



## Characterization of $\beta$ -carotene Extracted from Orange Peels and its Use as a Natural Colorant and Antioxidant in Ice Cream

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THE orange manufacturing sector produces great quantities of waste, mostly orange peels that generate environmental issues. These residues are rich sources of pigments and bioactive chemicals.  $\beta$ -carotene is one of the carotenoids, an antioxidant fat-soluble pigment. This study was performed to extract  $\beta$ -carotene from orange peels and investigate its stability to some treatments, including temperature, storage, light, pH and its antioxidant characteristics. Carotenoids were determined and identified using high performance liquid chromatography. The total content of phenolic compounds and flavonoids in the dried orange peels were found to be 16.12 and 2.06 mg / g, and  $\beta$ -carotene 14.51 mg/100 g, respectively. Additionally, antioxidant activity of orange peels powder was found to be 88.65% based on DPPH assay. As storage duration and the exposure to high temperature and light increased, beta-carotene degradation increased, while it was more stable in the alkaline medium. The antioxidant activity of  $\beta$ -carotene extract was found to be comparable to that of industrial BHT based on oil oxidation stability assay. In the sensory evaluation, panelists preferred ice cream incorporated with 1%  $\beta$ -carotene extract. During storage of ice cream for 21 days at -18°C, the  $\beta$ -carotene content decreased by about 5%.

**Keywords:** Orange peels;  $\beta$ -carotene; Stability; Ice Cream.

### Introduction

In all stages of the food life cycle, *i.e.* throughout agricultural production, industrial production, storage and delivery, food waste is generated. Household operations produce up to 42 percent of food waste, 39 percent losses in the food industry and 14 percent losses in the food service sector (prepared to consume food, restaurants and hotels), while 5 percent are wasted during production. It is anticipated to increase to approximately 126 Mt by 2020 if there is no avoidance policy or activity (Mirabella et al., 2014). In particular, the fast-growing food manufacturing industry in most nations around the globe produces enormous quantities of by-products, along with pomace, hull, husk, pods, peels, shells, flowers, stems,

bran, washings, pulp waste, press cakes and so on, it has less use and cause significant pollution of the environment (Sharma et al., 2016).

Orange peels are about half the complete weight of the fruit and are very pigment-rich (Oroian and Escriche, 2015). The orange peel color is usually derived from phenolic compounds and carotenoids (Oreopoulou and Tzia, 2006). Significant quantities of orange peel waste have been tracked in the technological circumstances when the juice is removed from the remainder of the fruit. This waste can be a very significant cause of distinct kinds of carotenoids: provitamin A, apocarotenoids and forms of epoxy. Carotenoids are accountable for many of the red, orange and yellow colors of fruits and vegetables as the

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primary types of coloring materials in nature (Cinar, 2004 and Chedea *et al.*, 2010).

Carotenoids are phytonutrients that occur in nature and are excreted as hydrocarbons that give their distinctive colour to fruits and vegetables. Carotenoids are lipid-soluble pigments that are orange to red and have a strong effect on the adoption of many products (Zhai *et al.*, 2014). Because of their photoprotection characteristics against UV radiation, carotenoids are also commonly used in cosmetic products. There are over 600 recognized carotenoids that can be categorized into two communities; (i) xanthophylls (initially recognized as phyloxanthins), comprising air as a functional band, including astaxanthin, lutein and zeaxanthin, and (ii) carotenes that form only one hydrocarbon chain without any functional range, including  $\beta$ -carotene and lycopene. Besides the operation of antioxidants, photoprotection and provitamin A (Ambati *et al.*, 2014).

This study was aimed to extract  $\beta$ -carotene from orange peels and identifying  $\beta$ -carotene using HPLC. Stability of  $\beta$ -carotene to different pH values, temperatures, and light was studied. There was also an assessment of the antioxidant activity of  $\beta$ -carotene produced from orange peels on sunflower oil and a sensory assessment of the prepared ice cream using various concentrations of  $\beta$ -carotene extract.

## Materials and Methods

### Materials:

Orange peels were obtained from El-Marwa Company for Preservative food, 6th October City, Egypt (2017). Most chemicals (analytical grade) were purchased from El-gomhouria pharmaceuticals Co., Cairo, Egypt. 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, quercetin and HPLC grade solvents were obtained from Sigma-Aldrich Chime, Steinheim, Germany. Refined sunflower oil (antioxidant free) was purchased from Misr Company for Oil and Soap, El Mansoura, Egypt.

### Methods and analysis

#### Preparation of orange peels

Orange peels were dehydrated at  $50 \pm 1^\circ\text{C}$  in a fan drying oven for 36 hours, then grinded and kept individually in polyethylene bags and stored in refrigerator at  $5 \pm 1^\circ\text{C}$  until used.

Manufacture of ice cream mix, according to the (Egyptian Standard, 2005), different amounts

of carotenes extract were presented into ice cream from orange peels powder (0%, 0.5%, 1%, 1.5%) and one sample arranged as an artificial color command (0.1). Samples of ice cream were prepared according to Arbuckle (1986).

