were 4.2 and 8%, respectively, for RC cupcakes vs 1.5 and 3.6 in the control cupcakes, respectively.

The results of the current study found 12% dietary fiber in the dry RC, similar results found in the work of Refs. [33–35]. On the other hand, Refs. [32,36] found the lowest level of fiber in the dry RC 2.3–5.6%, respectively. These differences may be due to different cultivars or differences in the methodologies for fiber determinations. The data in Table 2 indicate that the fat

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Table 2
Nutritional characteristics of Roselle calyces, extracts and cupcakes.

Measurement	Roselle calyces	Roselle extract	Control cupcakes	Roselle cupcakes
Moisture	9.5 ± 1	60 ± 3	29 ± 2[a]	29.2 ± 2[b]
Protein	5.3 ± 0.2	2 ± 0.04	11 ± 0.6[a]	10.3 ± 0.4[a]
CHO	65 ± 3	25 ± 0.9	46.4 ± 2[a]	41.2 ± 3[b]
Fat	3.2 ± 0.03	1.5 ± 0.09	8.5 ± 0.08[a]	7.1 ± 0.2[b]
Ash	5 ± 0.04	2.5 ± 0.03	1.5 ± 0.04[b]	4.2 ± 0.1[a]
Fiber	12 ± 0.5	9 ± 0.2	3.6 ± 0.5[b]	8 ± 0.4[a]
Titratable acidity[B]	19 ± 0.4	13 ± 0.2	1.2 ± 0.02[b]	14 ± 0.5[a]
pH	NA	5.5 ± 0.3	7.9 ± 0.1[a]	7.5 ± 0.07[a]
Ascorbic acid	16 ± 0.4	11 ± 0.3	ND	8 ± 0.06
Total anthocyanins	565 ± 9	535 ± 6 (5.3%)↓	ND	435 ± 7 (23%)↓
Anthocyanins[A]	580 ± 7	550 ± 9 (5.2%)↓	ND	465 ± 8 (20%)↓
Anthocyanin retaining %	NA	94.6 ± 8	NA	77 ± 4

All values are means with standard deviation (n = 4).
[A] Anthocyanins were analyzed by HPLC, mg/100 g as the total of individual anthocyanins; delphinidin-3-sambubioside, cyanindin-3-sambubioside, and delphinidin-3-glucoside. Total anthocyanins were analyzed colorimetrically, mg/100 g cyanidind-3-glucoside dry matter. Different letters within the same row differ significantly from each other (P < 0.05).
[B] Titratable acidity was expressed as g/100 g citric acid, ND: not detected, CHO: carbohydrates, total carbohydrate calculated by difference. Each sample was analyzed in triplicate ± standard deviation (SD). NA = none applicable. ↓ = reduction percentage.

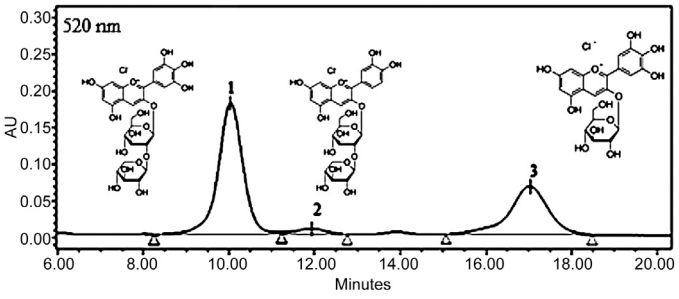

Fig. 1. HPLC chromatogram from HPLC analysis of dried Roselle calyces. 1 = delphinidin-3-sambubioside, 2 = cyanidin-3-sambubioside, and 3 = delphinidin-3-glucoside.

