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STUDIES ON THE UTILIZATION OF NATURAL CAROTENOIDS EXTRACTED FROM BALADY MANGO WASTES

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Abstract

This study was performed to extract, identify and determine the natural pigments in Balady mango wastes. The study also aimed at utilizing the separated natural pigments in coloring both fat base foods (butter) and water base-foods (ice cream). The obtained results reveal that it was successful and economic to separate natural pigments from Balady mango wastes. These natural pigments were carotenoids (5.6 g/ 100g of Balady mango wastes, on dry weights basis).

It was also ascertained that B-carotene was the predominant fraction among the seven fractions of carotenoids identified by thin layer chromatography. The results indicated the possibility and suitability of utilizing the separated natural pigments in coloring fat base foods i.e. butter and water base foods such as ice cream instead of the artifical colorants which proved to be harmful to the human health.

INTRODUCTION

Mangoes are considered among one of the most popular fruits grown in Egypt. The average total annual area cultivated with mango trees in Egypt is about 68356 feddans producing about 230873 tons of fruits (Anonymous, 1997).

Balady mangoes are among the oldest fruits grown in Egypt. This aforementioned variety of mango is highly prefered in processing for its distinctive color, delicious flavor, besides being a good source of carotenoids, sugars and minerals. It is well known that large quantities of Balady mangoes are widely processed either alone and/ or mixed with other suitable varieties to produce many products such as canned mango nectar, concentrated mango syrup and recently dehydrated mango sheets were introduced. (Hansen 1996). All these processed mango products are locally consumed and widely exported.

Jain (1961) reported that kernels and peels are the most important wastes remaining after the processing of mango fruits. These wastes represent about 50% of the fresh fruits. Many investigators demonstrated that these wastes are rich sources for carotenoids [Charles *et al* (1971) and Gould (1983)]. Thus attempts for exploring technical and economic feasibility in the utilization of these wastes would be of great

importance. Carotenoids are among the main groups of natural coloring substances in foods, being responsible for the brilliant orange and yellow color of edible fruits (Hansen, 1996). Color is one of the most important factors affecting quality and palatability of the foods among different consumers. Therefore, natural carotenoids are widely used in most developed countries as natural colorants for foods instead of the artificial ones which proved to have undesirable effects on human health (Smith, 1981). Some carotenoid fractions are precursors of vitamin (A) which is very essential in human nutrition. Since, most of Carotenoid fractions have much conjugated double bonds, they play a very important role as antioxidants. On the other hand, Miller *et al.* (1996) reported that the antioxidant activity of carotenoids are greatly influenced by the number of conjugated double bonds, the matter which would add great significance to this group of natural colorants. The present study aims at investigating the possibility of the utilization of wastes remaining after processing large quantities of Balady mango 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

1. Materials

Source and preparation of Balady mango wastes:

Balady mango wastes including kernels and peels were obtained from juice extraction unit (Bertuzi) in the pilot plant of the Food Technology Research Institue, Agric. Res. Center, Giza. These wastes were dried at 40^{o}C in a drying oven, then stored at room temperature (25^{o}C) for further uses.

2. Analytical Methods:

2.1 Isolation of natural pigments from Balady mango wastes:

2.1.1.Extraction with acetone (95%) then concentration and purification of natural pigments of natural pigments from Balady mango wastes were performed as described byTing and Hendrickson (1969).

2.1.2. Identification using spectropto metric analysis:

An ultraviolet spectrophotometer "Unicam sp 1800" was used for the identification of natural pigments. The absorbance (A) was measured at wavelengths ranging from 400 to 550 nm at intervals of 5 nm.

2.1.2.2. Identification by thin Layer Chromatography (T.L.C.):

The fractions of natural pigments isolated from Balady mango wastes were identified according to Davies (1976) using thin layer chromatography.

2.1.3. Determination of isolated natural pigments:

Total content of isolated natural pigments was determined according to Ranganna (1977). The moisture content of Balady mango wastes was determined according to A.O.A.C (1990).