### Gross chemical composition and minerals content:

They were determined based on the methods described in the AOAC (2012). Total carbohydrates were calculated based on differences Howard and Leonard (1963). After dry ashing, the mineral content was determined using the technique outlined in the AOAC (2012), using atomic absorption spectrophotometer (Perkin – Elmer, Model 3300, USA).

### Determination of total phenolic compounds

It was determined using the Folin-Ciocalteu reagent as outlined by Maurya and Singh (2010).

### Determination of total flavonoids

It was determined according to the method described by Jia *et al.*, (1999).

### Determination of antioxidant activity

The antioxidant activity of samples was determined by the 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity according to the colorimetric method of Brand-Williams *et al.*, (1995). The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994).

$$\text{Inhibition\%} = \frac{[Ac - At]}{Ac} \times 100$$

Where: Ac is the absorbance of the control at time = 0 min.

At is the absorbance of the antioxidant at time = 1hr

Rancimat method, the antioxidant activity was determined by the Rancimat method using Rancimat Metrohm 679 as described by Hasenhuttl and Wan (1992) and the induction period (I.P.) was conducted with Rancimat at  $110^\circ\text{C}$  and calculated at  $25 \pm 1^\circ\text{C}$  using the temperature coefficient of 2.2 for induction period according to the method reported by Hadorn and Zurcher (1974) and 2.5 for expired period according to the method reported by Pardun and Kroll (1972). The antioxidant activity and increasing index were calculated using the following equations:

$$\text{Antioxidant activity} = \frac{IPS \times 100}{IPC}$$

$$\text{Increasing index} = \frac{IPS - IPC}{IPC \times 100}$$

Where: IPS = induction period of sample and IPC = induction period of control.

*Extraction of  $\beta$ -carotene*

$\beta$ -carotene was extracted from orange peels, according to the procedure of Megahed (1985) as follows: 500 grams of orange peels powder were mixed and macerated in 1 L of acetone, in the presence of 0.1% ascorbic acid in a blender. The extract was filtrated, and the residue was re-extracted twice with the same extracting solvent. The crude colored extract was rapidly concentrated in a rotary vacuum evaporator at  $40 \pm 1^\circ\text{C}$ . Saponification is carried out to remove oil and chlorophylls extracted with the natural yellow pigments. The concentrated extract was washed twice with 200 mL portion of methanolic potassium hydroxide solution (100 g potassium hydroxide plus 750 mL of methanol) plus 250 mL water in a separator funnel. The suitable amount of hexane was added, and the upper layer was washed several times with 10% dihydrogen potassium phosphate solution. The extract was dried over anhydrous sodium sulphate and kept in dark bottles under refrigeration at  $5 \pm 2^\circ\text{C}$  until used.

*Determination of total carotenoids*

The total carotenoids of samples were determined according to the methods described by Holm (1954) and Wetstein (1957).

*Identification and quantification of carotenoids ( $\beta$ -carotene)*

HPLC analysis was conducted using the method described by Chiosa et al. (2005). The analyses were carried out on a HPLC (Agilent), model-LC 1100 series, equipped with a degasser, an auto sampler automatic injector, a high-pressure pump and a UV/Visible detector at multiple wavelengths wave. The mobile phase was acetonitrile - tetrahydrofuran - methanol - ammonium-acetate (68, 4 % (v: v): THF 22.0 % (v:v) : 6.8 % (v:v) : 2.8 % (v:v) (1% (w:v))). Detection wavelength was set at 450 nm with flow rate at 1.5 ml/min.

*Stability of carotenoids ( $\beta$ -carotene)**Effect of pH*

a preliminary study was conducted to test the stability of  $\beta$ -Carotene extract at different pH ranged from 2.0 to 10.0 for 30 min and then the color loss proportion was calculated.

*Effect of different temperature degrees on  $\beta$ -Carotene*

To study the stability of carotenoids extracted from orange peels against pH value, temperature and time exposed to heat. Different media with

pH value ranged from 2 to 10 at room temperature were used. To investigate the effect of heating on stability of carotenoids, different temperature ranged from 30 to  $100^\circ\text{C}$  for 30 min at pH 8, 10 were used. To study the effect of time exposed to heat on stability of carotenoids, different periods ranged from 0 to 120 min with interval of 30 min at  $100 \pm 1^\circ\text{C}$  and pH 8,10 were used.

*Effect of light*

a preliminary study was conducted to test the stability of carotenoids extract of orange peels the effect of exposure at dark and light condition on the stability of carotenoids pigment was measured every hour for six hours under different weather conditions. Retention of pigment of all the above parameters were estimated according to Du and Francis (1973) the following equation:

$$\text{Retention of pigment (\%)} = \frac{A_t \times 100}{A_i}$$

Where:  $A_t$  = Pigment, Absorbance after treatment.  $A_i$  = Pigment, Initial absorbance ( $A_{\text{max}}$ ).

