Extraction, Encapsulation and Utilization of Red Pigments from Roselle (*Hibiscus sabdariffa* L.) as Natural Food Colourants

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The present work was carried out to produce a dry powder of red colour from roselle calyces for colouring some food products using microencapsulation technique. The stability of the encapsulated pigments was investigated during storage under different water activities. The results showed that ethanol acidified with 1.5N HCl (85:15) had the greatest efficiency in extracting roselle pigments followed by 2% citric acid solution while distilled water was the less effective. The results indicated that at low pH values (1.5, 2, and 3); the pigment extracts exhibited their greatest stabilities during the entire holding time. Data showed that roselle pigment extracts heated for 30 min at temperatures of 60, 70, 80, 90, and 100°C, retained 99.87, 99.24, 94.49, 86.35, and 78.59% of their anthocyanins contents, respectively. As heating time was extended to 60 min., the retention values decreased to 96.99, 86.75, 82.10, 76.72, and 57.69%, respectively. The effect of three different encapsulating agents i.e. Maltodextrin D.E. 10, Maltodextrin D.E. 20, and gum Arabic on pigments stability was investigated. The degradation followed first – order reaction kinetics and was strongly dependent on a_w and the matrix. Among the polymeric matrices, which largely elongated the half-life of roselle anthocyanins, maltodextrin DE 20 was found as the most effective carrier in stabilizing the pigments under all storage conditions examined. The half-life periods for anthocyanins encapsulated in maltodextrin DE 20 were 433.1, 238.96, 80.58, and 94.93 days for storage water activities of 0.43, 0.53, 0.64 and 0.75, respectively. The prepared encapsulated pigments were utilized in colouring some food products included strawberry jam and hard candy.

Key words: *Rroselle (Hibiscus subdariffa L), anthocyanins, encapsulation, maltodextrin, gum Arabic, water activity, degradation kinetic, stability, hard candy, jam, shelf-life.*

INTRODUCTION

Colour is one of the most important quality attributes affecting the consumer's acceptance of food since it gives the first impression of food quality. There is a worldwide trend towards the use of natural additives in general, and food colourant in particular, in food applications (Woo *et al*., 1980, Shi *et al.,* 1992, and Ghorpade *et al.,* 1995). Anthocyanins (Greek anthos, flower and kyanos, blue) are part of a very large and widespread group of plant constituents known collectively as flavonoids, but they are differ from other flavonoids by strongly absorbing visible light. These intensely coloured water-soluble pigments are responsible for all the pink, magneta, red, violet, and blue colours in the petals, leaves, and fruits of higher plants (Timberlake and Brile, 1980; Brouillard, 1982; Francis, 1985; Mazza and Miniati, 1993).

Roselle (*Hibiscus subdariffa* L.) is a tropical plant which belongs to the family *Malvaceae* and is known in Egypt as *Karkadah*. It is probably a native of West Africa and is now widely cultivated throughout the tropics and subtropics e. g. Sudan, China, Thailand, Egypt, Mexico, and the West India (Purseglove, 1974; El-Saidy *et al*., 1992). Water extract prepared from roselle calyces is characterized by a brilliant red colour and pleasant

acid taste. Roselle extract is rich in anthocyanins, and could be used as a good source for producing a brilliant red colourant for many foods (Clydesdale *et al.,* 1979a and Pouget *et al*., 1990). Anthocyanins, as with most natural food colourants, suffer from inherent instability. (Mazza and Brouillard, 1987a). Colour stability of anthocyanins depends on a combination of various factors including: structure of anthocyanins, pH, temperature, oxygen, and 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 (Markikes, 1982; and Jackman and Simith, 1996).

Microencapsulation is a technique by which liquid droplets, solid particles or gas bubbles of core-material are coated with a thin film of protective materials. (Jackson and Lee, 1991; Shahidi and Han, 1993; Hogan *et al.,* 2001) . The coating film or wall protects the core against deterioration, reduces the evaporation of volatile compounds, and releases the core under desired conditions (Kibry, 1991). There are several microencapsulation techniques that have been used commercially or being evaluated by food industry. These techniques include spray-drying, freeze-drying, air suspension, coating, extrusion, spray cooling and spray chilling, centrifugal extrusion, rotational suspension separation, and inclusion complexing (Dziezak, 1988; O'Boyle *et a*l., 1992, and Desobery *et al*., 1997).

Several materials have been used as wall materials such as: starches, gum Arabic, methylcollulose, gelatin, whey proteins, corn syrup, ß-cyclodextrin, maltodextrins, disaccharides, pullunan, and sodium caseinate (Szente and Szejtli, 1986; Shahidi and Pegg, 1991; Bhandari *et al*., 1992; Onwulata *et al.* 1995; Sheu and Rosenberg, 1995; Hardas *et al.,* 2000; Kim *et al.,* 2000; Selim *et al.,* 2000; Bertolini *et al*., 2001; and Hogan *et al.*, 2001.

Microencapsulation has numerous applications in chemical processing and in the food industry for coating aroma compounds and oleoresins, vitamins, colourants and enzymes. Encapsulated colours are easier to handle and offer improved stability. Al-Kahtani and Hassan, (1990) produced roselle powder by pilot scale spray drying using single strength and vacuum concentrated water extract of the calyces. Clydesdale, *et al.* (1979b) prepared a spray-dried powder from a roselle liquid concentrate and added the powder to a dry beverage mix, and dry gelatin dessert mix. The objectives of the present study were: extracting of roselle pigments, studying the effects of pH values and heat treatments on the stability of the investigated pigments, studding the degradation kinetic of the encapsulated roselle pigments under various water activity conditions and utilizing of the encapsulated pigments as food colourants in some processed foods.

Materials and methods

Raw materials

Calyces of roselle (*Hibiscus subdariffa* L.) were used as source of the natural pigments in the present study. The calyces were obtained in a dried form (sun-dried). The dried calyces were kept at low temperature (4°C) till used. Maltodextrin DE 10, Maltodextrin DE 20, and Gum Arabic were used as encapsulating agents, and were purchased from Sigma Chemical Co. (USA). Ethanol, citric acid, hydrochloric acid, magnesium chloride, sodium nitrate, magnesium nitrate, and sodium chloride were of analytical grade and purchased from chemical suppliers.

