

## CHARACTERIZATION OF RED PIGMENTS EXTRACTED FROM RED BEET (BETA VULGARIS, L.) AND ITS POTENTIAL USES AS ANTIOXIDANT AND NATURAL FOOD COLORANTS

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### **Abstract**

Red pigments extracted from red beet (*Beta vulgaris*,L.) were identified for major constituents by using HPLC, these pigments were used as alternative natural red colorants in some processed foods, i.e. jelly and ice sherbets. Also, the effect of using red beet extract as natural antioxidant on corn oil was studied.

The results showed that red beet had 380 mg betalain /100g on fresh weight. The major constituents' of red beet pigments were betalain and isobetalain by analyzing with HPLC. The best carriers for red beet pigments were dextrin followed by soluble starch, lactose and glucose, respectively. On the other hand, color and higher pigment stability of betalain pigments extracted from red beet were at pH ranged between 3.0 to 7.0 and temperature ranged between 40.0 to 50.0°C. Meanwhile, the degradation of red pigments were 82.0% of the total pigments after 180 min at 90°C. Antioxidant activity of red beet extracts were assessed by measuring peroxide value in corn oil during 7 days at 60°C corn oil contained 1000 ppm was able to lower the peroxide value than using 200 ppm synthetic antioxidant butylated hydroxyl toluene (BHT). Analysis of variance for sensory evaluation of prepared jelly and ice sherbets indicated that, jelly samples containing 0.30% and ice sherbets containing 0.20% red beet pigments were given the highest scores of color, taste and overall acceptability similar with synthetic color carmine.

#### **Key Words:**

Betalain, natural red colorants, natural antioxidant, jelly, ice sherbets, red beet.

### **INTRODUCTION**

Color is one of the most important attributes of foods, being considered as a quality indicator and determining frequently their acceptance. Many naturally colored foods, such as fruit products, are submitted to color losses during processing, requiring

the use of colorants to restore their color. Natural colorants have many disadvantages when compared to synthetic ones, including higher cost in-use and lower stability. However, people have increasingly avoided synthetic colorants, preferring natural pigments, which are considered to be harmless or even healthy. Betalains are not found in plants containing anthocyanin pigments based upon their molecular structure. Henritte (2009). Betacyanins generally appear red to red violet in color they absorb in the 535-550nm range - hence our choice of filter in the colorimeter) Betaxanthins generally appear yellow in color (absorb in the 475-480nm range). They cause color in both flowers, fruits and sometimes vegetative organs. They are found in the vacuole and they are water-soluble.

Betanins or betalains are natural dyes extracted from different fruits and vegetables. They are largely used as food colorants in food products like yogurts, ice cream and other products (Zhong *et.al.* 2005 ) and (Stintzing *et.al.* 2002). Recent studies have shown that betanines have antioxidant, antimicrobial and antiviral activity (Pedreno and Escribano, 2001). Beetroot (*Beta vulgaris*) is the main source of natural red dye, known as "beetroot red". Betanine is the main component of the red colorant extracted from *Beta vulgaris*. Immediately after extraction, betanine is exposed to degradation. The pigment stability is influenced by factors such as enzymes, temperature, oxygen and pH (Pedreno and Escribano, 2001). Betalain pigments extracted from red beet (*Beta vulgaris*) roots provide a natural alternative to synthetic red dyes. Betalains have been successfully used in commercial food coloring operations for a number of years (Goldman *et al.*, 1996) and continue to be an important source of red color in the food industry. Betalains are derivatives of betalamic acid and can be classified into two groups: the red-violet betacyanins (BC) and the yellow betaxanthins (BX). These differ by conjugation of a substituted aromatic nucleus to the 1, 7-diazaheptamethinium chromophore, which is present in betacyanin. Betalains are water-soluble nitrogen-containing pigments, which are synthesised from the amino acid tyrosine into two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins. Beetroot pigment is used commercially as a food dye. It changes color when heated so can only be used in ice-cream, sweets and other confectionary, but it is cheap and has no known allergic side-effects. Beetroot itself, of course, is a common salad ingredient – when cooked, vinegar is added to the water to lower the pH. Betalains have several applications in foods, such as desserts, confectioneries, dry mixes, dairy and meat products.