Degradation of  $\beta$ - carotene (%) =  $100 - \text{Remained of } \beta\text{- carotene (\%)}$ .

*Sensory evaluation*

Sensory attributes (color, taste, odor, texture and overall acceptability) of ice cream was according to the method of Lindley et al., (1993).

*Color measurements*

The color was determined with a Chroma meter at three distinct points of the sample (Minolta CR 400, Minolta Camera, Co., Osaka, Japan). Corresponding  $L^*$  value (lightness of color from zero (black) to 100 (white);  $a^*$  value (degree of redness (0–60) or greenness (0 to -60); and  $b^*$  values (yellowness (0–60) or blueness (0 to - 60), while chroma ( $C^*$ ) distinguishes between vivid and dull colors were measured for all the samples were calculated according to Abonyi et al. (2002) by the following equation:

$$C^* = \sqrt{a^2 + b^2}$$

*Effect of storage at  $-18^\circ\text{C}$  on  $\beta$ - carotene stability*

Ice cream was studied by measuring the color intensity of those investigated samples every two days for 21 days applied as described by Francis (1989).

*Statistical analysis*

The statistical assessment was conducted using a one-way variance assessment (ANOVA) at a substantial rate of 0.05 for the whole results using the statistical program CoStat (Ver. 6.400)

according to Steel *et al.* (1997). To ascertain the significant among means of different samples, LSD test was applied.

### **Results and Discussion**

*Chemical composition of orange peels* is presented in Table 1. Carbohydrates and crude fiber are the major component of orange peel, followed by fat and protein. These findings are consistent with the findings of Abd El-Aal *et al.* (2017).

#### *Minerals content of orange peels*

The data presented in Table 2 show the minerals content of orange peels. Calcium, nitrogen, phosphorus, potassium, sodium, manganese, copper, zinc and iron were detected. The results also showed that the calcium and potassium content of orange peels was larger amount (1340 and 480 mg / 100 g) followed by nitrogen (230 mg / 100 g). At the same time, sodium and phosphorus content was found in moderate amounts (55 and 51 mg / 100 g). In addition, it contained the lowest amount of iron,

copper, zinc and manganese. These results agree with those recorded by Egbunu and Amadi (2016) and Romelle *et al.* (2016). The mineral content of plants can be significantly influenced by variety, location and environmental conditions (Rao, 1996).

#### *Bioactive compounds and antioxidant activity of orange peels*

Some bioactive compounds namely, polyphenols, flavonoids contents and antioxidant activity, as well as,  $\beta$ - carotene content were determined, and the results are presented in Table 3. Data show that total phenolic compounds and total flavonoids contents were 16.12 and 2.06 mg/g. as well as, antioxidant activity of orange peels was 88.65%. These results agree with those reported by Hussein *et al.* (2017) who found that total phenolic and flavonoids content of orange peels were 5.46 and 0.114 mg/g, respectively. On the other hand, orange peels are considered as a good source for  $\beta$ -carotene which amounted to 14.51 mg/100g, as total carotenoids content on dry weight basis (Sankalpa *et al.*, 2017)

**TABLE 1. Chemical composition of orange peels**

Chemical composition	Orange peels
Moisture	10.65
Ash*	3.70
Crude protein*	7.93
Crude Fat*	9.62
Crude fiber*	10.73
Carbohydrates <sup>oo*</sup>	68.02

\* Calculated on dry weight basis.

<sup>oo</sup> carbohydrate calculated by difference.

**TABLE 2. Minerals content (mg/100g) of orange peels\***

Sample	Minerals (mg/100g)								
	Ca	N	P	K	Na	Mn	Cu	Zn	Fe
Orange peels	1340	230	51	480	55	0.4	3.27	0.3	9.37

\* calculated on dry weight basis.

**TABLE 3. Bioactive compounds and carotenoids content of orange peels**

Sample	Constituents			
	Total phenolic compounds as Gallic acid mg/g	Total flavonoids (mg/g)	Antioxidant activity % as DPPH	$\beta$ -carotene mg/100g
Orange peels	16.12	2.06	88.65	14.51

### Identification of $\beta$ -carotene

HPLC devices have recognized carotenoid pigments obtained from orange peels.  $\beta$ -carotene pigments extracted from orange peels were separated and identified by HPLC are shown in Fig. 1. Spectral measurement and HPLC separation indicated that there are one major carotene components for orange peels namely  $\beta$ -carotene. These results are in agreement with those reported by Gama and Sylos (2005).

