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Identification of pumpkin Carotenoids and its utilizations as a natural antioxidant and colorants in some foods

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Abstract

The study structure of carotenoid pigments extracted from pumpkin by using thin layer chromatography (TLC) and High Performance Liquid Chromatography (HPLC) and the relation between adding of these carotenoid pigments in some processed food utilization as antioxidants and natural coloring materials. The obtained data showed that, the best carries of pumpkin carotenoid was lactose followed by dextrin. Five peaks were identified by TLC and HPLC in carotenoids from pumpkin namely lutein, cryptoxanthin, γ carotene, αcarotene and B carotene. The major carotenoids extracted from pumpkin were B carotene37% followed by lutein 25%, α carotene19%, γcarotene 12% and cryptoxanthin7% respectively. On the other hand, the sunflower oil contains 300 to 500 ppm pumpkin carotenoid recorded high stability (higher induction period) than sunflower oil contain 200 ppm synthetic antioxidant (BHT) in the Rancimat test. Addition of 0.30 to 0.40% pumpkin carotenoid pigments (as natural colors) improved the color, taste, overall acceptability of drops compared to other tested samples.

Key words Natural yellow pigments, carotenoids, pumpkin, Natural antioxidant, drops.

Introduction

Pumpkin fruit, the most commonly squash fruit, is orange in color when ripe and has been traditionally used as human and animal feed. In culinary terms, it is widely regarded as a vegetable. It has numerous culinary uses either as a vegetable or as an ingredient in pies, soup, stews, bread and many other dishes. Pumpkin is a seasonal crop, and since fresh pumpkins are very sensitive to microbial spoilage, even at refrigerated conditions, they must be frozen or dried (**Doymaz, 2007**). Pumpkin has a good shelf-life and it contains carotene, water soluble vitamins and amino acids. It is very important to have knowledge about its nutritive value in order to encourage the increase in its consumption and usage for nutritional and technological applications. Pumpkins are relatively low in total solids, usually ranging between 7% and 10% (**Alibas, 2007**). Its unique constituents, rich in antioxidants and vitamins, allow the pumpkin to have an important health-protecting effect. In fact, the higher values of lipophilic substances such as carotenoids, present in pumpkin varieties can significantly contribute in increasing the uptake of pro vitamin A and lutein, one of the carotenoids with special physiological functions (**Murkovic *et al*., 2005**). Carotenoids present in pumpkin are among the phytochemical components believed to reduce the risk of developing some degenerative diseases and are responsible for the attractive color of many fruits and vegetables. The yellow to orange color of the pumpkin flesh arises from this group of substances. Additionally, the good performance of the pumpkin-fiber products in relation to water and glucose highlights the possibility of their usage as food ingredients (**Escalada *et al.*, 2006**).

B carotene has been used many years as health food product under the claim "antioxidant". Many epidemiological and oenological studies suggest that humans feed on a diet high in carotenoid – rich vegetables and fruits, who maintain higher than average levels of serum carotenoids, have and lower incidence of several type of cancer and cardiovascular disease (**Albances *et al,* 1996**) and (**El – Sherefa 2004**).

The intake of foods rich in carotenoids appears to be associated with optimal health, and a reduction in the risk of cancer, cardiovascular disease, macular degeneration and cataract formation. Hydrocarbon carotenoids such as alpha, beta carotenes and lycopene may reduce the risk of cancer and heart disease, whereas oxygenated carotenoids, such as lutein and zeaxanthin, may be important to the protection of the eye. (**Yeum 1996**).

Consumer interest in B carotene has been developed as the media attention is focusing on the protective role of B carotene. The evidence that B carotene may act as a cancer preventive agent derives from a number of epidemiological studies which showed that high intake of B carotene correlates to a later observed lower incidence of certain type of cancer (**Charleux, 1991**). It has been suggested that, B carotene may have a role independent of its vitamin A activity. B carotene in addition to its pro vitamin A activity appears to play a role in supporting the body's defense mechanism against cancer and chronic degenerative disease.

Carotenoids have been used as food colors for countries. Color of carotenoids, together with beneficial properties such as vitamin A precursor and anti oxidant; have led to their wide application in the food industry. Preparations to apply them in oily or aqueous media have been produced including emulsions, colloidal suspensions and complexes with proteins. These preparations have found applications to pigment margarine, butter, fruit juices and beverages, canned soups, dairy and related products, deserts and mixes, preserves and syrups, sugar and flour confectionary, salad dressing, egg products, among other and interestingly other important areas of application of carotenoids have been emerged (**Delgado Vargas *et al,* 2000 and Sayed 2008**).