The concentration of pure pigment required to obtain the desired hue is relatively small, rarely exceeding 50 mg/kg, calculated as betanin (Delegado *et al.*, 2000). According to the (Codex Alimentarius Commission. 2004), betalain - limited only by good manufacturing practice. The food colorant known as 'beetroot red' extracted from beetroots are commercialized in European Union and USA as food colorant (Castellar *et al.*, 2003). Betanin (C<sub>24</sub>H<sub>27</sub>N<sub>2</sub>O<sub>13</sub>) makes up 75-95% of the total coloring matter found in the beet root, therefore it is used as a natural food coloring agent (Azeredo *et al.*, 2007). This pigment, like other betacyanins, is highly susceptible to changes induced by both pH and temperature (Pedreno and Escribano, 2001). As a powerful antioxidant pigment, betanin may provide protection and reduce risk of cardiovascular disease and cancer (Rakin *et al.*, 2007). Betalains are nitrogen-containing plant pigments whose colors range from red-violet betacyanins to yellow betaxanthins. They are used for coloring dairy products, meat and frozen desserts. Betalains have attracted additional interest because of their antioxidative, anti-inflammatory and anticarcinogenic properties.

The main source of commercially produced betalains is red beet root, but alternative sources are found in plants from the Amaranthaceae and Cactaceae families (Dubravko and Marijana 2011).

The aim of this research work was to obtain identify and characterize the pigments obtained from the red beet (*Beta vulgaris* L.), and using of these pigments as alternative natural red colorants in some processed foods (i.e. Jelly and Ice. Sherbet ). Also, to investigate the antioxidant activity of the extract through the addition to oil. Also, sensory evaluation was determined in jelly and ice sherbets after using the red beet extract as food colorant .

## **MATERIALS AND METHODS**

### **Materials**

Red beets (*Beta vulgaris* L.) were purchased from local market, Cairo, Egypt.

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

Refined corn oil was obtained from Arma Food Industries 10<sup>th</sup> of Ramadan.

Synthetic antioxidants namely butylated hydroxyl toluene (BHT) and synthetic red colorants namely carmine (alumlack of carminic acid) were purchased from Aldrich Chemical Company, USA.

**Analytical Methods:**

**Extraction and concentration of betalains pigment from red beet:**

About 200 g of red beet was mixed in blender with 1 liter of ethanol (acidified with 2% citric acid) for 15 min at room temperature and left for 24 hours. The extract were filtered and concentrated under vacuum by a rotary vacuum evaporator at 40 °C. as reported by Francis (2000).

**Determination of total betalains:**

The concentrated red beet was diluted with distilled water and measurement was carried out at wavelength of 535 nm and the quantification was expressed as mg betalains/ 100g using the following equation as determined by Castellar *et al.*, ( 2003)

$$\text{Total betalains content (mg / 100 g)} = A \times DF \times MW \times 1000 / \epsilon L$$

Where:

- A : Absorption value at 535 nm density.
- DF : Dilution volume.
- L : Path length of cuvette.
- MW : Molecular weight of betalain (550g/mol).
- € : The extinction coefficient for betalain 60000 L/mol.

**Selection of appropriate carrier:**

The concentrated betalains pigments were adsorbed on various supports using different ratios up to 7:1 (pigments: matrix (**carrier**) namely dextrin, soluble starch, anhydrous glucose and lactose and lately dried in oven at 40°C for 24 hours.

**Identification of betalains pigments by High performance Liquid Chromatography (HPLC).**

The identified of betalains pigments by HPLC Merck Pump L- 7100 according to the method reported by (Stintzing *et.al.*, 2002) using a LC18 column (250 mm × 4.6 mm, i.d). The flow rate was 1.0 ml / min., the elutes were monitored by visible spectrometry at 538nm and 476nm for betalain. Identification was performed within a standard sample as described by the same author.

