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Effect of Extraction Condition, Heat Treatments and Spay-drying on Stability of Roselle Anthocyanins as Natural Food Colorant

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Abstract

The present study was designed to investigate the effect of spray-drying as microencapsulation technique on the stability of roselle calyces (R.C.) anthocyanins. Different extraction conditions were evaluated to identify the best extraction presider for extracting roselle anthocyanins. The results showed that using 2% citric acid solution by 1: 10 solids: solvent ratio with crushed flower at 85°C for 20min. was the best condition for extraction of the red pigments from roselle calyces and recorded the highest anthocyanin yield of 1229mg/100g R.C. Total phenolic contents (TPC) and antioxidant activity was determined and the results showed that roselle calyces had TPC ranged from 12.16 to 14.45mg. gallic acid equivalent/g. depending on the extraction solvent. Methanol: water (80:20 V/V) recorded the highest TPC. Results also reflected that R.C had a strong antiradical efficiency of 0.727 and EC_{50} of 1.37µg roselle extract/µg DPPH. Thermal stability of roselle anthocyanins was investigated and the results showed that roselle extract heated at 95°C for 30 min. recorded retention value of 80.017%. The effect of three different encapsulating agents i.e. maltodextrin D.E. 18.7, gum Arabic and whey protein isolate on pigments stability was investigated. Maltodextrin DE 18.7 was found as the most effective carrier in stabilizing the pigments under the storage conditions examined with half-life of 577.62days. The application results proved that the addition of encapsulated roselle anthocyanins as a natural color with level of 0.3 % in a strawberry drink model system and 0.5% to jelly formulation was acceptable and can replace the synthetic color.

Keywords

Roselle (*Hibiscus subdariffa* L), Anthocyanins, Encapsulation, whey protein, shelf-life, jelly

1. Introduction

Today the food industries have an extensive range of both natural and synthetic colors. In line with the generally observed trend from synthetic dyes towards natural colorants [1]. However, because of the problems of the synthetic pigments that cause toxicity and carcinogenicity in the human body, the use of them is gradually has decreased. Therefore interest in natural pigments, that can replace synthetic ones, which caused many side effects, is increasing [2]. Recently in response to this trend, tend to use natural pigments as adding natural materials in the natural dyeing, healthy functional foods, cosmetic products for human health and safety have been gradually expanded [3]. Currently pigments of various kinds and forms have been used as additives or supplements in the food industry, cosmetics, pharmaceuticals, livestock feed and other applications. Roselle (*Hibiscus subdariffa* L.) is a tropical plant belongs to the family *Malvaceae* and is known by Egyptian consumers as Karkadah. Water extract of the Roselle calyces produces a brilliant red color and a pleasant acid test, rich in anthocyanins, and ideal for producing brilliant red colorings in many foods. Roselle anthocyanins as with most natural food colorants, suffer from inherent instability. Color stability of anthocyanins was found to be depending on a combination of various factors including: structure of anthocyanins, pH, temperature, oxygen, light, and water activity. Enzymatic degradation and interactions with food components such as ascorbic acid, sugars, metal ions, sulfur dioxide and copigments are no less important [4].

Microencapsulation can be used for many different products such as encapsulation of liquid flavors, enzymes, artificial sweeteners, coloring agents, vitamins and minerals [5]. Various techniques have been developed for encapsulation of both food ingredients and nutraceuticals, including spray drying, spray cooling/chilling, freeze drying, extrusion, fluidized bed coating, coacervation, liposome entrapment, inclusion complexation, centrifugal suspension separation, co-crystallization and emulsions [6].Spray-drying is the most commonly used technique, on account of it being a continuous, low cost process that produces dry particles of good quality, and for which the machinery required is readily available [7] and [8].

The main targets of the present work are: First, to investigate the effect of different extraction conditions (solvents type, solvent-to-solid ratio, temperature, extraction time, and particle size) on extraction efficiency of anthocyanins from roselle calyces. Second: to produce dry red powder of roselle pigments by using spray-drying technique and three different encapsulating agents (maltodextrin 18.7, arabic gum and whey protein) and study the stability of the encapsulated pigments during storage. Finally: utilization of the encapsulated roselle extract as food colorants in a model drink and jelly.

2. MATERIALS AND METHODS

2.1. Materials

The roselle calyces were obtained in a dried form (sun-dried) in summer 2013. The dried calyces were divided to two parts: The first part was kept as it is while the second part was crushed for 5 second using a blender (Braun type 4249, CombiMax (Germany). Both of the two parts were immediately packed in polyethylene bags kept away from light at low temperature (4°C) and till used. All chemicals used were purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Determination of total phenolic content

The Folin–Ciocalteu method was used to determine total phenolic compounds according to the methods described by [9]. The total phenolic content was determined by comparing with a standard curve prepared using gallic acid (10–200 µg/ml; $Y = 0.025X + 0.2347$; $R^2 = 0.9986$). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents (mg GAE)/100 g of roselle calyces.