### Effect of pH on retention of $\beta$ -carotene

The effect of different pH values on retention of  $\beta$ -carotene pigments derived from orange peels was determined using the pH-differential method at nine different pH ranged from 2 to 10 and the results are presented in Table 4. The highest O.D values at 420 nm. Observed at pH 9, and 10 (1.114 and 1.100, respectively) while; at pH 2 recorded

the lowest O.D (0.755). As well as, decreasing of pH values from 10 to 6 caused 17.41% little degradation of  $\beta$ -carotene content. However, the degradation of color was not significant till pH 6, meanwhile, the degradation of color reached to 28.64 and 32.23% at pH 3 and 2, respectively. Therefore, it could be observed that the stability of  $\beta$ -carotene pigment was more pronounced at alkaline pH values (7 to 10). While, highest degradation was occurred at pH 2.

These results are in accordance with those obtained by Rizk (2002) and Sheashea et al., (2002) most carotenoids are trans-isomeric in crops, while trans-isomer isomerization to cis-isomers happens during food processing (Khoo et al., 2011). Heat and light as well as acids are main factors that can promote isomerization of carotenoids from trans-isomer to cis-isomers (Provesi and Amante, 2015).

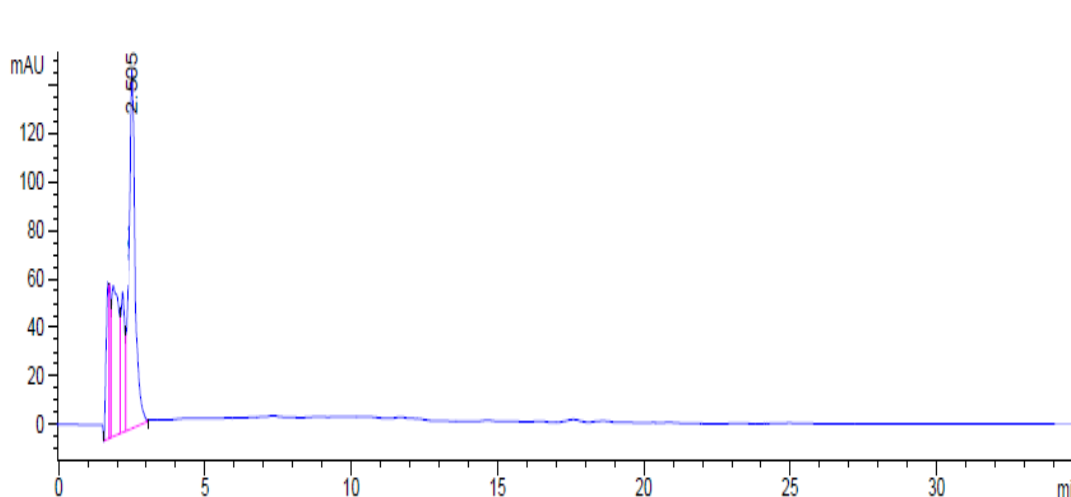


Fig.1. Identification of  $\beta$ -carotene pigment compounds extracted from orange peels by HPLC

TABLE 4. Effect of pH on retention of  $\beta$ -carotene pigment extracted from orange peels

pH	Absorbance $\lambda=420$ nm	Retained $\beta$ -carotene %	Degradation of $\beta$ -carotene %
2	0.755	67.77	32.23
3	0.795	71.36	28.64
4	0.840	75.40	24.60
5	0.895	80.34	19.66
6	0.920	82.59	17.41
7	0.985	88.42	11.58
8	1.020	91.56	8.44
9	1.114	100.00	0.00
10	1.100	98.74	1.26

*Effect of different temperatures on stability of  $\beta$ -Carotene dissolved in media with different pH*

Temperature and pH values are two factors that affect the stability of carotene pigments. The effect of different temperature (at room temperature (25°C  $\pm$ 3), 30, 40, 50, 60, 70, 80, 90 and 100 °C) at pH 8 and 10 was studied in Table 5. The results reveal that the highest O.D values at 420 nm. Observed at pH 8 and 10 (1.620 and 1.630, respectively) at ambient temperature while, at 100°C recorded the lowest OD (1.400 and 1.430, respectively). Meanwhile, the degradation rate of  $\beta$ -carotene pigment increased with temperature increasing at pH 8 and 10 which reached to 13.58 and 12.27 %; respectively at 100°C. On the other hand, the remained of  $\beta$ - carotene pigment after exposure to 100°C, at pH 8 and 10 was 86.58 and 87.73%, respectively. These results agree with those reported by Hamed *et al.* (2000) and Rizk *et al.* (2008). However, the obtained data in the same table showed that there was high degradation of carotenoid contents occurred at low pH values, while, it was more stable in alkaline solution comparing with acidic solution at different temperature degrees. These results are consistent with the findings of Abd El Fattah (2013) who demonstrated that carotenoids are more stable in the alkaline media.

*Effect of time exposed to heat on  $\beta$ -carotene stability at different pH values at 100°C*

To study the effect of heat-exposed time on  $\beta$ -carotene stability, different periods were used from 0 to 120 min at 100 ° C and pH 8 and 10, the results were tabulated in Table 6.