The synthetic food colorants have led to the prohibition of the use of some of the synthetic colors in food due to the discovery of possible toxic substances in them. On top of that, the less stringent tests necessary for the use of natural colorants and the increase in the demand for natural ingredients by increasingly health conscious consumers have led food manufactures to take another look into the use of natural food colorants. This has also resulted in a proliferation of interest in the development of natural food colorants, as can be seen from the vast number of patents field in recent years (**Francis, 1987 and Sayed 2008**).

The use of synthetic antioxidant such as butylated hydroxy toluene (BHT) and butylated hydroxyl anisol (BHA) cause harmful effects on humans (Farag et al 2003). In this connection, and easily decompose at high temperature In addition *(***Change *et al,* 1977**) showed that BHT and BHA are quite volatile, these synthetic antioxidant are not effective in preventing the development of initial off flavor.

The use of natural antioxidants is highly desirable to replace the synthetic antioxidants. In this respect, the extracts of several plants have been reported to possess wide degrees of antioxidant activities (**Kim *et al*,1994, Rizk *et al*, 2008, Azouz *et al,* 2007**).

The aims of this study were identification and structure of carotenoids from pumpkin and utilization as natural anti oxidant and food colorants.

Materials and Methods

Samples:

Pumpkins (*Cucurbita moschat*) were brought from Kafr El-Sheakh governorate-Egypt, were purchased at 2011-2012 season were used in this study.

Chemical and Regents:

The solvents used for spectral and HPLC analysis were of HPLC grade and all others solvents were of ACS grade. Refined sunflower oil free from antioxidants was obtained from Arma Food Industries, 10th of Ramadan. Synthetic antioxidant, namely butylated hydroxyl toluene (BHT) was purchased from sigma chemical co. St Lewis, U.S.A.

Chemical analysis:

Extraction and concentration of carotenoids.

The pumpkin was extracted and concentrated by the method reported by (**Nilzu and Rodriguez 2005**). Extraction and filtration on Buckner was repeated unite the residue was devoid of color (about 3 time), the total amount of acetone used being 400ml. The carotenoids were partitioned to 100 ml petroleum ether and saponefied overnight with an equal volume of 10% KOH in methanol. After washing, the carotenoid solution was concentrated in a rotary vacuum evaporator at 40^{o}C

The concentrated yellow pigments of pumpkin were adsorbed with different ratio of solid matrixes i.e. (Lactose, soluble starch, wheat flour and dextrin) up to 6: 1 pigment matrix and dried in oven at 40^{o}c for 24hr.

Determination of total carotenoids:

Total carotenoids were determined by the method of (**Reddy and Sistrunk 1980*).*** The O.D was measured at 440 nm and compared to B carotene standard curve.

Identification of carotenoid extracted from pumpkin.

Thin layer chromatography (TLC) analysis TLC was applied using silica gel 254 for identification of pumpkin carotenoids by the method reported by (**Eder, 1996**). Extracted carotenoid was dissolved in a small amount of acetone and spotting in TLC. The plate was developed with solvent system methyl chloride, ethyl acetate (4:1). Then dried at room temperature. For visualization of color spots panisaldehde was used and RF value was calculated.

High Performance liquid chromatography (HPLC) analysis.

The carotenoids of pumpkin were identification according to the method described by (***Gaylek et al 1987***).

Antioxidant activity:

Determination of induction period with Rancimat method.

Rancimat 679 (Metrom Ltd 9100 Herisau, Switzerland) was used for the de termination of oxidative stability of oils. The induction time was automatically determined, i.e. the time from the start of the experiment to the intersection point (**Mendez *et al*,1996**).

Technological Methods:

Drops was prepared by mixing sucrose (242.4 g), corn syrup (129.5 g) and citric and (0.75g) then heated unit 157 ^{o}C. Quickly, it was cooled to 110 ^{o}C and added (1.05g) flavoring agent ad natural yellow color (0 -1 to 0.5%) from carotenoid pigment extracted from pumpkin). These contents should be mixed very well and put in forming blocks unit it became solid, and then packed in special foil (**Sayed 2008**).

Sensory evaluation:

Ten panelists were asked to evaluate color, taste and overall acceptability. Sensory evolution was carried out according to the procedures described by (**Reitmeier and Nonnecke 1991**).

Statistical analysis:

Means of data obtained for sensory evaluation of samples were evaluated using Duncan's multiple range test to identify significant differences at the 0.05 probability ($p \leq 0.05$) using the statistical Analysis system (SAS) (SAS Institute Inc.1999).

