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ORIGINAL ARTICLE

Evaluation of red pigment extracted from purple carrots and its utilization as antioxidant and natural food colorants

M.T.M. Assous *, M.M. Abdel-Hady, Ghada M. Medany

Food Technology Research Institute, Agriculture Research Center, Giza, Egypt

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Abstract Anthocyanins derived from purple carrots were extracted, and identified by using HPLC. Extracted pigments from purple carrots are used as alternative natural red colorants for preparing hard candy and sweet jelly and also red carrot pigment used as natural antioxidant on sunflower oil to delay the rancidity of sunflower oil. Purple carrots contain 168.7 mg anthocyanin/100 g on fresh weight basis, where the major constituents were Cyanidin-3-xylosyl-glucosyl-galactoside acylated with ferulic acid (33.65%) followed by Cyanidin-3-xylosyl-glucosyl-galactoside acylated with coumaric acid (29.85%) and Cyanidin-3-xylosyl-galactoside (28.70%) as determined by HPLC. Dextrin was the best carrier for purple carrots anthocyanin pigment followed by cellulose, soluble starch and glucose respectively. On the other hand, the highest pigment color stability of anthocyanin derived from purple carrots was obtained at pH values ranged between 1.0 and 4.0 and temperatures ranged between 40 and 80 °C, while the degradation ratio of anthocyanin being 15% of total pigments after 180 min at 100 °C. Antioxidant activities of anthocyanin from purple carrots were assessed by determining peroxide value on sunflower oil during 7 days at 60 °C. Sunflower oil contained 1000 ppm purple carrots extract showed lower peroxide value being (7.90) than using 200 ppm synthetic antioxidant (BHT) (8.38) meq/kg. Analysis of variance for sensory evaluation of prepared hard candy and sweet jelly indicated that there were no significant differences for hard candy contains 0.30% and sweet jelly 0.20% anthocyanins pigments from purple carrots and control hard candy and sweet jelly.

Introduction

Recently, there has been an increased interest in the development of food colorants from natural sources as alternatives to synthetic dyes because of both legislative action and consumer concern (Giusti and Wrolstad, 1996).

Anthocyanins are the more important plant pigments visible to the human eye. They belong to the widespread class of phenolic compounds collectively named flavonoids. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (MingKong et al., 2003); furthermore, they are characterized by a wide spectrum of color tones, ranging from orange through red, to purple

* Corresponding author.
E-mail address: assous2010@yahoo.com (M.T.M. Assous).
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and blue, depending on the molecular structure and pH value (Dorota and Janusz, 2007).Anthocyanins are mainly distributed among flowers, fruits and vegetables and are responsible for their bright colors such as purple, red and blue (Xianli and Roland, 2005).

The interest of anthocyanins derives not only from their coloring effect but also from their beneficial properties, including antioxidizing activity due to their ability for preventing lipid oxidation in different lipid sources (Marja and Marina, 2003) Also, anthocyanins lead to improve the tightness of capillary blood vessels and prevention of thrombosis aggregation, all of which reduce the risk of circulatory diseases (Giusti and Wrolstad, 2003).

Anthocyanins are widely used in the food industry as an alternative of synthetic colorants, e.g., they can replace Allure red (FD&C Red No. 40) (Fabre et al., 1993). Meanwhile, anthocyanin pigments are water-soluble and this property facilitates their incorporation into numerous aqueous food systems, which made it as attractive natural colorants (Luigia and Giuseppe, 2006).

Anthocyanins of black carrots are characterized by a high ratio of mono-acylated structures with three sugar moieties increasing the color retention at pH of food and also showing increased thermal stability in addition to exerting strong antioxidant activity (Gizir et al., 2008). Purple or black carrots pigments can consider to be a good choice for coloring fruit juices, nectars, soft drinks, jellies and confectioneries (Downham and Collins, 2000).

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 (El-Gharably, 2005).

