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EXTRACTION OF CAROTENOIDS FROM NEW SOURCE OF CITRUS PEELS AS NATURAL COLORANTS IN BEVERAGES

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ABSTRACT

This study aims to investigate the extraction of carotenoids from clementine and balady mandarin peels by different solvents and the utilization of natural extracted carotenoides in coloring prepared beverages as a substitute of the synthetic colorants that clearly proved to be harmful to the human health. Some compounds of tested extracts were separated with thin layer chromatography to identify the major components. The components of the tested extracts were identified based on R_f value, color test under UV lamp. The study also aimed to fuond the effect of storage at room temperature for 8 weeks on carotenoids content of these beverages. It could be concluded that acetone was the first and superior solvent for the extraction of carotenoids from balady and Clementine mandarin peels. Carotenoides content was higher in Clementine peels compared to balady mandarin peels.Carotenoides content were decreased in samples of extracted carotenoides in acetone solvent and carotenoides content of the prepared colored beverages with the natural extracted carotenoids of mandarin peels during storage.

INTRODUCTION

Citrus is a complex source of carotenoids, with the largest number of carotenoids found in any fruit (Gross, 1987). Carotenoid concentration and composition vary greatly among citrus varieties and according to the growing conditions (Gross, 1987). Among citrus the oranges are the specie most cultivated following by mandarin, lemon and grapefruit. Mandarin is considered among one of the most popular fruits grown in Egypt. Clementine mandarin is a new cultivar of citrus mandarins grown especially in El-Aresh farms, North Sinai Governorate. having flavor, red in color and Trends in food legislation are to reduce the number of synthetic dyes permitted as food colorants (Henry, 1980). The importance of beta - acetone in a normal healthy diet has been well documented and accepted (N.A.S; 1980). In general, the citrus peel has 2 to 6 times the carotenogenic capacity of the pulp; thus 70% of the fruit carotenoids are in the peel (Erikson & Brannaman, 1960).

In food science and nutrition, big interest in the carotenoids comes from their many functions, such as natural coloring agents, either as inherent constituents or as additives, provitamin A activity of some carotenoids, role in lipid oxidation, precursors of some desirable and undesirable flavor components and anticarciogenic properties which make the consumption of carotenoid-rich foods recommendable. As natural food colorants, the carotenoid pigments can be used in coloring shortenings, margarine, butter, butter sauce, eggs, baked goods, macaroni, salad dressings, salad oils, cheese, ice-cream, yogurt, meat and fish products, gelatin, and desserts. New biological functions include activation of genes which encode the message for protein production and modulation of the enzymatic activities of lipoxygenases (Inci Çinar,2004). Carotenoid, and especially beta-carotene, supplementation significantly reduces the progression of cardiovascular disease, certain type of cancers, risk of cataracts and light sensitivity disorders, and enhances immune markers in HIV-infected patients (Canfield *et al.,* 1993).

Commercial interest in methods for production of natural carotenoid pigments is increasing. Since carotenoids are widely distributed throughout plants with a large variety of types of tissue, no one method of extraction can be used universally as a standard technique (Goodwin, 1976). Traditional methods of carotenoid extraction concentrate on the use of one solvent or combinations. Pesek and Warthesen (1987) used petroleum ether: acetone (50:50 v/v) as solvents. Sadler *et al.* (1990) introduced a rapid extraction method (15 minutes) for lycopene and β-carotene. Tomato paste and pink grapefruit homogenates were extracted using hexane: acetone: methanol (50:25:25 v/v/v) followed by addition of water to separate the solution into polar and non-polar layers. Hexane: acetone: methanol (80:10:10 v/v/v) ternary system in the AOAC method for xanthophylls was modified to hexane: acetone: methanol (70:20:10 v/v/v) to increase the solubility of pigments by Chen and Yang (1992), since the AOAC method was inadequate for dissolving polar xanthophylls. Bassi *et al.* (1993) achieved the fractionation of thylakoid membranes in which availability, ability to use at low temperatures and non-toxicity were the advantages of the method. contain pigment binding complexes. De Sio *et al.* (2001) used dichloromethane/methanol (2:1, v/v) for the vegetable carotenoid extraction.