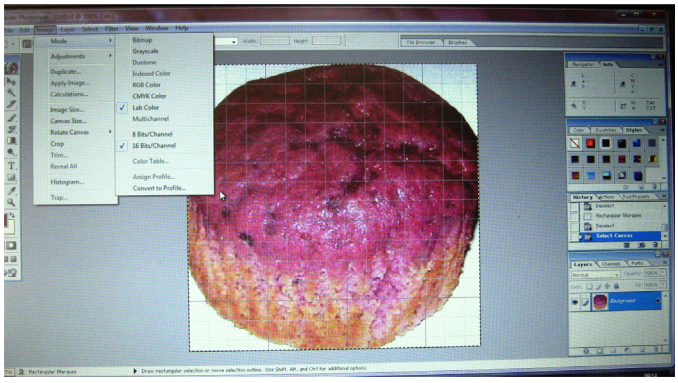

Fig. 2. The Photoshop Window Screen of Roselle cupcake.

was reduced from 8.5 to 7.1% in the control cupcakes vs RC cupcakes.

3.2. Anthocyanin content

Monomeric colorimetric anthocyanins in the dry RC were 565 mg/100 g Cyd-3-Glu dry matter (D.M.) vs 580 mg/100 g D.M. based on HPLC analysis, as a total of individual anthocyanins; delphinidin-3-sambubioside, cyanindin-3-sambubioside, and delphinidin-3-glucoside (Fig. 1, Table 2). The results of anthocyanins based HPLC were similar to those from colorimetric results. In this sense, the total concentration of anthocyanins in the RC cupcakes were 465 mg/100 g D.M. vs 435 mg/100 g Cyd-3-Glu D.M., with retaining 77% of anthocyanins.

In the current study, 100 g of the RC cupcakes would provide more than 400 mg of anthocyanins/100 g D.M. compared to the minimum average of the daily intake of anthocyanins for Americans (12.5–215 mg) [37] and also ~1/3 of the daily dietary fiber intake to achieve fiber adequacy [38]. The enriched cupcakes can provide this amount of anthocyanins in case of consumption ~2 cupcakes/day (each cupcake is ~50 g).

Peng-Kong et al. [39] reported high concentration of total anthocyanins 2520 mg/100 g fine powder of Roselle (expressed as delphinidin-3-glucoside). This was higher than our study probably the total colorimetric anthocyanins was expressed as cyaniding-3-glucoside. They also mentioned that, according to the analysis by high performance liquid chromatography (HPLC), the main anthocyanins in the RC were delphinidin-3-sambubioside (71.4%) and cyanidin-3-sambubioside (26.6%). In our study, the average percentage of delphinidin-3-sambubioside (64.8%), delphinidin-3-glucoside (3.5%) and cyanidin-3-sambubioside (31.7%) were in the line with earlier report [39] (Fig. 1). The results of Peng-Kong et al. [39] showed that delphinidin-3-sambubioside were the major anthocyanins present and this corresponded to those reported [40] and our study. However, cyanindin-3-glucoside was undetected [39] in and our study too.

Galicia-Flores et al. [41] reported total anthocyanins content between 365 and 607 mg/100 g dry ground sample in extract

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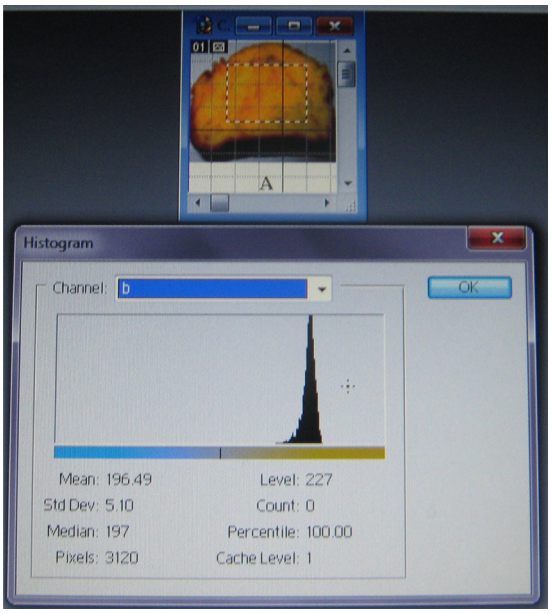

Fig. 3. The Photoshop Window Screen, including Histogram Window of cupcake crumb for control showing *b** (yellowness) mean value.

obtained using methanol acidified with 1% trifluoroacetic acid and between 172.6 and 297 mg/100 g of dry calyces using DW as the extracting agent was reported [41]. Because the experimenter enriched the cupcakes with RCE that contain (535–550 anthocyanin mg/100 D.M.), planning for sensory evaluation therefore RC was extracted with DW and dry RC was optimized with best particle size for anthocyanin content. The extraction was done with DW on 2 stages, soaking RC in DW for 12 h and then heating the RCE up to 80 °C for 1 h. Similar results [42] found the total anthocyanins content (colorimetric method) of 623 ± 2.0 mg cyanidin-3-glucoside/100 g of dry sample.