In recent years, it has an increasing effort 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 with 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 (Fu-kumoto and Mazza, 2000).

The aim of this study was to extract the anthocyanin pigment from purple carrot and to evaluate the major constituents of pigments and their color stability, selecting the appropriate matrix as a carrier material for anthocyanin pigment. The antioxidant activity for anthocyanin extracted from purple carrots by using sunflower oil was assessed. Pigment as a food using the natural colorants in preparation of hard candy and jelly prepared was also under taken.

Materials and methods

Materials

(a) Purple carrots (*Daucus carota* L. sp. *Sativus var. atrorubens.*) were purchased from the local market in Giza governorate, Egypt. Refined sunflower oil without adding antioxidants was obtained from Safola Saiem Company, 10th of Ramadan, Egypt. Carmine (red color, C.I. 75470) was obtained from Aldrich Chemical Company, USA.

Anthocyanin standards: Cyanidin-3-diglucoside-5 glucoside and Cyanidin 3, 5 diglucoside were obtained from Carl Roth GmbH D-76185 Karlsruhe, Germany.

Butylated hydroxy toluene (BHT) and solvents used for spectral and HPLC analyses were of HPLC grade and were purchased from Sigma Chemical Company USA.

Methods

Extraction and concentration of anthocyanins pigment from purple carrots

Anthocyanins from purple carrots were extracted by using the method of Fuleki and Francis (1968) modified by Colin and Peter (1980).Twenty gm of purple carrots was mixed with 100 ml ethanol acidified with 0.01% citric acid and macerated in a warring blender at full speed for 5 min. The mixture was filtered by Whatman No. 1 filter paper through a Buchner funnel. The residue on the filter paper was washed rapidly with the extracting solvent until collecting of about 450 ml from extracted pigment carrot. The extract was concentrated in a rotary vacuum evaporator at >40 °C until the solvent evaporated.

Determination of total anthocyanin

A small liquate 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 2 h and absorbance was measured at 520 nm.

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

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

whereas

OD = optical density
DV = diluted volume for the OD measurement
TEV = total extract volume
SV = sample volume
SW = sample weigh in grams
51.56 = E. value for which the major constituent (Cyanidin).

Analysis of anthocyanins of purple carrots extract

(a) Purification of anthocyanins

The concentrated extract of anthocyanins was purified according to the method described by Attoe and Van-Elbe (1981). The concentrate of anthocyanin was purified with 1:1 petroleum ether and ethyl acetate to remove nonpolar impurities. After phase separation, residual solvent was removed from the aqueous phase with rotary evaporator.

(b) Identification of anthocyanins pigments by High-Performance Liquid Chromatography (HPLC):

The purified anthocyanin of purple carrots was identified by HPLC according to the method reported by Andersen (1989) using supelcosil LC 18 column. 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 1.5 ml/min. The elutes were monitored by visible spectrometry at maximum wavelength 520 nm.

Properties anthocyanin pigment for purple carrots

(a) Effect of pH on the efficiency of anthocyanin color

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

(b) Effect of temperature on the efficiency of anthocyanin color

A preliminary study was conducted to determine the heat tolerance of anthocyanin for purple carrots at different temperature ranged between 40 and 100 °C for 30 min, and then percentage of color loss was calculated.

(c) Thermal stability of anthocyanin for purple carrots

Holding red colorant solution (anthocyanin pigment for purple carrots) was extended at 80–100 °C for 180 min and samples were taken each 30 min. and then cooled immediately in an ice bath followed by measuring the absorption spectra of the solution at 520 nm.

(d) Antioxidant activity testing

Antioxidant activity was tested by determining the peroxide value (POV) during incubation of sunflower oil containing purple carrots extract at 60 °C for 7 days according to the method of A.O.A.C., (2005). Ten grams of dried purple carrots was exhaustively extracted with ethanol (100 ml). The extracts 200, 500, and 1000 ppm were mixed with 25 g of sunflower oil in a flask against control 25 g of sunflower oil mixed with 200 ppm (BHT in a flask) as a control for each one and the mixtures were placed in an oven at 60 °C for 3 h daily, till 7 days. The peroxide value was determined daily.