The obtained data in Table 6 showed that the duration of exposure to heat had a significant impact on the deterioration of  $\beta$  -carotene,

especially at temperatures as high as 100 °C.  $\beta$ - Carotene can also be observed to be heated at 100 °C for a long time, causing a significant deterioration compared with short-term events. The degradation of  $\beta$  -carotene gradually increased with increased exposure to heat the degradation rate was (25.95 and 23.57%) at pH 8 and 10, respectively, at 100 ° C for 120 minutes. Meanwhile, at pH 8, 10 at 100 ° C for 30 minutes, the degradation rate was the lowest value were 13.16 % and 12.19 %, respectively. It was noticed from the mentioned data that the degradation rate of  $\beta$  -carotene pigments was high at the lowest pH value and high temperature degree.

While, the lower degradation rate was recorded at high pH value at the same temperature degree (100°C). In conclusion, at the higher temperatures and longer heating time lead to a larger percentage of  $\beta$  -carotene degradation. These results may be due to the molecular structural changes during heating, which lead to break down molecules into small fractions.

*Effect of light on stability of  $\beta$ - carotene*

Light is another factor, which affects the stability of  $\beta$ - carotene. The effect of exposure at dark and light condition on the stability of  $\beta$ -carotene pigment was measured everyone hour for six hours and the results are presented in Table 7. Data in Table 7 reveal that the samples which were exposed to light showed more reduction in the absorbance value compared with the samples kept in the dark. As well as, the remained of  $\beta$ -carotene (93.75%) was higher in the dark than the light (90.28%). Comparatively  $\beta$ - carotene degradation under dark condition is very low than degradation under light which reached to 6.25 % compared with light condition (9.72%).

**TABLE 5. Effect of temperature on stability of  $\beta$ - carotene dissolved in media with different pH**

C°/ 30min	pH 8			pH 10		
	Absorbance $\lambda=420$ nm	R*	D**	Absorbance $\lambda=420$ nm	R*	D**
At R*	1.620a	100	0.00	1.630 <sup>a</sup>	100.00	0.00
30 °C	1.600b	98.77	1.23	1.615 <sup>a</sup>	99.08	0.92
40 °C	1.570c	96.16	3.84	1.590 <sup>b</sup>	97.55	2.45
50 °C	1.545 <sup>d</sup>	95.37	4.63	1.560 <sup>c</sup>	95.71	4.29
60 °C	1.520 <sup>e</sup>	93.83	6.17	1.535 <sup>d</sup>	94.18	5.82
70 °C	1.475 <sup>f</sup>	91.05	8.95	1.500 <sup>e</sup>	92.02	7.98
80 °C	1.450 <sup>g</sup>	89.51	10.49	1.465 <sup>f</sup>	89.88	10.12
90 °C	1.430 <sup>h</sup>	88.27	11.73	1.450 <sup>f</sup>	88.96	11.04
100°C	1.400 <sup>i</sup>	86.58	13.58	1.430 <sup>g</sup>	87.73	12.27
LSD	0.013			0.015		

At R\*: At room temperature. (R\*): Remained%. (D\*\*): Degradation %. LSD  $\square$  0.05

TABLE 6. Effect of exposure to heat (100°C) on stability of  $\beta$ -carotene at different pH

Time (min) /100°C	pH 8			pH 10		
	Absorbance $\lambda=420$ nm	R*	D**	Absorbance $\lambda=420$ nm	R*	D**
0	1.215 <sup>a</sup>	100	0.00	1.230 <sup>a</sup>	100	0.00
30	1.055 <sup>b</sup>	86.84	13.16	1.080 <sup>b</sup>	87.81	12.19
60	1.000 <sup>c</sup>	82.31	17.69	1.020 <sup>c</sup>	82.93	17.07
90	0.950 <sup>d</sup>	78.19	21.81	0.970 <sup>d</sup>	78.87	21.13
120	0.900 <sup>e</sup>	74.08	25.95	0.940 <sup>e</sup>	76.43	23.57
LSD	0.016			0.015		

(R\*): Remained%. (D\*\*): Degradation %. LSD  $\leq$  0.05

TABLE 7. Effect of light on stability of  $\beta$ - carotene

Time (Hour)	Dark			Light		
	Absorbance $\lambda=420$ nm	R*	D**	Absorbance $\lambda=420$ nm	R*	D**
Control	1.44 <sup>a</sup>	100	0.00	1.44 <sup>a</sup>	100	0.00
1	1.410 <sup>b</sup>	97.92	2.08	1.390 <sup>b</sup>	96.53	3.47
2	1.390 <sup>c</sup>	96.53	3.47	1.375 <sup>c</sup>	95.49	4.51
3	1.38 <sup>c</sup>	95.84	4.16	1.355 <sup>d</sup>	94.10	5.90
4	1.365 <sup>d</sup>	94.80	5.20	1.340 <sup>e</sup>	93.06	6.94
5	1.360 <sup>de</sup>	94.45	5.55	1.335 <sup>e</sup>	92.71	7.29
6	1.35 <sup>e</sup>	93.75	6.25	1.300 <sup>f</sup>	90.28	9.72
LSD	0.013			0.015		

(R\*): Remained%. (D\*\*): Degradation %. LSD  $\leq$  0.05

Generally, it could be clearly concluded that  $\beta$ - carotene is slowly degraded in dark and highly degraded under light due to the photo degradation reactions predominated over the isomerization reactions and 9-cis isomer was the major formed isomer. While, the 13-cis isomer was formed in greater amount under dark conditions and at rate approximately 3 times faster than that of 9-cis isomer (Pesek et al.,1990).