In Table 2, anthocyanins retaining percentage were 77% in the RC cupcakes. By calculating the amount of anthocyanins between HPLC and colorimetric measurement, there is a better results of percentage reduction of the anthocyanin pigment in the RC cupcakes that improved 20–23% (HPLC vs colorimetric method).

Selim et al. [43] showed that Roselle calyx extracts heated for 30 min at temperatures of 60, 70, 80, 90, and 100 °C, retained 99.9%, 99.2%, 94%, 86%, and 79% of their anthocyanins contents, respectively. In the current study, the RCE exhibited their greatest stabilities (94.4%) during the entire holding time. The results showed that RCE heated for the above 50 °C occurred at 2 stages: heating the RCE at 80 °C for 1 h, and baking the cupcakes at 175 °C for ~20 min. As heating time was extended to 20 min, the retention values decreased to 77% in the RC cupcakes.

3.3. Titratable acidity and vitamin C

Results of titratable acidity and vitamin C are reported in Table 2, both values were in agreement with literature for example, Ref. [11] found 20% malic acid g/100 g in the RC that is similar to the present study (19%). It was also reported in Ref. [44] that the RC extracts also contain a high percentage of organic acids, including citric acid, hydroxycitric acid, hibiscus acid, malic and tartaric acids as major compounds, and oxalic and ascorbic acid as minor compounds. In our study, the RC extract's pH was 5.5 that may indicate to the presence of organic acids. The vitamin C in this study (16 mg/100 g D.M.) was similar to those reported 6.7–15 mg/100 g D.M. in Refs. [34,35], respectively and also similar with that reported in Ref. [44] (0.02%–0.05%).

3.4. Crust and crumb color of cupcakes

Results in Table 3 and Figs. 2 and 3 showed that the crust color was dependent on the RCE compared to the control cupcake. It seems that RCE and baking temperature were the main factors that influenced the color of the crust to be different than the crumb. The crumb of the control cupcake was lighter than the crust as the Maillard reactions occur mainly on the surface. The parameter *a** was significantly redder in the RC cupcakes compared to control cupcakes. While, the *b** was significantly ($P < 0.05$) reduced by addition of RCE. The pinkish color increased significantly ($P < 0.05$) when RCE was added (Table 3 and Figs. 2 and 3).

A recent study evaluated the effect of various organic acids on color retention during fruit juice storage, and found that acetic acid improved the color stability in both elderberry and black currant juices, whilst the citric and tartaric acids only improved color stability in elderberry juice [45]. It could be concluded that presence of organic acids in RCE helped to stabilize the red color of RC cupcakes probably by lowering the pH. Ref. [46] noted that the efficiency of extracting solvents for anthocyanins increased with increasing concentration of citric acid [45,47].

3.5. Sensory evaluation

The sensory evaluation scores for color, appearance, texture, taste, volume, lightness, aroma and overall liking were obtained

Table 3
Color measurements of crust and crumb for control and Roselle cupcakes.

Sample	Crust			Crumb		
	*L**	*a**	*b**	*L**	*a**	*b**
Control	71 ± 3^a	10 ± 0.2^b	61 ± 3^a	89 ± 3^a	-0.5 ± 0.01^b	65 ± 3^a
Roselle	18 ± 1^b	20 ± 1^a	8 ± 0.2^b	60 ± 2^b	50 ± 3^a	30 ± 1^b

Means that do not share the same letter in a column are significantly different according to Tukey HSD test ($P < 0.05$). (n = 3 cupcakes/sample). The surface and crumb color of cupcakes are reported as average *L** (lightness), *a** (redness), *b** (yellowness) values.