Selection of suitable carrier for anthocyanin pigments

The concentrated anthocyanin pigments were adsorbed on various supports according to the method described by Rizk (1997) using different ratios up to 10:1 (pigments:carrier) namely cellulose, glucose, dextrin and soluble starch and lately dried in oven at 40 °C for 24 h.

Application of anthocyanin pigments extracted from purple carrots

(a) Preparing hard candy

Hard candies were manufactured by using the formula shown in Table 1 with adding different levels of red color (purple carrots anthocyanin) 0.10, 0.20, 0.30, 0.40 and 0.50% w/w to the formula using the traditional procedure as described by Counsell (1980). The control sample was prepared using 0.10% synthetic color (carmine).

(b) Preparation of sweet jelly powder

Sweet jelly powder was prepared by mixing the ingredients illustrated in Table 2 with adding different levels of anthocyanins for purple carrots color by 0.10, 0.20, 0.30, 0.40 and 0.50% w/w using the traditional procedure. The control sample was prepared as the same ingredients using 0.10% synthetic color (carmine).

Both hard candy and sweet jellies powder were wrapped by polyethylene and aluminum foil and packed in carton bags and were stored at room temperature (25 ± 5 °C).

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 and sweet Jelly according to the method described by Reitmeier and Nonnecke (1991).

Statistical analysis

Means of data obtained for sensory attributes of hard candy and jelly 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 Inc., 1999).

Results and discussion

Total anthocyanins content of purple carrots

Results indicated that the extracted purple carrots contained about 168.70 mg/100 g on fresh weight of anthocyanins pigments; the results declared that purple carrots contained high concentration of total anthocyanins. These results are in agreement with that obtained by Mazza and Miniati (1993).

Identification of anthocyanins for purple carrots by HPLC

Anthocyanins pigments extracted from purple carrots were separated and identified by HPLC are shown in Table 3 and Fig. 1.

Spectral measurements and HPLC separation indicated the presence of five anthocyanins for purple carrots. Results in Fig. 1 and Table 3 indicated the detected types of anthocyanin pigments for purple carrots namely Cyanidin-3-xylosyl-glucosyl-galactoside (peak 1), Cyanidin-3-xylosyl-galactoside (peak 2), Cyanidin-3-xylosyl-glucosyl-galactoside acylated with sinapic acid (peak 3), Cyanidin-3-xylosyl-glucosyl-galactoside acylated with coumaric acid (peak 4) and Cyanidin-3-xylosyl-glucosyl-galactoside acylated with ferulic acid (peak 5), respectively.

Table 1 Hard candy formula (100 g).

Ingredients	g
Sucrose	48.48
Corn syrup	25.40
Water	25.26 ml
Flavoring oil	0.21
Citric acid	0.15
Color (purple carrots anthocyanin)	0.10–0.50

Table 2 Sweet jelly powder formula (100 g).

Ingredients	g
Sucrose	84.0
Gelatin	15.0
Citric acid	0.20
Flavoring agent	0.10
Sodium benzoate	0. 05
Potassium citrate	0.05
Ascorbic acid	0.10
Color (purple carrots anthocyanin)	0.1–0.5

The major anthocyanin pigments extracted from purple carrots were Cyanidin-3-xylosyl-glucosyl-galactoside acylated with ferulic acid (peak 5) and represent 33.65% of the total area, while peaks 4, 2, 1 and 3 represent 29.85, 28.70, 4.65% and 3.24% of the total area, respectively. These results are in agreement with Gizir et al. (2008) and Crig et al. (2009) and they found that five anthocyanins were identified in the purple carrots juice. Anthocyanins present in black carrots are mainly Cyanidin-3-xylosyl-galactoside and Cyanidin-3-xylosylglucosyl-galactoside, which is also further mono-acylated with ferulic, sinapic and p-coumaric acids Kammerer et al. (2004). Narayan and Venkataraman (2000) found five anthocyanins with only cyanidin as aglycon in black carrots (*D. carota*, L.).

