STABILITY OF SPRAY-DRIED CAROTENOIDS EXTRACTED FROM ORANGE PEELS WASTE

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ABSTRACT

This study was conducted to explore the possibility of utilization of waste residues (orange peels) obtained during orange juice extraction for the preparation of a value added product namely, encapsulated carotenoids in a powder form, high stable and suitable for use in food industry. Results showed that the most appropriate condition for preparing carotenoids powder by spraying-drying consists of 14% solid content of feed, with inlet air temperature of 135-145°C and outlet air temperature of 90-100 °C. The total amount of *all-trans* plus *cis* forms of lutein, α or β-carotene in the carotenoids powder decreased with increasing storage time and temperature, and the degradation rate of each one fits the first-order model. The major *cis* isomers formed in the dark were 13-*cis*-α and 13-*cis*-β-carotene, whereas, 9-*cis* isomers of both α-and β-carotene predominated under light. A high correlation was also observed between color changes and carotenoid content.

INTRODUCTION

The food processing industry generates approximately 45 % of the total organic industrial pollution (Akerberg and Zacchi 2000) Fruits processing industries in Egypt generate many tons of wastes (eg peel, seed, rag, core, pulp, and others.) per year, since in some fruits the discarded portion can be very high (eg orange 30-50%, banana 20%, and mango 30-50%). Therefore, there is often serious of environmental problems. Bioconversion of wastes is receiving increased attention in view of the fact that these wastes can act as substrates for the production of useful biomaterials (Jin

et al., 2005).The annual production of orange in Egypt amounted to be 1.64 million tons (Abdi and Ibrahim, 2001). Orange peels waste is rich in carotenoids which can serve as a cheap substrate for natural food colorants. Carotenoids are important factors in human health and essential for vision. The role of β-carotene and other carotenoids as the main dietary source of vitamin A has been known since long period (Moeller *et al.*, 2000). More recently, protective effects of carotenoids against serious disorders such as cancer, heart disease and degenerative eye disease have been recognized, and have stimulated intensive research into the role of carotenoids as antioxidants and as regulators of the immune response system (Cinar, 2004, Nunes and Mercadante, 2007) In addition, carotenoids add color to foods and beverages (e.g. orange juice, shortenings, margarine, butter, ice-cream, gelatin, and desserts.). Also, carotenoids are the precursors of many important chemicals responsible for the flavor of foods and the fragrance of flowers (Mozaffarieh *et al.*, 2003). The polyenic chain of carotenoids is responsible both for visible light absorption and for high molecule reactivity (Nunes and Mercadante, 2007). Most carotenoids in the nature are found in the *trans* configuration (Mercadante and Egeland, 2004) due to its higher stability compared to the *cis* isomer. However, during the processing and storage, carotenoids can easily rearrange in different geometric isomers and also be oxidized, with the consequent decrease or loss of the colorant and biological properties. The most applied alternative to increase stability of carotenoids and to allow them suitable for use as colorants or additives for food, beverages, cosmetics and drugs is the microencapsulation technique, which provides a physical barrier protecting the pigment. Among the available microencapsulation techniques, the most used process is spray-drying (Nunes and Mercadante, 2007). The aim of the present study was utilization of orange peels waste obtained during orange juice extraction for the preparation of a value added product (carotenoids in a powder form) and studying it's stability under different storage conditions.

MATERIALS AND METHODS

Materials :

All-trans-α- and β-carotenes standards, Lutein standard and gelatin were purchased from Sigma Chemicals Co., St. Louis, Missouri, USA. The other used chemicals were from El-Nasr Chemicals Co., Cairo, Egypt.

Method:

Waste resource and carotenoids extraction

carotenoids were extracted from peels following the methods described by Chen *et al.,* (1996) ; Ping and Gwendoline, (2006). Orange peels waste was obtained from a factory for juice extraction located in Giza city. Orange peels waste was washed and the surface layer (color part) was manually dehulled, then thirty grams of color part was blended in kitchen blender for 1 min with a mixture of 30 ml of acetone and 45 ml of hexane. The resulted suspension was filtered and then the filtrate was poured into a separating Funnel. The precipitate was washed twice with 15 ml of acetone and once with 15 ml of hexane and filtered. The filtrate was pooled and poured into the same separating funnel. The combined filtrates were washed four times with 100 ml of water and the lower layer was discarded. The upper layer containing the carotenoids was collected and mixed with 30 ml of methanolic potassium hydroxide (40%) in a separating funnel which was shaken vigorously at room temperature for 2 h for saponification. The solution was then washed with water several times and the lower layer was discarded. The upper layer containing the carotenoids was collected and filtered through anhydrous sodium sulfate. The solution was evaporated to dryness in a rotary evaporator (Buchi EL 130 brand) at 40°C. For HPLC analysis appropriate amount of dry carotenoids preparation was dissolved in methanol/methylene chloride (45: 55 v/v) and filtered through a 0.2 μm membrane filter.