**Properties of betalains:****Effect of pH:**

A preliminary study was conducted to test the stability of betalain pigments in different pH media that ranged from 2.0 to 10.0 for 30 min and then percentage of color loss was calculated.

**Effect of temperature:**

A preliminary study was conducted to test heat tolerance of betalains pigments at different temperature ranging from 40 to 100°C for 30 min and then percentage for color loss was calculated.

**Thermal stability:**

Holding red colorant (betalain pigments) at 80°C and 90°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 535nm.

**Antioxidant activity:**

**Determination of antioxidant activity:**

Corn oil was used as substrate for oxidant studies .Natural antioxidant extracted from red beet 10g of dried red beet extract and synthetic antioxidant butylated hydroxyl toluene (BHT) were added to oil at 200,500, and1000 ppm on dry weight basis to test their antioxidant effectiveness, according to (Matthaus, 2002). Control sample without additives was prepared in the same condition.

Corn oil with and without antioxidant (natural or synthetic) was heated in 500 ml/g beaker at 60°C (in an oven )for 3h daily, the experiment was continued for 7days. The peroxide value was determined as antioxidant, for each according to the method described in (A.O.A.C .2000).

**Technological methods:**

**Preparation of jelly:**

Jelly was prepared in laboratory by adding different levels of red beet pigments 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 (1).

Table 1. 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 beet pigments extract)	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 jelly was prepared with 0.10% synthetic color (carmine).

### 3) - Ice sherbets:

Ice sherbets was prepared in laboratory by adding different levels of red beet pigments extract ranging from 0.1 to 0.5% (w/w) using the traditional procedure. The formulation of ice sherbets are shown in Table (2).

Table 2. The formulation of sherbets.

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

These contents should be mixed very well and heated at  $90^{\circ}\text{C}$  for 15 min., cooling until reaches  $80^{\circ}\text{C}$  and then put in polyethylene bouchs and placed in deep freezer at  $-18^{\circ}\text{C}$ .

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

### Sensory evaluation:

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

### Statistical analysis:

Data were statistically analyzed to facilitate comparing the least significant differences (LSD) between means of different values according to (Snedecor and Cochran 1973).

## RESULTS AND DISCUSSION

### Extraction and Identification of betalains from red beet:-

Results of the extraction of betalains from red beet indicate that, the content of total betalains was 380 mg / 100 g on fresh weights. The result obtained in general were in accordance with (Zakharova and Petrova .1997), who found that, the total betalain content of red beet were 250 to 850 mg/100g on fresh weight while (*Delgado et.al.*, 2000) found that, red pigment content in red beet could reach 500 mg /100g on fresh weight.

### Identification of betalains extracted from red beet:-

Separation and identification of betalain pigments from red beet was done by HPLC as seen in Table (3). Three components were identified by HPLC, the major constituent of red beet were Betalain 82.79% followed by isobetalain 11.40% and vulgaxanthin 1.34% respectively. These results are coinciding with that of (Wybraniec,2005), who mentioned that the major components of pigments in red beet are betalain and isobetalain.

Table 3. Identification of red beet pigments.

Retention time(min)	Relative abundance area %	Identified pigment
10.50	1.34	Vulgaxanthin
23.0	82.79	Betalain
25.7	11.40	Isobetalain
28.9	4.47	Unidentified pigments

\*Total betalins was 380 mg/100g on fresh weight.



### Selection of appropriate carrier:

The adsorption materials used as carrier for betalain pigments extracted from red beet are shown in Table (4). It could be noticed that, the presence of betalains with carriers increased gradually by increasing the level of betalain pigments in all tested carrier but disappeared or become very low by using glucose, lactose and soluble starch at 7:1 betalain: carrier (g /100g). Results also indicated that, dextrin had the most effective adsorbent coated carrier material for betalain pigments (at high levels) which ranked as first order followed by soluble starch, lactose and glucose, where ranked in second, third and fourth orders respectively to act as a carrier for pigments of red beet.

