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Isolation of xanthophylls from Taiwanese orange peels and their effects on the oxidation stability of soybean oil

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The various xanthophylls present in Taiwanese orange peels were analyzed using liquid chromatography and their effects on the oxidation stability of soybean oil under light storage were determined. A mobile phase of hexane–acetone in different proportions and a mixture of cellulose and diatomaceous earth (1:1) as adsorbent were used to isolate the various pigments in orange peels by open-column chromatography. Each separated band was further analyzed by HPLC to determine the probable purity, and each pigment was identified by spectrum analysis. Results showed that violeoxanthin (and its epimers) was present in largest amount in orange peels, followed by violaxanthin, violaxanthin epimer, neoxanthin, sinesiaxanthin, β-cryptoxanthin, lutein epoxide and lutein. The antioxidant effectiveness of each pigment towards soybean oil was dependent upon concentration.

INTRODUCTION

The consumption of orange juice in Taiwan has been increasing steadily in recent years. According to a report by The Commission on Fruits and Vegetables of Taiwan (1992), consumption of orange juice reached 62,740,334 ton for the year 1991–1992. As orange peels are by-products obtained during processing of orange juice, an estimated 170,000 ton of orange peels can be produced annually in Taiwan because it constitutes about 30% of the total weight of orange. It has been reported that orange peels are good sources of carotenoids, of which xanthophylls constitute a large portion (Lawrence & Bailey, 1956; Molnar & Szabolcs, 1980; Noga & Lenz, 1983). The possible role of some carotenoids such as β-carotene in the treatment of human disease such as skin cancer has been reported (Mathews-Roth, 1981, 1982, 1985). Furthermore, β-carotene has been shown to be an effective radical-trapping antioxidant (Burton & Ingold, 1984). Therefore, the application of xanthophylls from orange peels to food products as colorants and antioxidants can be of great value to food manufacturers.

Fakourelis *et al.* (1985) reported that β-carotene minimized lipid oxidation of purified olive oil under light storage by its light-filtering effect. A similar result was found by Warner and Frankel (1987), who studied the effect of β-carotene on light stability of soybean oil. Lee and Min (1990) compared the effects of lutein, zeaxanthin, lycopene, isozeaxanthin and astaxanthin on the photooxidation of soybean oil and found that the antioxidant effectiveness of carotenoids increased as the number of the conjugated double bonds of carotenoids increased. Obviously the antioxidant ability of carotene and xanthophyll is due to the fact that they possess similar numbers of conjugated carbon–carbon double bonds.

The separation of carotenoids in orange peels by liquid chromatography has been difficult because most pigments are present in *cis* forms (Lawrence & Bailey, 1956; Molnar & Szabolcs, 1980; Noga & Lenz, 1983). Nevertheless, using column chromatography to isolate a large quantity of xanthophylls from orange peels is still an ideal method to choose. Lawrence and Bailey (1956) determined carotenoids in Valencia orange peels by open-column chromatography. Although 24 pigments were identified, the identification of most pigments is only tentative because of lack of advanced instruments such as photodiode-array detectors. Molnar and Szabolcs (1980) determined carotenoids present in Valencia orange peels using column chromatography and a total of 22 pigments were identified. However, the purity of each pigment was not determined. Rosenberg *et al.* (1983) used TLC to separate 10 bands from

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purified Valencia orange peel extract, and five of them were found to contain epoxides. The authors also reported that each separated band can be further separated, indicating that orange peel contains more than 10 pigments.

As most reports deal with the determination of carotenoids in Valencia orange peels, the composition of carotenoids present in Taiwanese orange peels needs to be investigated. The purpose of this study was to isolate xanthophylls from Taiwanese orange peels by open-column chromatography, to identify them by HPLC with photodiode-array detection, and to determine their effects on the oxidation stability of soybean oil under light storage.

MATERIALS AND METHODS

Materials

Twenty Taiwanese oranges were purchased from a local supermarket and were peeled in the laboratory. The orange peels were cut into small pieces and freeze-dried before grinding into fine powder with a grinder. Approximately 500 g of powdered orange peels was collected.

