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CAROTENOID PIGMENTS COMPOSITION OF CALENDULA FLOWER AND ITS POTENTIAL USES AS ANTIOXIDANT AND NATURAL COLORANT IN MANUFACTURING OF HARD CANDY

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ABSTRACT

The composition profile of carotenoid pigment compounds of Calendula flower were identified by thin layer chromatography (TLC) and HPLC analysis it was found the these compounds were β-carotene, creptoxanthin, lutein, flavoxanthin, leuteoxanthin, violaxanthin and zeaxanthin. The main carotenoids were β-carotene, lutein and flovoxathin. The suitable carrier matrix for calendula flower carotenoid pigments were lactose, followed by dextrin, wheat flour and glucose. Consequently, the higher stability of calendula carotenoid pigments as a colorant material was observed in alkaline pH at pH ranging from 7 to 10 and temperature ranging from 40 to 70ºC. Meanwhile, the degradation of calendula carotenoids did not exceed than 19.3% of total pigments after 180min at 100ºC. On the other hand, the antioxidant activity of calendula carotenoids was also studied by the rancimat test at 110ºC on sunflower oil by adding 100 to 500 ppm calendula carotenoids. However, sunflower oil containing 200 to 500 ppm recorded or higher induction period than 200 ppm BHT. On the other hand, analysis of variance for sensory evaluation of prepared hard candy indicated that, hard candy containing 0.3 and 0.35% natural calendula carotenoid color had received the highest scores for color, taste and overall acceptability compared with those prepared with 0.1 and 0.5% which recorded the lowest scores in all tested quality attributes.

INTRODUCTION

Chemical and pharmacological studies involving medicinal plants have increased in the last decades, not only related to the isolation of active principles, but also the characterization of new components with therapeutic activity and nutraceutic characteristics, important for the use in food industries, as well as in cosmetology and pharmacology **(Danielski, *et al* 2007).** Today there is unprecedented interest by consumers, public health organization, and the medical community to improve health and wellness through dietary means. This interest arises in large part from the increased rates in western society of adverse diet-related health condition such as obesity, late-onset diabetes, and cardiovascular disease, and their associated social and economic costs **(IFIC, 2004).**

Calendula *(Calendula officinalis L.* Asteraceae) a bright yellow and orange flower, is an annual herbaceous plant, native of Mediterranean countries. It is a well known medicinal herb. It is common knowledge that its medicinal properties are conditioned on biologically active complex substances of Carotene (Provitamin A), Stearin, Triterpinoid, Flavonoid, Kumarin, macro and micro compound elements **(Korakhashvili *et al* 2007).** The importance of carotenoids is based on their provitamin A activity and their antioxidant capacity **(Britton, 1995).** Phytopharmacological studies of different Calendula extracts have shown anti-inflammatory, anti-viral, Anti-tumoral activity and anti-genotoxic properties of therapeutic interest **(Jimenez-Medina *et al* 2006),** and also anti-Oedematous effect **(Zitterl-Eglseer *et al* 1997).** The flower is normally used as food additive to confer both color and flavour to food **(Hense *et al* 2006).**

Principal natural food colorants used in modern food manufacture are anthocyanins, betalains, carotenoids, chlorophylls, riboflavin and caramel.

Carotenoids (carotenes and xanthophylls) occur naturally in some foods such as carrots, red

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tomatoes, butter, cheese, paprika, palm oil, corn kernels, marigold petals, annatto, and red salmon. Carotenoids (alpha-or beta carotene and xanthophylls) are excellent antioxidants and inhibit some types of cancers **(Gonzalez de Mejia *et al* 1997).**

Carotenoids are present in many biological systems, often decreasing the formation of products of oxidative damage to biological molecules. In the macula their concentration is so high that it has been believed that the yellow color filters out damaging blue light. Recent reports that dietary lutein reduces the risk of cataract in the eye lens suggested that the antioxidant action of carotenoids, which has been inferred from decreased oxidative damage, warranted further direct investigation **(Trevithick-Sutton *et al* 2006).**