Determination of solvents efficiency in the extraction of roselle pigments.

Four solvents were compared in the extraction of pigments from roselle calyces. The evaluated solvents were: ethanol acidified with 1.5N HCl (85:15, V/V), ethanol acidified with 1% citric acid; **2**% citric acid solution, and distilled water. Extraction of pigments was carried out according to the procedures described by Pouget *et al.* (1990).

Absorption spectrum

Absorption spectrum of roselle anthocyanins was made according to the method of Von-Elbe and Schwartz (1996). According to this method, one ml of the pigment extract was mixed with 9 ml of puffer solution of the desired pH value in the range of 1.5 to 9 in screw test tube. The UV - visible spectra 200 – 850 nm) of the pigment solutions were recorded using Spectronic 2000, Spectrophotometer, Busch and Lomb (USA).

Total pigment content

Anthocyanins content of roselle extract was determined colourimetrically according to the procedure described by Du and Francis (1973). A known volume of the filtered extract was diluted to 100 ml with the extracting solvent. The colour intensity was measured at 520 nm for water and citric acid solution extracts and 535 nm for acidified ethanol using Spectronic 2000, Spectrophotometer. The total anthocyanins content referred to delphenidin-3,5-sumboside was calculated using the following equation:

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

Effect of pH

The effect of pH variation on the stability of roselle anthocyanins was studied on a wide range of pH values viz; 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9. The procedures were followed as described by Von Elbe, *et al.* (1974). In this method, two ml of roselle anthocyanins extract were mixed with 20 ml of prepared puffer of the

desired pH value in each of the three screw capped test tubes (2 x 20 mm). The tubes were wrapped with aluminum foil to provide full darkness and kept at room temperature (25 ±2°C). The degradation of anthocyanins was followed by periodic measurements of colouring power of the samples at 520 nm in the different buffered anthocyanin extracts.

Effect of heat treatment

Thermal stability of roselle anthocyanis was determined according to Mok and Hettiarachchy (1991). In this method, appropriate amount of roselle anthocyanins extract was diluted with citric phosphate buffer at pH 2.0 and the total anthocyanins were determined before heating. For heat stability, 10 ml of the extract were placed in a screw capped test tubes (2 x 20 mm) and heated in a thermostatically controlled water bath at 60, 70, 80, 90 and 100°C for 30, 45 and 60 min. The tubes were cooled down immediately in an ice bath and total anthocyanins were determined. Retention value of anthocyanins was estimated according to the following equation:

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

Encapsulation of roselle pigments in various matrices

Maltodextrin 10 DE, Maltodextrin 20 DE and Gum arabic were evaluated as encapsulating agents (matrices) and the ratio between the freeze-dried roselle extract and the matrix was 1: 20. Twenty grams of matrix were dissolved in 80 ml of distilled water in 100 ml volumetric flask and the pH was adjusted to 2.0. Then, 1.0 g of the freeze-dried roselle extract was quantitatively transferred to the flask and the volume was adjusted to the mark with distilled water. The mixture was magnetically stirred for 15 min and aliquots of 5 ml of the solution were distributed in series of non – transparent plastic containers which were frozen and kept at – 80°C. The samples were subsequently freeze-dried. The freeze-dried encapsulated samples were transferred to the incubators for the kinetic studies.

Degradation kinetic of encapsulated pigments

Saturated solutions were prepared from four different salts (i.e. $K_2CO_3\,_2H_2O$, Mg $(NO_3)_2\,6H_2O$, $NaNO_2$, and NaCl) and used to achieve different levels of water activity environments (i. e. a_w of: 0.43, 0.53, 0.64, and 0.75, respectively) as described by Winston and Bates 1960, and Kitic *et al.,* 1986. The saturated salt solutions were transferred into desiccators and kept at 35°C for 2 days in order to create atmosphere conditions of the desired water activities. Then, the encapsulated samples were kept at the above-mentioned relative humidity conditions at 35ºC in the dark using Gallenkamp incubator (Gallenkamp GmbH, UK). The degradation of the roselle

anthocyanins was followed periodically by measuring the colouring intensity of the stored samples. Each sample was quantitatively transferred with 80 ml of distilled water to a 100 ml beaker and magnetically stirred for 10 min. The solution was transferred to a 100 ml volumetric flask, the pH was adjusted to 2.0 and the volume was made up to the mark with distilled water. After filtration, the absorbance was measured with UV-Vis Spectrophotometer (Spectronic 2000, Busch and Lomb, USA), at 520 nm and the colouring 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_λ: Absorbance measured at a particular wavelength λ,, C: Concentration of the anthocyanin, (g per 100 ml of the solution), and L: Length of the cell, in cm

Degradation rate constants and the half-life values.

The degradation rate constants and the half-life value ($t_{1/2}$) for the encapsulated roselle anthocyanins were calculated by applying a first-order reaction model according to Tsimidou and Tsatsaroni, (1993).

Utilization of encapsulated pigments

Trials were made to utilize the encapsulated pigments in improving colour of some food products included strawberry jam and hard candy.

Strawberry jam

Encapsulated roselle anthocyanins powder was added to strawberry jam at concentrations of 0.5, 1, 1.5, and 2 g/kg jam mixture. The jam was prepared according to Egyptian Standard 129, (1986)). Control sample of strawberry jam without addition of roselle anthocyanins was prepared under the same conditions. The samples of jam were evaluated for their quality attributes at interval periods up to 9 months of storage at 25°C.

Hard candy:

The hard candy was prepared in a candy factory at Fayoum governorate. Roselle pigment was used to colouring the candy with different levels i.e. 0.05, 0.1, 0.2, and 0.3 %. The control sample was coloured with 0.019% synthetic colour (Raspberry red, E124). The candy was manufactured as described by Mabrouk, (1999). The samples were evaluated for their sensory quality attributes. Based on data collected from sensory evaluation in the preliminary studies, storage stability studies were carried out on the samples fortified with the more suitable level of roselle anthocyanins . The samples were stored at room temperature and sensory evaluated periodically for 4 months.

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 of sensory evaluation were analyzed by the analysis of variance.