Soybean oil was purchased from a local supermarket, and was further purified by passing 100 ml through a chromatographic column (30 cm × 25 mm i.d.) packed with 1:1 mixture of active carbon and diatomaceous earth. The purified soybean oil was eluted with 100 ml hexane. The same procedure was repeated five times and a total of approximately 500 ml purified soybean oil was collected.

Some pigment standards such as α-carotene, β-carotene, and lutein (75% purity) were purchased from Sigma Chem. Co. (St Louis, MO, USA). Lutein was further purified using open-column chromatography as described by Chen *et al.* (1991). β-cryptoxanthin was prepared from yellow corn by open-column chromatography using a method described by Quackenbush *et al.* (1961). HPLC-grade solvents such as methanol, chloroform, ethyl acetate and acetonitrile were purchased from Merck (Darmstadt, Germany) and filtered through a 0·2 μm membrane filter prior to use. Solvents used for extraction of pigments such as hexane, acetone, toluene and ethanol were ACS grade.

Extraction of carotenoids

Two grammes of powdered orange peel was mixed with 30 ml extractant (hexane–acetone–ethanol–toluene, 10:7:6:7) and 2 ml methanolic KOH (40%) in a 100 ml volumetric flask. The mixture was left standing in the dark at ambient temperature for 16 h to allow extraction and saponification to proceed simultaneously (Chen & Yang, 1992). Then 30 ml hexane was added to the flask, which was swirled gently for 1 min. The flask was then diluted to volume with 10% Na_2SO_4. The solution was shaken vigorously for 1 min and stood in the dark for 1 h until two phases were separated. The upper phase containing carotenoids was collected, evaporated under vacuum and dissolved in hexane for open-column chromatography. For HPLC analysis, 1·0 ml carotenoid extractant was further purified through a silica gel Sep-Pak cartridge (Millipore Cooperation, MS, USA) and eluted with 5 ml hexane–acetone–methanol (70:20:10, v/v/v). The eluate was evaporated under vacuum and dissolved in 3·0 ml ethyl acetate for HPLC analysis.

Separation of carotenoids by open-column chromatography

A mixture of cellulose and diatomaceous earth (1:1) was used as the major adsorbent to separate carotenoids in orange peels. The glass column (30 cm × 25 mm i.d.) was prepared by dissolving a mixture of cellulose (8 g) and diatomaceous earth (8 g) in 50 ml hexane. After swirling gently for 1 min the solution was poured into the column with suction at the bottom to allow the adsorbent to be packed firmly. A 2 cm layer of anhydrous sodium sulfate was then placed above the adsorbent to absorb residual moisture present in the carotenoid extractant. Three ml carotenoid extract was poured onto the column and the various fractions of pigments were eluted with solvent systems in increasing order of polarity. The elution process started with 60 ml 100% hexane, followed by 60 ml hexane–acetone (98:2), 40 ml hexane–acetone (96:4), 60 ml hexane–acetone (90:10), and 60 ml hexane–acetone (87:13). Each separated band was evaporated under vacuum and dissolved in an appropriate solvent for spectra and probable purity analyses.

Separation of carotenoids in orange peel by HPLC

Three ml purified carotenoid extract (dissolved in ethyl acetate) was filtered through a 0·2 μm membrane filter, and 20 μl of extractant was injected into the HPLC. A Phenomenex stainless-steel C_{18} column packed with ultremex C_{18} 5 μm particle size (Torrance, CA, USA), and a ternary solvent system of acetonitrile–methanol–ethyl acetate (80:10:10) with sensitivity at 0·2 AUFS and detection wavelength at 450 nm was used. The flow rate was set at 0·7 ml/min for the first 20 min, and then increased to 2·0 ml/min thereafter until completion of the run. Each separated band obtained by open-column chromatography was injected into the HPLC to determine the probable purity. The probable purity was calculated by dividing the area of the major peak over the total peak area of the separated band.

Identification of carotenoids in orange peels by open-column chromatography

Separated bands collected from the column were evaporated to dryness and dissolved in an appropriate solvent to determine the absorption spectra with a Beckman DU-70 Spectrophotometer. The epoxy-containing carotenoids such as neoxanthin, violaxanthin

and lutein epoxide were identified by a hypsochromic shift of 17, 38 and 17 nm and colour change from yellow to green, blue and green, respectively, upon acidification with a few drops of 0·1 M HCl to the pigment solution.

