The effect of Roselle calyces extract on the chemical and sensory properties of cupcakes

V T Thanh¹, Nhi Y T Tran²*, N T V Linh², Tran Anh Vy³, and Tran Thanh Truc⁴

¹NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
²Faculty of Chemical Engineering and Food Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
³Department of Chemical and Biochemical Engineering, Gachon University, South Korea
⁴College of Agriculture, Can Tho University, Vietnam

* ttnhi@ntt.edu.vn, labasm2013@gmail.com

Abstract Roselle calyces (RC) are a valuable crop for export and manufacture of a typical drink in Vietnam. The RC is widely applied as a natural colorant in different fields such as food and pharmaceutical industries. The objective of this research was to determine the effect of extraction parameters on the nutritional value and sensibility of cupcake added with RC. Examined indicators included lipid, moisture, pH, protein, and ash. Moreover, the sensory properties of cupcakes formulated with Roselle calyces extract were also determined. Compared with the control cupcake, the RC-added cupcake exhibited higher content of moisture, protein, and ascorbic acid, achieving 30.16 ± 0.25, 2.76 ± 0.07 and 37.65 ± 0.64 respectively. In contrast, the level of pH, ash, and lipid experienced a marginal drop, achieving 7.02 ± 0.25, 1.05 ± 0.13 and 14.78 ± 1.01 respectively. Moreover, the Roselle cupcake had 3.74 ± 0.02 mg/L anthocyanin content. The percentage of retention of anthocyanin content at 180°C for 20 minutes was 60.53 ± 0.48. RC cupcakes have high sensory scores (P<0.05) comparing with control RC cupcakes. RC cupcake can be a functional food which has a cost-effective advantage.

1. Introduction

Natural extraction from medicinal plants more and more popular due to the abundant content of bioactive molecules including vitamins, proteins, phenolic compounds [1–6]. These bioactive compounds play an essential role in different industrial fields such as cosmetics, pharmaceutical, food industries. Roselle is herbaceous plant. The scientific name is Hibiscus sabdariffa L., origin from Asia or West Africa [7]. In Roselle, calyces contain compounds with high antioxidant resistance as Anthocyanins, Flavonoid and Phenolic compounds [8]. The previous study demonstrated the Phenolic compounds found in Roselle calyces is Protocatechuic acid. Moreover, hibiscetin-3-glucoside and quercetin is Flavonoid also determined [9].

Special red pigments in calyces specified by the Anthocyanin compound have been commonly used as natural food coloring agents [4]. Cyanidine and delphinidin regulate Anthocyanin include delphinidin-3-sambubioside (hibiscin), cyanidin-3-sambubioside (gossypicycin), cyanidin-3,5-diglucoside. Besides the coloring effect, Antioxidation capacity of anthocyanins be reported such as grape [10], cherry, cranberry [11], Clitoria ternatea flower, Strawberry, anthocyanins also have...
biological effects in low density lipoprotein biosynthesis and lecithin-liposome systems [12]. The chromaticity of anthocyanin extract from plants is highly influenced by pH, showed from red to blue [13], due to that regulate the color of plants. The potential biological, medical and food values of Anthocyanins need to be studied. The previous study shows that ingestion of infusions of RC may aid to reduce chronic diseases such as dyslipidemia, diabetes, hypertension [12]. Numerous studies illustrate that RC and its extracts possess functional properties which provide health benefits to consumers.

The development of new value-added products has been receiving plenty of consumers due to cost-effective products with clean label. Currently, food processing technology is increasingly concerned, not only finding nutritious food but also studying the application of natural compounds to add value to products. Cupcake is one of the convenient cakes for daily consumption and preservation. Improving the nutritional value (free-radical scavenging capacity) is necessary. The study aims to apply Roselle calyces extracts as a natural source of color with its health benefits to enrich cupcakes. Also, the effects of Roselle calyces extract on the chemical and sensory properties of Cupcakes.

