



EVALUATION OF RED CABBAGE ANTHOCYANIN PIGMENTS AND ITS POTENTIAL USES AS ANTIOXIDANT AND NATURAL FOOD COLORANTS

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ABSTRACT

Anthocyanins derived from red cabbage were extracted, and identified by using HPLC. These pigments are used as alternative natural red colorants in some processed foods i.e.; hard candy, jelly and ice sherbets. They are also effect of using as natural antioxidant on sunflower oil. Red cabbage has 90.5 mg anthocyanin /100 gm on fresh weights. Where the major constituents were cyaniding-3-diglucoside-5-glucoside (80%) and cyanidin 3, 5 diglucoside (20%) with HPLC. The best carrier for red cabbage anthocyanin pigment was found to be dextrin followed by cellulose, soluble starch and glucose respectively. On the other hand, color and higher pigment stability of anthocyanin derived from red cabbage were in acidic condition at pH ranged between 1.0 to 4.0 and in temperatures ranged between 40 to 80°C. Meanwhile, the degradation of anthocyanin being 10% of total pigments after 180 min at 100°C. Antioxidant activities of red cabbage anthocyanin were assessed by determining peroxide value on sunflower oil during 7 days at 60°C. Sunflower oil contained 200 ppm red cabbage extract showed lower peroxide value being (9.92) than using 200 ppm synthetic antioxidant (BHT) (10.12) meq/Kg. Analysis of variance for sensory evaluation of prepared hard candy, jelly and ice sherbets indicated that, hard candy, jelly contained 0.10% and ice sherbets contained 0.20% red cabbage anthocyanin pigments revealed the highest score of color, taste, odor and overall acceptability similar with synthetic color (carmine).

INTRODUCTION

Anthocyanin (in Greek means flower, and kyanos means blue) are the more important plant pigments visible to the human eye. They belong to the widespread class of phenolic compound collectively named flavonoids. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (MingKong *et al* 2003).

Anthocyanins, as natural colorants, are widely used in the food industry as an alternative to synthetic colorants, e.g., they can replace FD&C Red No.40 (Allure red). They are characterized by a wide spectrum of color tones, ranging from orange through red, to purple and blue, depending on the molecular structure and pH value. (Dorota and Janusz, 2007).

The interest of anthocyanins derives not only from their coloring effect but also from their beneficial properties, including antioxidizing activity, improvement in the tightness of capillary blood vessels and prevention of thrombosis aggregation, all of which reduce the risk of circulatory diseases (Giusti and Wrolstad, 2003).

Red cabbage pigments are a natural extract mainly used as a food color. A colorful class of compounds called anthocyanins attributes to this color. Currently, red cabbage pigment are being used in beverages, candies, dry mixes, chewing gum, a variety of sauces, and yogurt (Chigurupati *et al* 2002).

There is considerable interest worldwide in the development of food colorants from natural sources as alternatives to synthetic dye (Fabre *et al* 1993).

Red cabbage (*Brassica oleracea L.*) is a promising source of anthocyanin for coloration of foods since its pigments are unique in being colored over a very broad pH range and high stability to heat and light compared to anthocyanins from other natural sources (Marianne *et al* 2001)

Anthocyanins are a group of widespread natural phenolic compounds in plants. They are mainly distributed among flowers, fruits and vegetables and are responsible for their bright colors such as purple, red and blue (Xianli and Ronald 2005, Dorota and Janusz, 2007).

Also, these pigments are water-soluble and this property facilitates their incorporation into numerous aqueous food systems. This specification makes anthocyanin attractive natural colorants (Luigia and Giuseppe, 2006).

The colors and stability of anthocyanin pigments from red cabbage is dependant on several factors, including structure and concentration of pigment, pH, temperature, metallic ions, co pigments, enzymes, oxygen, ascorbic acid, and sugars (Dyrby *et al* 2001).

Anthocyanins showed that, the ability of preventing lipid oxidation in different lipid sources (Marja and Marine, 2003).