2.1.4. Utilization of isolated natural pigments:

2.1.4.1. Coloring fat base foods

Table butter:

Table butter made from Bafflao milk (white in color) was obtained from the dairy products processing line in the Food Technology Research Institute, Agric. Res. Center, Giza, in winter of 1998 and heated at 50^{o}C to separate the butter fat. The extracted natural pigments were added to the white warm butter (37^{o}C) at concentrations of 0.05, 0.1, 0.15 and 0.2 gm/100 gm of white butter respectively.

Table butter made from cow's milk (yellow in color) brought from local market, was used as control sample. All the aforementioned five samples were stored for six months in a refrigrator at 4^{o}C. Samples were taken monthly to determine the color losses to study the effect of cold storage on color stability.

Organoleptic evaluation and statistical analysis:

Representative samples of each treatment of butter colored with the isolated natural pigments as well as the control were evalutated for their color. The organoleptic evaluation was carried out by 20 panelists.

Statistical analysis for the aforementioned organoleptic evaluation was performed according to Roscoe (1969).

2.1.4.2. Coloring water base foods ICE cream or stick:

The water ice compounds were prepared according to the method applied in Imbaba Factory from sucrose, glucose, water, artificial flavors, stabilizers (soluble starch as carrier) and coloring agent. The coloring agent was replaced by mixing different lev-

els of prepared natural pigment extract (1 gm, 2gm, and 3 gm). The natural pigments extracted from Balady mango wastes were suspended on 1 gm soluble starch in 500 ml water ice.

The blank portion was 0.3 ml of annato (Water soluble) 500 ml water ice. The formula was frozen at-18^{o}C then packed in wax coated paper to protect the product from melting down and to have the required shape. All samples were stored under freezing condition (-18^{o}C). Samples were taken at one week intervals to determine the stability of the color.

RESULTS AND DISCUSSION

1. Idntification of natural pigments isolated from Balady mango wastes:

1.1. Identification using spectrophotometric analysis:

Natural pigments isolated from Balady mango wastes were identified by spectrophotometric analysis measuring absorbaces at different wavelengths ranging from 400 and 500 nm. These absorances at the aforementioned wavelength are illustrated in Fig. (1). From this figure, it can be observed that the maximum absorptions of the natural pigments isolated from Balady mango wastes were recorded at 410, 420, 428,440,452,465 and 475 nm. idicating that the separated pigments were carotenoids. These results are in agreement with that reported by Gross *et al.* (1971), Davies (1976) and El-Seesy and Hamed (1998) who reported that the spectral absorption curves of carotenoids in the visible region (400 to 500 nm) were widely used for purposes of identification. From the same figure, it could be clearly observed that the maximum absorption was obtained at 452 nm. Yokoyama and White (1967) and Davies (1976) demonstrated that β-carotene gave its maximum absorption at 452 nm. This indicates that β-carotene is the predominant fraction in the carotenoids extract isolated from Balady mango wastes.

1.2. Identification by thin layer chromatography:

The natural pigments isolated from Balady mango wastes were identified by thin layer chromatogrphy (T.L.C.). The R_f values of the separarated fractions were calculated. Data are shown in Table 1.

From Table 1, it could be noticed that seven carotenoid fractions were identified

in the natural pigments isolated from Balady mango wastes. These fractions were Antheraxanthin, Zeaxanthin, Canxanthin, Echinenone, Rodoxanthin, cryptoxnthin and β-carotene.

The calculated R_f values of the aformentioned separated carotenoid fractions were similar to the standard ones given by Davies (1976).

2. Moisture and carotenoid contents of Balady mango wastes:

From Table 2, it could be observed that the moisture content of Balady mango wastes was 88.6%. From the same table, it could also be observed that total carotenoids content in these aforementioned wastes was 5.6%. These results are in agreement with those reported by Francis (1995).

