J. Agric. Sci. Mansoura Univ., 32 (2): 1159 - 1164, 2007

EXTRACTION OF ANTHOCYANINS PIGMENTS FROM *Pelargonium zonal* PETALS AS NATURAL COLORANTS IN SOME FOODS.

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ABSTRACT

The use of the synthetic pigments as a color of foods restricted. Anthocyanins extracted from pelargonia petals. Stability of these extracted anthocyanins was investigated. Anthocyanins extracted by different extraction solvents. Ethanolic hydrochloric acid solution, distilled water acidified with citric acid (3 gm/100 ml) or acetic acid (3 ml/100ml) used for the extraction. Anthocyanins content was higher in red pelargonia petals compare to pink pelargonia petals. The acidified ethanolic solution washed superior for the extraction compared with the acidified distilled water citric acid or acetic acid. Anthocyanins content was slightly decreased by heat treatment. Anthocyanins were slightly decreased during storage at temperature. Biologically results revealed that there were no effects on the morphological properties and the rate of death of white rates treated by pelargonica anthocyanins extracted from red pelargonia petals which preferred than the pink petals. These rates treated by 5 and 10% concentration of these extracted anthocyanins. Pelargonia red petals was the superior for anthocyanins extraction and distilled water acidified with citric acid was recommended for anthocyanins extraction on the economic bases.

INTRODUCTION

Pelargonia is a perennials ornamental plant has various colored flowers. It needs fewer costs for cultivation and giving its flowers. It needs a short time for the flowering after planting (Badr *et al.*, 1998). Now many countries prevented the use of synthetic colorants in foods. Most of the natural colorants are extracted from plants, animals or microorganisms (Wong and Koehler, 1983). With respect to anthocyanins, anthocyanins have been consumed for many years without apparent adverse effects, have bright attractive colors, and are water facilitating their incorporation into aqueous food systems. Interest in anthocyanins has increased because of their potential use as natural colorants and possible beneficial health effects. Anthocyanins extracted by El- Zoghbi *et al.* (1993) they extracted anthocyanins from roselle and reported that anthocanins were used succefully for coloring strawberry nectar. Mazza & Miniati (1993) reported that, interested in anthocyanins has increased because of their potential use as natural colorants and possible beneficial health effects. Lewis *et al.* (1995) extracted anthocyanins from delphinium and mallow petals. Labib (1996) extract anthocyanins from roselle leaves and observed a gradually decreased of these anthocyanins during storage at 20°C. Monica Guisti & Wrolstad (1996) extracted anthocyanins from red radish (*Raphanuis satiuus L.*). They found that acylation increased anthocyanins resistance against acid

hydrolysis. Mohamed *et al.* (2000) revealed that anthocyanins decreased by the blender during the extraction of pomegranate juice. They reported that this decrease may be due to oxidation of anthocyanins by oxygen. This investigation aims to study effect of variation of extraction solvents, heat treatment and storage at ambient temperature an anthocyanin pigments of pelargonia petals.

During storage of strawberry nectars at room temperature for 6 months anthocyanins content was gradually decreased of all samples by time Ibrahim (2006).

The object was extended to study anthocyanins stability in the prepared beverages colored with these extracted anthocyanins during storage, besides investigate the biological effect of the extracted anthocyanins on the experimental rates with respect to toxicity.

MATERIAL AND METHODS

1-Materials:-

Pelargonidia flowers (*Pelargonium zonal*) were obtained from EL- Qassassin Horticultural station, Ismalia Governorate, Egypt. Two petals of pelargonia flowers were used the first had red petals color and the second had pink petals color. These petals were handily separated, weighed and the petals weight and the flowers weight were determined.

2-Methods:-

I- **Anthocyanin extracted** from pelargonia petals according to Fuleki and Francis (1968) method. Various extraction solvents were used as the following:-

1. **Distilled water**.
2. Distilled water acidified with citric acid (3%).
3. Distilled water acidified with acetic acid (3%).
4. Ethanolic hydrochloric acid solution (95% ethanol: 1.5N HCl (85:15).