#### *Effect of $\beta$ -carotene extract on oxidative stability of sunflower oil*

Different levels of crude  $\beta$ -carotene extracted from orange peels were added to sunflower oil and its oxidative stability and the induction period were compared with those of BHT (200 mg/L).

Data in Table 8 show the effect of addition of crude  $\beta$ -carotene extract with levels 100, 200 and 300 mg/L on the oxidative stability of sunflower oil. The addition of BHT resulted in the highest

induction period (5.05 hrs.) and increased expired period at 25°C to 6.16 months. On the other hand, crude  $\beta$ -carotene extracted as pro-oxidant in higher concentrations (200 and 300 mg/L), which had induction periods 4.18 and 4.40 hrs.; respectively. Meanwhile, lower levels of crude  $\beta$ -carotene extract 100 mg/L had an antioxidant effects on the stability of sunflower oil which had induction periods of 3.90 hrs. Also, the levels of crude  $\beta$ -carotene extract 100, 200 and 300 mg/L had a relative stability were 0.84, 0.90 and 0.95, respectively compared with those value of BHT (1.09) (Table 8). Burton and Ingold (1984) stated that carotenoids pigments could be react with free radical responsible for oil rancidity. This would lead to give protection against rancidity and prolonging the oil induction period. Also, it was revealed that carotenoids appeared to be efficient in retarding photo-oxidation oil peroxidation while demonstrating pro-oxidant

activity under oven auto oxidation and showing slight antioxidant activity under Rancimat auto-oxidation tests (Maforimbo, 2002).

*Sensory evaluation of ice cream incorporated with  $\beta$ -carotene extract*

Five different treatments of ice cream were prepared with different levels of artificial color and natural  $\beta$ -carotene pigments (0.1 % artificial color, 0, 0.5, 1.0 and 1.5 % natural  $\beta$ -carotene pigments).

These treatments were sensory evaluated for taste, color, texture, odor, palatability and overall. The information has been evaluated statistically and the outcomes are shown in Table 9. From the data in Table 9, it could be clearly observed that addition of natural  $\beta$ -carotene pigments with 1.0% (T4) was more acceptable and recorded the highest scores for taste, palatability and overall acceptably (8.20, 8.40 and 40.10, respectively) compared with other treatments. Meanwhile, the lowest score was given to treatment (2) (without addition).

Finally, from the aforementioned data it

could be clearly concluded that addition of natural  $\beta$ -carotene pigments with 1.0% (T4) had the highest scores and were preferred by the panelists. Therefore, these treatments were selected being the most favorite ones to continue the study. Selected one was stored under freezing temperature. ( $-18 \pm 1$  °C) then, the effect of storage on color intensity was studied every two days for 21 days.

*Color measurements of ice cream*

The natural  $\beta$ -carotene pigments ( $\beta$ -carotene from orange peels) were used to enhancement of ice cream color and the results in Table 10 shows that the lightness values ( $L^*$ ) were significantly differed between all ice cream treatments obtained using  $\beta$ -carotene pigments in different concentrations 0, 0.5, 1.0 and 1.5% of  $\beta$ -carotene pigments in addition of artificial color 0.1% as control sample. At first, storage period or at zero-time lightness ( $L^*$ ) of ice cream with  $\beta$ -carotene pigments ranged from 81.66 to 85.29, in comparison with ice cream with artificial color which had ( $L^*$ ) values of (80.54). Meanwhile, the treatment ( $T_2$ ) without any addition recorded 87.15.

**TABLE 8. Effect of addition of crude  $\beta$ -carotene extract on oxidative stability of sunflower oil**

Extracts levels	Oxidative stability			
	Added weight (mg/L)	Stability at 110°C (hours)	Stability at 25°C (months)	Relative stability %
Control	-	4.63	5.65	100
BHT	200	5.05	6.16	1.09
$\beta$ -carotene extract	100	3.90	4.76	0.84
	200	4.18	5.10	0.90
	300	4.40	5.36	0.95