3. Utilization of natural pigments isolated from Balady mango wastes:

3.1 Coloring fat base foods, coloring of butter:

Statistical analysis of organoleptic evaluation:

Data concerning statistical analysis of the organoleptic evaluation for butter colored with different concentrations of isolated natural colorant as well as butter colored with an artificial coloring matter are shown in Table 3. From this table, it could be observed that; the higher the concentrations of the added natural colorant to the butter; the higher the summations of ranks. This means that the highest palatability of panelists for butter's color was obtained by increasing the natural colorant concentrations. On the other hand, the summation of ranks for butter colored with the artificial colorant (70) was less than those given for butter colored with 0.15 and 0.2 g natural colorant/1.0g of butter (71 and77.5 respectively), indicating that the aformentioned butter has less palability than the butter colored with the two mentioned concentrations of natural colorant.

Effect of cold storage on stability of butter's color

From the results shown in Table 4, it could be noticed that during storage at $4^{o}C$ the color losses decreased as the concentrations added were increased. These results are in agreement with Arad *et al.* (1996) who reported that natural carotenoids are more stable at lower temperatures and that these lower temperatures are very suitable for protecting natural pigments against any undesirable changes or discoloration.

3.2. Coloring water base foods Ice Cream or stick Stability of the color:

Results concerning the effect of storage of ice cream colored with different concentrations of natural carotenoids isolated from Balady mango wastes as well as the control one at - 18^{o}C are given in Table 5.

From these results, it could be observed that concentration of 3 gm natural carotenoids/500 gm ice cream was the best among other concentrations as the loss of its color was less compared to the other losses and it was closer to the control one indicating that this concentration appeared to be the most suitable for commerical uses.

Finally, it could be concluded that successful and economic utilization of Balady mango wastes in producing natural colorants such as carotenoids could be recommended. This utilization will be performed at low cost as the acetone used for extraction will be highly recovered with percenhtage of 90% recovery besides, this good utilization of natural pigments from Balady mango wastes will help to a great extent to stop using artificial colorants which are very harmful to human helath. As a results of these advantages the problem of contamination with these wastes inside factories will be solved at very low cost.

Table 1. Identification of carotenoids extracted from Balady mango wastes by different systems of T.L.C.

T.L.C. System			Fraction	R_f Value	Color
	Adsorbent	Solvent system			
1	Activated Silica Gel G	Benzene: ethylacetate: methanol 75: 20:5	Antheraxanthin	0.40	Yellow
			Zeaxanthin	0.52	Light-orange
			Canxanthin	0.87	Yellow
			Echinenone	0.98	Dark-red
2	Activated * $Ca(oH)_2$: Silica gel 6:1	Benzene	Rodoxanthin	0.17	Yellow
			Cryptoxanthin	0.37	Light orange
3	$Ca(oH)_2$ Silica ge 6:1	Petroleum ether: Benzene 49:1	B-carotene	0.83	Orange

Table 2. Moisture and carotenoids content (%) in Balady mango wastes (on dry weight basis).

Item	Moisture %	Carotenoids (%)
Balady mango wastes	88.6	5.6

Table 3. Statistical analysis of Values given through evaluating butter colored by natural carotenoids extract.

	A		B		C		D		E	
Panelists	Values	Ranks	Values	Ranks	Values	Ranks	Values	Ranks	Values	Ranks
1	4	4.5	0	1	2	2	3	3	4	4.5
2	5	4.5	3	1	4	2.5	4	2.5	5	4.5
3	2	2	1	1	2.5	3	3	4	4	5
4	3	4	0.5	1	1	2	2	3.0	4	5
5	4	3.5	1	1	2	2	4	3.5	5	5
6	3	3.5	1	1	2	2	3	3.5	4	5
7	5	5	1	1	4	4	3	3	3	2
8	5	5	0	1.5	0	1.5	2	3	3	4
9	3.5	2	3.5	1	4.5	4.5	4.5	4.5	4	3
10	5	5	3	1.5	4.5	3.5	4	3.5	3	1.5
11	3	3.5	1	1	2	2	4	5	3	3.5
12	3	3.5	2	2	4	5	3	3.5	1	1
13	3	4	0	1	1	2	2	3	4	5
14	5	5	2	2	4	4	3	3	1	1
15	2	2	1	1	3	3	4	4	5	5
16	4	4	1	1	2	2	3	3	5	5
17	2	1.5	2	1.5	4	3.5	4	3.5	5	5
18	2.5	3.5	4	1.5	5	5	4	1.5	2.5	3.5
19	3	2.5	1	1	3	2.5	4	4	5	5
20	2	1.5	2	1.5	3	3	5	5	4	4
ERanks		70		24.5		57		71		77.5

