# EFFECT OF EXTRACTION CONDITIONS, HEAT TREATMENTS AND SPRAY-DRYING ON STABILITY OF ROSELLE CALYCES ANTHOCYANINS AS NATURAL FOOD COLORANTS

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## ABSTRACT

The present study was designed to investigate effect of spray-drying as microencapsulation technique on the stability of Roselle calyces' anthocyanins. Twelve 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.. 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. Roselle calyces. Methanol: water (80:20) recorded the highest TPC followed by ethanol: water (50:50) while, distilled water recorded the lowest TPC of 12.16 mg Gallic acid equivalent/g. Roselle calyces. Results also reflected that Roselle calyces 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 70°C for 30min retained 89.01% of their anthocyanin content. With increasing the temperature to 95°C the retention value was decreased and recorded 80.017%, respectively. 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. The degradation followed first – order reaction kinetics and was strongly dependent on the matrix. Maltodextrin DE 18.7 was found as the most effective carrier in stabilizing the pigments. The obtained results showed that degradation rate constant values were 5.3, 1.2; 2.2 and 2.6 X $10^3$ for the control, Maltodextrin DE 18.7; Gum Arabic Whey protein isolate respectively. Half–life period of the encapsulated Roselle anthocyanins were increased feom130.78 for the control sample to; 266.60; 315.07and 577.62days for Whey protein isolate; Gum Arabic and Maltodextrin DE 18.7 respectively. The encapsulated pigments were used as coloring agent in strawberry drink model system and jelly. The results proved that addition of Roselle anthocyanins at concentration of 0.3 % in the drink model system and 0.5% to the jelly formulation, as a natural color was acceptable and can replace the synthetic color.

**Key words:**Roselle anthocyanins, Encapsulationc, shelf-life, jelly.

## 1.INTRODUCTION:

Color additives have long been a part of human culture. Archaeologists date cosmetics colors as far as 5000 B.C. Ancient Egyptian writings mention the use of drug colorants and historians estimate that food colors likely emerged around 1500 B.C. Color is an essential criterion for food choice and of the most important quality attributes affecting the consumers acceptance of food (Selim et al., 2008).

Anthocyanins (Greek anthos, flower and kyanos, blue) are part of very large and widespread group of plant constituents known collectively as flavonoids, but they are differ from other flavonoids by strongly absorbing visible light. They are the most important group of coloring matters in plants. These intensely colored water-soluble pigments are among the best known of the natural pigments, being responsible for all the pink, magenta, red, violet, and blue colors in the petals, leaves, and fruits of higher plants (Brouillard, 1982; Francis, 1985; Mazza and Miniati, 1993; Jackman and Smith, 1996 and Mahdavi, et al., 2016).

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.. There are many research have been conducted on *H. sabdariffa* extract, however most focused on its antioxidant activity (Tsai and Huang, 2004; Hirunpanich et al., 2006) rather than on their anthocyanins pigment stability and application in food products.

Roselle anthocyanins as with most natural food colorants, suffer from inherent instability. Degradation of anthocyanins may occur during extraction, purification, and normal food processing and storage. They may degrade into colorless and/or undesirable brown colored compounds (Mazza and Brouillard, 1990). 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 (Mazza and Brouillard, 1987a, Mazza and Miniati, 1993 and Duangmal et al*.,* 2004). Microencapsulation is a technique by which liquid droplets, solid particles or gas bubbles of core-material are coated with a thin film of encapsulating agents. The microencapsulated material is termed internal phase or the core. The encapsulating materials are called the shell, coating or wall materials (Shahidi and Han, 1993 and Hogan *et al*., 2001). The coating film protects the core against deterioration, reduces the evaporation of volatile compounds, and releases the core under desired conditions (Kibri, 1991).

Microencapsulation can be used for many different products such as encapsulation of liquid flavors, enzymes, artificial sweeteners, coloring agents,

vitamins and minerals (Reineccius, 1989; Jackson and Lee, 1991 and Mahdavi et al., 2014).

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 (Gibbs et al., 1999; Augustin and Hemar, 2009 and Desai and Park, 2005). Selection of an encapsulation technique depends upon specific applications and parameters such as physical/chemical properties of both core and coating materials, required particle size and coating materials, release mechanisms and acceptable process cost (Ré Mi, 1998). However none of the existing techniques can be considered as a universally applicable process, as individual food components demonstrate extreme differences in molecular weight, polarity, solubility, and stability (Augustin and Hemar, 2009).

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 (Arueya et al., 2014). Spray drying encapsulation has been successfully used for a number of colorants rich materials (Selim et al., 2008; Mahdavi et al., 2014).
Different types of wall materials have been used for microencapsulation including polysaccharides (starches, maltodextrins, ß-cyclodextrin, corn syrups and gum Arabic), lipids (stearic acid, mono- and diglycerides), pullunan, and proteins (gelatin, sodium casein, milk serum, soy and wheat) (Selim et al., 2000; Hogan et al., 2001, Bertolini et al., 2001; Desai and Park, 2005; Tonon et al., 2010 ).