**TABLE 9. Mean organoleptic scores\* for ice cream incorporated with natural  $\beta$ -carotene extract.**

T*	Properties					Overall acceptability
	Color	Taste	Odor	Texture	Palatability	
T1	7.50 <sup>ab</sup> ±1.78	7.80 <sup>a</sup> ±1.14	7.60 <sup>a</sup> ±1.58	7.70 <sup>a</sup> ±1.34	7.55 <sup>a</sup> ±1.26	38.15 <sup>a</sup> ±7.68
T2	6.65 <sup>b</sup> ±2.45	7.65 <sup>a</sup> ±1.56	7.15 <sup>a</sup> ±1.80	7.45 <sup>a</sup> ±1.64	7.50 <sup>a</sup> ±1.58	36.40 <sup>a</sup> ±8.15
T3	8.50 <sup>a</sup> ±1.52	7.60 <sup>a</sup> ±1.34	8.20 <sup>a</sup> ±1.34	7.90 <sup>a</sup> ±1.25	7.65 <sup>a</sup> ±1.14	39.85 <sup>a</sup> ±5.94
T4	7.90 <sup>ab</sup> ±1.07	8.20 <sup>a</sup> ±1.34	7.80 <sup>a</sup> ±1.57	7.80 <sup>a</sup> ±1.25	8.40 <sup>a</sup> ±1.44	40.10 <sup>a</sup> ±6.48
T5	7.50 <sup>ab</sup> ±1.78	7.80 <sup>a</sup> ±1.14	7.60 <sup>a</sup> ±1.58	7.70 <sup>a</sup> ±1.34	7.55 <sup>a</sup> ±1.26	38.15 <sup>a</sup> ±6.88

Values are mean  $\pm$ SD of three replicates. Different letters in each column show significant difference. All statistical analysis was performed at  $P \leq 0.05$ . T\* = treatments, scores according to a 10 point by 10 panelists. T1: (Control) Ice cream colored with artificial color 0.1 %. T2: Ice cream without addition. T3, T4 and T5: Ice cream enhanced with 0.5%, 1% and 1.5%, respectively of natural  $\beta$ -carotene pigment.



Ice cream with  $\beta$ -carotene pigments displayed lighter less than ice cream without  $\beta$ -carotene pigment. All ice cream with  $\beta$ -carotene pigments had low  $L^*$  value while the ice cream without  $\beta$ -carotene pigments appeared to have high ( $L^*$ ) value. It seems that the addition of  $\beta$ -carotene pigment, throw producing of ice cream has less enhancement and decreased the ( $L^*$ ) values. On the other hand, the darker color of the ice cream with  $\beta$ -carotene pigments can be attributed to red color and the processing temperature. Meanwhile, the results in Table 10 after three months of storage, show that ice cream with  $\beta$ -carotene pigments had a significant high in ( $L^*$ ) values compared to control sample. The value averaged from 79.50 to 82.59 in ice cream with  $\beta$ -carotene pigments. While, a dramatic reduction in ( $L^*$ ) values (80.62) as a result of absence of  $\beta$ -carotene pigments were observed. These results may be due to the storage temperature degrees which effect on the stability of  $\beta$ -carotene pigments. Several variables influence the whiteness of milk goods, including light dispersion by fat and protein particles (Metzger et al., 2000). In our study, low temperature storage at, delayed changes in  $L^*$  values of ice cream with minimization of milk fat and protein denaturation

The obtained results in Table 10 show that ice cream with  $\beta$ -carotene pigments had a significant difference in red value ( $a^*$ ). The value at zero-time averaged from -3.62 to 4.32 in ice cream with  $\beta$ -carotene pigments, however, ( $T_1$ ) with artificial

color presented to be (-12.19). On the other hand, a dramatic reduction in ( $a^*$ ) values (-3.13) as a result of the absence of natural red pigments were observed. In fact, red value ( $a^*$ ) decreased when the ( $\beta$ -carotene pigments) concentrations were decreased. Moreover, the obtained results after three months of storage had the same trend, and these results might be due to addition of  $\beta$ -carotene pigments.

The dominant color for some kinds of ice cream is yellow and hence can be best represented by Hunter color  $b^*$  (yellowness) to distinguish the color difference of the resulting ice cream as affected by the addition of  $\beta$ -carotene pigment. Highly significant differences were observed in  $b^*$  value (yellowness) between ice cream with and without addition of natural yellow color. Chroma value or vividness in yellow color of ice cream considerably affected by  $\beta$ -carotene pigments addition and its ratio as well as storage period. Similarly, the Chroma values of ice cream with addition of  $\beta$ -carotene pigments treatments were observed in the same trends of a more vivid yellow color than samples of ice cream without addition of  $\beta$ -carotene pigment which recorded (15.09) (Table 10).

*Effect of storage at -18 °C on stability of  $\beta$ -carotene in ice cream*

Stability of added  $\beta$ -carotene pigments in ice cream with 1.0% during storage at -18°C was studied every two days for 21 days and the results are presented in Table 11.