Selection of suitable matrix as a carrier for anthocyanin

The adsorption matrixes used as a carrier for anthocyanin pigments extracted from purple carrots are shown in Table 4. Results indicated that dextrin and cellulose had the most effective adsorbent carrier material for pigments for purple carrots (at high level) followed by soluble starch and glucose respectively. For instance, the best carrier for anthocyanin of purple carrots was dextrin which came in the first order followed by 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 extracted from purple carrots. 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 occurs during immobilization of anthocyanin (Colin and Peter, 1980 and El-Gharably, 2005) On other hand, the unsuitability of soluble starch and glucose as a carrier could be related to its capability to break down the anthocyanins during the immobilization as reported by Swain (1976) who indicated that the presence of sugar enhanced the breakdown rate of anthocyanins.

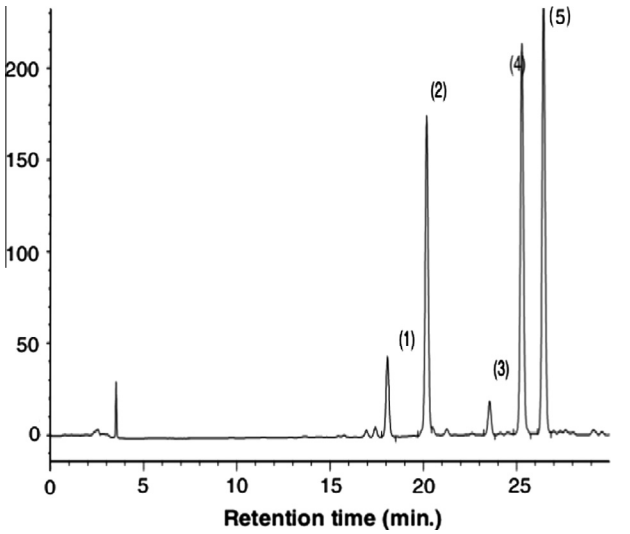

Fig. 1 Identification of compounds for anthocyanin pigments extracted from purple carrots.

Properties of anthocyanin pigments for purple carrots

Effect of pH values

The effect of different pH values on retention of anthocyanin pigments derived from purple carrots is illustrated in Table 5. Increasing pH values from 1 to 3 caused little degradation of anthocyanins pigment with about 3%. However, the degradation of color does not exceed 19% till pH value 5.0, while the degradation of color reached to 34% and 46% at pH (6 and 7.0), respectively. It could be observed that the stability of anthocyanins pigment was more pronounced at acidic pH values ranged from 1 to 5, while the highest degradation was occurred at pH 7. Stintzing et al. (2002) found that black carrots anthocyanins comprise high amount of acylated cyanidin derivatives (41.0%) which exhibit remarkable stability to pH changes. 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 flavylium cation which is the most intensely colored.

Effect of temperature

The effect of temperature on the degradation of anthocyanin extracted from purple carrots after holding for 30 min at pH 2.0 is shown in Table 6. There is no degradation of purple carrots anthocyanin was observed at 40–70 °C, while the rate of

Table 3 Identification of compounds for anthocyanin pigments extracted from purple carrots.

Peaks	Retention time	Area %	Identification of anthocyanin compounds
1	18.25	4.65	Cyanidin-3-xylosyl-glucosyl-galactoside
2	20.42	28.70	Cyanidin-3-xylosyl-galactoside
3	23.50	3.24	Cyanidin-3-xylosyl-glucosyl-galactoside acylated with sinapic acid
4	25.10	29.85	Cyanidin-3-xylosyl-glucosyl-galactoside acylated with coumaric acid
5	26.70	33.65	Cyanidin-3-xylosyl-glucosyl-galactoside acylated with ferulic acid

Table 4 Distribution pattern of anthocyanin extracted from purple carrots within selected carrier.