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:

#### 2.1.1. Raw materials:

Calyces of roselle (*Hibiscus subdariffa*, L.) are the raw materials used in this study and were collected from Upper Egypt. The 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.

**2.1.2. Chemicals:**

Maltodextrin DE 18.7, whey protein isolate (90 %) and gum arabic were used as encapsulating agents (wall materials) were purchased from MBD, Donated from Dreem SAE & MIFAD. Anhydrous sodium carbonate ($Na_2CO_3$), folin–ciocalteu, hydrochloric acid (HCl), gallic acid, methanol, citric acid were purchased from Merck (Darmstadt, Germany).

**2. 2. Methods**

**2. 2.1.Chemical analysis of Roselle calyces:**

Moisture, ash, fat, protein, fiber and carbohydrate of roselle calyces were determined according to the methods described in the AOAC (2005).

**2.2.2. Determination of total phenolic content.**

The Folin–Ciocalteu method was used to determine total phenolic compounds (Singleton, *et al.,* 1999). Roselle calyces sample (4g) was execrate with 100 ml of methanol: water (80:20) for 24h. at 4°C. the extract was filtered through whatman No. 1. A 50 µL aliquot of the extract solution was mixed with 500 µL 0.2 N Folin– Ciocalteu reagents and 1.950 µL of distilled water was added. The solution was thoroughly mixed by overtaxing and incubated for 5min at ambient temperature. 1500 µL of sodium carbonate (20% $Na_2CO_3$) was added to the reaction mixture. After incubation at room temperature for 2 h, the absorbance of reaction mixture was measured at 765 nm against a methanol blank. 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.

**2.2.3. 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 Abba Pacôme *et al.,* (2014). DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH solution. The antioxidant activity is then measured by the decrease in absorption at 515 nm. In this method, a 0.1 mM solution of DPPH in methanol is prepared (6 mg DPPH/100 ml methanol), and 2 ml of this solution are added to different concentration of the anthocyanin solution in methanol. The mixture was left to stand at room temperature for 30 min in the dark before absorbance measurement at 515 nm to assess the stability of the coloured reactive action. Control sample was prepared, which contained the same volume without any extract and methanol was used as the blank. The scavenging or inhibition percentage was calculated according to the following equation:

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

Where: abs. is absorbance at 515nm

The actual decrease in absorption induced by the tested compounds was compared with the positive control. 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.

**2.2.4. 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 **Cissé *at al.*, (2012)** 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 |
|---|---|---|---|---|---|
| Treatment1 | 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 |
| 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 |

**The extracts were collected and filtered through a filter paper (Whatman No.1) before analysis.**

**2.2.5. Physio-chemical analysis of the extracts:**

**2.2.5.1. Determination of pH:**

The pH of roselle calyx were measured using pH meter (model Cyber Scan 500) standardized with buffer solutions of 4.0 and 7.0 according to the method of AOAC (2005)

**2.2.5.2. Total titratable acidity (TTA):**

Total titratable acidity was expressed as % citric acid was determined by standard AOAC (2005) using 0.1 N NaOH and phenolphthalein as an indicator.

**2.2.5.3. The total soluble solids (T.S.S):**

The total soluble solids (T.S.S) of samples extracts were determined according to A.O.A.C (2005) at room temperature (25 ºC± 1) by Refractometer (ATAGO, Japan) and expressed as ºBrix (0 - 90),

**2.2.6. Total pigment content:**

Total anthocyanins content of roselle extracts were determined calorimetrically according to the procedure described by Du and Francis, (1973) where a known volume of the filtered extract was diluted to 100 ml. with the extracting solvent. The color intensity was measured at the wave length of 520 nm using Spectronic 2000, Spectrophotometer, (Busch and Lomb, USA). The total anthocyanins content referred to delphenidin-3,5-sumboside was calculated using the following equation:

$$\text{Total anthocyanins (mg/100g)} = \frac{\text{Absorbance x dilution factor x 100}}{\text{Sample weight x 55.9}}$$

**2.2.6.1. 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). The system provides the values of three color components: L* (black–white component) and the chromaticity coordinates, a* (red to green component) and b* (yellow to blue component). The samples were placed in a 34mm optical glass cell and illuminated with D65-artificial daylight (10 standard angle) in accordance with the instructions of the manufacturer.