2.2.2. Determination of radical scavenging activity

The free radical scavenging activity of the anthocyanins was analyzed by using the 1, 1-dipheny-2-picrylhydrazyl (DPPH) assay according to [10]. The scavenging or inhibition percentage was calculated according to the following equation:

$$\text{Inhibition}(\%) = \frac{(\text{abs.control} - \text{abs.sample}) \times 100}{\text{abs.control}}$$

Where: abs. is absorbance at 515nm

Measurement was performed at least in triplicate. Inhibition of coloration was expressed as a percentage, and the effective concentration 50 % (EC_{50}) was obtained from the inhibition curve.

2.2.3. Extraction of anthocyanins

To investigate the efficiency of extracting condition in the yield of anthocyanince from roselle calyces two different solid-to-solvent ratio (1:10 & 1:20), two temperature values (4°C & 85°C), and two extraction time 20 min & 24h.) were studied. Distilled water & citric acid 2% was used as solvents and the extraction of pigments was carried out according to the procedures described by [11] with some modification as described in (Table 1).

Table 1. Conditions for extracting of pigments from roselle calyces

Sample	Roselle Calyces	Solvent	Solvent: sample ratio	Temperature	Extraction time
Treatment 1	Crushed flowers	Hot water	1:10	85 °C	20 min
Treatment 2	Crushed flowers	Hot water	1:20	85 °C	20 min
Treatment 3	Whole Flowers	Hot water	1:10	85 °C	20 min
Treatment 4	Whole Flowers	Hot water	1:20	85 °C	20 min
Treatment 5	Crushed flowers	Cold water	1:10	5 °C	Overnight
Treatment 6	Crushed flowers	Cold water	1:20	5 °C	Overnight

Table 1. *Cont.*

Sample	Roselle Calyces	Solvent	Solvent: sample ratio	Temperature	Extraction time
Treatment 7	Whole Flowers	Cold water	1:10	5 °C	Overnight
Treatment 8	Whole Flowers	Cold water	1:20	5 °C	Overnight
Treatment 9	Crushed flowers	2 % citric acid	1:10	85°C	20 min
Treatment 10	Crushed flowers	2 % citric acid	1:20	85°C	20 min
Treatment11	Whole Flowers	2 % citric acid	1:10	85°C	20 min
Treatment12	Whole Flowers	2 % citric acid	1:20	85°C	20 min

2.2.4. Total pigment content

Total anthocyanins content of roselle extracts were determined calorimetrically according to the procedure described by [12]

2.2.5. Color diminution of roselle calyces extract (L*, a*, and b*)

The color of different samples was determined using a Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) according to the CIE Lab scale (CIE Colorimetric Committee 1974).

2.2.6. Effect of heat treatments on roselle anthocyanins

Thermal stability of Roselle anthocyanis was determined according to the method carried out by [13]. Appropriate amount of roselle anthocyanins extract was diluted with distilled water, total anthocyanins absorbance at 520 nm. was determined before heating. For heat stability study, 10% solution of the extract were placed in screw capped test tubes (2 × 15 mm) and heated in a thermostatically controlled water bath for heat treatment at 65, 75, 85 and 95°C for 10, 20 and 30 min. The tubes were cooled down immediately in an ambient water bath and total anthocyanins were determined by measuring the absorbance at 520 nm. Retention of anthocyanins was estimated according to the following equation:

$$\text{Retention of anthocyanin's (\%)} = \frac{\text{Total anthocyanins after heating}}{\text{Total anthocyanis before heating}} \times 100$$

2.2.7. Microencapsulation processes

For encapsulation purposes, maltodextrin 18, whey protein concentrate and gum arabic were evaluated as wall materials the process was cured out according to [14] with some modification. Twenty grams of each carrier were dispersed in 150 ml of the pigment extract (5°Brix) and the pH was maintained at the range 2.6. Then, the mixtures were vigorously homogenized at 10,000 rpm for 15min at room temperature. The resulting mixtures were subsequently were fed into the pilot plant spray dryer (Mini Spray Dryer B-290, BÜCHI Labortechnik, Switzerland) with a nozzle atomization system with 1.5 mm diameter nozzle and main spray chamber of 500 × 215 mm. at feed flow rate of 5 cm3/min. The prepared microcapsules were collected in a cyclone and packaged to prevent light incidence and stored at room temperature for further experiments.