A: Control

B: Butter colored with 0.05 % natural carotenoids

C: Butter colored with 0.1 % natural carotenoids

D: Butter colored with 0.15 % natural carotenoids

E: Butter colored with 0.2 % natural carotenoids

Table 4. Color absorbances of butter colored with different concentrations of natural carotenoids extracted from Balady mango wasts at 452 nm stored for 6 months at 4°C.

Period of stroge	Concentrations (%)			
Months	0.05	0.01	0.15	0.2
Zero time	0.370	0.650	0.780	0.890
1	0.365	0.642	0.766	0.875
Loss %	1.35	1.23	1.79	1.69
2	0.359	0.626	0.750	0.863
Loos %	2.88	3.69	3.85	3.00
3	0.330	0.567	0.77	0.796
Loss %	10.81	12.77	13.21	10.56
4	0.309	0.507	0.605	0.720
Loss %	16.49	22	22.44	19.10
5	0.236	0.437	0.521	0.610
Loss %	36.22	32.77	33.21	31.46
6	0.136	0.388	0.470	0.590
Loss %	63.24	40.30	39.74	33.71

Table 5. Stability of ice cream colored with different concentrations of natural carotids extracted from Balady mango wastes stored for 6 weeks at-18°C. (at 452 nm).

Storage period (Weeks)	Control	1 gm/5ooml	2 gm/5ooml	3 gm/5ooml
0	1.400	0.140	0.270	0.650
1	1.400	0.138	0.269	0.650
2	1.390	0.136	0.268	0.648
3	1.386	0.134	0.266	0.646
4	1.384	0.132	0.264	0.644
5	1.380	0.130	0.263	0.640
6	1.378	0.127	0.250	0.632
Loss %	2.2	9.29	7.4	2.77