**2.2.7. Effect of heat treatments on roselle anthocyanins:**

Thermal stability of Roselle anthocyanis was determined according to the method carried out by Mok and Hettiarachchy (1991). 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 x 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:

$$\text{Retention of anthocyanins (\%)} = \frac{\text{Total anthocyanins after heating}}{\text{Total anthocyanis before heating}} \text{ X 100}$$

**2.2.8. Preparation of encapsulated pigments:**

Roselle pigments were extracted with distilled water according to Mattuk, (1998). One hundred grams of ground dried calyces were thoroughly mixed with 1000 ml and heated up 85 °C for 20 minutes. The water-soluble extract was filtered using Whatman No.1 filter paper to obtain roselle pigment extract.

**2.2.8.1. 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 Idham, et al., (2012) 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. The solutions were fed into the main chamber through a peristaltic pump and the feed flow rate was controlled by the pump rotation speed. Drying air flow rate was 2.5 m3/min and compressor air pressure was 0.06 MPa. Inlet and outlet air temperature were 180-195°C and 71-75°C, respectively, and feed flow rate was 5 cm3/min. to prepare the powder form. The prepared microcapsules were collected in a cyclone and packaged to prevent light incidence and stored at room temperature for further experiments.

**2.2.8.2. Degradation kinetics of the encapsulated pigments:**

To evaluate the stability of encapsulated pigments, immediately after capsules preparation, the spray-dried encapsulated samples were stored in brown bottles with screw caps and placed at 35°C for the kinetic studies and effects of storage time on the stability of anthocyanin's powders. 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 solution was transferred to a 25 ml volumetric flask, 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:

$$E_{cm}^{1\%} = \frac{A_{\lambda}}{C\,L}$$

Where:

$E_{cm}^{1\%}$ : Extinction coefficient (55.9)

$A_{\lambda}$ : Absorbance measured at a particular wavelength, $\lambda$

C: Concentration of the anthocyanin, (g per 100 ml of the solution)

L: Length of the cell, in cm

**2.2.8.3.. Degradation rates constants and the half-life values:**

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 according to Tsimidou and Tsatsaroni (1993).

**2.2.9. Applications of encapsulated pigments:**

Trials were made to utilize the encapsulated pigments in improving color of some food products including drink and jelly.

**2.2.9.1. A model system of a drink**

A model system of a drink were prepared according to Duangmal, et al., (2004) 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. The ingredients for the drink were sugar (10% w/v), citric acid (0.5% w/v) and colorant. The drink was pasteurized at 85°C for 20 minutes. Each drink was then aseptically hot filled into

glass bottles (200 ml) with 1-ml headspace. Their 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.

**2.2.9.2. Color measurements of drink:**

The color of different drink treatments were measured using a Spectro-Colorimeter ( Tristimulus color machine) with CIE lab color scale ( Hunter, Lab Scan XE, Reston VA.) calibrated with a white standard tile of Hunter Lab color standard (LXNO. 16379): L*, a*,and b* values were recorded for the treatments.

**2.2.9.3. Addition of roselle extract to strawberry jelly formulation**

Jelly powders were prepared according to Egyptian Standard ES: 800/ 2007. 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). Samples have been prepared by dissolving powders in boiling water then colorants have been added and stirred. Jelly samples have been cold in a water path then kept in the fridge for 24 hours then sensory evaluation has been done.

**2.2.9.4. Sensory evaluation of Jelly samples**

The jelly samples were subjected to sensory evaluation after preparing samples to determine the more preferred level of added color and compare to the synthetic color. The panelists measured the selected critical jelly attributes such as color, flavor, texture, Transparency, and overall acceptability according to the 9-hedonic scale from "dislike extremely" to "like extremely. Data collected from the sensory evaluation were statistically analyzed.

**2.2.9.5.. Statistical analysis:**

Linear regression analysis was used to obtain the degradation rate constants in the kinetic studies of the encapsulated samples. Significance of differences among various rate constants was analyzed using t-test at the 95% confidence level according to Steel and Torrie, (1980). Data from sensory evaluation were analyzed by the analysis of variance using the statistical analysis system (SAS, 1996).

**3. Results and dissection**

**3.1. Chemical analysis of raw materials:**

Roselle calyces used in this investigation were obtained in a dry form from the local market in Egypt. The calyces were subjected for chemical analysis and obtained data is given in Tables (2). The results indicated that moisture content of sun dried calyces was 12.75 %. It is well known that roselle extract is characterized by its sour taste. This is confirmed by the total acidity of roselle calyces, which was as high as 14.68%. In the same ways, pH value was as low as 2.69. Similar result was reported by Wills et al., (1998). They indicated that the pH value of Roselle calyces was 2.62. The pH depends on the concentration of free H ions or mirrored the changes in total organic acids. The free state of H ions is due to dissociation of H ions from the carboxylic group (- COOH) of organic acid. Chemical analysis also showed that roselle calyces contained 6.19, 10.5 and 0.35, of protein, fiber and fat. Results

given in table (2) showed that roselle calyces had a high ash content which reached 11.34 %.