2.2.8. Degradation kinetics of the encapsulated pigments

The degradation of roselle anthocyanins was followed periodically by measuring the coloring power of the stored samples. 0.5 gram of each encapsulated sample was dissolved in 20 mls. of distilled water on 50ml. beaker and magnetically stirred for 10 mins. The pH was adjusted to 2.6 and the volume was made up to the mark with distilled water. After filtration, absorbance was measured at 520 nm using a Spectronic 2000, Spectrophotometer, Busch and Lomb, (USA) and the coloring strength of the extract was expressed using the following formula:

$$E_{cm}^{1\%} = \frac{A\lambda}{CL}$$

Where: $E_{cm}^{1\%}$: Extinction coefficient (55.9), A_{λ}: Absorbance measured at a particular wavelength, λ; C: Concentration of the anthocyanin, (g./ 100 ml of the solution) and L: Length of the cell, in cm

Degradation parameters including degradation rate constants (k) were obtained from slope of a plot of the natural log of anthocyanins retention and half-life value (T1/2) for the encapsulated roselle anthocyanins were calculated by applying a first-order reaction model.

2.2.9. Morphological properties of the encapsulated powders

A Scanning Electron Microscopy (JEOL JSM6300 SEM, Tokyo, Japan) was used to acquire the morphology of the encapsulated anthocyanins powder. Samples were lightly gold sputter coated (Sputter coater, Agar Aids, England) for 45 seconds and imaged under scanning electron microscope operated at 10 kv and low beam current

2.3. Applications of encapsulated pigments

2.3.1. Addition of roselle extract to a model system of a drink

A model system of a drink were prepared according to [4] with added roselle extract at three different concentrations (0.1, 0.2, and 0.3 % w/v, 0.1% w/v, carmine and 0.05% w/v Carmoisine) was studied. Anthocyanins content and color were measured at zero time. The bottles were divided into two groups: the first group was stored in the refrigerator at 4-5ºC, the second was stored at room temperature (40 ± 2 ºC). For each sample group, one bottle was randomly selected for analysis every week for a period of 10 weeks.

2.3.2. Addition of roselle extract to strawberry jelly formulation

Jelly powders were prepared according to [15]. Three treatments of jelly with different adding reoselle extract, were prepared with added roselle extract at concentrations of 0.167, 0.33 and 0.50%, while carmine treatments was colored with carmin at concentration of 0.167%. Control treatment was a commercial jelly powder including synthetic color (carmoisine). Jelly samples have been sensory evaluated for jelly attributes such as color, flavor, texture, Transparency, and overall acceptability.

2.4. Statistical analysis

Data were expressed as the mean ± SD for five rats in each group. The data were analyzed using SPSS software package version 20.0 and values were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range tests at 5% level of significance was used to compare between means.

3. Results and dissection

3.1. Total phenolic and antioxidant activity of roselle extracts

Regarding to the results in (Table 2), the highest phenolic compounds found when methanol: water (80:20) was used as extracting solvent (14.245mg /g roselle calyces) followed by citric acid solution which gave 13.71mg/g roselle calyces) While, distilled water extracted the lowest quantity of phenolic compounds. This finding could attribute to the polar character of the phenolic compounds which makes them soluble in the polar solvents such as methanol, ethanol, and water. Also the present of acid solvent extraction of anthocyanins is the initial step in the determination of total and individual anthocyanins prior to quantification, purification, separation, and characterization [16]. Citric acid 2 % solution indicating phenolic compounds yield of 13.71mg/ g. roselle calyces). The difference is probably due to the characteristic of the solvent; this could affect which compounds are extracted from the plant matrix. This phenomenon can be explained by a change in polarity of the antioxidant compound due to the particular solvent used for extraction. [12] obtained dried roselle calyces contained 37.42mg/g dry weight sample of total phenolic compounds

Table 2. Total phenolic contents of roselle calyces as affected by extraction solvents

Extraction solvent	Total phenolic content mg/g extract. D.W.
Methanol: water 80:20	14.45
Ethanol: water 50:50	12.83
Citric acid solution 2%	13.71
Distilled water	12.12

Total phenolic compounds contents of roselle calyces ranged from 24.36 (in water) to 44.43 (in ethanol -methanol) mg of gallic acid 100 g^{-1} of dried calyces [17]. [18] Estimated the total phenolic content in roselle calyces using ethanol 70% as extracting solvent and found it to be 41.07 mg Gallic acid equivalent /g roselle calyces. The content of phenolic compounds and other phytochemicals present in medicinal plants, as well as in fruits and vegetables, is largely influenced by the type of cultivation, genetic factors, environmental conditions, in addition to the degree of maturation and the variety of the plant [19].