There have an increasing effort in recent years to develop effective natural antioxidants for edible oils in order to retard lipid oxidation. The demand for natural antioxidants has been increased due to consumer concerns about the safety than using of synthetic antioxidants (Hudson, 1990). Anthocyanin, as well as other phenolics compounds could acts as antioxidants by donating hydrogen to highly reactive radicals thereby preventing further formation of oxidation products (Fukumato and Mazza, 2000).

The objective of this study was to extract and evaluate the major constituents of red cabbage pigments affecting color stability and to select the appropriate carrier materials.

Assessment antioxidant activity for red cabbage extract using sunflower oil as well as sensory evaluation of hard candy, Jelly and ice sherbets prepared with various levels of anthocyanin pigments as potential natural food colorants was also undertaken.

MATERIALS AND METHODS

Materials

Red cabbage (*Brassica oleracea var capitata L.*) was obtained from Agricultural development market. Giza, Egypt.

The solvents used for spectral and HPLC analysis were of HPLC grade and all other solvents were of ACS grade.

Refined sunflower oil without adding antioxidant was obtained from Safola Saime, Egypt.

Synthetic antioxidant i.e. butylated hydroxy toluene (BHT) were purchased from Sigma Chemical Company U.S.A.

Carminic acid of carminic acid, C.I. 75470 was obtained from Aldrich Chemical Company, USA.

The anthocyanin standards Cyanidin-3-diglucoside-5 glucoside and Cyanidin 3,5 diglucoside were obtained from Carl Roth GmbH (D-76185 Karlsruhe, Germany).

Methods

Chemical analysis

A- Anthocyanin pigments

1) Extraction and concentration of anthocyanin pigment

Anthocyanins from red cabbage were extracted by using the method of (Fuleki and Francis, 1968). Twenty gm of red cabbages were mixed with 100ml ethanol acidified with 0.01% citric acid (instead of HCL) according to the method of (Colin and Peter, 1980). The previous mixture was macerated in a warring blender at full speed for 5min and filtered on the filter paper Whatman No. 1 through a Buchner funnel. The residue on the filter paper was washed rapidly with the extracting solvent until approximately 450 ml of the extract was collected in a 500 ml volumetric flask and made up to volume with the extracting solvent. The previous collected extract was concentrated in rotary vacuum evaporator at <40°C.

2) Determination of total anthocyanin

A small aliquate of the filtered extract was diluted with the extracting solvent to yield an optical density within the optimum range of the instrument. The diluted extract was stored in the dark for 2h and absorbance was measured at 520 nm.

The total anthocyanin content was calculated using the equation reported by (Du and Francis, 1973).

$$\text{Total anthocyanin content (mg/100g)} = \frac{\text{OD} \times \text{DV} \times \text{TEV} \times 100}{\text{SV} \times \text{SW} \times 51.56}$$

OD	=	Optical density
DV	=	Diluted volume for the O.D measurement
TEV	=	Total extract volume
SV	=	Sample volume
SW	=	Sample weigh in grams
51.56	=	E. value for which the major constituent red cabbage

3) Selection of appropriate carrier

The concentrated anthocyanin pigments were adsorbed on various supports according to the method described by (Rizk, 1987) using different ratios up to 6:1 (pigments: matrix) namely cellulose, glucose, dextrin and soluble starch and lately dried in oven at 40°C for 24h.

B- Analysis of anthocyanin of red cabbage extract

1) Purification of anthocyanins

The anthocyanin concentrated extract was purified according to the method of (Attoe and Van-Elbe, 1981). The concentrate of anthocyanin was purified with petroleum ether and with ethyl acetate to remove non polar impurities. After phase separation; residual solvent was removed from the aqueous phase with rotary evaporator.

2) Identification of anthocyanin pigments by High-Performance Liquid Chromatography (HPLC)

The purified anthocyanin of red cabbage were identified by Kanuer HPLC pump 64 according to the method reported by (Andersen, 1989), using suplecosil LC 18 column (15cm x4.6 mm, 3 µm, Supelco). For both HPLC column of two solvents were used for elution (1) formic acid, water (1:9) and (2) formic acid, water, methanol (1:4:5). The flow rate was 1.5ml/min. The elutes were monitored by visible spectrometry at max. Wavelength 520 nm. Identification was performed within a standard sample as described by the same author.