Results and dissection

Absorption spectrum of roselle pigments

The absorption spectrum of roselle pigments is shown in Fig. (1). Data indicated that absorption maximum of water extract of roselle anthocyanins was found to be at wave length of 520 nm. Data also showed that absorption maximum did not change over the pH values between 1.5 to 5.however, intensity of absorption decreased with increasing pH value. These results indicated that anthocyanins show their greatest strength at approximately pH value of 1.5, when the pigment molecules were mostly in the unionized form (oxonium) which is coloured and more stable. As pH increased up to 4.5, the anthocyanins changed into colourless form (hemiketal). Similar results were reported by Von Elbe and Schwartz (1996) and Delgado-Vargas and Paredes-Lopez (2003).

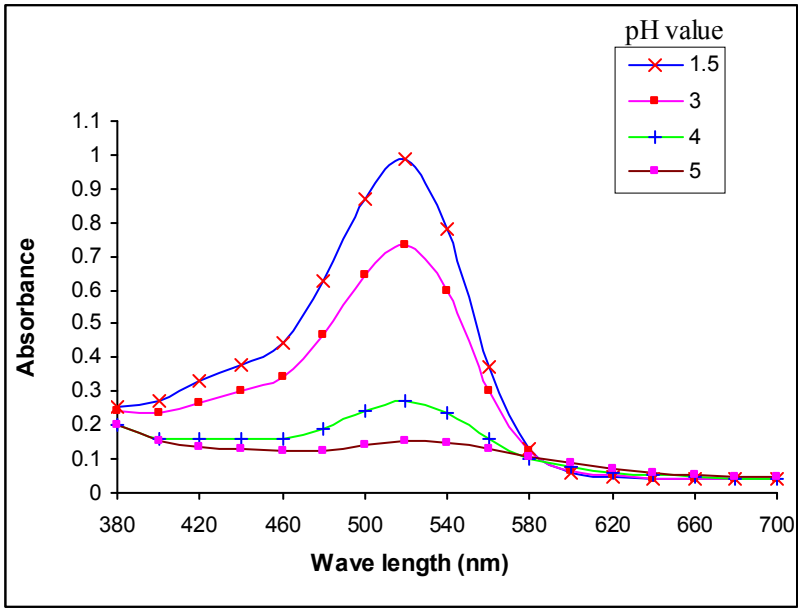

Fig. (1): Absorption spectrum of roselle anthocyanins

Extraction efficiency of pigments

Ethanol acidified with 1.5N HCl (85:15), ethanol acidified with 1% citric acid, 2% citric acid solution and distilled water were used in extracting pigments from roselle calyces. The yields of pigments

recovered with the different solvents are shown in Table (1). Addition of acids to water or ethanol increased the efficiency of anthocyanins extraction compared with the distilled water. In general, HCl was more effective than citric acid. The results indicated that ethanol acidified with HCl showed the strongest influence on the amount of anthocyanins extracted followed by 2 % citric acid solution and ethanol acidified with 1% citric acid. Similar data were reported by Tulyathan *et al*.(1989) ; Abou Rayan (1997), and Mattuk (1998).

Table (1): Extraction efficiency of pigments from roselle calyces as affected by the type of solvent.

Extracting solvent	Yield of pigments*(Mg/100g)
Ethanol acidified with 1.5N HCl (85:15)	1457
Ethanol acidified with 1% citric acid	799
2% citric acid solution	1051
Distilled water	576

- Based on dry weight

It could be concluded that adding acid to the extraction medium had a great effect in stabilizing anthocyanins, and increasing extraction efficiency. These observations revealed that pH value is a very important factor affecting extraction of anthocyanins indicating that at lower pH value, anthocyanins yield was the highest. Bronnum-Hansen, *et al*. (1985) noted that the efficiency of extracting solvent was increased with increasing the concentration of citric acid and concluded that pH of extracting medium is considered as a determining factor for anthocyanins extractability. Calvi and Francis (1978) recommended methanol acidified with 0.01% citric acid for extraction of concord grape anthocyanins.

In fact, the highest yield of pigment recovered is considered the main goal in extraction process. However, in addition to economic consideration, safety should be considered. Accordingly, water acidified with citric acid giving anthocyanins yield of 1051mg/100g may be choice as the more preferable solvent comparing with ethanol acidified with HCl that showed the highest yield i. e. 1457 mg/100g dry weight.

Effect of the pH

Colour stability of pigment extracts was determined at a wide range of pH values between 1.5 and 9 at a temperature of 25°C by measuring the absorbance values of roselle extracts at wave length of 520 nm at different holding periods. The results obtained are illustrated in Fig. (2). Data showed that roselle anthocyanins were almost stable under acidic conditions. It was observed that anthocyanin-containing roselle extracts displayed their most intense red colouration at acidic pH (e.g.≤ 3.0). Even holding roselle extracts at the acid pH

values for a period as long as 96 h, the red colour of the extracts retained its high stability. With increasing pH value of the extract up to 4-5, the red colour greatly faded and almost appeared colourless at pH value of 6.0. By continuous increasing pH up to 7.0, the colour of the extracts changed to violet and finally became blue at pH 8-9. Holding roselle pigments extracts at the different pH values for 96h. showed the same trend.