Oxidative degradation of natural carotenoids could be accelerated by light, metals, peroxides and lipids oxidizing enzymes (Lozas & Kalathenas, 1988). Natural carotenoids were extracted from mango wastes and carrot roots. It can used successfully in coloring fat base foods i.e butter and water base foods such as ice cream (Hand, 2000 and Hand *et al*; 2000). The present study aims to investigate the possibility of utilizing peels as wastes remaining after processing of balady and Clementine mandarin citrus fruits in producing natural colorants which may be used in coloring foods as a substitute for the synthetic colorants that are harmful to health.

MATERIALS AND METHODS

Sample Preparation

The Clementine variety (*Citrus reticulate l. tangerine*) was obtained from EL–Aresh, Sinai Governorate. Balady mandarin fruits (*Citrus reticulate l. mandarin*) were obtained from EL-Kassassin Horticultural research station, Ismailia Governorate, Egypt. Ethyl alcohol, Methyl alcohol, Diethyl ether, Acetone and Hexane obtained from El.Nasr Pharmaceutical Chemicals Company (ADWIC).

Fruits were picked at the ripe stage of maturity sorted, and hand peeled. Mandarin peels were washed under tap water and diced into approximately 0.5cm^3, thoroughly mixed, weighed and used immediately for further processing. The samples were homogenized in a laboratory Waring blender for 2 minutes to increase the surface area for efficient extraction treatment.

Pigment Extraction

Carotenoids were extracted according to the methods of Wettestein (1957) and Megahed (1985) with using of the following solvents:

1- Ethyl alcohol.
2- Methanol.
3- Diethyl ether.
4- Acetone.
5- Hexane.

One part of homogenized peels was mixed with one solvent (1:10 w\v) of each previous treatment. Each of these mixtures was blended carefully in the electrical blender. The mixtures were put at the refrigerator for 12 hours. Extraction was repeated until no color was extracted.

The obtained carotenoid extracts were concentrated in a vacuum evaporator. The obtained extract was mixed with 10% dehydrogenated potassium phosphate solution, dried over anhydrous sodium phosphate and concentrated in rotary evaporator then weighed and kept in dark bottles under refrigeration. The carotenoids content was determined calorimetrically at 420 nm. by the spectrophotometer according to(Asker and Treptow, 1983).

Thin layer chromatography (TLC):

Balady and Clementine mandarin peels extracts were spotted on TLC silica GF_{254} plates (sizes 20×20, thickness 0.25mm and activation at 105°C for 2 hours) and using benzene: ethyl acetate: methanol (75: 20: 5 v/v/v) as a solvent system . The plates were examined under ultra violet (UV) lamp (365 nm) according to Pratt and Miller, (1984) and the components were marked for R_f (Rat of flow) value by the following equation :-

$$R_f \text{ value} = \frac{\text{The distance of sample}}{\text{The distance of solvent}}$$

Total soluble solids:

Total soluble solids were determined according to the A.O.A.C. (1990).

Preparing of the beverages and storage:

The beverages were prepared by using sucrose solution with total soluble solids 50%, sodium benzoate 0.1% and the extracted carotenoids were suspended on soluble starch, 2.5% gm of the colorant were suspended on 0.5 gm of soluble starch and added lately to sucrose solution and the total soluble solids were adjusted to 16%.The bottles of 100ml volume were filled with beverages, closed tightly and pasteurized at 80°c for 5 minutes and stored for 8 weeks at room temperature.

RESULTS AND DISCUSSION

Table (1) shows the physical properties of balady and Clementine mandarin fruits. It could be noticed that, fruits weight was 84.59g and 102.25 grams balady and Clementine fruits respectively. Balady and Clementine mandarin fruits volume was 82.50 and 83.32 cm^2 respectively. With respect to mandarin juice weight, Clementine juice weight represented higher values compared to balady juice weight also fruit peels weight showed the same trend as where balady peels weight was 22.15 grams but Clementine peels weight was 24.39 grams.