**TABLE10. Color measurements of ice cream**

Treatments	Properties				
	$L^*$	$a^*$	$b^*$	Chroma	Hue
<b>Zero time</b>					
T1	80.54	-12.19	58.21	59.48	102.48
T2	87.15	-3.13	14.76	15.09	101.99
T3	85.29	-3.62	27.52	27.67	97.49
T4	83.08	-1.99	37.56	37.70	93.03
T5	81.66	4.32	58.05	58.21	85.74
<b>After 21 days</b>					
T1	78.14	-12.21	55.18	56.51	101.82
T2	83.80	-1.98	12.04	12.20	99.35
T3	82.59	-3.23	25.81	26.01	97.13
T4	80.62	-1.45	36.81	36.84	92.20
T5	79.50	4.28	56.08	56.26	85.64

$L^*$ : degree of lightness       $a^*$ : degree of redness       $b^*$ : degree of yellowness      T1: (Control) ice cream colored with artificial color 0.1 %.      T2: ice cream without addition.      T3, T4 and T5: ice cream enhanced with 0.5%, 1% and 1.5%, respectively of natural  $\beta$ -carotene pigment.

Data reveal that the amount and remained of  $\beta$ -carotene pigments decreased from 1.280 to 1.220 mg/100g and 100 to 95.31% during storage period and it considered not significant differences. Meanwhile,  $\beta$ -carotene degradation rate was increased from 0.0 to 4.69%.

From the aforementioned data, it could be clearly seen that results are not in complete different in  $\beta$ -carotene amount and degradation rate were observed during storage period for 21 days under freezing. These results could be due to the fact of carotenoids are very sensitive to heat so processes involving heat treatments should be kept at minimum both or for peak carotenoid stability, temperature, time and storage at frozen temperatures are recommended (Wenzel *et al.*, 2010 and Cervantes-Paz *et al.*, 2014).

### Conclusion

The manufacture of oranges produces large quantities of residues, most of which are peels,

which contain important chemical and biological active compounds.  $\beta$ -carotene extract from orange peels showed high antioxidant activity based on DPPH and oil oxidative stability assays. Considering that beta-carotene is not exposed to high temperatures as well as not being exposed to light or storage for long periods because it causes the deterioration of beta-carotene, while increasing stability as the higher the degree of pH in the food. The best percentage of natural beta carotene extract for ice cream was 1%. Therefore, orange peels can be suggested as a good source of  $\beta$ -carotene, which can be used as functional ingredient and natural colorant in the food industry.

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**TABLE 11. Effect of storage at -18 °C on stability of  $\beta$ -carotene in ice cream**

Storage duration (days)	$\beta$ -carotene content(mg/100g)	Properties	
		Retained $\beta$ -carotene (%)	Degradation (%)
Control	1.280	100.00	0.00
2	1.275	99.61	0.39
4	1.268	99.06	0.94
6	1.260	98.44	1.56
8	1.255	98.05	1.95
10	1.250	97.66	2.34
12	1.247	97.42	2.58
14	1.245	97.27	2.73
16	1.240	96.88	3.12
18	1.230	96.10	3.90
20	1.225	95.70	4.30
21	1.220	95.31	4.69

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## توصيف للبيتاكاروتين المستخلص من قشور البرتقال واستخدامه كملون طبيعي ومضاد للأكسدة في الأيس كريم

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ينتج عن قطاع تصنيع البرتقال الكميات الكبيرة من النفايات، والتي يكون معظمها من قشور البرتقال والتي تنسب مشاكل بيئية. هذا لخلفات هي مصادر جيدة للصبغات والمواد الكيميائية النشطة بيولوجيا. من هذه المواد البيتاكاروتين وهو احد الكاروتينويدات القابلة للذوبان في الدهون. أجريت هذه الدراسة لاستخلاص البيتاكاروتين من قشور البرتقال ودراسة مدى ثباته وتأثره ببعض المعاملات. بما في ذلك درجة الحرارة والتخزين والضوء ودرجة الحموضة وخصائصها لمضادة للأكسدة. تمت تقييم وتحديد مركب اتالكاروتينويدات وتفريدها بواسطة HPLC. كان المحتوى الكلي للمركبات الفينولية والفلافونيدات في قشور البرتقال المجففة 16.12 و 2.06 ملجم / جم. وكان البيتاكاروتين 14.51 ملجم / 100 جم. بالإضافة إلى ذلك، كان نشاط المضادة للأكسدة لمطحون قشور البرتقال 88.15% (على أساس تقدير DPPH). مع زيادة مدة التخزين والتعرض لدرجات الحرارة والضوء المرتفعة. زاد تدهور بيتاكاروتين. بينما كان أكثر استقرارًا في الوسط القلوي. كانت نسبة نشاط مضادات الأكسدة للبيتاكاروتين قريبة من مادة ال-BHT الصناعية المعروفة على أساس قياس الثبات الاكسيدي للزيت. في التقييم الحسي. فضل القائمين بالتذوق الأيسكريم المدمج بمستخلص البيتاكاروتين 1%. أثناء تخزين الأيسكريم لمدة 21 يومًا على 18- درجة مئوية، انخفض محتوى  $\beta$ -كاروتين بنحو 5%.