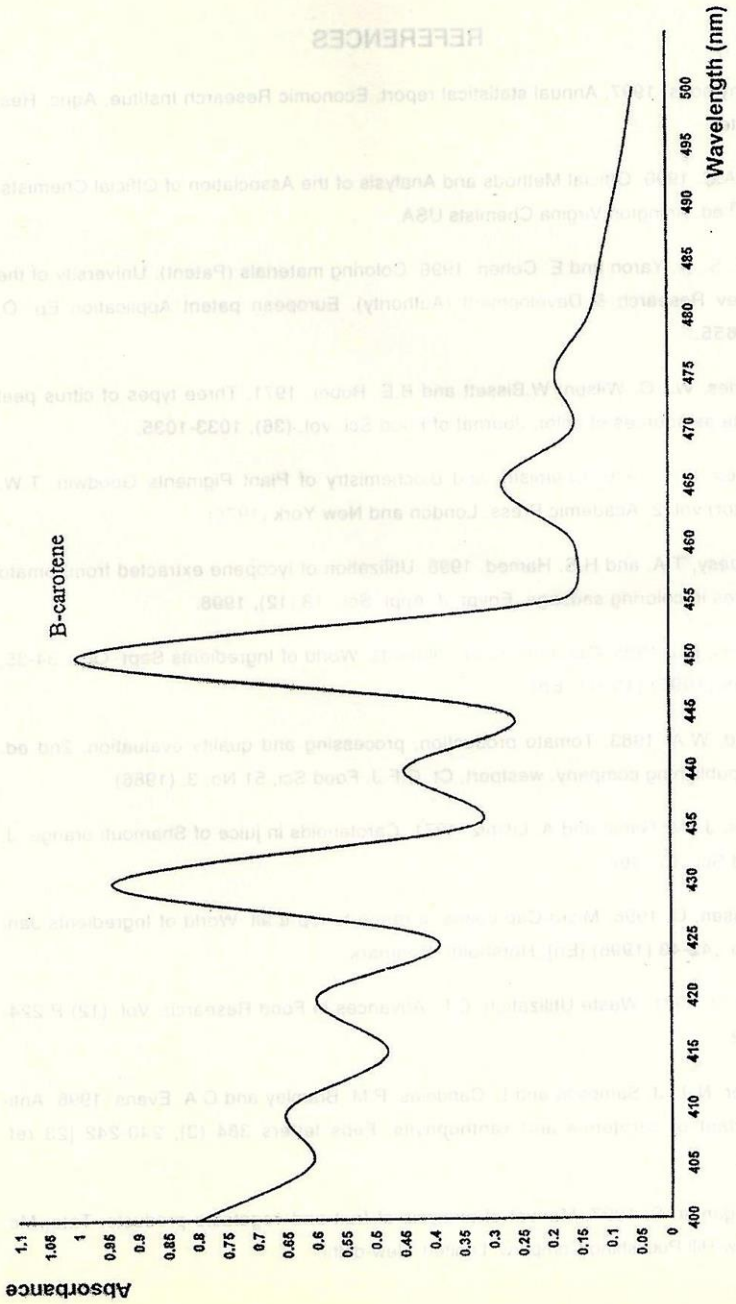

Fig. 1. Qualitative spectra of natural pigments from Balady mango wastes.

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دراسات علي إستخدام مستخلص الكاروتنويدات الطبيعية من مخلفات ثمار المانجو

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- أجريت هذه الدراسة بغرض إستخلاص والتعرف وتقدير الصبغات الطبيعية الموجودة في مخلفات ثمار المانجو البلدي. وتهدف الدراسة ايضاً إلي دراسة امكانية استخدامهذه الصبغات الطبيعية في تلوين الاغذية مثل الزبد والأيس كريم وذلك بتركيزات مختلفة من هذه الصبغات.

وقد اثبتت النتائج المتحصل عليها ان مخلفات ثمار المانجو البلدي الناتجة عن تصنيع المانجو البلدي تحتوي علي ٥.٦ جرام كاروتنويدات لكل ١٠٠ جرام من المخلفات المذكورة (علي الوزن الجاف) وقد ثبت من الدراسة من خلال التحليل الطيفي أن الكاروتنويدات هي الصبغات الموجودة في هذه المخلفات وان مركب البيتاكاروتين هو المكون السائد او الرئيسي من ضمن سبع مكونات امكن التعرف عليها بإستخدام التحليل الكروماتوجرافي ذو الطبقة الرقيقة. كما ثبت أيضا نجاح استخدام الصبغات الطبيعية المفصولة المعروفة باسم الكاروتنويدات في تلوين الزبد وانه بزيادة تركيز اللون يقل الفقد الحادث في لون الزبد اثناء التخزين علي درجة ٥٤ م كذلك أمكن بنجاح استخدام الصبغات الطبعية المفصولة بعد تحميلها علي نشا ذائب لضمان جودة انتشار الصبغة في تلوين الايس كريم كذلك كان الفقد في اللون قليلاً بزيادة تركيز اللون اثناء تخزين الايس كريم علي درجة - ١٨ه م. وفي النهاية ثبت انه يمكن وبأسلوب اقتصادي غير مكلف فصل الصبغات الطبيعية بمذيب الاسيتون الذي كان نسبة الاسترجاع له بعد استخدامه في الاستخلاص قد وصلت إلي ٩٠٪ وبذلك يمكن الاستفادة من مخلفات ثمار المانجو البلدي التي تترك دون استخدام بالمصانع وتسبب تلوثا للبيئة وجلبا للحشرات في انتاج الوان طبيعية يمكن استخدامها كبدائل للالوان الصناعية التي ثبت ضررها لصحة الانسان وتوضع دائما القيود علي تركيزاتها وحدودها المستعملة.