One hundred grams of pelargondia petals mixed in the blender (Warring) with 100 ml of the extraction solvent of the aforementioned solvents used respectively. The obtained extract was filtered on Whitman No.1 filter papers and make up to 500 ml by the same solvent at the volumetric flask. The extraction of anthocyanin pigments awash repeated on the residue on the filter paper. The optical density values of the extracted suitable diluted extracts were obtained using Spectrophotometer (spectronic 21) at 535 nm.

II-**The moisture content** was determined according to Rangana (1979).

III- The total soluble solids were determined according to A.O.A.C. (1990).

V- **Effect of heating on anthocyanin pigments content** was determined according to the method of Tinsley and Bockian (1960).

VI-**Preparation of the beverages** was achieved by using sucrose solution 50% total soluble solids. Sodium benzoate 0.1 and suitable amount of the extracted anthocyanins were added to the aforementioned sucrose solution to give the best color, since this is the range of a commercial artificial drink. The total soluble solids were adjusted to 13% by the refractometer. These

beverages were filled in white bottles, pasteurized at 80°C for 5 minuete and stored for 6 weeks at room temperature. Anthocyanin content was determined calorimetrically during storage.

The biological effect of anthocyanins on rats:

The effect of anthocyanins extracts of pelargonia red and pink petals was determined. The white rats at 150 gm weight were treated by feed from the mouth to the stomach by the stomach tube. The rats were treated by pelargonia petals anthocyanins extracts at 5 and 10% concentration in two groups. The rate of death and the morphological changes on the rats were determined during and after 25 days of the treatments by feeding of the white rats.

RESULTS AND DISCUSSION

Data in Tables (1) showed the physical characteristics of pelargonia petals and extracts. It could be noticed that the weight of ten floweret disk was 12.6 and 46.8 gm pelargonia red and pink petals varieties respectively. The weight of ten flowers was 0.9 and 1.2 gm respectively. The flowers petals extracts color was red and pink of the acidified ethanolic extracts of red and pink petals varieties respectively.

Table (1): Some physical characteristics of pelargonia red and pink flowers.

Plant flower / Characters	Red flowers	Pink flowers
Weight of 10 floweret disks (gm)	12.6	46.8
Weight of 10 flowers (gm)	0.9	1.2
The flower color	Red	Pink
Weight of petals from 10 flowers (gm)	0.6	0.8

Results in Table (2) represented effect of the extraction solvents of the obtained anthocyanin contents. It could be observed that the extraction by the acidified ethanol solvent was the superior compared to extraction by the distilled water or the acidified distilled water by citric acid (3%) or distilled water acidified by acetic acid (3%). Anthocyanin content of acidified ethanolic extracts was 73.01 and 64.43 (mg/100gm) extracted from pelargonia red and pink petals respectively.

Table (2): Effect of solvent extraction on the anthocyanins content (mg/100gm) of pelargonia red and pink petals (on dry wt. basis).

Plant petals / Extraction solvent	Anthocyanins content	
	Red petals	Pink petals
Distilled water	10.34	9.83
Distilled water with 3% citric acid	51.08	45.21
Distilled water with 3% acetic acid	43.58	41.43
Acidified ethanol	73.01	64.43

Data in Table (3) showed effect of the acidication of distilled water at different levels of pH values on anthocyanin content extracted from pelargonia red and pink petals. It could be noticed that the acidification increased anthocyanin content these pelargonia red and pink varieties. The highest anthocyanin content observed at pH 3.1 followed with pH 4.5. The lowest anthocyanin content observed at pH 6.1 for red petals.

Table (3): Effect of pH values on anthocyanin content (mg/100gm) of pelargonia red and pink petals (on dry wt. basis).

Plant petals / pH value	Anthocyanins content	
	Red petals	Pink petals
8.0	42.40	37.71
6.1	41.76	38.43
4.5	43.58	40.16
3.1	49.12	35.42

Table (4) showed the effect of heating treatment at 70°C and 80°C for various times on anthocyanin content extracted from red and pink pelargonia petals. anthocyanin content affected slightly by heat treatment. It was slightly decreased by increasing of heat treatment time at 70°C and 80 ° C.This decreases may be due to heat degradation and oxidation effect on anthocyanins extracted from pelargonia petals. Red petals anthocyanins extract are stable against the heating at 70 and 80°C than that extracted from pink petals. These results are in agreement with those reported by Harzdina *et al.* (1970) and Abd EL-Latif *et al.* (1992). Thus red petals are preferred than pink petals.