Encapsulation of carotenoids by spray-dryer

The carotenoids were encapsulated following the methods described by Bhandari *et al.,* (1992) and Barbosa *et al.,* (2005).

Sucrose and gelatin (7:5), total of 60 g were solubilized in 200 ml water at 45°C and kept under stirring. The microcapsules were prepared by gradually adding 25 ml concentrated carotenoids extract prepared from 90 g peels waste to the previous solution and vigorously homogenized at 5000 rpm for 35 min at room temperature using polytron PT10-35 homogenizer. The solid content of the emulsion was controlled at 15% (w/v) by adding distilled water. The emulsion was maintained under slow agitation during the spray-drying process. The spray-dryer (Mobile Minor of GEA Niro A/S, DK-2860 Söborg) was operated at air flow rate of 30 ml/min, entrance and exit air temperatures of 1135-145 °C and 90-100 °C, respectively. Approximately 40 g of carotenoids powder was obtained. The above process was repeated until about 600 g concentrated carotenoids were accumulated

Storage of carotenoids preparation under different conditions :

The carotenoids preparation was divided into two parts. The first part was stored under dark and nitrogen gas conditions in 25ml-brown bottles containing 5g carotenoids preparation in each. The filled bottles were incubated (Percival scientific Model 1-30VL) at temperatures 4°C, 25°C and 45°C for 12 weeks, while the other part was stored in translucent bottles under light (light intensity 1500 LX) and nitrogen gas at 25°C. Two bottles were randomly removed from each temperature treatment every 2 weeks and 4 g preparation powder was taken from each bottle for carotenoids analysis according to the method of Nunes and Mercadante, (2007).

Preparation of stored carotenoids for analysis :

Four grams of powder was mixed with 40 ml of water in blender and 80 ml of petroleum ether/acetone (1: 1 v/v) was added. The solution was blended for 25 second and then centrifuged at 10 000 rpm for 15 min. The upper layer was collected and placed in a separating funnel then washed three times with 40 ml of saturated saline. The upper phase containing the carotenoids was then collected and the solvent was evaporated to dryness. The residue

was dissolved in 400 µl of methanol/methylene chloride (45: 55 v/v) and centrifuged at 4000 g for 30 min. The supernatant was filtered through a 0.2 µm membrane filter for carotenoids analysis according to the method of Nunes and Mercadante, (2007).

Standard :

Six *all-trans*-α-carotene concentrations, 1, 5, 10, 25, 50 and 70 µg/ml ; six *all-trans*-β-carotene concentrations, 10, 25, 50, 75, 100 and 130 µg/ml and Six lutein concentrations, 1, 2, 5, 10, 15 and 20 µg/ml were prepared by dissolving an appropriate amount from each in 100 ml of methanol/methylene chloride (99: 1 v/v) according to the method of Chen *et al.*, (1996).

HPLC analysis :

Carotenoids, either as fresh extracts or from dried powder were analyzed by HPLC as previously described by Nunes and Mercadante (2007). The analysis was carried out using a Waters HPLC system equipped with a photo-diode array detector (Waters, model 996). The equipment also included an on line degasser, a Rheodyne injection valve with a 20 ml loop and an external oven. The chromatograms were processed at 470 nm and the spectra were obtained between 250 and 600 nm, according to the method of Nunes and Mercadante, (2007.

Color stability of carotenoids powder during storage:

The color difference meter was used to *L, a* and *b* values of which *L* (+) stands for brightness *a* "+" stands for redness and *b* "+" stands for yellowness. The overall color change of carotenoids powder was monitored using *ΔE* values as described by (Chen *et al.*, 1996) with the formula.

$$\Delta E = \sqrt{(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2}$$

Where L_0, a_0 and b_0 are the hunter *L, a* and *b* values of carotenoids powder before storage and L_1, a_1 and b_1 are the hunter *L, a* and *b* values of carotenoids powder after storage for *n* weeks.

Statistical analysis:
The data were subjected to analysis of variance and Duncan's multiple-range test (SAS, 1985).