2. Methods and materials

2.1 Sample preparation

2.1.1 Roselle calyces extract
A 500g calyces are convection dried to constant mass, then ground and through 400 mesh sieve, 30g of dried samples add 200ml of water, heat at 80°C for 1 hour and cool to room temperature, overnight solution, 450ml more water stirred, then filter the residue, anthocyanin solution is contained in dark glass bottles.

2.1.2 Cupcake
In this study, the Electrolux 21-liter oven (model EOT4805K) with a maximum capacity of 1500W and dimensions of 299 x 472 x 350 mm. 40g mixed egg 21g butter was placed in the whisk, add 55g sugar, salt, vanilla powder and baking powder, add 50ml anthocyanin extract. Complete solution blended with 80g wheat flour, then pour the mixture for each cup equally in volume, bake at 20 minutes at 180°C, analyze and cool.

2.2 Chemicals
Gallic acid was bought at Sigma-Aldrich Chemical, Co Ltd (USA), and 2,6-dichlorophenolindophenol (DCPIP) was imported from India. Other chemicals such as distilled water (with pH between 6.5 and 8), diethyl ether (99.5% purity), Na₂CO₃ (99.5% purity), ascorbic acid (99.7% purity), NaHCO₃ (99.5% purity), FeSO₄·7H₂O (99.0-101.0% purity), CuSO₄·4H₂O ( ), K₂SO₄ (99.5% purity), H₂SO₄ were originated from China.

2.3 Analytical method

2.3.1 Determination of total Anthocyanins
The total Anthocyanins content was measured by the colorimetric methods Monomeric anthocyanin pigments reversibly change color in pH. Described by Giusti & Wrolstad. (2001) [14], after adjusted to pH 1.0 and 4.5 using 0.2 M KCl and 0.1 M acetate buffer, respectively, the absorbance was measured at 520 nm and 700 nm. The results were expressed as mg cyanidin-3-glucoside equivalent per volume of the sample (mg/L).

2.3.2 Determination of Vitamin C
The ascorbic acid content in the sample determined according to AOAC 967.21 based on oxidation of ascorbic acid with 2,6-dichlorophenolindophenol (DCPIP) to dehydroascorbic acid and the colorless
lenco derivatives [15]. The optimized reaction is at pH between 3 and 4. In this environment, a drop of excess blue DCPIP will make solution turn pink.

A 5-gram of the sample was ground and extracted with metaphosphoric acid. A 5 ml of extract was titrated with 2, 6-dichlorophenolindophenol (DCPIP). The titration stopped when a drop of excess blue DCPIP makes solution turn pink in acidic medium and last for 30 seconds. Indophenol solution was titrated with the standard ascorbic acid solution. The ascorbic acid content was expressed in mg per gram of dry matter (mg/g dry matter).

2.3.3 Determination of Lipid
The mass of total lipid was measured by using the Soxhlet method [16] from the difference in the flask mass before and after extraction. 5 g of dried sample to constant mass placed in the tube with diethyl ether solvent. The system is heated at 60°C within 48 hours. Check the volume of solution in a glass flask. Solvent recovery when the extraction process is complete. Drying containers, record the mass difference. Lipid content was determined by mg per gram of dry matter (mg / g dry matter).

2.3.4 Determination of Protein
Total nitrogen was measured by Kjeldahl analysis [17]. 1g of potassium sulfate is added to 1 g of solid sample to increase the boiling temperature of 3 ml of concentrated sulfuric acid. 0.1g copper sulfate and ferrous sulfate were added before the chemical sample process. The catalytic effect of several drops of hydrogen peroxide accelerates acid oxidation. After 6 hours at 370°C, 10ml of fluid was calibrated with boric acid. Protein content was expressed in total percentage of nitrogen per gram of dry matter.