Dietary antioxidants may reduce the risk of oxidative tissue damage. As part of an European multicentre project, several studies were undertaken with the aim of testing whether the consumption of foods rich in carotenoids reduces oxidative damage to human tissue components **(Olmedilla *et al* 2002).**

Carotenoids are used as safe natural colorants for foods, animal feeds, are considered the main source of vitamin A in human. Free carotenoid pigments together with xanthophyll esters and other lipophilic substances (mainly triglycerides) are extracted on industrial scale using organic solvents from seeds, fruits, or flower petals **(Nishino, 1999).**

The main carotenoids of the petals and pollens of Calendula by analyzing with HPLC were flavoxanthin and auroxanthin while the stem and leaves mostly contained lutein and beta-carotene as reported by **Bako *et al* (2002)**. The stability of carotenoids during their commercial shelf life is very important if the final products is to be attractive and acceptable. The isomerization and oxidation of carotenoids result in a loss of color, which is one of the most important attributes related to quality affecting choice of purchase **(Calvo *et al* 2001 & Baker and Gunter, 2004).**

Color alongside freshness, is one of the main criteria for food selection by consumer. For this reason, coloring are added to many foods to make them appear healthier and more appealing. The objective of the present study was to extract the carotenoid pigments from Calendula to investigate its composition by TLC and HPLC and to identify the most effective carotenoids for orange color and also to obtain more information about the suitable carrier matrix used for increasing its stability at different pH and temperature. Also, assessment of antioxidant activity of calendula carotenoids on sunflower oil. Sensory evaluation of prepared hard candy using various levels of natural orange pigment of calendula was also undertaken.

MATERIALS AND METHODS

Samples

Calendula flower *(Calendula officinalis L.)* was obtained from the Agricultural Research Station of Cairo Food Flavors and Essences Company, Giza, Egypt during the season 2006.

Chemicals and reagents

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

Refined sunflower free from antioxidants was obtained from Arma Food Industries, 10th of Ramadan. Synthetic antioxidant, namely butylated hydroxytoluene (BHT) was purchased from Sigma Chemical Co., St Lewis, USA.

Methods

Chemical analysis

Extraction and concentration of carotenoids

The flower sample was extracted and concentrated by the method reported by **Nilzu and Rodriguez-Amaya (2005)** with cold acetone using a mortar and pestle. Extraction and filtration on Buchner was repeated until the residue was devoid of color (about 3 times), the total amount of acetone used being 300 ml. The carotenoids were partitioned to 100ml/peteroleum ether and saponified overnight with an equal volume of 10% KOH in methanol. After washing, the carotenoid solution was concentrated in a rotary vaccum evaporator at 30ºC.

a- Stabilization of yellow colorants in solid matrixes

The concentrated yellow pigments of Calendula flower were adsorbed with different ratio of solid matrixes i.e., (lactose, glucose, dextrin and wheat flour) up to 4 : 1 pigment matrix and dried in oven at 40ºC for 24 hr.

b- Determination of total carrotenoids

Total carotenoids were determined by the method of **Raddy and Sistrunk (1980)** 5g of the Calendula flower were extracted with 40 ml hex-

ane, 60ml ethanol. After blending, the hexane layer was separated by adding 2% cold NaCl solution. Sample was diluted to (1: 20) and the O.D was measured at 440 nm and compared to β-carotene standard curve.

c- Properties of stabilized pigament

1) Effect of pH

A preliminary study was conducted to test the stability of yellow Calendula flower pigments in different pH media that ranged from 2.0 to 10.0 for 30min and then percentage of color loss was calculated according to the method described by **Rizk and Tolba (2002).**

2) Effect of temperature

A preliminary study was conducted to test heat tolerance of yellow Calendula pigment at different temperature ranging from 40ºC - 100ºC for 30min and then percentage of color loss was calculated as mentioned by **Rizk and Tolba (2002).**