3.2. DPPH radical-scavenging activity of the roselle calyces extract

The results of the antioxidant activity of different concentrations of the roselle extract are shown in (Figure 1). The results indicated that the DPPH radical-scavenging activity of roselle calyces was occur in a dose-dependent manner. With increasing

the concentrations of roselle extract the inhibitory activity against the DPPH radical increased. The lower EC_{50} value reflects better protection action against oxidation. We determined the concentration required to inhibit 50 % radical-scavenging effect (EC_{50}) using a series of concentrations tested. A lower EC_{50} value corresponds to a larger scavenging activity. The EC_{50} values of the roselle extract was EC_{50} = 1.375µg roselle extract /µg DPPH and antiradical efficiency AE (0.7272).

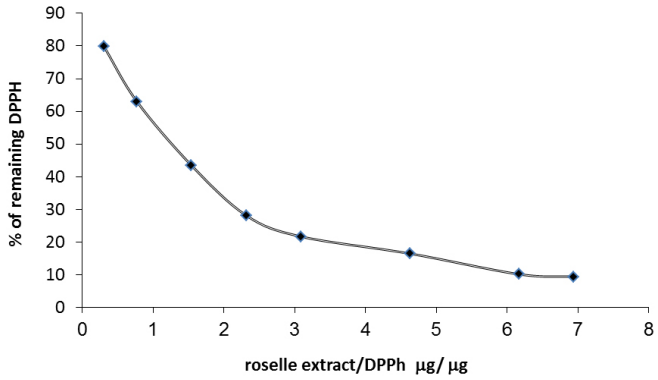

Figure 1. Percent of remaining DPPH as function of µg of roselle extract per µg DPPH

These results showed that when roselle extracts used with a concentration of 0.31µg roselle extract /µg of DPPH the inhibition ratio was 20.22% while 6.94µg roselle extract /µg of DPPH recorded inhibition ratio of 90.69%.

These results indicated that there are abundant antioxidative phytochemicals presents in the clyces extracts of roselle. Our results are similar to that reported by [10]; [20] and [18]. The strong antioxidant activity of roselle extract could be due to exist of polyphenol compounds. [12] Investigated antioxidant activity of roselle plant extracted by different solvents and indicated that the ethanol acidified with 1% citric acid extracts exhibited higher value in total antioxidant activity and recorded (EC_{50}) of 42.77 (µg/ml).

3.3. Effect of extraction conditions on the extraction efficiency of roselle anthocyanins

The effect of extraction condition on the yield of pigments recovered, the total soluble solid and the pH of the extracts are showed in (Table 3). By reducing the particle size, the yield of anthocyanins increased in all the extraction conditions examined. The crushed flower gave total anthocyanins of (0.994.18mg. /100g. DW.) While whole flower gave 912.96mg/100 dry weight under the same conditions. It could be also notes that adding citric acid to the extraction medium had a great effect in stabilizing anthocyanins, thus the extraction efficiency increased. When 2% citric acid solution used as extraction solvent with crushed flower and 1:10 solid: solvent ratio, total anthocyanins recorded 1229.62 mg. /100g dry weight. These observations show that the pH value is a very important factor affecting extraction of anthocyanins. It was reported that the extracting solution should be slightly acidic to maintain the flavylium cation form, which is red and stable in highly acidic medium [21 & 12]. Likewise [22] reported that and the mixture of 50% (v/v) ethanol and acidified water resulted in largest anthocyanin content (390.6 mg/L). Similar results reported by[11,23 & 2]. Extracting the anthocyanins at 85°C for 20min recorded higher anthocyanin content than extracting at 5°C for overnight in all condition investigated [1].

Table 3. Extraction efficiency of different extraction conditions and color diminution of the extracts

Sample	T.S.S	pH	Total anthocyanins Mg./ 100 g/ D.W.	L*	a*	b*
Treatment 1	5	2.8	994.18	1	42.2	1.48
Treatment 2	2	2.84	760.80	11.37	75.97	19.29
Treatment 3	5	2.81	912.96	2.21	57.17	3.59
Treatment 4	2	2.83	791.64	11.51	77.3	19.53
Treatment 5	5	2.79	827.63	3	65.77	4.95
Treatment 6	2	2.92	546.95	13.6	40.33	25.25
Treatment 7	5	2.80	796.78	3.52	68.25	5.84
Treatment 8	2	2.86	567.52	13.8	39.21	23.81
Treatment 9	6.3	2.48	1,229	1.14	3.61	1.15
Treatment 10	4.1	2.58	1,079	1.68	3.46	1.16
Treatment 11	5.9	2.51	944.66	1.84	2.48	0.45
Treatment 12	4.2	2.54	902.03	1.02	2.77	0.73