RESULTS AND DISCUSSION
Influence of the encapsulation process on Carotenoids:
The recovery of *all-trans-α-*, β- and lutein presented in carotenoids powder ranged form 85 to 90 %, this value was lower than those of encapsulated lycopene (from 96.4 to 98.1%) using spray-dryer (Lab Plant SD-04, United Kingdom), with Gum arabic and sucrose (8:2) at 20% of total soluble solids, inlet and exit air temperatures of 170 ± 2 and $113 \pm 2°C$, respectively (Nunes and Mercadante, 2007). Results in Table (1) show the influence of the encapsulation process on carotenoids compared with that freshly extracted. The amount of each carotenoids in spray-dried carotenoids powder was found to be substantially higher than those in freshly extracted, except for *all-trans*-β-carotene. This result implied that part of *all-trans*-β-carotene may be converted to other *cis* isomers during spray-drying. The results also reveal that both carotenoids isomers *15-cis-α*-carotene and, *15-di-cis*-β-carotene were not found in the freshly extract while they were detected in carotenoids powder. These results were in agreement with the findings of Polyakov *et al*, (2004).

Table (1): Carotenoids from orange peels waste and powder (µg/g)

Carotenoids	Peels waste	Powder
9-cis-lutein	0.41	0.77
13-cis-lutein	0.51	1.22
All-trans-lutein	3.72	5.97
9-cis-α-	0.56	1.38
13-cis-α-	0.80	2.03
15-cis-α-	-	1.05
All-trans-α-	27.32	36.50
9-cis-β-	0.91	2.07
13-cis-β-	2.56	6.35
15-cis-β-	1.06	2.46
15-di-cis-β	-	1.10
All-trans-β	54.26	50.87

Stability of *all-trans*-lutein and its *cis* isomers in carotenoids powder during storage:

Results in Table 2 show the changes in concentration of *all-trans* lutein and its *cis* isomers in powder during storage. In the samples stored in the dark the loss of *all-trans*-lutein increased with increasing storage temperature and time. The losses after 12 week at 4, 25 and 45°C were 0.74, 0.76 and 0.79µg/g respectively. In contrast, the concentration of *13-cis*-lutein increased with increasing storage temperature and time were 0.27, 0.33 and 0.39 µg/g, respectively. There were no significant changes (*P < 0.05*) in the concentration of *9-cis* lutein at 4 and 25°C. By comparison of the results described above, it was observed that the higher storage temperature, the faster the degradation of *all-trans*-β-carotene. Also, *13-cis*-lutein was more abundant than *9-cis* lutein during storage of carotenoids powder in the dark. This phenomenon may be explained as follows, the low activation energy of the *13-cis* type isomer could be favored during dark storage because of the low energy provided (Zechmeister, 1994). In a similar study (Pesek *et al.,* 1990, Tang and Chen, 2000) also reported that the *13-cis*-β carotene formation was faster than that of *9-cis* β-carotene when *all-trans*-β-carotene was held in the dark. During illumination at 25°C the concentration of *all-trans*-lutein declined by 1.69µg/g during 12 weeks of storage and *13-cis*-lutein increased by 0.32µg/g, but there was only a minor change in *9-cis*-lutein. This result implied that light storage can be more destructive to *all-trans*-lutein than dark storage. The degradation rate constants (day^{-1}) of the total amount of *all-trans* and *cis* forms of lutein during storage under light at 25°C and in the dark at 4, 25 and 45°C for 12 weeks were 0.015, 0.005, 0.008 and 0.011, respectively (Table 3). Also, all of the degradation at various temperatures fit the first-order model since a linear correlation was observed for the plot of the logarithm of the total lutein (*all-trans* and *cis* forms) concentration versus time.

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Table (2): Stability of *all-trans*-lutein and its *cis* isomers in carotenoids powder during storage