Table (4): Effect of heating at 70 and 80°C on anthocyanins content of pelargonia red and pink petals (on dry wt. basis).

Temperature / Time (minutes)	Anthocyanins content			
	70°C		80°C	
	Red petals	Pink petals	Red petals	Pink petals
0	51.08	45.21	51.08	45.21
5	50.96	39.87	50.32	39.51
10	50.27	39.54	49.23	39.18
15	50.13	38.82	49.15	38.63

Biologically, Table (5) revealed the effect of feeding by anthocyanin extracts on the white rats at the weight of 150gm.

Table (5): Effect of feeding by pelargonia red petals extracts on white rats (morphological and rate death).

Extracts concentration (%)	Numbers of Rats	Time of feeding (day)	Morphological changes	Rats of death
5	5	25	-	-
10	5	25	-	-

It could be seen that the treatment of these rats with this extracts concentrations of 5% and 10% for 25 days of feeding revealed that there were no effect on any of the treated rats morphologically of the body and the rats of death.

Data represented in Table (6) showed the effect of storage at room temperature for 12 weeks on the anthocyanin content in the colored beverages by pelargonia anthocyanin pigments. From this Table it could be reported that a slight gradually decreased was observed during storage at room temperature by acidity conditions and storage temperature. These results are in agreement with those observed by Star & Francis (1973), Riboh (1976), Labib (1996), Mohamed *et al.* (2000) and Ibrahim (2006).

Table (6): Effect of storage at room temperature on the anthocyanins content (mg/100gm) (on dry wt.basis) of the prepared beverages (at 535nm.).

Plant petals / Storage(week)	Red petals	Pink petals
1	44.32	38.95
2	43.61	38.13
3	42.83	37.35
4	42.15	36.68
5	41.32	35.84
6	40.48	35.12
7	39.85	34.21
8	39.07	33.46
9	38.26	32.79
10	37.49	31.92
11	36.82	31.25
12	36.04	30.46

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استخلاص صبغات الانثوسيانينات من بتلات نباتات البلارجونيا لاستخدامها كملونات طبيعية في بعض الأغذية.

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استعمال الصبغات الصناعية تم الحد منة في تلوين الأغذية. تم استخلاص صبغات الانثوسيانين من بتلات نبات البلارجونيا (الخبيزة الافرنجي). حيث تم تقدير ثبات هذه الصبغات. تم استخلاص الانثوسيانينات بطرق استخلاص مختلفة: ايثانول محمض بحامض الهيدروكلوريك و ماء مقطر محمض بحامض الستريك(٣ جم/١٠٠مل) أو حامض خليك (٣مل/١٠٠مل). محتوى الانثوسيانينات كان عالي في المستخلص الناتج من الأوراق الحمراء مقارنة بالمستخلص من الأوراق الوردية. محاليل الايثانول المحمض بحامض الهيدروكلوريك المستخدمة في الاستخلاص تم مقارنتها بالماء المقطر المحمض بحامض الستريك او حامض الخليك. محتوى الانثوسيانينات نقص بعض الشئ بالمعاملة الحرارية. الانثوسيانينات نقصت بعض الشيء أثناء التخزين على درجة حرارة عالية نسبيا.ومن الناحية البيولوجية فقد وجد أن استخدام مستخلصات الأنثوسيانينات من بتلات نبات البلارجونيا (الخبيزة الأفرنجي) الحمراء والتي فضلت عن البتلات الوردية اللون بتركيزات ٥ و ١٠ % في تغذية من الفم للفئران البيضاء لم تؤثر على صفات الشكل والمظهر الخارجي ولم تسبب أي معدل موت لفئران التجارب. أوراق البلارجونيا الحمراء كانت الأفضل في استخلاص صبغات الانثوسيانين كما أنة يوصى باستخدام الماء مع ٣% حامض الستريك في الاستخلاص على أساس اقتصادي.