3.4. Effect of heat treatment on retention of roselle anthocyanins

As with most of chemical reactions, the stability of anthocyanins markedly influenced by heat treatment. The retention of roselle anthocyanin as related to heating temperature and time are given in (Figure 2). The results showed that at 65°C no significant loss occurred in anthocyanins content of roselle extract since retention values were 95.92, 93.77 and 92.34 after heating times of 10, 20, and 30 min. respectively. As heating temperature increased to 85°C, retention of anthocyanins was still as high as 91.84% after 30 min. of heating. When heat treatment was occurred at 95°C for 30 mins, roselle extract retained more than 80% of its original content of anthocyanins before heating. It may be concluded that roselle anthocyanins have relatively high stability at high temperature particularly when heating period was relatively short (30 min). Similar observations were found by [24 &25].

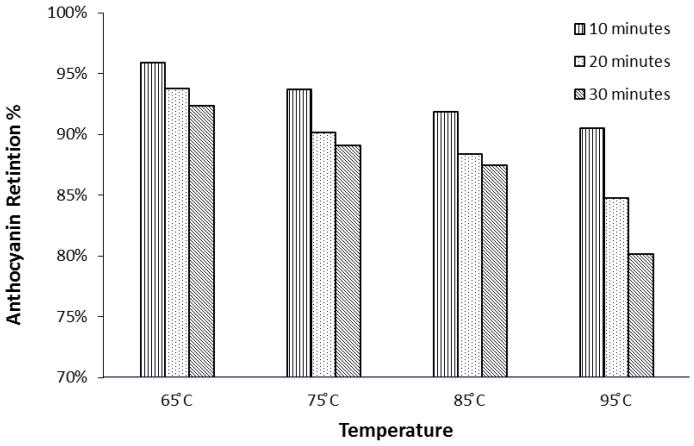

Fig 2. Effect of heat treatments on roselle anthocyanins stability.

Many authors have studied the influence of temperature in the anthocyanins stability from different sources proving that heating have a harmful effect on the anthocyanin content [26; 27; 28 & 11]. The thermal degradation of anthocyanins have to be occur via two mechanisms: first, hydrolysis of the 3-glycoside linkage to form the more labile aglycone and second, hydrolytic opening of the pyridium ring to form a substituted chalcone, which degrades to a brown, insoluble compound of polyphenolic nature [14]. [29] studied the anthocyanin degradation of blueberry puree in novel hydro-thermodynamic processing and found that anthocyanin degradation was non-significant in the range of temperatures from 25 to 80 °C, becoming significant above 80 °C.

3.5. Degradation kinetics and storage stability of encapsulated roselle anthocyanins.

Degradation kinetic studies the of roselle anthocyanins encapsulated in Maltodextrin DE 18.7, Whey protein isolate (90 %) and Gum Arabic, and control sample without carrier carried out in dark at 30ºC. changes in color strength for the different encapsulated roselle anthocyanin powders followed by periodical measurements of absorbance to define the order of anthocyanins degradation reaction. As illustrated in (Figure 3), plotting color strength values (Ln E_{520}) vs storage time (days) gave straight lines for the different encapsulating agents and control. Linear regression analysis showed that the degradation of roselle anthocyanins encapsulated in the three evaluated coating materials followed first – order reaction kinetics. Similar kinetic responses reported by [29 &30] for the degradation of pelargonidin based anthocyanins at different water activity conditions.

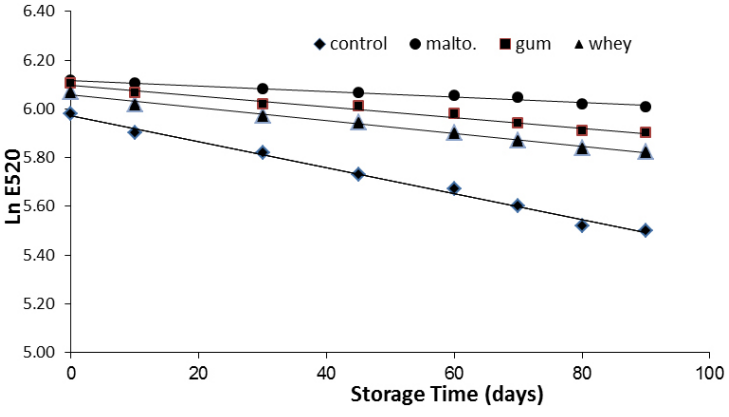

Figure 3. First-order degradation plots for spray-dried roselle anthocyanins with different encapsulating agents during storage at 30°C in dark containers.