Temp. (°C)	Time (weeks)	Lutein[a] (µg/g)		
		9-cis	*13-cis*	*All-trans*
4 (dark)	0	0.73 [a]	1.23 [a]	5.95 [a]
	2	0.73 [a]	1.27 [a]	5.85 [b]
	4	0.72 [a]	1.33 [b]	5.77 [b]
	6	0.74 [a]	1.38 [c]	5.59 [c]
	8	0.74 [a]	1.43 [d]	5.45 [d]
	10	0.74 [a]	1.47 [de]	5.32 [e]
	12	0.74 [a]	1.50 [e]	5.22 [f]
25 (dark)	0	073 [a]	1.23 [a]	5.95 [a]
	2	0.74 [a]	1.29 [b]	5.83 [b]
	4	0.74 [a]	1.38 [c]	5.65 [c]
	6	0.75 [a]	1.45 [d]	5.44 [d]
	8	0.74 [a]	1.49 [de]	5.24 [e]
	10	0.75 [a]	1.53 [ef]	5.09 [f]
	12	0.76 [a]	1.56 [f]	4.91 [g]
45 (dark)	0	0.73 [a]	1.23 [a]	5.95 [a]
	2	0.74 [a]	1.29 [b]	5.74 [b]
	4	0.75 [ab]	1.39 [c]	5.52 [c]
	6	0.77 [ab]	1.51 [d]	5.22 [d]
	8	0.78 [ab]	1.56 [de]	4.97 [e]
	10	0.79 [b]	1.59 [ef]	4.73 [f]
	12	0.79 [b]	1.62 [f]	4.50 [g]
25 (light)	0	0.73 [a]	1.23 [a]	5.93 [a]
	2	0.73 [a]	1.26 [a]	5.73 [b]
	4	0.77 [ab]	1.31 [b]	5.28 [c]
	6	074 [ab]	1.37 [c]	4.97 [d]
	8	076 [abc]	1.44 [d]	4.69 [e]
	10	0.79 [bc]	1.49 [e]	4.55 [f]
	12	0.80 [bc]	1.55 [f]	4.25 [g]

[a] Mean of duplicate analysis. Values in the same column bearing different letters are significantly different $(P < 0.05)$.

Table (3): Rate constants of lutein, α-and β-carotene in spray-dried carotenoids powder during storage at various temperature

Temp. (oC)	Rate constant (day-1)		
	Lutein a	α-caroteneb	β-carotenec
4 (dark)	0.005	0.014	0.018
25 (dark)	0.008	0.024	0.031
45 (dark)	0.011	0.043	0.050
25 (light)	0.015	0.049	0.058

Lutein includes *all-trans*-lutein and its *cis* isomers [a]

[b] α-carotene includes *all-trans* α-carotene and its *cis* isomers.

[c] β-carotene includes *all-trans*-β-carotene and its *cis* isomers.

Stability of *all-trans*-α-carotene and its *cis* isomers in carotenoids powder during storage:

Results in Table 4 show the changes in the concentration of *all-trans*-α-carotene and its *cis* isomers in powder during storage. In the samples stored in the dark the loss of *all-trans*-α-carotene increased with both increasing storage temperature and time. The losses after 12 weeks at 4, 25 and 45°C were 6.86, 11.86 and 18.37µg/g, respectively. In contrast, *13-cis*-α-carotene increased under the same storage conditions and periods by 0.32, 0.67 and 1.11µg/g, respectively. The concentration change of *9-cis*-α-carotene showed the same trend, with increases of 0.14, 0.32 and 0.59 µg/g respectively. There were no significant changes ($P < 0.05$) in the concentration of *15-cis*-α-carotene at 4°C. From the above results it can be observed that both the isomerization and degradation of *all-trans*-α-carotene was greater at 45°C than at 4 or 25°C. These results indicated that the higher storage temperature, the faster the isomerization and degradation of *all-trans*-α-carotene. It was also noticed that *13-cis*-α-carotene was more readily formed than *9-cis*-α-carotene in the dark. Apparently this phenomenon can be attributed to activation energy differences between *9-cis*-α-carotene and *13-cis*-α-carotene as explained before. During illumination at 25°C, the concentration of *all-trans*-α-carotene declined by 20.05 µg/g during 12 weeks of storage, but *9-cis*-α-carotene, *13-cis*-α-carotene and *15-cis*-α-carotene increased by 1.05, 1.60 and 0.31 µg/g, respectively. With the exception of *13-cis*-α-carotene, the amounts of both 9 and *15-cis*-α-carotene formed under light were higher than those in the dark. These results implied that the *9-cis* type isomer of α-carotene was favored during illumination both heat and light energies can be provided. In other studies Chandler and Schwartz, (1987) observed that the *9-cis* type isomer of β-carotene was favored during illumination also Pesek and Worthesen, (1990) found that the *9-cis* isomer of β-carotene was formed in larger amount under light storage. The degradation rate constants (day^{-1}) of the total amount of *all-trans* and *cis* forms of α-carotene during storage under light at 25°C and in the dark at 4, 25 and 45°C for 12 weeks were 0.049, 0.014, 0.026 and 0.043, respectively and all of the degradation fit the first-order model

since a linear correlation was observed for the plot of the logarithm of the total α-carotene (*all-trans* and *cis* forms) concentration versus time (Table 2).