C- Properties of red cabbage anthocyanin pigment

1) Effect of pH on the efficiency of anthocyanin color

A preliminary study was conducted to test the stability of red cabbage anthocyanin at different pH media ranged from 1.0 to 10.0 for 30 min and then percentage of color loss was calculated.

2) Effect of temperature on the efficiency of anthocyanin color

A preliminary study was conducted to heat tolerance of red cabbage anthocyanin at different temperature ranged from 40-100°C for 30 min and then percentage of color loss was calculated.

3) Thermal stability of red cabbage anthocyanin

Holding red colorant solution (red cabbage anthocyanin) at 80 to 100°C was extended for 180 min through which they were removed each 30 min. and cooled immediately in an ice bath followed by measuring absorption spectra of the solution at 520 nm.

D- Antioxidant activity testing

Antioxidant activity was tested by the determination of peroxide value (POV) during incubation of sunflower oil containing red cabbage extract at 60°C for 7 days as described by (Matthous, 2002). Ten grams of dried red cabbage were exhaustively extracted with ethanol (100ml). 200, 500, 1000 ppm of the extract were mixed with 25g of sunflower oil in a flask against control 25g of sunflower oil mixed with 200, 500, 1000 ppm (BHT in a flask) as a control for each one and the mixtures were placed in an oven at 60°C for 3h daily, the experiment was continued for 7 days. The peroxide value was determined for each according to the method described in (A.O.A.C., 2000).

E- Technical performance

1) Hard candy

Hard candies were manufactured in laboratory with adding different levels of red color (red cabbage anthocyanin) 0.10, 0.20, 0.30, 0.40 and 0.50% w/w to the formula using the traditional procedure as described by (Counsel, 1980). The formulation of the hard candies sample are shown in Table (1).

Table 1. Formulation of hard candy samples

Ingredients	%
Sucrose	48.48
Corn syrup	25.40
Water	25.26
Flavoring oil	0.21
Citric acid	0.15
Color (red cabbage anthocyanin)	0.10 – 0.50

The control of hard candy prepared with 0.10% synthetic color (carmine)

2) Preparation of jelly

Jelly was prepared in laboratory by adding different levels of red cabbage anthocyanin color i.e. 0.10, 0.20, 0.30, 0.40 and 0.50% w/w in laboratory using the traditional procedure. The formulation of Jelly is shown in **Table (2)**.

Table 2. The formulation of Jelly

Ingredients	%
Sucrose	84.0
Gelatin	15.0
Citric acid	0.20
Flavoring agent	0.10
Sodium benzoate and potassium citrate	0.10
Ascorbic acid	0.10
Color (red cabbage anthocyanin)	0.1-0.5

Jellies were wrapped by polyethylene and aluminum foil and packed in carton bags and were stored at room temperature $25 \pm 5^\circ\text{C}$.

The control of hard candy prepared with 0.10% synthetic color (carmine)

3) Ice sherbets

Ice sherbets was prepared in laboratory by adding different levels of red cabbage anthocyanin ranging from 0.1 to 0.5% (w/w) using the traditional procedure. The formulation of ice sherbets are shown in **Table (3)**.

Table 3. The formulation of sherbets

Ingredients	%
Sugars	15.0
Water	84.20
Citric acid	0.20
Flavoring agent	0.10
Red cabbage anthocyanin (natural red color).	0.10- 0.5

These contents should be mixed very well and heated at 90°C for 15 min., cooling until 80°C and put in polyethylene bouchs and placed in deep freezer at -18°C .

The control of ice sherbets prepared with 0.10% synthetic colors (carmine)

F- Sensory evaluation

Sensory evaluation was carried out by ten panelists. The panelists were asked to evaluate color, taste odor and overall acceptability for prepared candy, Jelly and ice sherbets according to the method described by (Reitmeier and Nonnecke, 1991).