Degradation rate constants for anthocyanins encapsulated in the different matrices were calculated with the correlation coefficients and half-life period and the data is presented in (Table 4). The obtained results showed that degradation rate constant values were 5.3, 1.2; 2.2 and 2.6 × 10^3 for the control, Maltodextrin DE 18.7; Gum Arabic Whey protein isolate respectively. The highest value of the rate constants for anthocyanins degradation was observed at for the control sample while the lowest rate constant was recorded for Maltodextrin DE 18.7. Among the polymeric matrices, which largely elongated the half-life of roselle anthocyanins, maltodextrin DE 18.7 was found as the most effective carrier in stabilizing the pigments during storage.

Table 4. Degradation rate constants for roselle anthocyanins encapsulated in different encapsulating agents during storage at 30°C in the dark

Encapsulating agent	Rate constant (days^{-1})	Correlation coefficient R^2	Half - life period (days)
Control	5.3 × 10^3	0.99	130.78
Whey protein	2.6 × 10^3	0.99	266.60
Gum arabic	2.2 × 10^3	0.98	315.07
Maltodextrin	1.2 × 10^3	0.97	577.62

The half–life period of the encapsulated roselle anthocyanins were increased feom130.78 for the control sample to; 266.60; 315.07and 577.62 days for Whey protein isolate; Gum Arabic and Maltodextrin DE 18.7 respectively. Similar kinetic responses were reported by [30 & 25]. The could be due to the open porous structure obtained in the freeze died final product, which makes it exposed to air if the encapsulated product is not packed under vacuum or inert atmospheric condition [31].

3.6. Particle size and microstructure

SEM photographs of microcapsules for the MD, GA and WP particles which were spray dried at 160 °C air inlet temperature with 20° Brix feed solid levels showed that the particle size of powders ranged from 3 µm to 20 µm approximately (Figure 4). The microcapsules obtained from MD were smooth spheres with hardly any surface cracks in the wall systems. No holes observed on the surface of any of the samples.

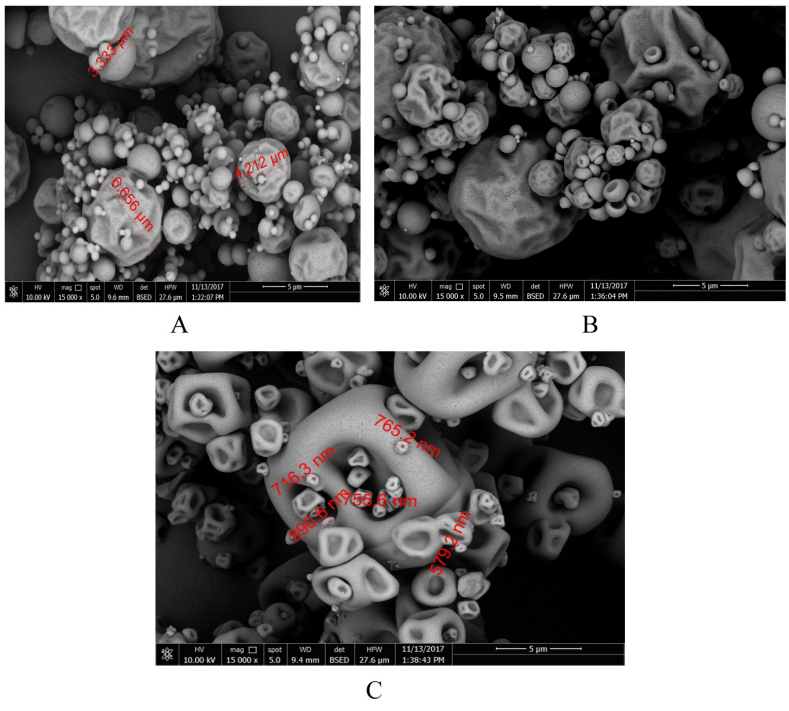

Figure 4. Micrographs of the microcapsules produced from spray-dried roselle pigments using three different encapsulating agents (A) Maltodextrin, (B) Gum Arabic and (C) Whey protein

3.6. Stability of roselle anthocyanin in a drink model of system

One of the most key factors affects the applications and usage of natural colorants in the food industry is the stability during storage and handling. Roselle anthocyanin stability in a drink model system has been investigated during storage of the drink model at 40 °C & 5 °C. we used three different concentration of roselle anthocyanin (0.1, 0.2 and 0.3%); Carmine E 120 dosage 0.1% and Carmoisine E124 dosage 0.05% were used for comparison as the most common used red colors; Natural and synthetic, respectively. The color retention was measured periodically by 10 days; the results shown in (Figure 5 and 6). When the drink samples stored at 5°C, the degradation rates for the five treatments were lower than the degradation when stored at 40°C. After 70 days storage at 5°C, the drink model samples colored with roselle anthocyanin retained 42.16, 45.37 and 68.65 % of anthocyanin content, while drink model samples with Carmine and Carmoisine retained 78.05 and 87.01 of the color content respectively.