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Table 4
Sensory evaluation scores for cupcakes.

Sample	Color	Appearance	Texture	Taste	Volume	Lightness	Aroma	Overall liking
Control cupcakes	4.8 ± 0.1^{a}	4.8 ± 0.3^{a}	4.9 ± 0.3^{a}	4.8 ± 0.3^{a}	5.0 ± 0.1^{a}	5.0 ± 0.2^{a}	4.8 ± 0.3^{a}	4.9 ± 0.3^{a}
Roselle cupcakes	4.8 ± 0.2^{a}	4.9 ± 0.2^{a}	4.6 ± 0.4^{a}	4.6 ± 0.2^{a}	4.8 ± 0.2^{a}	4.5 ± 0.2^{b}	4.8 ± 0.4^{a}	4.6 ± 0.2^{b}

Means that do not share the same letter in a row are significantly different according to Tukey HSD test ($P < 0.05$). A 5-point hedonic scale ranging from 1 = dislike very much; 3 = neither like or dislike; 5 = like very much was used to evaluate the attributes in the table.

from untrained panelists are shown in Table 4. RC cupcakes had closer acceptance scores for the attributes compared to the control cupcakes (with milk) specifically, color (4.5) and aroma (4.8). Most panelists liked the color, appearance, and texture of Roselle cupcakes compared to the control cupcake. The data in this area are singular, Ref. [36] studied the effect of substitution of wheat flour with 0%, 5%, 10%, 15% and 20% Roselle calyces powder found that sensory evaluation of muffin with 10% substitution of RC was rated the most acceptable. In the current study the investigator enriched the cupcakes with Roselle calyces extract (not powder) at 20% that is less sour as mixed with water.

3.6. Cost effective product

Cupcake is popular in Egypt and hundreds of bakery and specialized shops produce cupcakes. The market of cupcakes in Egypt is large, targeting most people through cupcake shops, TV cooking programs and specialized cooking channels. However, our target in this study is children as they like colored cupcakes and they are the most sensitive target group because over consumption of synthetic food colorants as these shops use them apparently without official control.

Our cupcakes are reddish to purple with clean label. Also our target is elderly who looking for healthy and soft products that can be made commercially to be labeled as functional cupcakes. Roselle cupcakes can provide food safety for children and protective agents for older people. Beneficial health effects associated with anthocyanin consumption include lowering the risk of cardiovascular disease [48].

The current study focused on using Roselle calyces to produce a new cupcake-like product (CLP); as a cost effective (Roselle calyces are available and cheap) and functional cupcake food with high nutritional value (specifically bioactive compounds). The cost effective in the new product is reasonably cheap compared to the control cupcake costs. One of the most important factors that influence the cost effectiveness of producing cupcake is the choice and the prices of ingredients such as flour. The price of flour based on a retailer price in Egypt is between L.E 7 and 8 (1 kg), while the average commercial cupcakes are L.E 10. The real calculation of each cupcake in this study as only ingredients for Roselle cupcakes is L.E 2.60 compared to control L.E 3.20. With the assumption of reducing the cost of cupcakes to ~20% as (~L.E 0.6) when Roselle calyces added, in addition to its higher nutritional value. As we have found in the literature, total phenols, anthocyanin, and fiber content exert their beneficial effects in reducing risks of coronary heart disease [49,50,48,51]. Additionally, its use could contribute to reducing the cost of national heath services in the treatment of heart diseases.

4. Conclusions

Roselle calyces (RC) are sources of acids that would enhance the stability of anthocyanins pigment and have numerous of phytochemicals. Roselle calyx extracts was used to enrich cupcake. The panelists gave a closer score (statistically significant at $P < 0.05$) to the RC cupcakes. The a* value was increased significantly while the b* value was significantly reduced. The consumption of 100 g of the RC cupcakes provided 465 mg/100 g anthocyanins, along 8% total dietary fiber and 4.2% ash.

Conflicts of interest

There is no conflicts of interest.

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