Table (4): Changes of *all-trans*-α-carotene concentration and its *cis* isomers during storage

Temp. (°C)	Time	α-carotene[a] (µg/g)			
	(weeks)	9-cis	13-cis	15-cis	All-trans
4 (dark)	0	1.97 [a]	1.98 [a]	1.00 [a]	36.45 [a]
	2	1.38 [a]	2.01 [ab]	1.00 [a]	35.40 [b]
	4	1.40 [ab]	2.06 [b]	1.01 [a]	34.35 [c]
	6	1.44 [bc]	2.13 [c]	1.02 [a]	33.15 [d]
	8	1.47 [cd]	2.21 [d]	1.02 [a]	31.85 [e]
	10	1.49 [d]	2.25 [d]	1.02 [a]	30.73 [f]
	12	1.51 [d]	2.30 [e]	1.04 [a]	29.95 [g]
25 (dark)	0	1.37 [a]	1.98 [a]	1.00 [a]	36.45 [a]
	2	1.40 [a]	2.06 [b]	1.00 [a]	34.98 [b]
	4	1.45 [b]	2.16 [c]	1.02 [ab]	32.95 [c]
	6	1.55 [c]	2.30 [d]	1.05 [bc]	30.85 [d]
	8	1.65 [d]	2.42 [e]	1.07 [cd]	28.80 [e]
	10	1.66 [de]	2.57 [f]	1.07 [cd]	26.72 [f]
	12	1.69 [e]	2.65 [g]	1.10 [d]	24.59 [g]
45 (dark)	0	1.37 [a]	1.98 [a]	1.00 [a]	36.45 [a]
	2	1.46 [b]	2.10 [b]	1.02 [ab]	33.74 [b]
	4	1.56 [c]	2.25 [c]	1.05 [bc]	30.70 [c]
	6	1.70 [d]	2.47 [d]	1.09 [cd]	27.52 [d]
	8	1.85 [e]	2.75 [e]	1.13 [de]	24.16 [e]
	10	1.92 [f]	2.95 [f]	1.16 [ef]	21.02 [f]
	12	1.96 [f]	3.09 [g]	1.18 [f]	18.08 [g]
25 (light)	0	1.37 [a]	1.98 [a]	1.00 [a]	36.45 [a]
	2	1.49 [b]	2.04 [a]	1.03 [a]	34.00 [b]
	4	1.64 [c]	2.14 [b]	1.08 [b]	31.05 [c]
	6	1.82 [d]	2.26 [c]	1.14 [c]	27.80 [d]
	8	2.05 [e]	2.38 [d]	1.22 [d]	23.87 [f]
	10	2.22 [f]	2.48 [e]	1.27 [e]	20.03 [f]
	12	2.42 [g]	2.58 [f]	1.31 [e]	16.43 [g]

[a] Mean of duplicate analysis. Values in the same column bearing different letters are significantly different ($P < 0.05$).

Stability of *all-trans*-β-carotene and its *cis* isomers in carotenoids powder during storage:

Results in Table 5 show the changes in the concentration of *all-trans*-β-carotene and its *cis* isomers in carotenoids powder during storage. In the samples stored in the dark the loss of *all-*

Table (5): Changes of *all-trans*-β-carotene concentration and its *cis* isomers during storage