2.3.5 Determination of pH
According to the method of Von Elbe et al. (1974) [18], pH of control cupcake, Roselle cupcake and anthocyanins extract were determined by Consort multi parameter analyzer (model C3010T). The sample (0.5-gram) was ground and extracted with 20ml diluted water and then filtered through Whatman No.1 and measured pH.

2.3.6 Determination of Lightness
Color measurements (CIE L*a*b* color space) of baked cupcakes were done using an image analysis technique [18]. Color measurements were performed using 0.3NH Scanner Chroma colorimeter (NR60CP model). Lightness value L* have ranged from 0-100, with two components is a* (from green to red) and b* value (from blue to yellow).

2.3.7 Sensory evaluation
Sensory evaluation acceptance was performed by description methods of Amerin et al [19]. The results were expressed by chart and acceptance level description.

2.3.8 Data Analysis
All experiments were conducted in duplicate. The mean and standard deviation of the results were calculated using Microsoft Excel program (Microsoft Inc., Redmond, WA, USA). Experiment data were analyzed using one-way analysis of variance (ANOVA) test in SPSS program (IBM Company, USA) with the level of significance at 5%.

3. Result and discussion

3.1 Nutritional characteristics of Roselle extract, Control and Roselle cupcake
The sensory evaluation score for color, texture, aroma, flavor and overall was obtained from untrained panelists are shown in table 1. The quality characteristic of the cupcake is presented for Ash, Moisture, Lipid, Protein content. There was no significant difference between control cake
and Roselle cupcake. This may be because they have the same recipe for making cakes. At the same time, milk replacement with Roselle extract does not affect these values. The change in pH value in Roselle extract and Roselle cupcake is significant from 3.41 ± 0.25 to 7.02 ± 0.25, respectively. This change may be affected by salt and baking powder added earlier, these two components are strongly alkaline, causing the pH of the cake to change in the direction of increase. Ash content in the cupcakes found about 1.05%. Incorporation of RCE in the cupcakes did not affect the ash content compared to the control cupcakes. Adding RCE to cupcakes significantly (P < 0.05) increased the moisture and Ascorbic acid content in the samples. The moisture and Ascorbic acid were 30.16% and 37.65%, respectively, for RC cupcakes vs 27.35% and 33.85% in the control cupcakes, respectively. The data in Table 1 also indicate that the fat was reduced from 17.91 to 714.78% in the control cupcakes vs RC cupcakes.

| Measurement          | Roselle extract | Control         | Roselle cupcake |
|----------------------|-----------------|-----------------|-----------------|
| pH                   | 3.41 ± 0.25a    | 7.63 ± 0.27b    | 7.02 ± 0.25b    |
| Ash %                | None            | 1.21 ± 0.19a    | 1.05 ± 0.13a    |
| Moisture %           | None            | 27.35 ± 0.35a   | 30.16 ± 0.25a   |
| Lipid %              | None            | 17.91 ± 0.55a   | 14.78 ± 1.01a   |
| Protein %N           | None            | 2.75 ± 0.27a    | 2.76 ± 0.07a    |
| Ascorbic Acid mg/g DM| None            | 33.85 ± 0.92a   | 37.65 ± 0.64a   |
| Anthocyanin mg/L     | 6.17 ± 0.02a    | None            | 3.74 ± 0.02b    |
| Anthocyanin Retention%| None            | None            | 60.53 ± 0.48    |

Moreover, Results indicated that the content of anthocyanins decreased significantly from 6.17 ± 0.02mg/L to 3.74 ± 0.02 mg/L (P < 0.05). This result can be explained as after bake at 180°C for 20 minutes, endothermic and endogenous enzymes in plant cells have accelerated the process of degradation anthocyanin pigments [20]. Thus, anthocyanin has been subjected to degradation by time and temperature. Previous reports by Patras et al (2010) demonstrated that Cyanidine 3-glucoside was significantly affected at temperature 70 for 2 minutes [21]. In this study, the retention of anthocyanin compound after bake processing is 60.53% ± 0.48. They are considered significant when applying compounds that are antioxidant into food through heat treatment. It is possible to explain when mixing the extract into the dough mixture, the gluten network can retain anthocyanin compound [22][23].