G- Statistical analysis

Means of data obtained for sensory attributes of hard candy, jelly and ice sherbets were evaluated using Duncan's Multiple range test to identify significant differences at the 0.05 probability ($P < 0.05$) using the statistical analysis system **SAS** (SAS Institute., 1999).

RESULTS AND DISCUSSION

A- Extraction and determination of total anthocyanin

Results of the extraction of anthocyanin from red cabbage indicated that, the content of total anthocyanin was 90.50 mg/100 gm fresh weigh. These results are in agreement with (Piccaglia *et al* 2002 and Loscalzo *et al* 2008).

In the present investigation anthocyanin extracted from red cabbage were determined and identified with HPLC. HPLC method has many advantages because with this method individual anthocyanin can be determined (Reyes *et al* 2007).

Determining the amount of individual anthocyanin is important owing to their specific properties.

1) Selection of appropriate carriers

The adsorption materials used as carriers for anthocyanin pigments extracted from red cabbage are shown in **Table (4)**. It could be noticed that, the presence of anthocyanins pigment within carrier increased gradually by increasing the level of anthocyanin pigments in all tested carriers but disappeared and became very low by using both soluble starch and glucose at levels 5 :1 and 6 : 1 anthocyanin a: carrier (g/100g). Results also indicated that, dextrin had the most effective adsorbent carrier material for red cabbage pigment (at high level) followed by cellulose, soluble starch and glucose respectively. For instance, the best carrier for red cabbage anthocyanin was dextrin which came in the first order and cellulose which came in the second order. While glucose and soluble starch were the inferior and out of order to act as a carrier for pigments of red cabbage. The unsuitability of soluble starch and glucose as a carrier could be related to its capability of breakdown the anthocyanin during immobilization. Also, as reported by **(Swain, 1976)** the presence of sugar enhanced the breakdown rate of anthocyanin. On the other hand, the positive influence of cellulose and dextrin as coated carrier for anthocyanin pigment may be due to its function of these carriers as an inhibitor of polyphenol oxidase, the enzyme which hydrolyze the anthocyanin and also, due to the strict interfering of such carrier within the condensation reaction that usually occur during immobilization of anthocyanin **(Colin and Peter, 1980; El-Gharably, 2005)**.

B- Separation and identification of anthocyanin

Pigments for red cabbage were done by HPLC as seen in **Fig. (1)**.

Two anthocyanins from red cabbage were separated by HPLC **Fig. (1)**. Peak 1 was the major anthocyanin and represented 80% of the total area, while peak 2 represented 20% of the total area. Spectral measurement and HPLC separation indicated the presence of two anthocyanins for red cabbage namely cyaniding-3-diglucoside-5-glucoside (80%) and cyaniding 3,5 diglucoside (20%). These results were in agreement with **(Dyrby et al 2001 and Mc-Doug et al 2007)**. They found that, anthocyanidin was the only aglycone and that only two different anthocyanins were pre-

sent in the extract cyaniding-3-diglucoside-5-glucoside and cyaniding 3, 5 diglucoside

C- Properties of red cabbage anthocyanin pigments

1) Effect of pH on retention of red cabbage anthocyanin

The effect of different pH on retention of pigments derived from red cabbage is illustrated in **Table (5)**. The color changes were induced by pH variation. Most striking was the effect of pH on the anthocyanin content which was about 100 to 85% at pH varied from 1.0 to 4.0 while, the degradation of color reached to 38% and 50% at pH 6 and pH 7.0.

Moreover, the degradation of color not exceeds than 20% between pH 1.0 and 5.0. The highest stability remained at higher acidic pH 1.0 and pH 5.0.

Subsequently, results have contributed to the apparent low level of the pH dropped approximately 15% to 50% at pH 4.0 to 7.0, although, the reduction of color pigments was approximately 8% at pH 3.0.