Table 4. Distribution pattern of betalains within selected carrier.

Selected carrier	Ratio of betalains to carrier g/100g	Concentration of betalains g/100 g carrier
Soluble starch	7:1	4.34
Lactose	7:1	3.46
Dextrin	7:1	20.63
Glucose	7:1	2.25

### Properties of betalains extracted from red beet:

#### Effect of pH:

Preliminary study was conducted to test the stability of betalains pigments derived from red beet in different pH media. The results obtained in Table (5) show that there are relationship between color changes and pH variation .Most striking was the effect of on betalains content which was about 93.0 to 100.0 % at pH varied from 2.0 to 7.0, while the degradation of color reached to 21.87 and 50.0 % at pH 8.0 and pH 10.0 respectively.

Moreover, the degradation of color does not exceed 7 % in the range of pH values 3.0 to 7.0. For instance, the highest stability remained at pH 3.0 to pH 7.0 since less betalain degradation were extracted from red beet These results are similar with that

of (Castellar *et al.*,2003). Below pH 3.0, the absorption maximum shifts toward lower wavelengths, and above pH 7.0 the change is toward upper ones, out of the pH range 3.0–7.0 the intensity of the visible spectra decreases.

Table 5. Retention% of betalain pigments extracted from red beet as a function of pH values.

pH values	% retained of betalain pigments	% degradation of betalain pigments
2	95.00	5.00
3	95.31	4.69
4	98.44	1.56
5	100.00	00.0
6	96.88	3.12
7	93.75	6.25
8	78.13	21.87
9	60.94	39.06
10	50.00	50.0

#### **Effect of temperature:**

The effect of temperature on the remained and degradation rates of red beet betalains is illustrated in Table (6). Prolonged exposure of pigment at moderate temperature ranged between 40 and 50°C, showed no degradation and similar stability were observed, whereas at above 50°C, the degradation of betalains increased gradually by increasing temperature. For instance, the degradation in betalains pigments, caused by its exposing at high temperature. The higher degradation of betalains was observed at 100°C followed by 90, 80 and 70°C, respectively. While the corresponding degradation rate 19.0, 36.0, 46.0 and 57.0 % after exposure at 70, 80, 90 and 100°C respectively.

The degradation rate of betalains increased gradually by increasing the temperature degree. The higher degradation rates of betalains were observed above 70°C and the lowest rate was below 50 °C while at 40°C no any degradation for betalains could

be observed. Thus the maximum stability of pigment could be in the range of 40°C up to 50°C .

Table 6. Effect of temperature on the degradation rate of betalains extracted from red beet at various temperatures for 30 min.

Temperature (°C / 30 min)	% retained of betalains pigments	% degradation of betalains pigment
40	100	0.0
50	98	2.0
60	92	8.0
70	81	19.0
80	64	36.0
90	54	46.0
100	43	57.0

#### **Thermal stability:**

The Thermal stability of betalains on duration time at various temperatures ranged between 80 and 90°C are evident in Table (7). Results indicate that, the increase of destruction occurred by increasing of the duration time. On the other hand, the remaining of betalains being 18.0% of the total pigments after holding for 180 min at 90°C. Consequently, the exposing red beet betalains to 80 - 90 °C for 120 min caused a destruction reached to about 69.0 % of betalains while after 180 min increased to 82 % of the total betalains pigments respectively. However the temperature is considered to be the most important factor on betalain stability during food processing and storage as reported by ( *Garci et al., 1998*).

Table 7. Thermal stability of betalains extracted from red beet.