3.2 Lightness of Roselle cupcake

Figure 1 and Table showed that Roselle extracts had affected to the lightness of the cupcakes. Red pigmentation of anthocyanins is altered in alkaline pH of cakes. Besides, the yellow and red bonding leads to the transformation of Roselle cupcake after baking does not retain the characteristic color of anthocyanins in calyces. The L * value of the two cakes is different but still in the middle of this default range. The parameter a* was significantly greener in the RC cupcakes compared to control cupcakes. In Table showed that the b * value at the control is 22.79 ± 0.01b reduced by 15.07 ± 0.02a by anthocyanins pigment. The previous study demonstrated that the a was significantly red in the RC cupcakes compared to control, achieved 77% retaining of anthocyanin [24].
Figure 1. The effect of Roselle's extract on the structure and color of cupcake (A1, B1, control cupcake; A2, B2, Roselle cupcake)

Table 2. Color measurements

| Sample               | L*          | a*          | b*          |
|----------------------|-------------|-------------|-------------|
| Control              | 51.28 ± 0.16b | -2.01 ± 0.02a | 22.79 ± 0.01b |
| Roselle cupcake      | 50.15 ± 0.15a | -1.42 ± 0.01b | 15.07 ± 0.02a |

3.3 Sensory evaluation of the cupcake

The sensory evaluation scores for color, appearance, texture, taste, volume, lightness, aroma and overall liking were obtained from untrained panelists are shown in Figure 2. Results were recorded from 30 untrained panelists. Each of these indicators is evaluated at the 5-point scale. The difference is significant in incense assessment 2.91 ± 1.04 and 4.18 ± 0.75 for control and Roselle cupcake, respectively. The heating at 180°C causes the transformation of the lipid present in the material into volatile aromatic compounds in both evaluation samples [22].

However, the level of the evaluation board for Roselle is higher than the 4-point scale (like moderately), indicating that they have commercial potential in the market. Figure 2 shows that the Roselle cupcake acceptance rate is higher than control sample, achieved at 3.91 ± 0.54 and 3.18 ± 0.6, respectively. A.R. Abdel-Moemin (2016) investigated the Roselle extract at a concentration of 20% for the same result [24]. Moreover, M.S.F. Amin (2014) studied the effect of 0%, 5%, 10%, 15% and 20% Roselle calyces powder in muffin and found that the acceptance score of the Roselle muffin was higher than the control cupcake and at 10% concentration of Roselle extract receive the highest score [25].
Figure 2: Sensory evaluation scores for Cupcakes (level 1: dislike extremely; level 2: dislike moderately; level 3: Neither like nor dislike; level 4: like moderately; level 5: Like extremely)

4. Conclusion
Roselle calyces are primary sources of acids which enhance the stability of anthocyanins pigment and have various of phytochemicals. Roselle calyx extracts were used to enrich cupcake. Dried Roselle calyces are commercially available and to obtain concentrated extracts which might be applied in the food and pharmaceutical industries for color and health benefits. In general, Roselle extract is added to have a significant influence on the nutritional and sensory value of cupcakes. There was an increase in the content of moisture, protein, and ascorbic acid which compares with control cupcake, achieved 30.16 ± 0.25, 2.76 ± 0.07, 37.65 ± 0.64 respectively. In contrast, the level of pH, ash, and lipid witnessed a reduction, achieved 7.02 ± 0.25, 1.05 ± 0.13, 14.78 ± 1.01 respectively. Ascorbic acid content and free-radical scavenging capacity tend to increase from 33.85 ± 0.92 to 37.65 ± 0.64. More than 50% of anthocyanin content is retained, achieved 3.74 ± 0.02. The sensory evaluation also showed that Roselle cupcake has an advantage over the traditional cake.

5. References
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