3) Thermal stability

Holding yellow colorant solution (Calendula carotenoid piagment) at 60ºC to 100ºC was extended for 180min through which they were removed each 30min and cooled immediately in an ice bath followed by measuring absorption spectra of the solution at 440nm **(Rizk and Tolba, 2002).**

d- Identification of the carotenoids

- Thin Layer Chromatography (TLC) analysis

TLC was applied using silica gel GF 254 for identification of Calendula carotenoids by the method reported by **(Eder, 1996).** Extracted carotenoid was dissolved in a small amount of acetone and spotting in TCL. The plate was developed with solvent system methylene chloride, ethylacetate (4 : 1). Then dried at room temperature. For visualization of color spots P-anisaldehyde was used and RF value was calculated.

- High Performance Liquid Chromatography (HPLC) analysis

The carotenoids of Calendula flower were identified by Knauer HPLC pump 64 according to the method reported by **Gaylek *et al* (1987)** using octadecyl silane C 18, 3.9 x 150mm. For both HPLC columns, two solvents were used for elution: (1) methanol (2) ethyl acetate. The flow rate was 1.8ml/ min and absorbance was measured at 475 nm.

e- Evaluation of antioxidant activity

Calendula flower extract carotenoid pigments, was tested as antioxidant by using the Rancimat method, 5g of dried Calendula flowers were exhaustively extracted with methanol (100ml). 100, 200, 300, 400 and 500 ppm of the extract were mixed with 25g of sunflower oil in a flask, against a sample 25g of sunflower oil mixed with 200 ppm of synthetic BHT in a flask. On the other hand, the control was sunflower oil without any additives. A 5g portion of each tested sample was loaded into the reaction vessel cylinder. The air supply was maintained at 20 ml/min and the heating temperature was described by **Laubli and Bruttel (1986).**

Technological methods

- Manufacturing of hard candy

The hard candy was manufactured in the laboratory by adding different levels of yellow colorants (Calendula carotenoids) 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50% (W/W) using the traditional procedure as described by **Counsell (1980).** The formulation of hard candy sample is shown in **Table (1).**

Table 1. Formulation of hard candy samples

Ingredients	%
Sucrose	48.48
Corn syrup	25.90
Water	25.26
Flavoring oil	0.21
Citric acid	0.15
Color	0.01 – 0.50

- Sensory evaluation

Sensory evaluation was carried out by ten panelists to evaluate color, taste and general acceptability of prepared candy according to the method 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

Carotenoids content of Calendula and its distribution within selected carrier

Total carotenoid concentration of fresh Calendula flowers was found to be 403.6 mg/100g. Which, was higher than those in dehydrated marigold flower meal (ranged from 180 to 247 mg/100g) as reported by **Delgado-Vargas and Paredes-Lopez *(*1997).** However, the extraction of the carotenoids depend on the conditions for silaging, drying, and solvent used for extraction **(Barazna *et al* 2002).**

It is known in that within the mechanism of adsorption, sprying of carotenoid should realize the separation of pigment in droplets in order to prevent their sticking together. This could be usually achieved by using suitable powder or liquid medium to catch the particles. The powder phase will adhere to the surface of the pigment particles within a liquid which will form a film around them **(Rizk and Tolba, 2002).**