**Table (2): Chemical analysis of roselle calyces.**

| Constituent | Content (%) | |
|---|---|---|
| | **Sun dried weight basis** | **Dry weight basis** |
| Moisture | 12.75 | - |
| Total acidity | 14.68 | 16.824 |
| pH | 2.69 | - |
| Protein | 6.19 | 7.094 |
| Ether extract | 0.35 | 0.401 |
| Fiber | 10.5 | 12.034 |
| Ash | 11.34 | 12.99 |
| Carbohydrate | 70.12 | 80.37 |

These results are in agreement with that obtained by selim et al., (2008). Likewise similar results also reported by Abou-Arab, et al., (2011) who investigated the Physico- chemical properties of natural pigments extracted from Roselle calyces and indicated that moisture content, protein, fat, fiber and ash were 12.81 %, 7.51%, 0.46 %, 11.17 % and 11.24 %, respectively. Jafarian et al., (2014) investigated the chemical analyses of dried roselle calyces and reported that moisture content of naturally dried roselle calyces was 11.35% .Chemical analysis also showed that roselle calyces contained 6.42, 0.33, 10.27 and 11.02 % of protein, fat, fiber, and ash respectively. The differences between our finding and the data reported by the other researchers may be due to genetic variety and type of soil.

**3.2. Total phenolic and antioxidant activity of roselle extract**

The screening of plants for nutritional and medicinal value has been carried out by numerous researchers with the help of preliminary phytochemical analysis (Abba Pacôme et al., 2014). In the present investigation, polyphenols were determined. Polyphenols have attracted a great attention in relation to their potential for beneficial effects on health. During the recent years, several experimental studies have revealed biological and pharmacological properties of polyphenols compounds, especially their antioxidant activity, anti-inflammatory activity, antiviral and cytotoxic activity and helpful in protection and prevention against many degenerative diseases, Azevedo et al., (2010) and Abba Pacôme et al., (2014).

In the present study total phenolic content (TPC) of roselle calyces was determined using Folin- Ciocalteu essay. A standard calibration plot was generated using known concentrations of gallic acid (10-60 µg/mL).TPC was calculated from the calibration splot and expressed as mg gallic acid equivalents (mg GAE) of phenol/g of roselle calyces.. The calibration equation for gallic acid was $y = 0.0248x + 0.237$, R2 = 0.997, where y is absorbance and x is concentration of gallic acid in µg/mL. All measures were performed in triplicate.

Regarding to the results in table (3). The highest phenolic compounds were 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 (Rivas-Gonzalo, 2003). 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. Abou –Arab et al., (2011) obtained dried roselle calyces contained 37.42mg/g dry weight sample of total phenolic compounds.

**Table (3): Total phenolic contents of roselle calyces as affected by extraction solvents.**

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

Jafarian et al., (2014) found that 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. Sirag et al., (2014) 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 (Koleva et al., (2002).

**3.3. DPPH radical-scavenging activity of the roselle calyces extract**

In this part of the study, the antioxidant activity of the sun dried roselle calyces extracts was determined using the 2, 2 diphenyl-2-picryl hydrazyl (DPPH) which is widely to determine the total antioxidant capacity of several natural compounds such as phenolic or crude extracts of plants (Mensor et al., 2001; Chinedu et al., 2011; Tsai et al., 2012 and Sirag et al., 2014).

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. The concentration required to inhibit 50 % radical-scavenging effect (EC50) was determined from the results of a series of concentrations tested. A lower EC50 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). These results reflated that

when roselle extract was use with a concentration of 0.31µg roselle extract /µg of DPPH the inhibition ratio was 20.22% while concentration of 6.94µg roselle extract /µg of DPPH recorded inhibition ratio of 90.69%.

These results indicated that there are abundant antioxidative phytochemicals present in the clyces extracts of roselle. Our results are similar to that reported by Abba Pacôme et al., (2014). They found that the antioxidant activity of the roselle extract has a scavenging ability of DPPH radical (around 97 %) with EC50 values of 0.24 mg/ml. According to Ersus and Yurdagel, (2006) the maximum inhibition varied between 80 and 90 % in the presence of 7µg roselle extract /µg DPPH. The strong antioxidant activity of roselle extract could be due to the presence of polyphenol compounds Abou-Arab et al., (2011) 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 (EC50) of 42.77 (µ/ml). Tee et al., (2002) reported that roselle extract had stronger antioxidant activity than BHA or tocopherol in a linoleic acid model system.