Betalain pigments	Tolerance periods in min						
	Temp °C	30	60	90	120	150	180
Retained	80	64	57	50	42	36	21
Degradation	80	36	43	50	58	64	79
Retained	90	54	41	36	31	26	18
Degradation	90	46	59	64	69	74	82

**Antioxidant activity of red beet extract on corn oil:**

The efficiency of natural antioxidant of red beet (betalains ) extract was compared with synthetic antioxidant (BHT) measured by peroxide value (PV) and the results are reported in Table (8). from the results it could be observed that as the concentration natural antioxidant (red beet) increased there was increase in the inhibitory effect of ( PV)

After 7 days of storage at 60°C, PV values of corn oil treated with 200, 500 and 1000 ppm of red beet extract were 10.9, 9.4 and 7.80 meq / kg<sup>-1</sup> ,while the PV value of corn oil treated with 200 ppm of BHT (synthetic antioxidant), was 8.40 meq / kg<sup>-1</sup>. On the other hand, the red beet extract (1000 ppm) was more effective for suppression development of PV value than BHT.

From aforementioned results, 1000 ppm red beet extract had higher effect on controlling the development of rancidity in corn oil than that of synthetic antioxidant (BHT). In addition, the natural antioxidant extract of red beet extracts would be preferred over synthetic antioxidant to minimize the adverse health effects.

Table 8. Effect of betalains and BHT as antioxidants on peroxide value (PV) of corn oil during storage at 60°C. for 7 days.

Storage time (days)	PV (meq / kg <sup>-1</sup> ) for corn oil treated with				
	Control without antioxidant	*BHT	Red beet extract		
		200 ppm	200 ppm	500 ppm	1000 ppm
0.0	1.18	1.18	1.18	1.18	1.18
1.0	3.52	2.73	3.42	3.28	2.80
2.0	5.40	3.16	4.90	4.28	3.10
3.0	7.30	3.94	5.30	4.60	3.80
4.0	8.25	4.76	6.77	6.12	4.70
5.0	10.33	6.21	8.44	7.11	6.11
6.0	14.65	7.30	9.86	8.78	6.98
7.0	17.40	8.40	10.90	9.40	7.80

\*BHT : Butylated Hydroxyl Toluene

**Sensory evaluation of jelly and ice sherbets:**

Sensory properties of jelly and ice sherbets prepared with adding different levels of betalain extracted from red beet as natural colorants compared with other products prepared with 0.10% synthetic red color (carmine) are given in Table (9). Analysis of variance showed mostly significant differences in color, taste, odor and overall acceptability for both jelly and ice sherbets as control or prepared by different levels of natural red color (red beet) in the range 0.1 to 0.5%. The addition of natural red color from red beet with different levels significantly affected color, taste, odor and overall acceptability. However, ice sherbets prepared with levels of betalains extracted from red beet in concentration 0.10, 0.40 and 0.5% and also concentrations 0.4, 0.50 % for jelly received the lowest score in all tested quality attributes.

The jelly prepared by adding natural color from red beet at 0.30 % had a highest score of investigated attributes followed by adding 0.4 and 0.2 respectively. On the other hand, the ice sherbets prepared by adding betalain extracted from red beet 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 Jellies and ice sherbets.

Table 9. Sensory evaluation of jelly and ice sherbets prepared with different levels of natural red colorants (betalain) from red beet.