The adsorption material used as stabilizing carriers for carotenoid pigments extracted from Calendula flower are shown in **Table (2)**. It could be noticed that, a total 100g of mixing cartenoids with coated carrier, i.e., lactose, dextrin, wheat flour and glucose (in the presence of 0.15% ascorbic acid) were 18.00, 13.00, 6.20 and 4.00g/100g carrier, respectively. This means that lactose was the most effective adsorbent coated carrier matrix for yellow colorant extracted from the investigated samples followed by dextrin, wheat flour and glucose. The reason for adding ascorbic acid during dispersion of extracted carotenoids from Calendula flower on the applied carrier is based on its higher capability of adsorbing the oxygen surrounding the extracted coloring substances. In other words, ascorbic acid could be oxidized more rapidly than the extracted coloring substances, a pattern which lead to more stabilization of the tested pigments **(Rizk, 1997).** Also, the aim of using carrier matrixes for Calendula carotenoid pigment to promote the utilization of these pigments during food processing may be due to the opinion of **Rodriguez-Amaya, (2003)** who mentioned that, during processing, isomerization of trans-carotenoids, the usual configuration in nature, to the cis-forms occurs, with consequent alteration of carotenoids bioavailability and biological activity. Isomerization is promoted by light, heat and acids. In addition, the principal cause of carotenoid loss during processing and storage of food is enzymatic or non-enzymatic oxidation of the highly unsaturated carotenoid molecules. Thus retention and stabilization of carotenoids has been the major concern in the preparation, processing and storage of foods.

The unsuitability of wheat flour and glucose as carriers could be related to their capability to breakdown the carotenoids during immobilization. These results are in agreement with **(El-Gharably, 2005).** On the other hand, the positive influence of dextrin and lactose as coated carriers for carotenoids of Calendula flower may be due to the strict interfering of such carriers within the condensation reaction that usually occur during immobilization **(Colin and Peter, 1980).**

Table 2. Distribution pattern of carotenoids within selected carrier

Selected carrier	Ratio of carotenoid to carrier g/100g	Concentration of pigment g/100g carrier
Glucose	4 : 1	4.00
Lactose	4 : 1	18.00
Wheat flour	4 : 1	6.20
Dextrin	4 : 1	13.00

Separation and identification of Calendula caroenoids by using TLC and HPLC.

Separation and identification of Calendula carotenoids pigment was done by TLC and HPLC as summarized in **Table (3)** and **Fig. (1).** The carotenoids extracted from Calendula flower were separated based on their functional group into seven fractions by thin layer chromatography (TLC) on silica gel. TLC was mainly used for preliminary examination of cartenoid constituents 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).**

The calculated Rf values for carotenoid fractions of Calendula flower were 92, 88, 78, 76, 62, 32 and 24 for cryptoxanthin, lutein, leuteoxanthin, β-carotene, flavoxanthin, violaxanthin and zeaxanthin, respectively.

On the other hand, seven carotenoids were identified in the Calendula flower by using HPLC namely β-carotene, cryptoxanthin, lutein, flavoxanthin, leuteoxanthin, violaxanthin and zeaxanthin,. The carotenoids from Calendula flowers contained 35.86% β-carotene, 7.91% crypotoxanthin, 24.82% lutein, 15.17% flavoxanthin, 6.37% leuteoxanthin,

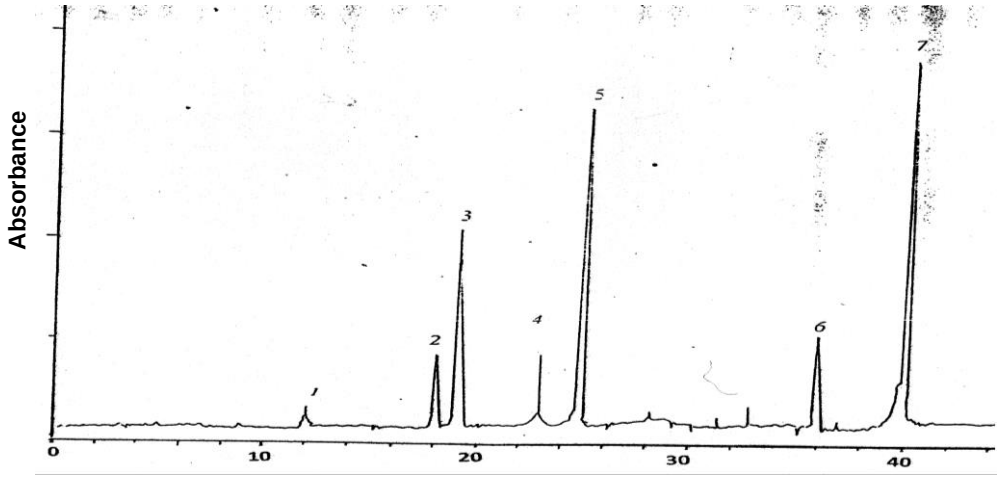

Fig. 1. HPLC separation of carotenoid pigment extracted from Calendulla petals
1 = Zeaxanthin 2 = Violaxanthin 3 = Leuteoxanthin
4 = Flavoxanthin 5 = Lutein 6 = Cryptoxanthin
7 = B carotene