Temp. (°C)	Time (weeks)	β-carotene[a] (µg/g)				
		9-cis	13-cis	15-cis	13-15-di-ds	All-trans
4 (dark)	0	2.09 [a]	6.32 [a]	2.39 [a]	1.07 [a]	50.80 [a]
	2	2.09 [a]	6.38 [a]	2.40 [a]	1.07 [a]	49.40 [b]
	4	2.12 [ab]	6.46 [a]	2.39 [a]	1.06 [a]	47.79 [c]
	6	2.17 [bc]	6.59 [b]	2.38 [a]	1.05 [a]	44.84 [d]
	8	2.20 [cd]	6.69 [bc]	2.38 [a]	1.05 [a]	41.77 [e]
	10	2.24 [de]	6.77 [cd]	2.38 [a]	1.05 [a]	39.95 [f]
	12	2.28 [e]	6.87 [d]	2.37 [a]	1.05 [a]	38.73 [g]
25 (dark)	0	2.08 [a]	6.32 [a]	2.39 [a]	1.07 [a]	50.80 [a]
	2	2.11 [a]	6.42 [a]	2.41 [a]	1.07 [a]	48.02 [b]
	4	2.17 [b]	6.57 [b]	2.43 [ab]	1.09 [a]	44.67 [c]
	6	2.30 [c]	6.76 [c]	2.46 [bc]	1.13 [ab]	40.64 [d]
	8	2.36 [d]	6.96 [d]	2.49 [cd]	1.14 [bc]	36.43 [e]
	10	2.44 [e]	7.13 [e]	2.52 [de]	1.15 [bc]	32.62 [f]
	12	2.52 [f]	7.29 [f]	2.54 [e]	1.17 [c]	30.13 [g]
45 (dark)	0	2.08 [a]	6.32 [a]	2.39 [a]	1.07 [a]	50.80 [a]
	2	2.11 [a]	6.51 [b]	2.42 [ab]	1.10 [ab]	47.38 [b]
	4	2.25 [b]	6.77 [c]	2.45 [bc]	1.13 [bc]	43.55 [c]
	6	2.40 [c]	7.01 [d]	2.49 [c]	1.15 [cd]	37.32 [d]
	8	2.54 [e]	7.33 [e]	2.56 [de]	1.18 [de]	30.47 [e]
	10	2.69 [e]	7.60 [f]	2.60 [ef]	1.21 [ef]	25.41 [f]
	12	2.78 [f]	7.82 [g]	2.64 [f]	1.25 [f]	20.47 [g]
25 (light)	0	2.08 [a]	6.32 [a]	2.39 [a]	1.07 [a]	50.80 [a]
	2	2.25 [b]	6.40 [a]	2.43 [ab]	1.10 [ab]	47.66 [b]
	4	2.47 [c]	6.52 [b]	2.48 [bc]	1.13 [bc]	42.56 [c]
	6	2.74 [d]	6.70 [c]	2.53 [cd]	1.18 [c]	36.38 [d]
	8	2.98 [e]	6.92 [d]	2.57 [de]	1.24 [de]	38.84 [e]
	10	2.18 [f]	7.10 [e]	2.62 [e]	1.29 [ef]	22.63 [f]
	12	3.34 [g]	7.23 [f]	2.74 [f]	1.33 [f]	17.46 [g]

[a] Mean of duplicate analysis. Values in the same column bearing different letters are significantly different ($P < 0.05$).

tans-β-carotene increased with both increasing storage temperature and time. The losses after 12 weeks at 4, 25 and 45°C were 12.07, 20.67 and 30.33µg/g, respectively. In contrast, the concentration of *13-cis*-β-carotene increased with increasing storage temperature and time, 0.55, 0.97 and 1.50µg/g, respectively. The concentration change of *9-cis*-β-carotene showed the same trend, with increasing of 0.20, 0.44 and 0.70 µg/g. There were no significant changes ($P < 0.05$) in the concentrations of both *15-cis*-β-carotene and 13, *15-cis*-β-carotene at 4°C, on the other hand a slight increase at 25 and 45°C was observed. The formation of 13-*15-di-cis*-β-carotene may be due to conversion of *13-cis*-β-carotene or 15-cis-β-carotene. The *di-cis*-isomer of *all-trans*-β-carotene can be formed only under drastic treatments such as canning, illumination or storage under high temperature (Chen *et al.*, 1994, 1995, Tang and Chen, 2000). The formation of *9-cis*-β-carotene may be due to conversion of *13*- or *15-cis*-β-carotene through *all-trans*-β-carotene (Pesek and Wathesen, 1990; Pesek *et al.*, 1990; Mercadante and Egeland, 2004). Likewise, the formation of *13-cis*-β-carotene may be due to conversion of 9-or *13-cis*-β-carotene through *all-trans*-β- carotene. It has been well established that each mono-*cis* isomer can be converted to other forms of *cis* isomers only after it changes to the *all-trans* (Pesek and Warthesen, 1990 and Pesek *et al.*, 1990, Mercadante and Egeland, 2004). From the above results it can be concluded that the formation of *mono-cis* and *di-cis* isomers of β-carotene increased as the storage temperature increased.

This phenomenon was also observed for dark storage of *all-trans*-lutein and *all-trans*-α-carotene. During illumination at 25°C the concentration of *all-trans*-β-carotene declined by 33.34 µg/g during 12 weeks of storage while, *9-cis*-β-carotene and *13-cis*-β-carotene were increased. Interestingly, the amount of *13-cis*-β-carotene formed under light storage was found to be lower than that in the dark. This result implied that illumination can facility the degradation rate constants (day^{-1}) of the total amount of all-*trans* and *cis* forms of β-carotene during storage under light at 25°C and in the dark at 4, 25 and 45°C for 12 weeks were found to be 0.058, 0.018, 0.031 and 0.050, respectively and all of the degradation fit a first-order model because a linear correlation was