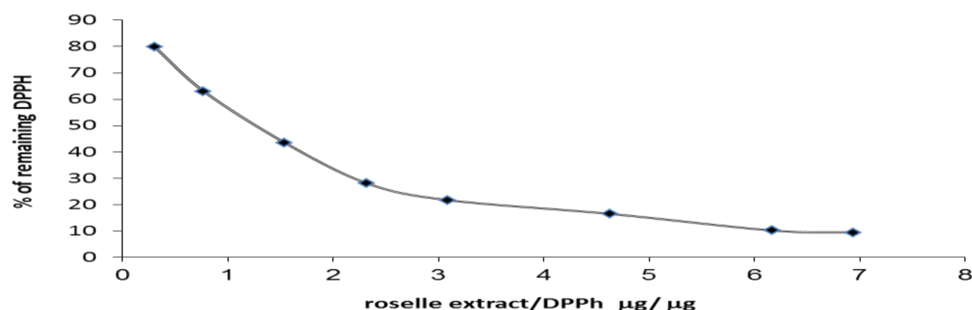


**Fig. (1). Percent of remaining DPPH as function of µg of roselle extract per µg DPPH**

**3.4. Effect of extraction conditions on the extraction efficiency of roselle anthocyanins**

The main objective of the present experiment was to decide the extraction condition that is more suitable for pigment extraction from roselle calyces on commercial applications. In fact, the highest yield of pigment recovered is considered the main goal in the extraction process. However, in addition to economic considerations, safety should be considered. We investigate the effect of temperature (5°C & 85°C), particle size (whole & crushed), contact time (20min & 24h), and solvent-to-solid ratio (1:10 & 1:20) and solvent type (distilled water and 2% citric acid solution) on the aqueous anthocyanins extraction from calyces. The yield of pigments recovered, the total soluble solid and the pH of the extracts are showed in table (4). By reducing the particle size, the yield of anthocyanins was increased in all the extraction conditions examined. The crushed flower gave total anthocyanins of 0.994.18mg./100g. dry weight while the 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 was used as extraction solvent with crushed flower and 1:10 solid: solvent ratio, total anthocyanins recorded 1229.62 mg. /100g dry weight while it recorded 994.18mg /100g when distilled water was used under the same conditions. These observations reveal that the pH value is a very important factor affecting the extraction of anthocyanins. Bronnum-Hansen, *et al.* (1985) reported similar results where they noted that the efficiency of extracting solvent increased with increasing the concentration of citric acid and concluded that pH of extracting medium was the determining factor for anthocyanins extractability. Likewise, Andersen and Markham, (2006) reported that the extracting solution should be slightly acidic to maintain the flavylium cation form, which is red and stable in highly acidic medium. Abou-Arab *et al.,* (2011) investigated the effect of different solvents on extraction efficiency of anthocyanins from roselle calyces and reported that water acidified with citric acid indicating anthocyanins yield of 1051mg/100g might be the best choice and the more preferable solvent compared with ethanol acidified with HCl which showed the highest yield i. e. 1457 mg/100g dry weight. Selim, *et al.* (2008) reported that addition of acids to water or ethanol increased the efficiency of anthocyanins extraction compared with the distilled water alone. Likewise Chandrasekhar et al., (2012) reported that and the mixture of 50% (v/v) ethanol and acidified water resulted in maximum anthocyanin content (390.6 mg/L). Rodrigues et al., (2015) used ultrasound for extraction of anthocyanin from jabuticaba peel. The results showed that the operating condition that has maximized the target compounds extraction required the sonicated of the peels for 10 min in a 46% (v/v) ethanol: water solution acidified at pH 1.

**Table (4): 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 | 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 |

It could also notes that increasing extraction temperature led to decrease the extraction time and by enhancing solubility of anthocyanins and increasing the diffusion coefficient which increase the yield of anthocyanins in the extract similar results was reported by (Cisse et al., 2012 and Oancea et al., 2012). Extracting the anthocyanins at 85°C for 20min recorded higher anthocyanin content than extracting at 5°C for overnight in all condition investigated. Lambri et al., (2015) studied

Influence of three different thermal treatment; two different red varieties in the colored grape juice production. The results showed that the use of high temperatures for short times was the most profitable time–temperature combination based on the extraction efficacy, color stability and energetic requirements. Effect of variations time (20–80 min), temperature (10–50°C), and solid–solvent ratio (1:15–1:45) on black rice pigments extraction were studied (Pedro *et al.*, 2016). The results indicated that extraction at 34.7°C for 80 min using a solid: solvent ratio of 1:30 rendered an extract with 116.58 mg 100 $g^{-1}$ of anthocyanins.

Regarding to L*, a, and b values of the color extraction with different prosudures, the results indicated that the lowest L*value was recorded for treatment No.9 followed by treatment No.1 treatment 10 and 11 and recorded L* values of 1.04, 1.09, 1.68 and 1.84 respectively. On the other hand treatment No. 8 recorded the highest L* value of 13.8. It could be notes that when citric acid was used as extracting solvent, a* and b* values was lower than all the other.

**3.5. Effect of heat treatment on retention of roselle anthocyanins**

As with most of chemical reactions, the stability of anthocyanins was markedly influenced by heat treatment. The retention of roselle anthocyanin as related to heating temperature and time are given in and Fig (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 Abo-Rayan (1996); Kirca and Cemeroglu (2003) and Sipahli, et al., 2017).