Table 3. Identification of carotenoid pigments from Calendula flower by TLC and HPLC

TLC	HPLC			Identified carotenoid
	Peak	Area %	Rt (min)	
24	1	1.28	12	Zeaxanthin
32	2	5.25	18	Violaxanthin
78	3	6.37	19	Leuteoxanthin
62	4	15.17	23	Flavoxanthin
88	5	24.82	25	Lutein
92	6	7.91	36	Cryptoxanthin
76	7	35.86	39	β-caroten

5.25% violaxanthin, and 1.28% zeaxanthin. Therefore, β-carotene had the highest % of Calendula total carotenoids followed by lutein, flavoxanthin, cryptoxanthin, leuteoxanthin, violaxanthin and zeaxanthin. **Nilzu and Rodriguez-Amaya (2005)** mentioned that, the carotenoids identified as violaxanthin had a visible absorption spectrum with well-defined spectral structure (III, II =98), typical of carotenoid with 9 conjugated double bond in the polyene chain. While zeaxanthin presented a visible spectrum with λ max higher than those of lutein and little definition of the peaks. **Tsao and Yang (2006)** mentioned that, structures of lutein, β-carotene and violaxanthin were identified by LC atmospheric pressure chemical, ionization MS in positive ion mode. Results also indicated that, the β-carotene (35.86%), lutein (24.82%) and flavoxanthin (15.17%) were the predominant consituent of carotenoids for Calendula flower which are considered highly yellow colored compounds. These results are similar with those of **Fisher and Rouseff, (1986)** they mentioned that, certain carotenoids such as β--cryptoxanthin, α-carotene, β-carotene are highly colored compounds that also exhibit porvitamin A activity. Also, they are the main carotenoids of Calendula petals, and it is clear that these carotenoids are responsible for the orange color of petals **(Kishimato *et al* 2005).** On the other hand, our results are in agreement with **Bako *et al* (2002)** who reported that, the stem and leaves of Calendula mostly contained lutein and β-carotene.

Properties of stabilized carotenoids

a- Effect of pH.

A preliminary study was conduced to test the stability of carotenoid pigments derived from Calendula flower in different pH media. The obtained results are illustrated in **Table (4)**, from which the color changes were induced by pH variation. Most striking was the effect of pH on the carotenoid content which was about 100.00 to 82.22 at pH varying from 10 to 5. While, the higher degradation of color reached 51.26 and 39.47% at pH 2.0 and 3.0, respectively. On the other hand, the degradation of color did not exceed 8% between pH 10.0 and 7.0 for instance, the highest stability and less degradation of carotenoid extracted from calendula flower was at higher alkalinity at pH 7.0 to 10.00. Subsequently, results have shown at pH 3.0 and 4.0 a degradation of 39.47% and 29.35% respectively.

For instance, the carotenoids of Calendula were more instable in the acid media than a alkaline media. These results may be due to the characteristic of conjugated double bond system of carotenoid produces the main problem associated with work and manipulation on carotenoids, that is their particular instability, especially towards light, heat, oxygen and acids **(Oliver and Palou, 2000).**