Results and Discussion

It is well know that within the mechanism of adsorption spraying of carotenoids should realize the separation of pigments in droplets in order to prevent their sticking together. This could be usually achieved by using a suitable powder or liquid medium to catch the particles. The powder phase will adhere to the surfaces of the pigment particles while; liquid will form a film around them (**Counsel 1980 and Rizk and Tolba 2002**). With this view in mind the average yield of extracted yellow colorants determined as Carotenoids is given in Table (1). The concentration of the extracted carotenoids was 4.65 mg / 100g fresh pumpkin sample. For the same table it could be concluded that, a total 100g of mixing carotenoids with coated carrier (6:1) i.e. lactose, soluble starch, wheat flour and dextrin represented (in the presence 0.1 %ascorbic acid) 11.60, 5.70, 2.30 and 7.50 g carotenoids extracted from pumpkin respectively. This means that, lactose was the most effective adsorbent coated carrier material for yellow colorant extracted from pumpkin followed by dextrin, soluble starch and wheat flour respectively. These results are confirmed by (**Rizk and Tolba 2002, Rizk and Attia 2009**).

Table (1) Distribution pattern of carotenoids extracted from pumpkin with in selected carrier

Rate of carotenoids to carrier g /100 g	Lactose	Soluble starch	Wheat flour	Dextrin
1 : 1	0.562	0.320	0.291	0.390
2 : 1	1.520	0.630	0.582	0.610
3 :1	3.272	0.864	0.735	1.023
4 : 1	5.341	1.232	0.975	2.541
5 :1	8.321	2.320	1.423	4.260
6 : 1	11.60	5.70	2.30	7.50

The reason of adding ascorbic acid during dispersion of extracted carotenoid (from pumpkin) on the applied carrier is based on its higher capability of absorbing the oxygen surrounding the extracted coloring substances. In other words, ascorbic could be oxidized more rapidly than the extracted coloring substances, patterns which lead to more stabilization of the tested pigment, (**Counsel 1980 and Rizk *et al,* 2008**).

Identification of carotenoids from pumpkin. A typical HPLC given in Table (2) summarized the carotenoid extracted from pumpkin. Carotenoid extract from pumpkin was separated based on their functional groups into four fractions by thin layer chromatography (TLC) on silica gel. In carotenoid analysis by TLC was mainly used for preliminary examination to give an indication of the number and variety of carotenoids present and to help in the selection of a suitable separation and purification procedure for the given mixture (**Eder 1996 and Rizk and Tolba 2002**).

Table (2) Identification of carotenoid pigments extracted from pumpkin

TLC	HPLC		Retention time (min)	Identification of carotenoids
RF X 100	Peak No.	Fractions %		
88	1	25%	25	Lutein
92	2	7%	36	Cryptoxanthin
56	3	12%	30	γ carotene
63	4	19%	32	α Carotene
76	5	37%	39	B carotene

The calculated RF values for carotenoid fraction of pumpkin 88, 92 56, 63 and 76 respectively. On the other hand, five peaks were identified by using HPLC in care of pumpkin carotenoid namely lutein, cryptoxanthin, γ carotene, α carotene and B carotene respectively.

The major carotenoids extracted from pumpkin were B carotene37% followed by lutein 25%, α carotene19%, γ carotene 12% and cryptoxanthin7% respectively.

Antioxidant activity of pumpkin carotenoids

The effect of different concentration (200, 300 , 400, 500 and 600 ppm) of carotenoid extracted from pumpkin on the stability of sunflower oil compared to the sunflower oil contain 200 ppm BHT and sunflower oil without any added are illustrated in Table (3). Stability of sunflower oil was measured at 110^{o}C by Rancimat method.

The induction period were increasing gradually by increasing the concentration of carotenoid pumpkin from 200 to 600 ppm. The induction period of sunflower oil contain 200ppmBHT and sunflower oil without any added were 5.20 and 2.80 h respectively. On the other hand, the induction periods of sunflower oil contain 200,300,400,500 and 600ppm carotenoid extracted from pumpkin were 5.25, 6.80, 7.90, 9.20 and 11.10 h respectively.

Table (3). The effect of different concentration of carotenoid extracted from pumpkin and BHT on the stability of sunflower oil

Treatments	Induction period (h)
Sunflower oil without any additives	2.80[e]
Sunflower oil containing 200 ppm BHT	5.20[d]
Sunflower oil containing 200 ppm carotenoid extract	5.25[d]
Sunflower oil containing 300 ppm carotenoid extract	6.80[cd]
Sunflower oil containing 400 pm carotenoid extract	7.90[c]
Sunflower oil containing 500 ppm carotenoid extract	9.20[b]
Sunflower oil containing 600 ppm carotenoid extract	11.10[a]

Values with different letters in the same column are significantly different at $P<0.05$.