In acidic media anthocyanin showed red color; while as the pH is progressively increased in alkaline side, they became more blue.

These results are similar with that of **(Kalt et al 2000)**. They reported that, the high level of anthocyanin at pH 1.0 is consistent with the presence of the flavylum cat ion which is most intensity colored.

2) Effect of temperature on retention of red cabbage anthocyanin

The effect of temperature on the degradation of anthocyanin extracted from red cabbage after holding for 30 min at pH 2.0 are shown in **Table (6)**. There is no degradation of red cabbage anthocyanin at 40 to 70°C. While the rate of degradation at 80°C, 90°C and 100°C being 1.0, 3.0 and 5.0% respectively.

3) Thermal stability of red cabbage anthocyanin

The stability of red cabbage anthocyanin on duration time at various temperatures 80°C to 100°C is evident in **Table (7)**. Results indicated that, no noticeable changes was observed for anthocyanin extracted from red cabbage after 90 min up

Table 4. Distribution pattern of anthocyanin extracted from red cabbage within selected carrier

Rate of anthocyanin to carrier g/100 g	Distribution of anthocyanin within selected carrier g/100g			
	Dextrin	Cellulose	Soluble starch	glucose
1:1	0.362	0.210	0.124	0.116
2:1	0.786	0.525	0.246	0.215
3:1	1.521	0.936	0.456	0.385
4:1	3.235	1.862	0.586	0.429
5:1	6.423	3.520	---	---
6:1	9.218	6.321	---	---

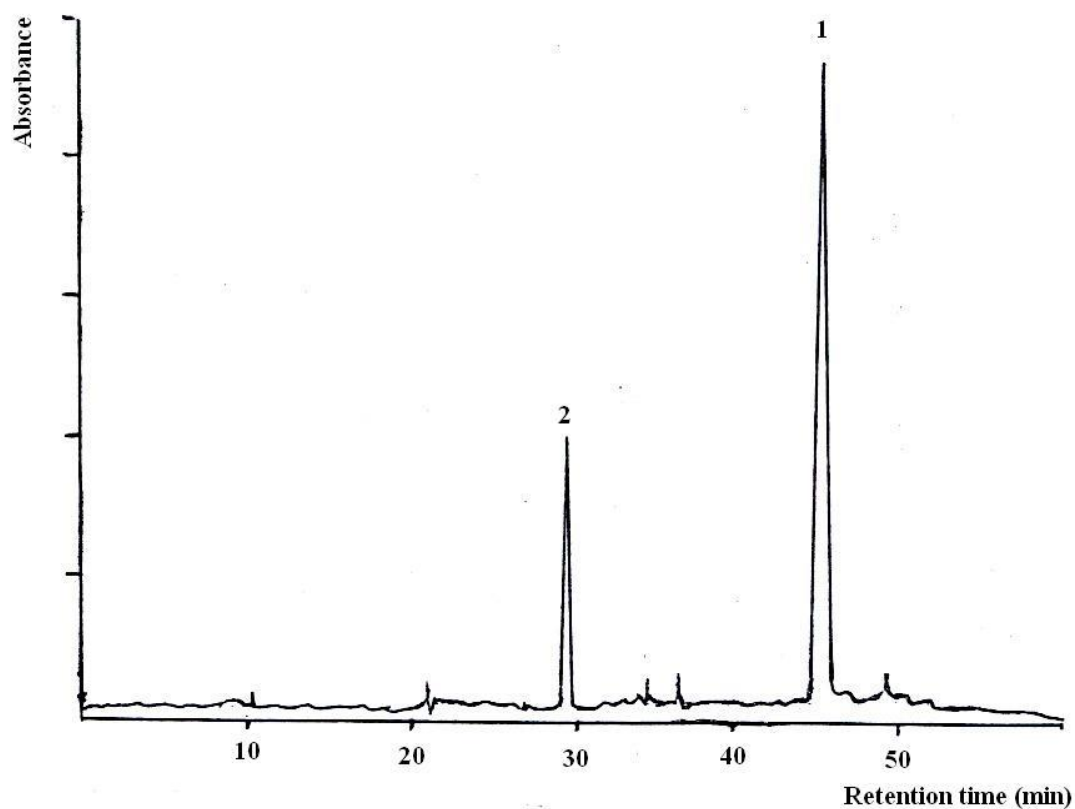


Fig. 1. Identification of anthocyanin pigments extracted from red cabbage

- 1- Cyanidin-3-diglucoside-5 glucoside
2- Cyanidin 3,5 diglucoside

Table 5. Effect of pH on retention % of anthocyanin pigment extracted from red cabbage