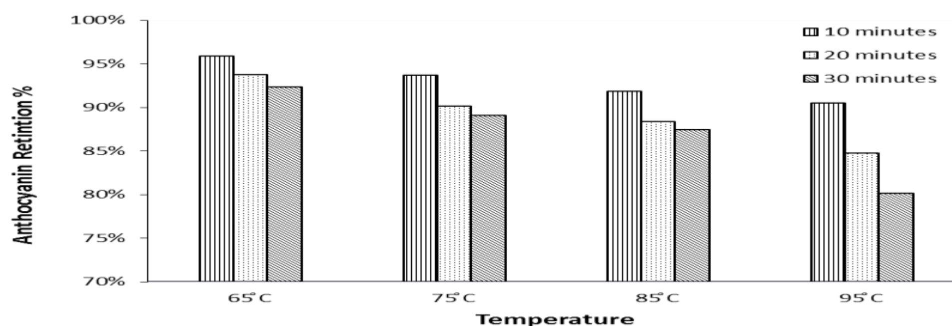


**Fig (2): Effect of heat treatments on roselle anthocyanins stability.**

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 (Selim et al., 2008; Ikeda et al., 2009; Jiménez et al., 2010; Lin and Chou, 2009; Sadilova, 2009; Cisse et al,.2012 and Idham et al., 2012). 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 (Idham et al., 2012). Martynenko and Chen (2016) 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.

**3.6. Degradation kinetics and storage stability of encapsulated roselle anthocyanins.**

Kinetic studies on the degradation of roselle anthocyanins encapsulated in Maltodextrin DE 18.7, Whey protein isolate (90 %) and Gum Arabic, were carried out in dark at30ºC and control sample without carrier. changes in color strength for the different encapsulated roselle anthocyanin powders were followed by periodical measurements of absorbance to define the order of anthocyanins degradation reaction. As illustrated in Fig. (8. 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 were reported by Garzon and Wrolstad (2001) for the degradation of pelargonidin based anthocyanins at different water activity conditions. The same degradation kinetics was also observed for anthocyanins from other sources such as; sunflower - hull (Mok and Hettiarachchy, 1991) and sour cherry (Cemeroglu et al., 1994).

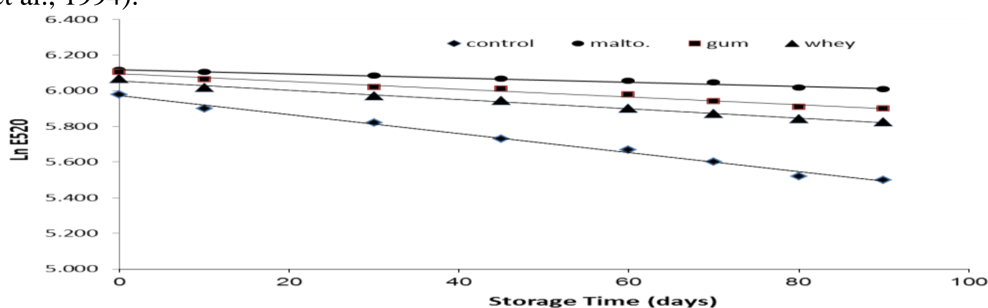


**Fig. (3): First-order degradation plots for spray-dried roselle anthocyanins with different encapsulating agents during storage at 30°C in dark containers**

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 (5). The obtained results showed that degradation rate constant values were 5.3, 1.2; 2.2 and 2.6 X $10^3$ 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(5):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 x $10^3$ | 0.99 | 130.78 |
| Whey protein | 2.6 x $10^3$ | 0.99 | 266.60 |
| Gum Arabic | 2.2 x $10^3$ | 0.98 | 315.07 |
| Maltodextrin | 1.2x $10^3$ | 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 Garzon and Wrolstad, (2001), Gradinaru et al., (2003) and Selim et al., (2008). As comparing with freeze-drying technique cured out by Selim et al., (2008), the results indicated that spray-drying technique was patter than freeze-drying technique in protection of the encapsulating pigment. 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 (Gómez-Carracedo et al., 2007). In addition, most freeze dried encapsulated bioactives only provide stability upon storage and not (or to a limited extent) in the gastrointestinal tract, as the high porous wall offers poor protection for prolonged release (Manojlovic et al., 2010; Zuidam and Heinrich, 2010).

**3.7. Roselle anthocyanin stability 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. Three different concentration of roselle anthocyanin (0.1, 0.2 and 0.3%) were used; 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 are shown in Figure (4 and 5). When the samples were stored at 40 °C, the drink model samples were 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.