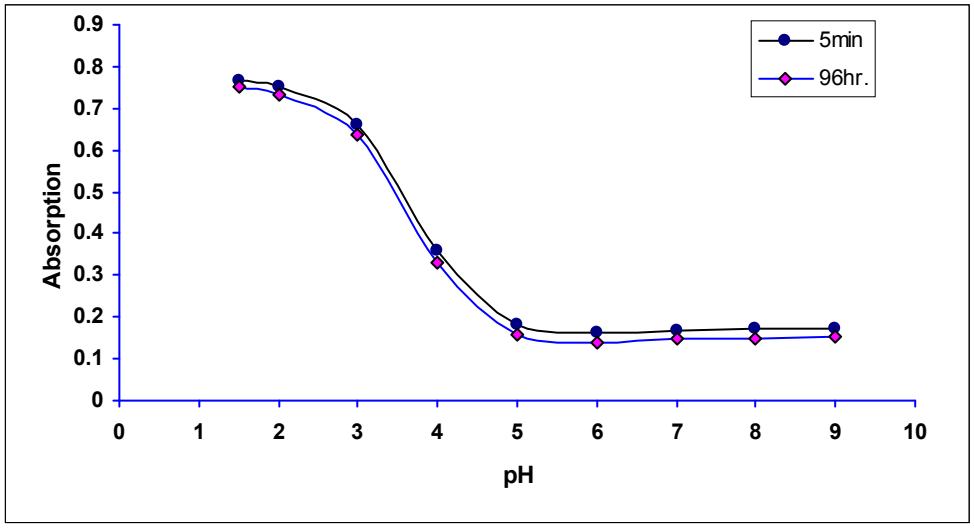

Fig. (2): Effect of pH on the stability of roselle anthocyanins

These results may be explained based on the structural transformations of anthocyanins as a function of pH (Jackman and Smith, 1996). In the aqueous medium, including foods, anthocyanins can exist in equilibrium of four possible structural forms depending on the pH: The red flavylium cation, the blue quinonoidal base, the colourless carbinol pseudobase and the colourless chalcone (Timberlake and Bridle, 1980; Francis, 1985; Mazza and Brouillard, 1987a; Mazza and miniati, 1993; Von Elbe and Schwartz, 1996; Lapidot *et al.,* 1999, and Clifford, 2000). At acidic pH $\leq$ 3, the flavylium structure was favoured; at pH values of 4 –6 the colourless carbinol was found to be dominant, while at pH $\geq$ 7, the quinonoidal form dominated. These results are in agreement with those obtained by Calvi and Francis (1978); Williams and Hrazdina (1979); Mazza and Brouillard (1987b) and Mok and Hettiarachchy (1991).

Effect of heat treatment:

Thermal stability of roselle anthocyanins was studied by heating the extracts at 60, 70, 80, 90 and 100°C for holding times of 30, 45, and 60 min. Before the heat treatment, roselle extracts were adjusted at pH value of 2.0. Based on absorbance values measured for roselle extracts before and after heat treatments, retentions of anthocyanins as related to heating temperature and time were calculated and data obtained are given in Table (2). The stability of anthocyanins was markedly influenced by heat treatment. At 60°C, no significant loss occurred in anthocyanins content of roselle extract since retention values were 99.87, 98.37 and

96.99% after heating times of 30, 45, and 60 mins. respectively. When heating temperature was elevated up to 80°C, retention of anthocyanins was still as high as 82.1% after 60 min. Even when heat treatment was carried out at 100°C for 30 min, roselle extract retained more than 78% of its content of anthocyanins. It may be concluded that roselle anthocyanins have relatively high stability at high temperature particularly when heating period was relatively short (30-45 min). Similar observations were found by Rizk, 1997; El-Dkak, 1998; Dyrby *et a*l., 2001, and Kirca and Cemeroglu , 2003.

Table (2): Effect of heat treatment on retention of roselle anthocyanins heated for different periods at pH (2).

Temperature ºC	Retention %			
	30 min.	**45 min.**	**60 min.**	**LSD**
60	99.87	98.37	96.99	0.342
70	99.24	96.74	86.75	0.879
80	94.49	91.74	82.10	1.115
90	86.35	83.23	76.72	0.977
100	78.59	71.96	57.69	0.741
LSD	0.648	0.788	0.772	

. Degradation kinetic and storage stability:

Kinetic studies on the degradation of roselle anthocyanins encapsulated in the different matrices, i. e. gum arabic, maltodextrin – DE 10 and maltodextrin – DE 20, were carried out at a temperature of 35ºC under various water activity levels (a_w i.e. 0.43, 0.53, 0.64 and 0.75). At each evaluated a_w, changes in colour strength for the different encapsulated roselle anthocyanins powders were followed by periodical measurements of absorbance to define the order of anthocyanins degradation reaction. As illustrated in Figures (3 - 6), plotting colour strength values (Ln E_{520}) vs storage time (days) gave straight lines at the different values of a_w for the different encapsulating agents. Linear regression analysis showed that the degradation of roselle anthocyanins encapsulated in the three evaluated coating materials followed first – order reaction kinetic for the different aw values. Similar kinetic responses were reported by Mok and Hettiarachchy, 1991); Cemeroglu *et al.,* 1994; and Garzon and Wrolstad, 2001.

Degradation rate constants for anthocyanins encapsulated in the different matrices were calculated at the various water activity values and presented in Table (3).The results indicate that at water activity of 0.43, the degradation rate constant values were very low and ranged between 6.2 x 10^3 $days^{-1}$ for the control sample and 1.6 – 2.0 x 10^3 $days^{-1}$ for the encapsulated samples. At a_w value of 0.53, reaction rate constants of the control sample and the encapsulated samples increased to 29.3 x 10^3 and 2.9 – 7.4 x 10^3 $days^{-1}$ respectively.

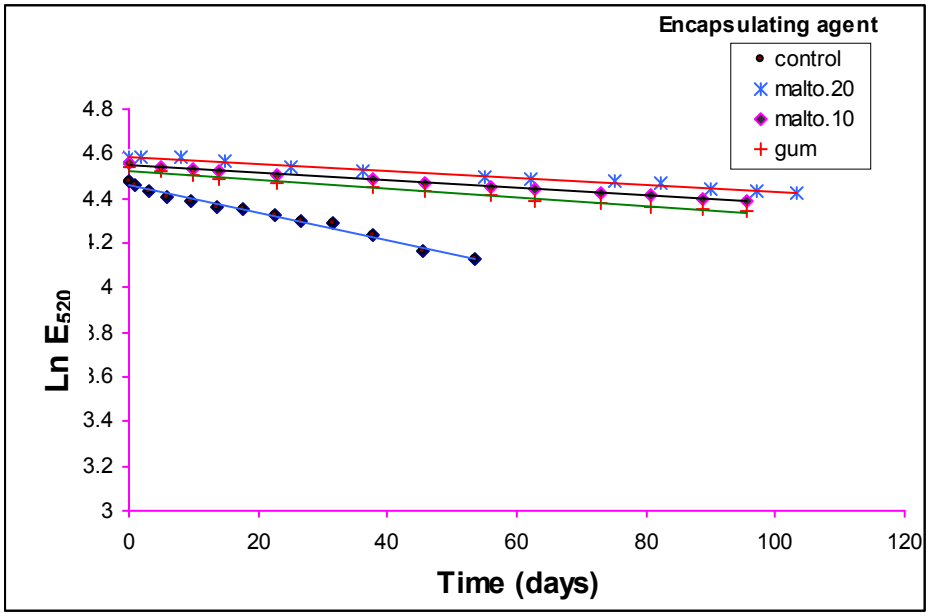

Fig.(3): First-order degradation plots for roselle anthocyanins encapsulated in different matrices during storage at a_w 0.43 and temperature 35ºC.