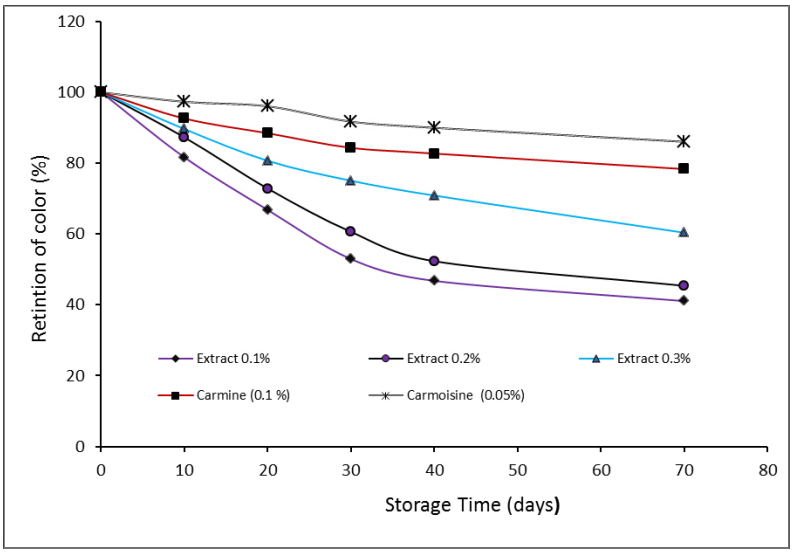

Figure 5. Stability of roselle anthocyanin in a drink model of system stored at 5°C compared with carmine and carmoisine

When the samples stored at 40 °C, the drink model samples colored by the roselle anthocyanin extract lost their color faster than the drink model samples colored with Carmoisine and Carmine. During storage at 40°C, the sample colored with roselle anthocyanin 0.1% was recorded the highest color losses and recorded a retention ratio of 26% after 70 days storage. The results reflected that the storage temperature had a very important effect on the roselle anthocyanin stability.

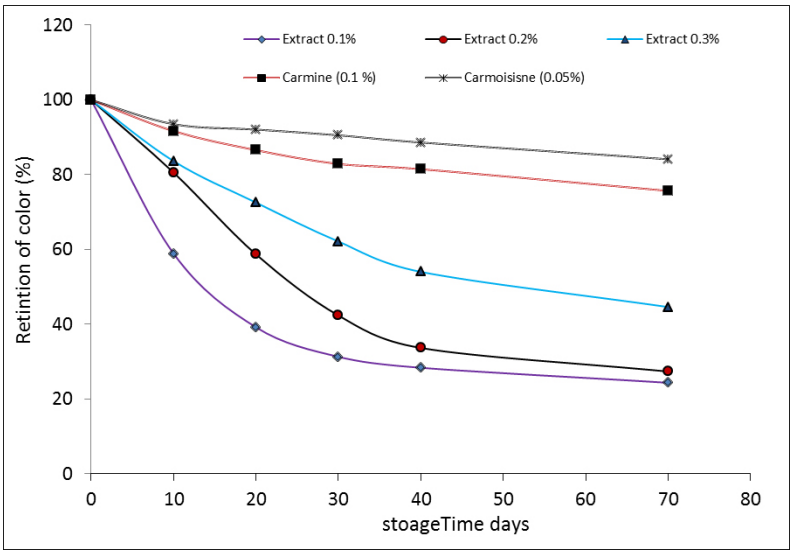

Figure 6. Stability of roselle anthocyanin in a drink model of system stored at 40°C.