pH values	% remained anthocyanin pigment	% degradation of anthocyanin pigment
1.0	100.0	0.00
2.0	98.0	2.00
3.0	92.0	8.00
4.0	85.0	15.00
5.0	80.0	20.00
6.0	62.0	38.00
7.0	50.0	50.00
8.0	60.0	40.00
9.0	70.0	30.00
10.0	79.0	21.00

Table 6. Effect of temperature on the degradation rate of anthocyanin extracted from red cabbage at various temperature for 30 min at pH 2.0

Temperature	% remained anthocyanin pigment	% degradation of anthocyanin pigment
40	100.0	0.0
50	100.0	0.0
60	100.0	0.0
70	100.0	0.0
80	99.0	1.0
90	97.0	3.0
100	95.0	5.0

Table 7. Thermal stability of anthocyanin extracted form red cabbage

Anthocyanin%	Temp. °C	Duration time (min)					
		30	60	90	120	150	180
Remained	80	99.0	99.0	99.0	98.0	98.0	97.0
Degradation	80	1.0	1.0	1.0	2.0	2.0	3.0
Remained	90	97.0	96.0	96.0	95.0	94.50	94.0
Degradation	90	3.0	4.0	4.0	5.0	5.50	6.0
Remained	100	95.0	94.0	93.0	92.50	92.0	90.0
Degradation	100	5.0	6.0	7.0	7.50	8.0	10.0

Fore instance, anthocyanins pigments of red cabbage were found to be stable up to 80°C for 120 min and more labile by increasing both the .holding time and temperature used.

to 80°C, the destruction of anthocyanin was 4.0% at 90°C after incubation 90 min of heating. On the other hand, the remaining of anthocyanin being 90.0% of the total anthocyanin pigments after holding for 180 min at 100°C. Consequently, the holding of pigments at 80, 90 and 100°C for 120 min caused a reduction of 2.0, 5.0 and 7.50% while, the corresponding results were 3.0, 6.0 and 10.0% after 180 min for the total anthocyanin pigments respectively.

The high stability of red cabbage anthocyanins may be due to the attribute of the nature their anthocyanins in their composition.

The degradation rates of anthocyanins are dependent on pigment concentration being slower with higher concentration (Merin *et al* 1987). They further added that, destruction color during heating is much more rapid when oxygen is present and hydrolysis of the aglycone-sugar bond (position, 3) can occurred at 100°C (pH 2-4). Also, thermal degradation leads to formation of the chalcone and its subsequent yield several degradation products which condense to form complex brown polymeric compounds known as melanoidin pigments (Piffaut *et al* 1994).

D- Antioxidant activity of red cabbage extract on sunflower oil

Both the addition of red cabbage extract and BHT as antioxidant for sunflower oil retarded the changes in peroxide value of sunflower oil during