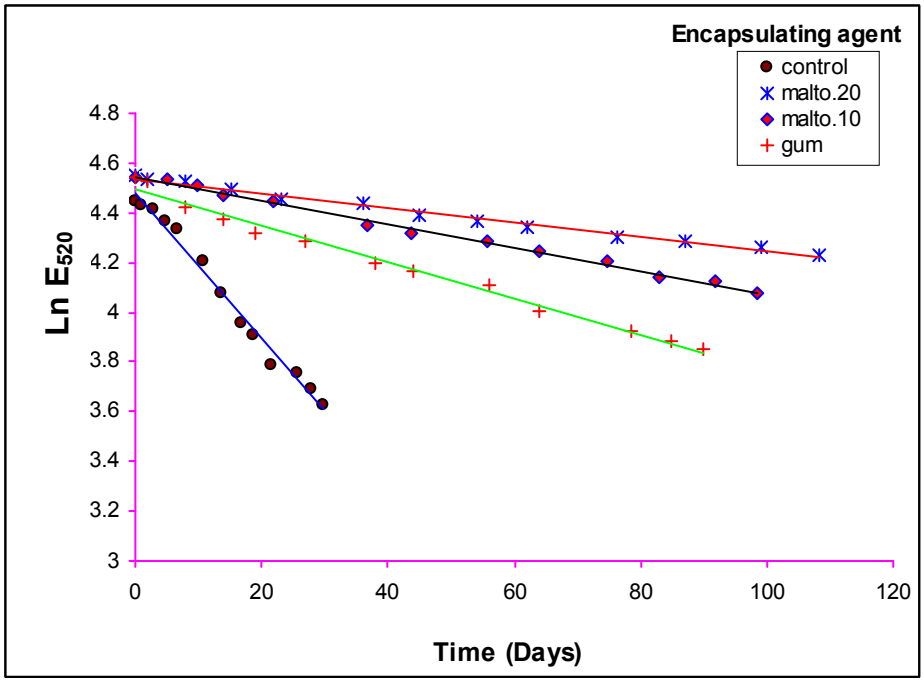

Fig. (4): First-order degradation plots for roselle anthocyanins encapsulated in different matrices during storage at a_w 0.53 and temperature 35ºC.

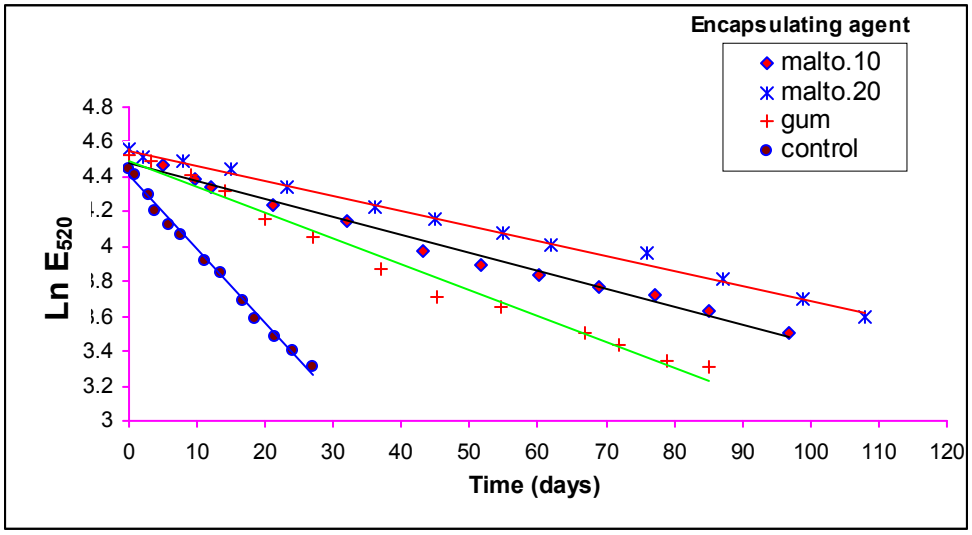

Fig.(5): First-order degradation plots for roselle anthocyanins encapsulated in different matrices during storage at a_w 0.64 and temperature 35ºC.

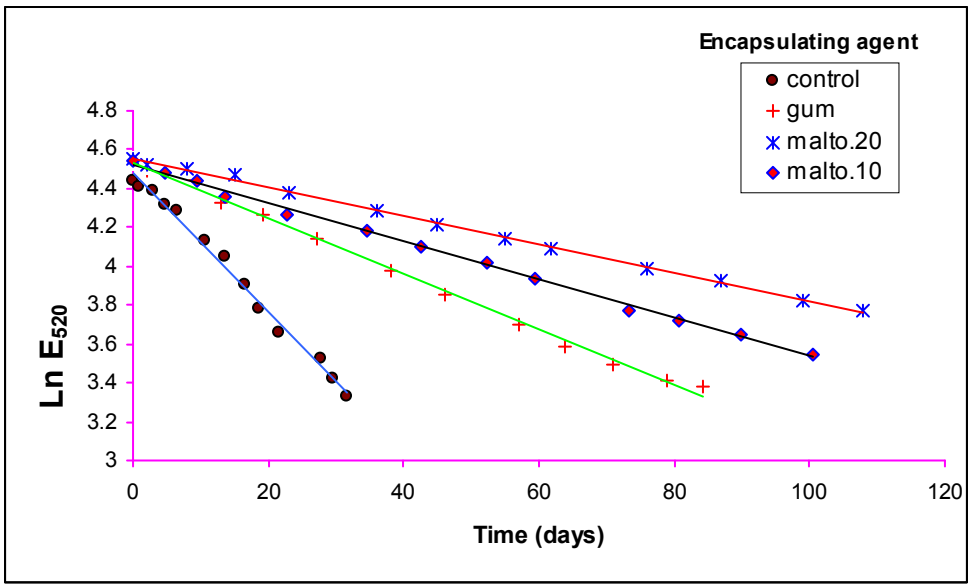

Fig. (6): First-order degradation plots for roselle anthocyanins encapsulated in different matrices during storage at a_w 0.75 and temperature 35ºC.