Selected carrier	Ratio of anthocyanin to carrier g/100 g	Concentration of anthocyanin g/100 g carrier
Soluble starch	10:1	5.40
Cellulose	10:1	38.65
Dextrin	10:1	40.82
Glucose	10:1	2.30

Table 5 Effect of pH on retention % of anthocyanin pigment extracted from purple carrots.

pH values	% Remained anthocyanin pigment	% Degradation of anthocyanin pigment
1.0	100.0	0.00
2.0	99.0	1.00
3.0	97.0	3.00
4.0	90.0	10.00
5.0	81.0	19.00
6.0	66.0	34.00
7.0	54.0	46.00
8.0	63.0	37.00
9.0	72.0	28.00
10.0	77.0	23.00

Table 6 Effect of temperature on the degradation rate of anthocyanins extracted from purple carrots at various temperature at pH 2.0 for 30 min.

Temperature (°C)	% Remained of 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	97.0	3.0
90	95.0	5.0
100	92.0	8.0

degradation at 80 °C, 90 °C and 100 °C being 3.0, 5.0 and 8.0% respectively.

Purple carrots anthocyanins comprise high amount of acylated cyanidin derivatives (41.0%) that exhibit remarkable stability to heat (Stintzing et al., 2002).

Effect of thermal stability

The stability of purple carrots anthocyanin on duration time at various temperatures ranged between 80 °C and 100 °C is evident in Table 7. Results indicated that little changes in anthocyanin extracted from purple carrots was observed after 90 min at 80 °C, and the destruction of anthocyanin was 9.0% at 90 °C after holding for 90 min at 90 °C. On the other hand, the remaining of anthocyanin were being 85.0% 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 6.0, 10. 0 and 12.0% while the corresponding results were 7.0, 12.0 and 15.0% after 180 min for the total anthocyanin pigments respectively.

For instance, anthocyanin pigments of purple carrots were found to be stable up to 80 °C for 120 min and more labile by increasing both the holding time and temperature used. The high stability of purple carrots anthocyanin may be due to the nature attribute of anthocyanins and 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 the destruction color during heating is much more rapid when oxygen is present and hydrolysis of the aglycon-sugar bond (position, 3) can occur at 100 °C (pH 2–4). Also, thermal degradation leads to the formation of the chalcone and its subsequent yield of several degradation products that condense to form complex brown polymeric compounds known as melanoid in pigments (Piffaut et al., 1994).

Antioxidant activity

Both the addition of anthocyanin extracted from purple carrots 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 as shown in Table 8. After 7 days of storage at 60 °C, peroxide values on sunflower oil treated with 200, 500 and 1000 ppm of purple carrots anthocyanin were 12.30, 9.60 and 7.90 meq/kg^{-1}, while the values of (BHT) were 8.38 meq/kg^{-1}. On the other hand, 1000 ppm of anthocyanin extracted from purple carrots extract was more effective for the suppression of the development of POV value than 200 ppm of BHT.

Actually, the addition of 1000 ppm of anthocyanin extracted from purple carrots had the highest effect on controlling the development of rancidity in sunflower oil than that of synthetic antioxidant (BHT). In addition, the natural antioxidant of extract purple carrot extracts would be preferred over synthetic antioxidants to minimize the adverse health effects.