Table 4. Retention % of carotenoids extracted from Calendula flower as a function of pH

pH value	% retained of carotenoid pigment	% degradation of carotenoid pigment
2	48.74	51.26
3	60.53	39.47
4	70.65	29.35
5	82.22	17.78
6	88.35	11.65
7	92.27	7.73
8	96.80	3.20
9	100.00	0.00
10	100.00	0.00

b- Effect of temperature

The main cause of carotenoids degradation in foods is oxidation. In processed foods, the mechanism of oxidation is a complicated, process and dependent on many factors. The rate of pigments autoxidize by reaction with atmospheric oxygen depends on light, heat and presence of pro and antioxidants **(Fennema, 1976).** Moreover, isomerization is promoted by light, heat and acids **(Rodriguez-Amaya, 2003).**

Table (5) represents the rate of remained and degradation % of carotenoids derived from Calendula flower. There is no degradation and also similar stability was observed as a result of exposing Calendula carotenoid pigment at moderate temperature between 40ºC - 60ºC, while at above 60ºC, the degradation of carotenoids increased gradually by increasing the temperature. For instance, the degradation of carotenoid pigment, caused by its exposing to high temperature.

The higher degradation of Calendula carotenoids pigments was observed at 100ºC followed by 90, 80, and 70ºC, respectively. Therefore, carotenoids extracted from Calendula flower, was more heat stable between 40ºC to 60ºC but lower degradation rate was noticed up to 100ºC. These results are confirmed with **Marimion (1984)** who mentioned that, the carotenoid pigments is relatively stable to heat. Similar findings were given by **Rayner (1991)** who proved that, the carotenoid pigment extracted from marigold exhibits good stability to heat. β-carotene is normally the index of deterioration due to its high instability to environmental factors (e.g., oxygen, temperature and light) **(Walter *et al* 1970).**

Table 5. Effect of temperature on the degradation rate of carotenoids extracted from Calendula flower

Temprature (ºC/30 min)	% remained of carotenoid	% degradation of carotenoid
40	100.00	0.00
50	100.00	0.00
60	100.00	0.00
70	97.32	2.68
80	95.76	4.24
90	92.25	7.75
100	90.80	9.20

Thermal stability

The stability of carotenoids extracted from Calendula flower on duration time at various temperature ranging between 60ºC to 100ºC is evident in **Table (6)**. No noticeable changes were observed for carotenoids extracted from Calendula flowers

Table 6. Thermal stability of carotenoid extracted from Calendula flower

Pigment carotenoid %	Temperature ºC	Duration time (min)					
		30	60	90	120	150	180
Remained	60	100.00	100.00	100.00	99.50	99.50	99.00
Degradation	60	0.00	0.00	0.00	00.50	00.50	1.00
Remained	70	97.32	97.00	97.00	96.60	99.00	95.50
Degradation	70	2.68	3.00	3.00	3.40	4.00	4.50
Remained	80	95.76	94.20	93.00	92.40	91.30	90.20
Degradation	80	4.24	5.80	7.00	7.60	8.70	9.80
Remained	90	92.25	91.00	89.60	88.80	88.00	87.00
Degradation	90	7.75	9.00	10.40	11.20	12.00	13.00
Remained	100	90.80	88.20	87.30	85.11	83.13	80.70
Degradation	100	9.20	11.80	12.70	14.89	16.87	19.30

after 60min. up to 80ºC, but the retention of carotenoid was 87.30% after 90min. at 100ºC. The destruction of carotinoid was 16.87% at 100ºC after 150min. These results may be due to its presence in the form of complexes with protein or lipoproteins, submicroscopic structure may be also a factor in their outstanding stability **(Rizk and Tolba, 2002 & Counsell, 1980). Antioxidations activity of Calendula flower carotenoid.**

Antioxidants are usually added to fats, oils and foods containing fats in order to inhibit the development of off-flavour arising from oxidation of unsaturated fatty acid. However, the commercial use of the synthetic antioxidants is strictly controlled and increasing. Consumer awareness of food additives and safety has promoted increased interest to the use of natural antioxidants e.g., carotenoid, ascorbic acid and tocopherol **(Laubli and Bruttel 1986 & Addis and Warner, 1991).**