The highest values of the rate constants for anthocyanins degradation were observed at an intermediate water activity level (a_w 0.64) for all matrices evaluated. Besides, data shown in Table (3), indicated that reaction rate constant of the control anthocyanins was as high as 42.36 x 10^3 days-^{1}, while those of encapsulated anthocyanins samples ranged between 8.6 x10^3 days^{-1} for maltodextrin –DE 20 and 14.8 x 10^3 days^{-1} for gum Arabic. A change in the kinetic responses of anthocyanin degradation was observed for samples stored at a_w of 0.75Table (3). At this a_w environment, the rate constant for all systems were lower than those observed for a_w 0.64, but they were still higher than those observed for the low a_w environments (a_w of 0.43 and 0.53).

Similar kinetic responses have been reported for β – carotene oxidation in an oleoresin / cellulose model (Goldman *et al.,* 1983), and for the degradation of bixin (Gloria *et al.,* 1994). Likewise, Bronnum- Hansen and Flink (1985) found that loss of freeze-dried elderberry anthocyanins was significant only when a_w values were above 0.51. These observations could be explained by the fact that the reaction of anthocyanins degradation like most of chemical reactions is controlled by the mobility of the reactants (Leung, 1987). At low a_w, water is tightly bound to the surface polar sites and is generally not available for any kind of reactions. Increasing moisture content results in faster mobility of the reactants, which leads to a greater reaction rate. An increase in the moisture content above a certain level may reduce the reaction rate by diluting the reactive elements. In this context, the mobility factor dominates the kinetics at low a_w levels, whereas the reactant dilution effect becomes predominant at the high a_w value. As a result, the rate of anthocyanins degradation increased up to its maximum value as a_w increased from 0.43 to 0.64 and then decreased at the higher a_w (0.75).

Table (3): Statistical comparison for degradation rate constant values of roselle anthocyanins encapsulated in the different matrices during storage at various water activities.

Encapsulating Agent	Rate constant x 10^3 ($days^{-1}$)			
	Water activity			
	0.43	**0.53**	**0.64**	**0.75**
Control	6.20 ± 0.1^{a1}	29.3 ± 1.3^{a2}	42.3 ± 2.3^{a3}	35.7 ± 0.91^{a4}
Gum arabic	2 ± 0.07^{b1}	7.4 ± 0.24^{b2}	14.8 ± 0.57^{b3}	14.3 ± 0.26^{b3}
Maltodextrin 10 DE	1.7 ± 0.04^{b1}	4.7 ± 0.12^{c2}	10.3 ± 0.34^{c3}	9.8 ± 0.17^{c3}
Maltodextrin 20 DE	1.6 ± 0.05^{c1}	2.9 ± 0.08^{d2}	8.6 ± 0.17^{d3}	7.3 ± 0.09^{d3}

* Different superscript letters for rate constant values (columns) indicated significant differences among matrices for each a_w environment ($p<0.05$).

\+ Different superscript numbers for rate constant values (rows) indicated significant differences for the same matrix at different a_w environment ($p<0.05$).

The effects of the encapsulating agent and water activity on storage stability of encapsulated roselle anthcyanins were statistically analyzed Table (3). At all storage a_w values, encapsulation showed a significant protective effect for anthocyanins against deteriorative reactions. It could be observed that degradation rate of the control sample ranged between 6.2 x 10^3 $days^{-1}$ at a_w 0.43 to 35.7 x 10^3 $days^{-1}$ at a_w 0.75. Under the same storage conditions, degradation rate constants of the encapsulated samples ranged between 1.6 – 2.0 x 10^3 and 7.3 – 14.3 x 10^3 $days^{-1}$ depending on the type of encapsulating agent. The protective effect of maltodextrins was significantly better compared with gum Arabic, but no significant differences could be detected between the two types of maltodextrins particularly at low a_w value (0.43). Berset, *et al.* (1985) studied the effect of different encapsulating agents on the stability of four different natural red colourants. Results showed that the

effectiveness of each support material depended on the chemical and physical structure of the support material. The results also showed that native or modified starch enhanced the anthocyanins stability, while cellulose did not protect the samples against thermal or photoreactive degradation.

Half – life periods of the encapsulated roselle anthocyanins stored under the different conditions of a_w were estimated (Figure 7). In general, increasing the rate constants was associated with decreasing the half-life periods (days) for the stored samples, where the control samples lost their stabilities sharply during storage under the various water activity values. It could be observed (Fig.7) that half – life period of un encapsulated anthocyanins decreased from 111.7 days at a_w of 0.43 to a value as a low as only 14.9 days fore samples stored at a_w of 0.75.

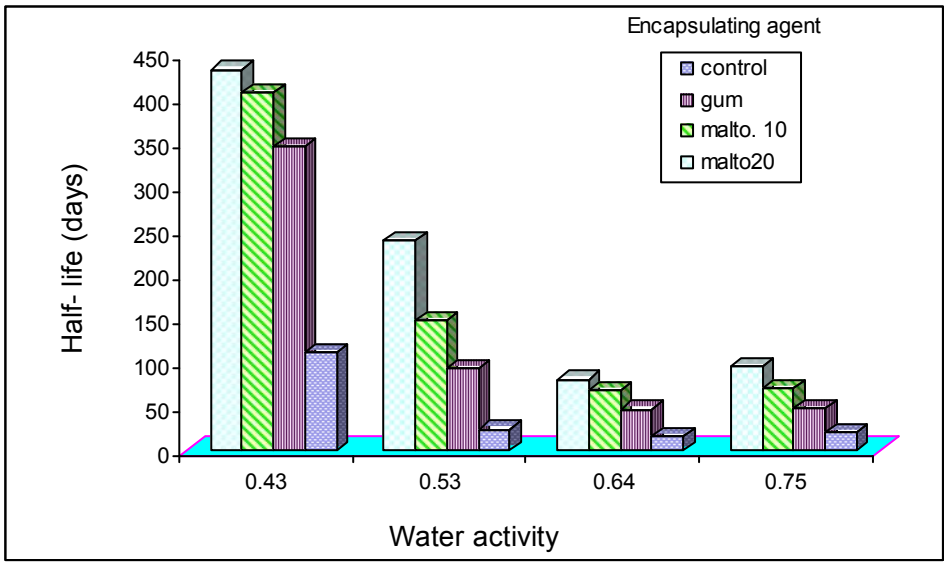

Fig.(7): Half-life periods (days) for roselle pigments encapsulated in different matrices during storage at 35°C as function of the encapsulating agent and water activity (a_w)