Sensory evaluation of hard candy and sweet jelly

Sensory properties of hard candy and sweet jelly prepared with adding different levels from anthocyanins pigment of purple carrots as natural colorants compared with control prepared with 0.10% synthetic red color (carmine) are given in Table 9. Analysis of variance showed no significant differences in color for both hard candy prepared by adding 0.30% anthocyanins pigment of purple carrots and sweet jelly contained 0.20% natural red color (purple carrots anthocyanin) and control with 0.10% carmine, while hard candy and sweet jelly prepared with 0.5% recorded the lowest score in color scores. Meanwhile, there is little significant difference in taste and odor scores of hard candy and sweet jelly prepared with adding different levels of anthocyanins pigment form purple carrots and control with 0.10% carmine. On the other hand, hard candy prepared by adding 0.305 and sweet jelly 0.20% anthocyanins pigment of purple carrots recorded the highest scores for tested parameters and came in the first order and equal with control followed by hand candy contains 0.40% and sweet jelly contains 030%. However, both hard candy that contains 0.10% and sweet jelly that contains 0.10 and 0.50% anthocyanins pigment of purple carrots were inferior than other tested samples recorded slightly significant differences compared with other samples.

Table 7 Thermal stability of anthocyanin extracted from purple carrots.

Reaming of anthocyanin (%)	Temp. °C	Duration time (min)					
		30	60	90	120	150	180
	80	97.0	96.0	96.0	94.0	93.0	93.0
	90	95.0	93.0	91.0	90.0	90.0	88.0
	100	92.0	90.0	90.0	88.0	87.0	85.0

Table 8 Effect of purple carrots 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[a]	BHT (200 ppm)	Purple carrots extract (ppm)		
			200	500	1000
0.0	0.79	0.79	0.79	0.79	0.79
1.0	1.90	1.45	1.70	1.60	1.40
2.0	3.95	2.26	3.44	3.00	2.15
3.0	7.53	3.42	5.38	4.60	3.20
4.0	9.89	4.20	6.70	5.00	4.00
5.0	13.30	5.21	8.33	6.30	4.96
6.0	17.27	7.10	10.57	8.80	6.82
7.0	20.20	8.38	12.30	9.60	7.90

[a] Without antioxidant.

Table 9 Mean score of sensory evaluation of hard candy and jelly prepared with different levels of natural red colorants (anthocyanin) from purple carrots.

Treatments	Hard candy				Sweet jelly			
	Color	Taste	Odor	Overall acceptability	Color	Taste	Odor	Overall acceptability
Control[A]	9.6^{a}	9.6^{a}	9.5^{a}	9.6^{a}	9.8^{a}	9.6^{a}	9.7^{a}	9.7^{a}
P.C.A. (0.1%)[B]	5.3 ^{e}	9.5^{a}	9.4^{a}	8.7^{bc}	7.4^{c}	9.2^{ab}	9.3^{ab}	7.3^{c}
P.C.A. (0.2%)[B]	7.7^{bc}	9.2^{ab}	9.0^{ab}	8.0^{bc}	9.8^{a}	9.6^{a}	9.5^{a}	9.7^{a}
P.C.A. (0.3%)[B]	9.5^{a}	9.6^{a}	9.6^{a}	9.6^{a}	9.5^{a}	9.4^{a}	9.4^{a}	9.5^{a}
P.C.A. (0.4%)[B]	9.3^{a}	9.1^{ab}	9.0^{ab}	9.2^{ab}	8.2^{b}	9.1^{ab}	9.2^{ab}	8.2^{b}
P.C.A. (0.5%)[B]	7.1 ^{c}	9.1^{ab}	9.2^{ab}	8.3^{bc}	7.5^{c}	9.0^{ab}	9.1^{ab}	7.4^{c}

Values with different letters in the same column are significant different at $P < .0.05$.
[A] Control (prepared with 0.10% carmine as synthetic red color).
[B] P.C.A.: Purple carrots anthocyanins.

Conclusions

Our results show that anthocyanins of Egyptian purple carrots have been a good characteristic as a natural source of food colorants for the preparation of hard candy and sweet jelly and antioxidant for sunflower oil instead of synthetic antioxidant for delaying the rancidity of sunflower oil than using synthetic antioxidant (BHT).

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