observed for the plot of the logarithm of the total β-carotene (*all-trans* and *cis* forms) concentration versus time. It has been well established that both degradation and isomerization of *all-trans*-β-carotene can proceed simultaneously during illumination, and the dominant reaction depends upon temperature, light intensity and the presence of catalyst (Pesek and Warthesen, 1990, Mercadante and Egeland, 2004, Pesek and Warthesen, 1990) reported that higher amounts of both *9-cis*-β-carotene and *13-cis*-β-carotene were found during illumination of *all-trans*-β-carotene solution. However, in this study less *9-cis*-β-carotene and *13-cis*-β-carotene was formed, mainly because dry powder was used under illumination and the stability of *all-trans*-β-carotene can thus be greatly enhanced. By comparison of the results mentioned above, it can be concluded that the degradation of *all-trans*-β-carotene was greater than the degradation of *all-trans*-α-carotene and *all-trans*-lutein, probably because the former possess a longer conjugated carbon-carbon double bond, which is more susceptible to temperature and illumination loss. The lesser of *all-trans*-lutein is probably due to the formation of a lutein-gelatin complex during spray-drying (Bryant *et al.,* 1992).

Color stability of carotenoids powder during storage:

Results in Table 6 show the Hunter *L, a* and *b* values in carotenoids powder during storage at various temperatures. The Hunter *L* value was found to decrease as the storage time increased. Also, the *L* value decreased by 3.5, 1.5, 2.0 and 2.7, respectively after storage under light at 25°C and in the dark at 4, 25 and 45°C for 12 weeks. This result implied that the brightness of carotenoids powder decreased with increasing storage time. The Hunter *b* value showed the same trend with decreases of 6.8, 2.7, 4.2 and 5.3, indicating that the yellow color of powder also decreased with increasing storage time. In contrast, insignificant (*P > 0.05*) change was observed for the Hunter *a* value, implying that red is not a major contributing factor to the color of carotenoids powder. From the above results it can be found that light storage can be more destructive to the color of carotenoids powder than dark storage. The decline of yellow color of carotenoids powder

during storage may be due to the degradation of *all-trans*-β-carotene and formation of its *cis* isomers. It has been well established that the formation of *cis* carotenoids can decrease color intensity (Khachike *et al.*, 1986 and Chen *et al.*, 1995). A high correlation (r^2) was observed between concentration change of carotenoids (*all-trans* and *cis* forms of α-and β-carotene) and Hunter *b* value, which amounted to 0.97, 0.97, 0.96 and 0.95 for dark storage at 4, 25 and 45°C and light storage at 25°C, respectively. To better understand the overall color change of carotenoids powder during storage, the *ΔE* value must be investigated. Figure 1 shows the *ΔE* value changes of carotenoids powder during storage at different temperature in the dark, the *ΔE* value shows greater change, implying that the color of carotenoids powder faded to a greater extent. This result also confirmed the previous finding that light storage can be more destructive to the overall color of carotenoids powder.
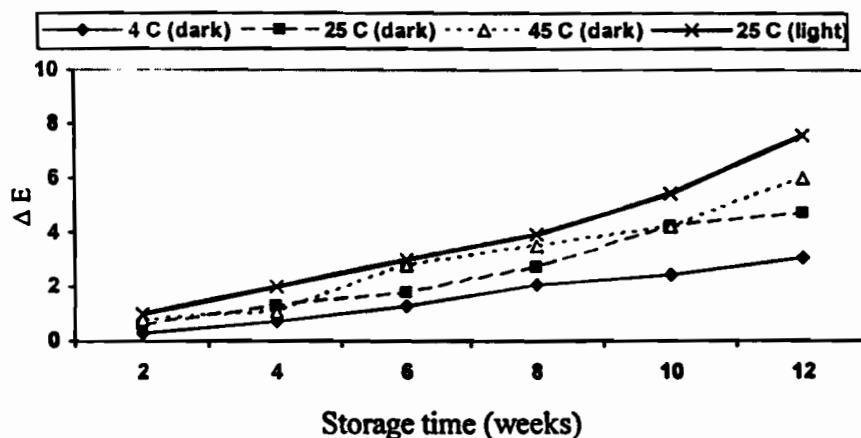

Figure (1): Changes in *ΔE* values of spray-dried carotenoids powder during storage.

In conclusion, the most appropriate condition for preparation of carotenoids powder by spray-drying consists of 15% solid content of feed material, with inlet air temperature of 135-145°C and outlet air temperature of 90-100°C. The degradations of *all-trans* and *cis* forms of lutein, α-and β-carotene

Table (6): Changes in hunter L, a and b values of spray-dried carotenoids powder during storage[a].