Among the encapsulating agents evaluated, maltodextrin DE 20 showed the greatest protecting effect indicating the longest half – life periods at the various storage water activities. At a_w 0.43 half – life period of roselle anthocyanins extended from 111.7 days for the uncoated powder (control) to 346.5, 407.6 and 433.1 days for anthocyanins encapsulated in gum Arabic, maltodextrin DE 10 and maltodextrin DE 20, respectively. The protective effect of maltodextrin DE 20 was also found to be more pronounced at lower values of water activity. These results are in agreement with those obtained by Anandaraman and Reineccius (1986). Similar results were also found by Beatus, *et al*. (1985). They reported that maltodextrin was the best agent to protect the encapsulated paprika pigments against oxidation during storage. Also, Wagner and Warthesen (1995) found that

hydrolyzed starch, of 36.5 DE, was superior to 4, 15, and 25 DE in improving the retention of encapsulated carrot carotenoids.

It could be suggested that maltodextrin 20 DE may provides high protection for the encapsulated pigments (roselle anthocyanins) because it collapses rapidly during storage and thereby, it becomes a more effective barrier to oxygen permeation. Collapse of freeze-dried materials is associated with the disappearance of micro-pores and cavities through which oxygen enters the matrix. On the other hand, gum Arabic matrix being more stable during storage is likely to be more permeable to oxygen and thus less effective than the maltodextrins systems in protecting the encapsulated pigments from the oxidation (Serris and Biliaderis, 2001).

Based on degradation kinetic data for roselle anthocyanins encapsulated in the different matrices, it could be concluded that water activity is an important factor controlling the degradation of the pigments during storage. It is also could be observed that the degradation of the pigments was occurred even at the low water activity (0.43), thus the encapsulated pigments should be stored at storage environmental conditions having water activity level less than 0.43 for effective stability.

Utilization of encapsulated roselle pigments.

Trials were made to utilize the encapsulated pigments powders prepared from roselle extracts in colouring and / or improving the colour of some food products included strawberry jam and hard candy.

Strawberry jam

Strawberry jam samples were prepared with added encapsulated roselle anthocyanin powders at concentrations of 0.0.5, 0.1, 0.15 and 0.2 %, while control sample was prepared without adding pigments. The different samples were subjected to sensory evaluation to determine the more preferred level of added pigment. Data showed that jam sample fortificated with anthocyanin powder at a level of 0.1% scored the highest value for colour indicating the greatest consumer preference. For studying stability of colour during storage, strawberry jam sample fortificated with encapsulated roselle anthocyanin at a level of 0.1% and control samples were stored for 9 months at room temperature (25 + 2 °C). The different samples of jam were sensory evaluated for their quality attributes at intervals of 3 months during storage and data are summarized in Table (4).

Table (4). Quality attributes of strawberry jam fortificated with roselle anthocyanins during storage at room temperature for 9 months.

Tested parameters	Colour		Taste		Odour		Over all acceptance	
Treatments / Storage time(month)	**S_1**	**S_2**	**S_1**	**S_2**	**S_1**	**S_2**	**S_1**	**S_2**
Zero	8.3C	9.4A	9.25	٩,٤	٩,٠٥	٩,١٥	٨,٥٥CD	٩,٣٥A
3	8.0C	9.1AB	8..8	٩,٢	٨,٩	٩,٠	٨,٣٥D	٩,١٥AB
6	٧,٠E	٨,٣٥C	٧,٧٥	٨,٨	٨,٥٥	٨,٦	٧,٧٥E	٨,٦CD
9	6.5F	٧,٦D	٧,٤٥	٨,٣	٨,٠	٨,٢	٧,٠٥F	٧,٩٥E
Mean	٧,٤٥a	٨,٦١b	٨,٣١a	٨,٩٤b	٨,٦a	٨,٧٥b	٧,٩a	٨,٧٦b
L.S.D.: treatments	Small letters ٠,١٧٩		0.1٥٨		0.140		0.156	
L.S.D.: treat. & storage interaction	Capital letters 0.358		N.S		N.S		0.311	

S_1= control
S_2= jam fortificated with 0.1% encapsulated anthocyanin

Data showed that after 6 months storage, colour of the fortificated samples scored higher values and was significantly preferred than control samples stored under the same conditions. Even at the end of 9 months storage, colour of fortificated jam sample was still highly accepted with a significant difference comparing to control sample.

No significant differences between all samples regarding their taste and odor over the entire period of 9 months storage Table (4). In general, based on sensory evaluation data, strawberry jam could be successfully fortificated with the natural colourant, i.e encapsulated roselle anthocyanin powder without any adverse effects and the samples retained their good colour during storage at room temperature for 9 months. Similar results were reported by Mattuk, 1998, and Garcia-Viguera *et a*l., 1998.

Hard candy

Roselle anthocyanins were evaluated as natural colourant for hard candy. To determine the more suitable concentrations of these natural colourants, the encapsulated pigment was used at levels ranged between 0.05 to 0.3 %. Control sample was coloured with 0.019% synthetic colourant (Raspberry red, E124). Candy samples were sensory evaluated for their quality attributes i. e. colour; flavour, mouth feel, and overall acceptability. The results showed that colour of control sample, which coloured with synthetic dye scored the highest values for colour while those coloured with low levels of roselle anthocyanins i. e. 0.05 and 0.1% were significantly different. However, adding roselle anthocyanis at a level of 0.2% was effective to produce hard candy with colour comparable to that of control sample. No significant differences could be found between control sample and samples coloured with different levels of anthocyanins regarding the flavor and mouth feel properties.