From the aforementioned results, it could be noticed that, the sunflower oil contain 300 to 500 ppm carotenoid from pumpkin recorded high stability (higher induction period in Rancimat test) than sun flower oil contain 200 ppm synthetic antioxidant (BHT).

These results are agreement with (**Nilson *et al*, 1999, Rizk and Attia 2009, Azouz *et al*, 2007 and Rizk *et al*, 2008**).

These compounds are considered to be beneficial to health act as since its antioxidants in the body inhibiting lipid by pro oxidation scavenging, free radicals and displaying anti mutagenic properties. These results suggest that the carotenoids extracted from pumpkin possess antioxidant properties could be used alternatives natural antioxidants with wide food applications.

Sensory Evaluation:-

Sensory evaluation of drops in the normal process, the sweet mass consisting principally of sugar, corn syrup, water and citric acid was build to the correct point and added flavoring agent and natural yellow color and mixed into the hot mass by kneading. When adequately mixed and when the correct temperature is reached, the mass was pressed into shapes and wrapped. The carotenoids give truly beautiful color (**Counsel, 1980**).

Table (4) Average values of various sensory parameters of the tested drops with different level of pumpkin carotenoid pigment

Overall acceptability	Taste	Color	Variable
			Drops +
4.60^{e}	4.40^{e}	4.30^{e}	0.10% pigments
6.30^{d}	6.20^{d}	6.40^{d}	0.20%
9.70^{a}	9.60^{a}	9.70^{a}	0.30
9.40^{a}	9.30^{a}	9.10^{ab}	0.40
8.20^{b}	8.80^{b}	7.80^{c}	0.50

Values with different letters in the same column are significantly different at $P<0.05$.

Sensory properties of drops prepared by adding different levels of carotenoid extracted from pumpkin in the range of 0.10 to 0.50% are given in Table (4). On using Duncan's multiple range test, it could be concluded that, in the presence 0.30% pumpkin carotenoid pigment a superior color of the tested samples. On contrary, the inferior color was recorded in samples prepared with 0.10% pumpkin carotenoid.

For the aforementioned results, it could be noticed that drops prepared by adding of 0.30 and 0.40% of pumpkin carotenoid pigment have an improved color, taste and overall acceptability, while more than 0.40 or less than 0.20% pumpkin carotenoids pigment led to unacceptable drops.

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التعرف على كاروتينويدات القرع العسلى واستخدامها كمضادات أكسدة طبيعية وملونات للأغذية

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الملخص:

تم دراسة تركيب الكاروتينويدات المستخلصة من القرع العسلى والتعرف على مكونات هذا المستخلص باستخدام جهاز الكروماتوجرافي TLC و HPLC وايضا تم دراسة تأثير إضافة الكاروتينويدات المستخلصة إلى بعض الأغذية المصنعة كمواد طبيعية مضادة للأكسدة ومواد ملونة طبيعية فى الحلوى

ولقد أظهرت النتائج المتحصل عليها أن :

١-أفضل مادة لتحميل الكاروتينويدات (اللون الأصفر) المستخلص من القرع العسلى هو اللاكتوز ثم الدكسترين.

٢-وباستخدام طرق التحليل الكروماتوجرافي أمكن التعرف على خمسة مركبات من الكاروتينويدات المستخلصة من القرع العسلى وكان ترتيبهم هو الليوتين - الكربثوكزانثين - جاما كاروتين - الألفاكاروتين - البيتاكاورتين.وكان البيتاكاروتين هو المركب السائد فى كاروتينويدات القرع العسلى بنسبة ٣٧% تلاه الليوتين بنسبة ٢٥% ثم الجاماكاروتين بنسبة ١٩% ثم الالفاكاروتين بنسبة ١٢%ثم الكربتوكزانثين بنسبة ٧% على التوالى.

٣-وعلى الجانب الآخر أظهرت النتائج أن إضافة ٣٠٠ إلى ٥٠٠ جزء في المليون من الكاروتينويدات المستخلصة من القرع العسلى إلى زيت عباد الشمس أعطت فترة نبات أعلى من الزيت المحتوى على ٢٠٠ جزء في المليون من مضاد الأكسدة الصناعية BHT.

٤-وكذلك أوضحت النتائج أن إضافة ٠.٣ إلى ٠.٤% من الكاروتينويدات المستخلصة من القرع العسلى (اللون الأصفر) إلى تحسين الخواص الحسية للحلوى المصنعة Drops من قبل المحكمين .