Table (7) showed the induction period of sunflower oil containing different concentrations of carotenoids extracted from Calendula as natural antioxidant by the Rancimat test at 110ºC. Results indicated that, induction period of sunflower oil was increased by increasing the concentration of extracted Calendula carotenoids. The induction period was 2.65 and 4.15 h for sunflower oils without adding antioxidant and that contained 200 ppm BHT, respectively, meanwhile, our results proved that the induction period was increased gradually by adding and increasing the concentration of natural β-carotene extracted from Calendual as antioxidants. These values of induction periods increased to 5.21, 6.96, 8.11 and 10.21 h for sunflower oil that contained 200, 300, 400 and 500 ppm Calendula carotenoids extract, respectively. These results, are similar to those given by **Nilson *et al* (1999)** who proved that, the carotenoids acts as antioxidants by destroying the free radicals. Also **Mubarak (2003)** reported that, natural antioxidant extracts of 0.1% concentration increased the induction period of sunflower oil assessed by Rancimat method. The replacment of synthetic antioxidant by Calendula carotenoid natural antioxidant may have benefits due to health implication of functional parameter such as stability in both oil and water **(Reglero *et al* 1999).**

Table 7. Relation between induction period of sunflower oil (SO) containing different levels of antioxidant extract from Calendula flower assessed by the Rancimat at 110ºC

Treatments	Induction period (h)
SO free from additives	2.65
SO containing 200 ppm BHT	4.15
SO containing 100 ppm carotenoid extract	3.00
SO containing 200 ppm carotenoid extract	5.21
SO containing 300 ppm carotenoid extract	6.96
SO containing 400 ppm caroenoid extract	8.11
So containing 500 ppm carotenoid extract	10.21

SO = sunflower oil

Sensory evaluation of prepared candy

Table (8) shows mean scores of sensory evaluation of hard candy prepared with different levels of Calendula carotenoids within range of 0.10 to 0.50%. Analysis of variance showed that, there is no significant differences in color for candy prepared by adding different levels of natural yellow colorants from 0.25 to 0.35% but above or less than these levels, the prepared candy had significant differences in color scores. Results also indicated that, no significant difference in taste and overall acceptability between candy prepared by adding 0.30 or 0.35% natural colorants but there were little differences between samples prepared with both 0.25 and/or 0.40% yellow natural colornts. On the other hand, it could be noticed that, hard candy prepared in the presence of 0.30 and/or 0.35% of Calendula carotenoids had recorded the highest scores of all investigated attributes and were considered the best prepared candy. However, hard candy prepared with 0.5% and 0.1% had inferiored and received the lowest scores in all tested quality attributes. For instance, adding of 0.30 and/or 0.35 Calendula carotenoids as natural colorants should be used as alternative yellow colorant instead of using synthetic colors. In general, consumer perception has been that natural food colorant ingredient instead of synthetic colors would be more safe, healthier and is considered as potential food colorants for manufacturing hard candies.

Table 8. Mean scores of sensory evaluation of hard candy treated with different levels of natural carotenoid extract from Calendula flower

% of natural carotenohds	Color	Attribute	General acceptability
		taste	
0.10	5.13e	5.14e	4.55f
0.15	6.02d	6.47d	5.58e
0.20	7.20b	6.47d	6.76d
0.25	9.32[a]	7.48c	8.95b
0.30	9.80 [a]	9.86a	9.63a
0.35	9.62 [a]	9.23a	9.11a
0.40	8.82 [a]	7.68c	7.65c
0.45	5.80d	5.14c	5.44c
0.50	4.41f	4.38f	4.82f

Therefore, the best level of Calendual yellow carotenoid pigments that improved the sensory quality properties of prepared candy was 0.30 and/or 0.35% but higher or lower than these levels of natural colorants will lead to obtain inferior qualities. These findings are in agreement with that of **Wiesenborn *et al* (1991)**. Also, there is a trend in the food industry towards functional food, which produce healthy effects based on their antioxidant properties **(Velioglu *et al* 1998, Köhkönen *et al* 1999 & Robards *et al* 1999).**

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