It could be noted that with increasing roselle anthocyanin concentrations led to increase of color stability. Therefore, colored sample with 0.1% roselle anthocyanins extract retained only 41.03 % while the sample colored with 0.3 % roselle anthocyanin

extract retained 60.35 % of its color after 70 days storage at 5°C. This is could be due to the copigmentation reaction which enhanced roselle anthocyanin stability. Similar findings reported by [32 &33]. Many authors investigated the effect of temperature on the anthocyanin stability [27 & 34]). The chemical stability of thermally treated anthocyanin aqueous solutions during storage at 4, 25, 45, and 65 °C was investigated [35]. The results showed that the degradation rate of anthocyanins in aqueous solutions was faster than those in real food. They also reported that the anthocyanin aqueous solutions stored at 4 °C had the best chemical stability. [4] reported that the rate of color changes in a drink containing roselle anthocyanins extract during the storage was higher than that in drinks containing either synthetic Carmoisine or natural carmine and the chroma decreased with increases of lightness

3.7. Application of encapsulated roselle extract in jelly formulation

The prepared jelly samples were sensory evaluated and means scores were statistically analyzed and the results presented in (Figure 7). The results indicated that no significant differences ($p > 0.05$) found between sample colored with carmoisine; carmine and sample colored with roselle anthocyanins at a level of 0.5% as coloring agent. On the other hand, there were significant differences between samples colored with roselle anthocyanins at low level 0.16 and 0.33 and the other samples. It could be notes that jelly sample colored carmoisine scored the highest value for color of (9.67) while the sample with 0.167roselle anthocyanins recorded the lowest value for color of 7.50.

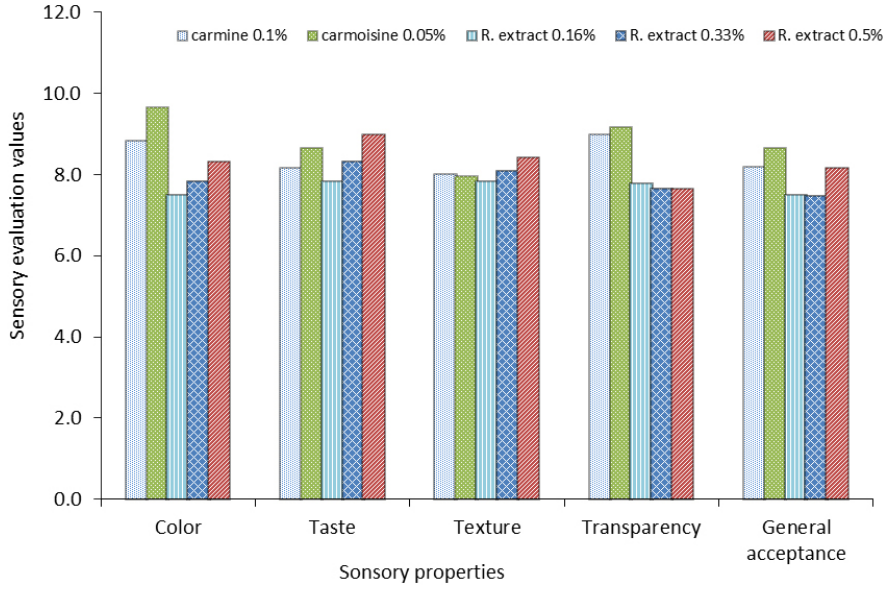

Figure 7. Sensory evaluation of jelly samples containing encapsulated roselle extracts, carmine and carmoisie

Regarding to the samples taste sample with roselle extract at a level of 0.5% recorded the highest taste value of 9.00. The results reflected that there were no significant differences ($p > 0.05$) between all samples except the sample colored with roselle extract at a level of 0.16% which scored the lowest value for taste (7.83). The results showed that no significant differences ($p > 0.05$) found in sample colored with carmoisine and carmine in transparency while those colored with different concentrations of roselle anthocyanins i. e. 0.16, 0.3 and 0.5% were not significantly different. Sensory data showed there were no significant differences based on texture of all samples. Concerning the overall acceptability, most of the panelists preferred the samples colored with carmoisine and carmine and samples produced with 0.5% roselle anthocyanin. Based on data collected from sensory evaluation in the studies, adding 0.5% of roselle anthocyanins to the jelly formula gave close scores to carmoisine and carmine samples thus, indicates that addition the natural color with level of 0.5% to the jelly formulation was acceptable and can replace the synthetic color. [8] reported similar results.

Conclusion

Using 2% citric acid solution by 1: 10 solids: solvent ratio with crushed flower at 85C for 20min. was the best condition for the red pigments from roselle calyces. Encapsulation of anthocyanins with polysaccharides enhanced anthocyanin stability for efficient use in food systems. The storage stability result supported that maltodextrin as wall material gave the longest shelf life and the smallest change in the pigment color. Storage period significantly affect the color changes of the spray dried powder. Hence, this study signifies that encapsulation process could stabilize and extend the shelf life of anthocyanins content. The results indicates that the addition of roselle anthocyanins as a natural color with level of 0.3 % in the drink model system and 0.5% to the jelly formulation was acceptable and can replace the synthetic color

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