7 days of storage at 60°C. It is evident from these results that, as the concentration of antioxidant increased, inhibitory effect on peroxides value increased considerably (Table 8). After 7 days of storage at 60°C, peroxide values on sunflower oil treated with 200, 500 and 1000 ppm of red cabbage anthocyanin were 9.92, 8.24 and 6.10 meq/kg⁻¹. While the corresponding values of (BHT) were 10.12, 8.92 and 6.19 meq/kg⁻¹. On the other hand, the red cabbage anthocyanin was more effective for suppression of the development of POV value than BHT. Results also indicated that, similar effect on inhibition of sunflower oil peroxidation was similar by using 1000 ppm (BHT) and 500 ppm red cabbage extracts but the greatly inhibited was observed in POVs values in the presence of red cabbage extract at concentration of 1000 ppm. Results of present work indicate that, the compositions of red cabbage contain cyaniding-3 glycoside, for instance, displayed twice in vitro as scavenging activity as that commercially available antioxidant such as butylated hydroxytoluene and α tocopherol which was in line with the findings of (Fu-Kumoto and Mazza, 2000). Actually, this study (Table 8) revealed that, red cabbage extract had higher effect on controlling the development of rancidity in sunflower oil than, that of synthetic antioxidant (BHT). In addition, the natural antioxidant extract red cabbage extracts would be preferred over synthetic antioxidants to minimize the adverse health effects.

Table 8. Effect of red cabbage extract and BHT as antioxidants on peroxide value (POV) of sunflower oil during storage at 60°C for 7 days

Storage period (days)	POV (meq/kg ⁻¹) for sunflower oil treated with						
	Control without (AA*)	BHT (ppm)			Red cabbage extract (ppm)		
		200	500	1000	200	500	1000
0.0	0.80	0.80	0.80	0.80	0.80	0.80	0.80
1.0	1.95	1.85	1.69	1.54	1.90	1.65	1.45
2.0	4.83	2.97	2.10	1.82	3.10	2.00	1.80
3.0	8.65	4.83	3.82	2.76	4.62	3.36	2.46
4.0	11.22	6.32	4.25	3.34	6.28	4.00	3.12
5.0	14.26	7.94	6.21	4.67	7.80	5.26	4.23
6.0	18.64	8.98	7.42	5.88	8.60	6.37	5.12
7.0	21.27	10.12	8.92	7.19	9.92	7.24	6.10

AA* = Antioxidant

E- Sensory evaluation of hard candy, jelly and ice sherbets

Sensory properties of hard candy, jelly and ice sherbets prepared with adding different levels of red cabbage anthocyanin as natural colorants compared with other products prepared with 0.10% synthetic red color (carmin) are given in **Table (9)**. Analysis of variance showed mostly significant differences in color, taste, odor and overall acceptability for both hard candy, jelly and ice sherbets as control or prepared by different levels of natural red color (red cabbage anthocyanin) in the range 0.1 to 0.5%. The addition of natural red color from red cabbage with different levels significantly affected color, taste, odor and overall acceptability. However, hard candy, jelly and ice sherbets prepared with 0.40 and 0.5% received the lowest score in all tested quality attributes. On the other hand, panelists gave samples prepared by adding red cabbage anthocyanin at 0.1% for hard candy, jelly and 0.2% for ice sherbets

The hard candy and jelly prepared by adding natural color from red cabbage at 0.1% had a highest score of investigated attributes followed by adding 0.20, 0.30, 0.40 and 0.50%, respectively. On the other hand, the ice sherbets prepared by adding red cabbage anthocyanin 0.20% had a highest score of investigated attributes followed by adding 0.30, 0.10, 0.40 and 0.50% respectively.

In general, consumer perception has been that natural food colorant ingredient would be safer, healthful and considered as potential food colorants for preparing hard candy and Jellies these findings are in accordance with that of **(El-Gharably, 2005)**. Also, there is a trend in the foodstuffs industry towards functional foods, which produce healthy effects based on their antioxidant properties **(Kahkonen et al 2001)**.

Conclusions

Our results shown that, anthocyanins of Egyptian red cabbage have been a good characteristics as a natural source of food colorants instead of synthetic colorants aiming to: (1) a wide range of coloration from red to blue depending on the pH media (2) Heat resistant without loss of its color at higher temperature and low sensitivity to photo degradation at lower pH from 1.0 to 5.0. (3) Possess a higher antioxidant activity therefore, it is interesting to use in manufacturing of hard candies, jellies and ice sherbets also, it can be used as a higher antioxidant activity for delaying the rancidity of sunflower oil than using synthetic antioxidant (BHT).

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