Treatments	Jelly				Ice sherbets			
	Color	Taste	Odor	Overall acceptability	Color	Taste	Odor	Overall acceptability
Control**	9.80 <sup>a</sup>	9.80 <sup>a</sup>	9.70 <sup>a</sup>	9.70 <sup>a</sup>	9.50 <sup>a</sup>	9.50 <sup>a</sup>	9.50 <sup>a</sup>	9.50 <sup>a</sup>
0.1% betalain pigments	7.40 <sup>c</sup>	7.10 <sup>c</sup>	7.20 <sup>c</sup>	7.20 <sup>c</sup>	7.30 <sup>c</sup>	7.20 <sup>c</sup>	7.20 <sup>c</sup>	7.20 <sup>c</sup>
0.20% betalain pigments	8.40 <sup>b</sup>	8.20 <sup>b</sup>	8.30 <sup>b</sup>	8.30 <sup>b</sup>	9.50 <sup>a</sup>	9.50 <sup>a</sup>	9.50 <sup>a</sup>	9.50 <sup>a</sup>
0.30% betalain pigments	9.80 <sup>a</sup>	9.70 <sup>a</sup>	9.60 <sup>c</sup>	7.60 <sup>c</sup>	8.30 <sup>b</sup>	8.20 <sup>b</sup>	8.30 <sup>b</sup>	8.30 <sup>b</sup>
0.40% betalain pigments	8.50 <sup>b</sup>	8.30 <sup>b</sup>	8.30 <sup>b</sup>	8.30 <sup>b</sup>	7.10 <sup>c</sup>	7.20 <sup>c</sup>	7.20 <sup>c</sup>	7.10 <sup>c</sup>
0.5% betalain pigments	7.10 <sup>c</sup>	7.00 <sup>c</sup>	7.00 <sup>c</sup>	7.00 <sup>c</sup>	6.00 <sup>d</sup>	6.20 <sup>d</sup>	6.10 <sup>d</sup>	6.10 <sup>d</sup>

\*values with different letters in the same column are significant different at P<.0.05.

\*\* control (Prepared with 0.10% carmine as synthetic red color.

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## خواص الصبغات الحمراء المستخلصة من البنجر الأحمر واستخدامها كمضاد للأكسدة وملون طبيعي للأغذية

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تم استخلاص الصبغات الحمراء من البنجر والتعرف على التركيب الأساسي لهذه الصبغات بالتحليل الكروماتوجرافي واستخدام تلك الصبغات كملونات طبيعية بديلة للملونات الصناعية في بعض الأغذية المصنعة مثل الجيلي والشراب المتلج ولقد أخذ في الاعتبار أيضا دراسة تأثير استخدام مستخلص البنجر الأحمر كمضاد أكسدة طبيعي في زيت الذرة.

ولقد أتضح من الدراسة أن البنجر الاحمر احتوى على ٣٨٠ ملليجرام بيتالين لكل ١٠٠ جرام بنجر طازج وكانت المكونات الرئيسية لتلك الصبغات الحمراء المستخلصة من البنجر الأحمر هي البيتالين ، الايزو بيتالين.

وأشارت النتائج أن أفضل مادة لتحميل الصبغات الحمراء المستخلصة من البنجر الاحمر كانت الدكسترين يليها النشا الذائب ثم اللاكتوز وأخيرا الجلوكوز على التوالي.

ومن ناحية أخرى فقد أوضحت الدراسة أن الثبات العالي لصبغات البيتالين المستخلصة من البنجر كانت عند أس هيدروجيني من ٣ الى ٧ ودرجات حرارة ٤٠ - ٥٠ م° بينما كان معدل هدم البيتالين بنسبة ٨٢ % من الصبغة الكلية كان على ٩٠ م° بعد ١٨٠ دقيقة.

هذا وتشير النتائج إلى أنه بتقييم مستخلص البنجر الاحمر كمضاد للأكسدة عن طريق تتبع التغير في رقم البيروكسيد لزيت الذرة خلال سبعة أيام على درجة حرارة ٦٠ م° حيث وجد أنه بإضافة ١٠٠٠ جزء في المليون من مستخلص البنجر الاحمر أدت إلى خفض رقم البيروكسيد بدرجة ملحوظة بالمقارنة بإضافة ٢٠٠ جزء في المليون من مضاد الأكسدة الصناعي بيوتيل هيدروكسي تولوين

ومن ناحية أخرى فقد أظهرت نتائج التقييم الحسي للجيلي المحتوية على ٠,٣ % والشراب المتلج المحتوى على ٠,٢٠ % لون احمر مستخلص من البنجر حصلت على أعلى القيم لصفات اللون والطعم والقبول العام متماثلة مع تلك المصنعة باستخدام اللون الصناعي الكارمين .