Colour stability of candy fortified with 0.2% encapsulated pigment was studied during storage at room temperature. The samples were evaluated for colour, flavour, mouth feel, and overall acceptance during storage for four months. Mean scores of the evaluated parameters were statistically analyzed and the results are given in Table (5). It could be seen that after 4 months, the candy coloured with roselle anthocyanins was still accepted for its colour and had a score value of 6.7.

Table (5) sensory data for hard candy coloured with synthetic dye , and roselle pigments during storage for four months

Tested parameters	Colour		Flavor		Mouth feel		Overall acceptability	
treatments / Storage time (month)	synthetic dye	Roselle extract	synthetic dye	Roselle extract	synthetic dye	Roselle extract	synthetic dye	Roselle extract
Zero	9.7A	9.1C	9.3	9.2	8.85	8.65	8.9A	8.6AB
2	9.55AB	7.65D	9.2	8.9	8.6	8.5	8.6AB	8.5AB
3	9.5ABC	7.4DE	8.9	8.8	8.55	8.25	7.9C	7.8C
4	9.2BC	6.79F	8.65	8.5	8.5	7.85	7.8C	7.7C
mean	9.4a	7.7b	9.0a	8.8b	8.6a	8.3b	8.3a	8.15a
L.S.D.: treatments	Small letters 0.185		0.140		0.159		0.159	
L.S.D.: treat. & storage interaction	Capit. letter 0.370		N.S		N.S		0.317	

Results given in Table (5) showed no significant differences between the control sample (synthetic dye) and the samples coloured with roselle anthocyanis regarding their overall acceptability. This indicates that the addition of the natural pigments to the candy improved the colour without adverse effects on the quality attributes. Concerning the overall acceptability, the results showed no significant difference between the control sample and that coloured with roselle pigments during the storage period (4 months) at room temperature.

These results indicated that encapsulated roselle anthocyanins could be successively used as a natural colourant for colouring hard candy products which retained their accepted colour for storage period of 4 months. Gadallah (2002) found that colouring the hard candy with acalypha leaves anthocyanins at a level of 0.1% gave the highest score values for colour and overall acceptability.

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الملخص العربى

استخلاص وكبسلة والاستفادة من الصبغات الحمراء من سبلات الكركديه كمواد ملونة طبيعية

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الملخص العربى

أجريت هذه الدراسة بهدف الحصول على الصبغات الحمراء من سبلات الكركديه فى صورة جافة عن طريق عملية الكبسلة encapsulation ودراسة ثبات هذه الصبغات تحت مستويات مختلفة من النشاط المائى واستخدامها فى تلوين بعض المنتجات الغذائية.

استخدمت أربعة مذيبات مختلفة لاستخلاص الصبغات من سبلات الكركديه ؛ وأظهرت النتائج أن كحول الإيثايل المحمض بحامض الهيدروكلوريك (١,٥ع) (٨٥ : ١٥) كان الأكثر كفاءة فى الاستخلاص ثم محلول حامض الستريك (٢ ٪) بينما كان الماء أقل المذيبات المستخدمة كفاءة .

تم تقدير التغير فى تركيز اللون الأحمر فى المستخلص على مدى من قيم الـ pH يتراوح بين ١,٥ إلى ٩ وأظهرت النتائج أن تركيز اللون الأحمر يتغير بتغير قيمة pH الوسط حيث كان أكثر تركيزا على قيم الـ pH المنخفضة التى تتراوح ما بين ١,٥الى٣ .

تم دراسة تأثير المعاملة الحرارية على ثبات الصبغات الحمراء فى مستخلص الكركديه. وأظهرت النتائج احتفاظ مستخلص الكركديه بتركيزات مرتفعة من الأنثوسيانين تصل إلى٩٩,٨٧ - ٩٩,٤٢ - ٩٤,٤٩ - ٨٦,٣٥ و ٧٨,٥٩ ٪. بعد التسخين لمدة ٣٠ دقيقة على درجات حرارة ٦٠ ،٧٠ ،٨٠، ٩٠ و ١٠٠م على الترتيب، و بزيادة مدة التسخين إلى ٦٠ دقيقة انخفضت نسب الاحتفاظ بالأنثوثيانين إلى ٩٦,٩٩ – ٨٦,٧٥ – ٨٢,١٠ – ٧٦,٧٢ و ٥٦,٦٩٪ على الترتيب.

تم دراسة تأثير استخدام ثلاث مواد حاملة (مالتودكسترين ١٠ و مالتودكسترين ٢٠ و الصمغ العربى) على ثبات صبغات الكركدية أثناء التخزين تحت مستويات مختلفة من النشاط المائى . أظهرت النتائج أن هدم صبغات الانثوسيانين المحملة على الأنواع الثلاثة من المواد الحاملة كان تفاعل من تفاعلات الدرجة ألاولى First - order reactions، كما أظهرت النتائج أن الأنواع الثلاثة من المواد الحاملة المستخدمة أدت إلى زيادة ثبات الانثوسيانين فى مساحيق الصبغات المكبسلة مقارنة بمسحوق الصبغات غير المكبسلة أثناء التخزين تحت الظروف المختلفة من النشاط المائى . وتشير النتائج إلى أن المالتو دكسترين ٢٠ كان أفضل المواد الحاملة المستخدمة فى توفير الحماية للصبغات المكبسلة أثناء التخزين تحت المستويات المختلفة من النشاط المائى وأظهرت النتائج أن ثبات الصبغات يزداد بانخفاض درجة النشاط المائى لوسط التخزين .

استُخدم مسحوق صبغة الكركديه المكبسلة لتحسين لون مربى الفراولة و الحلوى الصلبة وأظهرت نتائج التقييم الحسى للعينات إن المربى المضاف إليها صبغات الكركديه بنسبة ٠,١ % والحلوى المضاف إليها صبغات الكركديه بتركيز ٠,٢٪ سجلتا أعلى قيم اللون والقبول العام مقارنة بالكنترول