Storage time (weeks)	Storage temp.			
	4°C (dark)	25°C (dark)	45°C (dark)	25°C (light
	Hunter L value			
0	89.8 ª	89.8 ª	89.8 ª	89.8 ª
2	89.5 ab, A	89.4 a, A	89.4 b, A	89.1 b, A
4	89.2 ab, A	89.0 b, A	89.0 b, A	89.0 b, A
6	89.0 c, A	88.8 b, AB	88.2 c, C	88.5 c, BC
8	88.8 cd, A	88.6 b, A	88.1 c, B	87.8 c, C
10	88.5 de, A	88.1 cd, B	87.5 c, C	86.5 c, B
12	88.3 c, A	87.8 d, B	87.1 c, C	86.2 c, B
	Hunter a value			
0	0.2 a, A	0.2 a, A	0.2 a, A	0.2 a, A
2	0.2 a, A	0.2 a, A	0.1 a, A	0.1 a, A
4	0.1 a, A	0.1 a, A	0.1 a, A	0.1 a, A
6	0.2 a, A	0.2 a, A	0.0 a, A	0.0 a, A
8	0.1 a, A	0.1 a, A	0.1 a, A	0.1 a, A
10	0.1 a, A	0.1 a, A	0.0 a, A	0.0 a, A
12	0.1 a, A	0.1 a, A	0.1 a, A	0.1 a, A
	Hunter b value			
0	27.0 ª	27.0 ª	27.0 ª	27.0 ª
2	27.2 a, A	26.6 b, AB	26.3 b, B	26.3 b, B
4	26.6 ª	25.9 c, B	26.1 b, B	25.2 c, C
6	26.0 b, A	25.5 c, B	24.6 c, C	24.1 d, D
8	25.2 c, A	24.6 d, B	23.8 cd, C	23.6 c, C
10	25.0 c, A	23.2 c, B	23.5 d, B	22.7 f, C
12	24.3 c, A	22.8 f, B	21.7 c, C	20.29 D

Mean of duplicate analysis. Values in the same column bearing different letters are significantly different ($P < 0.05$). Values in the same row bearing different capital letters are significantly different ($P < 0.05$).

fit the first order model. The *13-cis* type isomers of carotenoids dominate during dark storage, while the *9-cis* type is favored during light storage, both the Hunter *L* and *b* values show decreases, whereas only insignificant change of the Hunter *a* value is observed.Finally, we have to point out here that the conditions used for preparing carotenoids powder in this study are only a preliminary step for possible commercial production in the future. Also, the problems of carotenoids losses during storage need to be overcome before the commercial viability can be properly assessed. It is quite possible that the stability of carotenoids powder can be greatly enhanced by employing appropriate packaging methods and storage conditions. For instance, the shelf lives of carotenoids powder may be substantially increased by vacuum packaging in foil laminated sachets and storage below $0^{\circ}C$. Thus further research is needed to evaluate the effects of various processing conditions on the commercial production of carotenoids powder. In addition, the possibility of increasing the stability of carotenoids powder by using various packaging techniques has to be studied.

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الملخص العربى

مدى ثبات الكاروتينات المجففة المستخلصه من مخلفات قشور البرتقال تحت ظروف التخزين المختلفة

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هدفت الدراسة إلى بحث إمكانية الإستفادة من القشور المتخلفة أثناء صناعة عصير البرتقال فى إنتاج مواد ذات قيمة عالية مثل الكاروتينات المجففة بالرزاز حيث أنها على هذه الصورة تكون أكثر ثباتا ومناسبة للإستخدام فى مجال الصناعات الغذائية ثم دراسة مدى ثبات المنتج الذى تم تحضيرة تحت ظروف التخزين المختلفة. اظهرت النتائج المتحصل عليها ان الظروف المثلى لتجفيف الرزازهى : تركيز المادة الصلبه فى معلق الكاروتينات المراد تجفيفة تكون ١٤% وزن/حجم و درجة حرارة الهواء الداخل تكون ما بين ١٣٥- ١٤٥ درجة مئوية ، درجة حرارة الهواء الخارج تكون ما بين ٩٠- ١٠٠ درجة مئوية. كما أوضحت النتائج أن الكميه الكليه من كاروتين-β ، -α-*trans*-*all* ومشابهه *cis* للكاروتينات المجففه انخفضت بزيادة وقت التخزين ودرجة الحرارة ، اكثر اشكال المشابه *cis* المتكونة بالتخزين فى الظلام هى β ,*α*-*cis*-*13* كاروتين ، أما فى حالة التخزين فى الضوء كانت β-*,α*-*cis*-*9*. ايضا أوضحت الدراسة أن هناك لرتباط قوى بين التغير فى اللون والمحتوى من الكاروتينات.