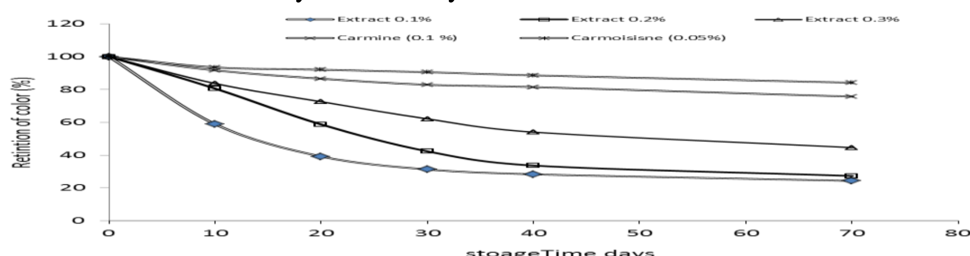


**Fig (4) Stability of roselle anthocyanin in a drink model of system stored at 40°C.**

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.

Many authors investigated the effect of temperature on the anthocyanin stability (**Sadilova** et al., 2009 and Cavalcanti et al., 2011). Sui et al., (2016) investigate the color, chemical stability of thermally treated anthocyanin aqueous solutions during storage at 4, 25, 45, and 65 °C. The results showed that the degradation rate of anthocyanins in aqueous solutions was much faster than those in real food. They also reported that the anthocyanin aqueous solutions stored at 4 °C had the best chemical stability. Duangmal et al., (2004) 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 was decreased with increases of lightness.

It could be noted that with increasing roselle anthocyanin concentrations, the stability of color was increased. Therefore, colored sample with 0.1% roselle anthocyanin 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 the stability of roselle anthocyanin. Similar findings were reported by Rein, (2005); Gris et al. (2007); Talcott et al., (2005) and González-Manzano et al., (2008). Cemeroglu et al., (1994) reported that the half-life values for the anthocyanin were 54.3; 22.5, and 8.1hr in sour cherry juice, while values were 24; 10.9, and 4.4hr in sour cherry concentrate of 45°Brix.

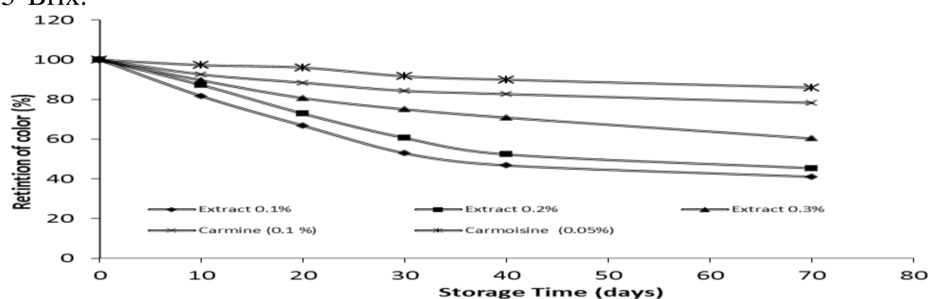


**Fig. (5). Stability of roselle anthocyanin in a drink model of system stored at 5°C. compared with carmine and carmoisine**

Kirca and Cemeroglu, (2003) Comparing the thermal stabilities of anthocyanins in blood orange juice and concentrate and found that after 60 min heating at 70, 80, and 90ºC, the losses were 21.7, 38.3, and 68.35% in the concentrate of 45ºBrix and 27.6, 54.8, and 85.95% in concentrate of 69ºBrix respectively.

### 3.7. Application of encapsulated roselle extract in jelly formulation

Strawberry jelly samples were prepared using three different concentrations of roselle extracts 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) 0.05% was used for comparing. The

prepared jelly samples were sensory evaluated and means scores were statistically analyzed and the results are presented in fig. (6). 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.

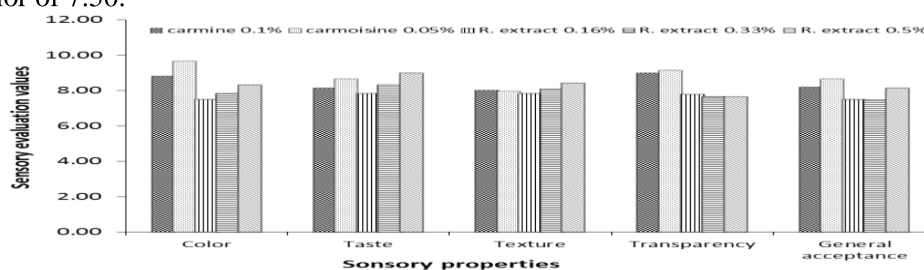


**Fig. (6). Sensory evaluation of jelly samples containing encapsulated roselle extracts, carmine and carmoisine**.

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, also control sample was significantly. 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 the addition of the natural color with level of 0.5% to the jelly formulation was acceptable and can replace the synthetic color. Similar results was reported by Mahdavi et al., 2016