Table (1): Some properties of balady and Clementine mandarin fruits.

Variety / Physical properties	Balady mandarin	Clementine
Fruit weight (g)	84.59	102.25
Fruit volume (cm^3)	82.50	83.32
Fruit peels weight (g)	22.15	24.39
Juice weight (g)	39.40	48.66

The effect of different extraction solvents on quantity of the extracted balady and Clementine mandarin varieties have shown in table (2). From this table, it could be clearly observed that acetone solution had the highly extracted carotenoids content form both of balady and Clementine mandarin peels compare to the other extraction solution used in this work followed by methanol solution.

Carotenoids content that extracted from balady and Clementine carotenoids peels were 2.562 and 7.180 mg /100gm respectively. Diethyl ether solution was the lowest effective solvent for carotenoids extracted from mandarin balady and Clementine peels. This table also represented that, carotenoids content in all extraction solvent cases was highest in case of Clementine peels compared to that extracted from balady mandarin carotenoids peels under the same conditions.

Data represented in table (3) shows anthocyanins pigments content extracted from balady and Clementine mandarin peels measured at various wavelengths. It could be noticed that, anthocyanin content was highest in the extracts obtained from Clementine peels compared to that extracted from balady peels at all measuring wavelengths. This may be due to the reddish color of Clementine peels while that of balady mandarin peels was yellow. This table also reveals that, anthocyanin content was high when being measured at 503nm absorbance wavelength in both balady and Clementine mandarin peels extracts and the solvent of extraction was ethyl alcohol while the lowest value of anthocyanin were observed when these extracted anthocyanin pigments were measured at 551nm wavelength . From this data it could be noticed that the wave length 503nm was the superior preferred wavelength for measuring anthocyanin pigments extracted from balady and Clementine mandarin peels using ethanol for the extraction. Anthocyanin content was 1.002 and 1.511 mg / 100gm of balady and Clementine mandarin peels respectively at 504nm wavelength.

Table (2): Effect of type of solvents on carotenoid content extracted from balady and Clementine mandarin peels.

No.	Extraction solvent	Carotenoid (mg / 100g)	
		Balady mandarin	Clementine
1	Ethyl alcohol	1.211	2.894
2	Methanol	1.337	2.915
3	Diethyl ether	1.236	3.753
4	Acetone	2.562	7.180
5	Hexane	1.663	4.832

Table (3): Effect of mandarin variety on the anthocyanin content extracted by ethanol and measured at different wavelengths (nm).

No.	wavelengths (nm)	Anthocyanin (mg / 100g)	
		Balady mandarin	Clementine
1	503.00	1.003	.511
2	510.00	0.926	.308
3	512.00	0.906	.252
4	519.00	0.835	.059
5	520.00	0.824	.038
6	522.00	0.809	0.998
7	530.00	0.743	0.830
8	532.00	0.733	0.794
9	536.00	0.712	0.723
10	538.00	0.687	0.697
11	543.00	0.636	0.651
12	545.00	0.610	0.631
13	546.00	0.585	0.626
14	547.00	0.575	0.616
15	551.00	0.534	0.611

Data in Table (4) show the identification of component of various extracts on TLC under ultra violet light at 365 nm using benzene: ethyl acetate: methanol (75: 20: 5 v/v/v) as a solvent system.

Data showed that ethanolic, methanolic, diethyl ether, acetonic and hexane extracts of balady mandarin peels contain about three components separated and high resolution appeared on TLC under UV different in its color and R_f value, whereas, the high molecular weight appears at R_f = 0.200, 0.114, 0126, 0.097 and 0154 (violet color), respectively, the lower molecular weight appears at R_f =0.971, 0.943, 0.954, 0.943 and 0.931 (violet color) respectively. While ethanolic, methanolic, diethyl ether, acetonic and hexane extracts of Clementine mandarin peels contain about two components appearing under UV different in its R_f value and color. The compounds having low molecular weight appear at R_f =0.314, 0.257, 0.286, 0.263 and 0.314 (violet color), high molecular weight appear at R_f = 0.200, 0.183, 0.229, 0.189 and 0.229 (violet color) respectively.