## 4. CONCLUSION

The search for optimal operating conditions to maximize the efficiency of the extraction process is necessary for industrial application. In this study, it has been found 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 velocity and anthocyanin extraction yield of the red pigments from roselle calyces. Storage stability and efficiency of the encapsulated roselle anthocyanin in three different encapsulating agents were investigated. The results improved that encapsulation process could stabilize and extend the shelf life of anthocyanins content. Under the conditions of this study, the microencapsulated anthocyanins with maltodextrin 18.7 had the

highest encapsulation efficiencies. A model system of a drink and formulating a jelly using encapsulated roselle anthocyanins powder as colorant at different levels compared to that of drinks containing carmine and synthetic carmoesine was carried out and the colour stability was determined. The results indicated that the addition of encapsulated 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. In conclusion, Roselle calyces are a good source of anthocyanin with several potential applications in the food industries.

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## تاثير ظروف الاستخلاص، المعاملات الحرارية والتجفيف بالرذاذ على ثبات الانثوسيانبنات من سبلات الكركدية كملون غذائي طبيعى

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تم تصميم هذة الدراسة لبحث تاثير عملية الكبسله باستخدام التجفيف الرزازى على ثبات صبغة الانثوسيانين المستخلصة من سبلات الكركدية. فى هذة الدراسة تم تقييم اثانتا عشرة طريقة لتحديد افضل الظروف المستخدمة لاستخلاص اعلى كمية من الانثوسيانين من سبلات زهرة الكركدية. اظهرة النتائج ان استخدام محلول حامض الستريك ٢% بنسبة ١ : ١٠(سبلات : محلول) مع استخدام السبلات المجروشة وعلى درجة حرارة استخلاص ٨٥°م ولمدة ٢٠ دقيقه كانت افضل الظروف لاستخلاص الانثوسيانين من سبلات الكركديه.

تم تقدير الفينولات الكلية والتأثير المضاد للاكسدة لبسبلات الكركديه واظهرة النتائج ان سبلات الكركدية تحتوى على فينولات كلية تتراوح بين ١٢.١٦ -١٤.٤٥ مجم (مكافئ حامض الجاليك لكل جم سبلات. كما بينت النتائج ان استخدام الميثانول والماء بنسبة (٨٠ :٢٠ ) كمحلول استخلاص اعطى اعلى كمية فينولات يليه استخدام الميثانول والماء بنسبة (٥٠ : ٥٠ ) بينما اعطى استخدام الماء المقطر اقل كمية من الفينولات الكلية . اظهرت النتائج ايضا ان مستخلص سبلات الكركدية كان لها تأثير قوى كمضادات اكسدة حث كانت الكمية الكافية لتكثير نصف تركيز مادة الـ DPPH ١.٣٧ ميكروجرام مستخلص كركدية جاف لكل ميكروجرام DPPH.

تم بحث الثبات الحراري للأنثوسيانين المستخلص من سبلات الكركديه وأظهرت النتائج أنه عند معاملة مستخلص الكركديه على ٧٠ درجة مئوية لمدة ٣٠دقيقة احتفظ المستخلص بـ ٨٩.٠١٪ من محتواه من الأنثوسيانين، وعند زيادة درجة الحرارة إلى ٩٥ درجة مئوية انخفضت قيمة االانثوسيانين فى المستخلص وسجلت ٨٠.٠١٧٪.

تمت دراسة تأثير ثلاثة مواد حامله مختلفة ( مالتوديكسترينD.E. 18.7 ، الصمغ العربي وبروتين الشرش المعزول ) على ثبات صبغات الانثوسيانين المستخلصه. واوضحت النتائج ان مالتوديكسترين D.E. 18.7 كان هو افضل ماده حامله فى الحفاظ على ثبات الصبغات المكبسله، حيث أظهرت النتائج التي تم الحصول عليها أن قيم ثابت معدل التحلل كانت ٥.٣، ١.٢، ٢.٢، ٢.٦ X $10^3$لل من الكنترول ، مالتوديكسترين D.E. 18.7 ،الصمغ العربي وبروتين الشرش المعزل على التوالي. وزادة فترة نصف العمر لانثوسيانين الكركديه المكبسل من ١٠.٧٨ يوم للكنترول الى٥٧٧.٦٢ يوم للعينات المكبسلة باستخدام المالتودكسترين.

تم استخدام ألصبغات المكبسلة كمادة ملونة في شراب الفراولة الصناعى وكذلك كمادة ملونة ضمن خلطة جيلى الفراولة، وأظهرت النتائج أن إضافة أنثوسيانين كركديه بتركيز ٠.٣٪ في شراب الفراولة الصناعى و ٠.٥٪ إلى خلطة جيلى الفراولة اعطت لون مقبول للمستهلكين. وبذلك يمكن استخدام هذة النسب لتحل محل اللون الاصطناعي.