Table (4): Separation and fractionation of compounds of crude extracts by different solvents on TLC and its characteristic under UV lamp at 365 nm.

plant material	Fraction On TLC	Extracts									
		Ethanolic		Methanolic		Diethyl ether		Acetonic		Hexane	
		R_f	Color	R_f	Color	R_f	Color	R_f	Color	R_f	Color
Balady mandarin peels	1	0.200	Violet	0.114	Violet	0.126	Violet	0.097	Violet	0.154	Violet
	2	0.314	Violet	0.189	Violet	0.171	Violet	0.183	Violet	0.217	Violet
	3	0.971	Violet	0.943	Violet	0.954	Violet	0.943	Violet	0.931	Violet
Clementine mandarin peels	1	0.200	Violet	0.183	Violet	0.229	Violet	0.189	Violet	0.229	Violet
	2	0.314	Violet	0.257	Violet	0.286	Violet	0.263	Violet	0.314	Violet
	3	-	-	-	-	-	-	-	-	-	-

Data reported in table (5) indicate that storage at room temperature for 8 weeks of pasteurized beverages prepared with carotenoid extracted from peels of balady and Clementine mandarin, affected sharply the carotenoid content of these beverages . From these data it could be noticed that, carotenoids from clementine peels were decreased from 3.398 to 3.026mg / 100gm after storage for two weeks at the same temperature. The same trend of carotenoids decrease was observed during storage period. The decrease of carotenoids during storage at room temperature may be due to the (cis – trans) conversions and oxidative destruction. These results are in agreement with those reported by Goldman *et al* (1993) and Ashour *et al.* (1990).

Table (5): Effect of storage at room temperature on acetonic carotenoids content of mandarin balady and Clementine used for prepared beverages.(mg/100g)

Mandarin variety / Storage period(week)	Balady mandarin		Clementine mandarin	
	Acetonic extract	Colored beverages	Acetonic extract	Colored beverages
0	1.532	2.038	1.860	3.398
2	1.259	1.721	1.624	3.026
4	1.165	1.485	1.415	2.814
6	1.083	1.387	1.328	2.569
8	0.921	1.245	1.137	2.315

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إستخلاص الكاروتينويدات من مصدر جديد لقشور الموالح كملونات طبيعية فى المشروبات

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يهدف هذا البحث إلى إمكانية استخدام الكاروتينويدات الطبيعية المستخلصة من قشور الكلمنتين واليوسفى البلدى فى تلوين المشروبات كبديل عن إستخدام الملونات الصناعية التى ثبت أنها تسبب ضرراً على صحة الإنسان. وقد تم استخدام كروماتوجرافيا الطبقة الرقيقة TLC في التعرف علي ما تحتويه من مركبات باستخدام معدل السريان (R_f) واختبارات اللون كما يهدف إلى دراسة تأثير التخزين على درجة حراره الغرفة لمدة ثمانيه أسابيع على محتوى المشروبات من هذه الكاروتينويدات.أظهرت النتائج أن مذيب الأسيتون هو أفضل المذيبات التى استخدمت فى إستخلاص الكاروتينويدات من قشور اليوسفى البلدى والكلمنتين. كما وجد ان المحتوى من الكاروتينويدات المستخلصة من قشور اليوسفى الكلمنتين كانت أعلى منها فى حاله الكاروتينويدات المستخلصه من قشور اليوسفى البلدى.

كما لوحظ خلال فتره التخزين إنخفاض المحتوى من الكاروتينويدات المحتفظ بها فى الأسيتون وكذلك الكاروتينويدات فى المشروبات التى تم تجهيزها.