Identification of carotenoids in orange peels by HPLC

Some peaks, such as β-cryptoxanthin, lutein and lutein epoxide were identified by cochromatography with added standards. In addition, each peak was scanned before and after addition of a few drops of 0·1 M HCl to the pigment solution by using a Linear 206 photodiode-array detector (Linear Instruments, Reno, NV, USA). The scanning range was between 190–365 and 366–800 nm, and the data were stored and processed with an Axxiom 727 dual-channel chromatography data system (Axxiom Chromatography Inc., Calabasas, CA, USA). The identification of *cis* carotenoids were based on the following rules:

1. *Cis* carotenoids will result in a hypsochromic shift of about 4 nm. (Zechmeister, 1944; Davies, 1976; Goodwin, 1981).
2. *Cis* carotenoids are accompanied by a reduction in fine structure and a hypochromic effect on absorbance (Goodwin, 1981).
3. The central *cis* carotenoids have a strong peak present in the UV region at about 340 nm (Zechmeister, 1944; Goodwin, 1981; Khachik *et al.*, 1986).
4. The di-*cis* carotenoids will be shifted to shorter wavelength than mono-*cis* carotenoids (Tsukida *et al.*, 1982).

Quantification of major carotenoids in orange peels

The eluate of each separated band from the column was evaporated to dryness and dissolved in ethanol. Concentrations of violeoxanthin and Taiwanese orange peel extract (TOPE) were determined using the following formula:

$$\text{Concentration (g/ml)} = E/E^{1\%}_{1\,cm} \cdot 100$$

where E = extinction at given wavelength (violeoxanthin 443 nm, TOPE 440 nm); and $E^{1\%}_{1\,cm}$ = extinction coefficient of 1% solution measured in cell with 1 cm light path (violeoxanthin 2250, TOPE 2250) (Davies, 1976).

Determination of oxidation stability of soybean oil

Fifty ml purified oil containing different levels of pigments was added to a 100 ml volumetric flask with a rubber cap sealing tightly on the top. Pigments including α-carotene, β-carotene, lutein, violeoxanthin and TOPE were used at concentrations of 10, 25 and 50 ppm. The flasks were placed in a chamber with two 20 W fluorescent tubes (General Electric) illuminating for 1, 2, 3, 4, 5, 6 and 7 days. The light intensity at the sample level was 4500 lux and the temperature was 30°C. All samples were analyzed in triplicate. The oxidation stability of soybean oil during light storage was determined by measuring its peroxide and TBA values according to the AOAC method (1984).

RESULTS AND DISCUSSION

Isolation of xanthophylls from orange peels

β-Cryptoxanthin was first eluted with 60 ml 100% hexane, followed by lutein 5,6-epoxide with 60 ml hexane–acetone (98:2), violeoxanthin (9-*cis*-violaxanthin) with 40 ml hexane–acetone (96:4) and violaxanthin with 40 ml hexane–acetone (90:10). The other minor pigments, such as neoxanthin and sinesiaxanthin, were eluted together with 40 ml hexane–acetone (87:13). Table 1 shows the identification data of various pigments isolated from orange peels by open-column chromatography. Each pigment was identified by comparing absorption spectra with reference values reported in the literature (Davies, 1976; Bauernfeind, 1981; Chen & Chen, 1993). The epoxy-containing carotenoids such as lutein 5,6-epoxide, violeoxanthin, 13-*cis*-violaxanthin, violaxanthin and sinesiaxanthin were identified based on hypsochromic shifts of 18, 30, 34, 38 and 20 nm and

Table 1. Identification data of carotenoids in Taiwanese orange peels by open-column chromatography with cellulose as the adsorbent

Pigment	Visible spectra			Probable purity[c] (%)	Epoxide test	
	Max. found	Solvent	Max. reported[b]		Hypsochromic shift	Colour
β-Cryptoxanthin	(426),[a] 448, 473	Ethanol	(428),[a] 449, 473	84	—	—
Lutein 5,6-epoxide	420, 442, 460	Ethanol	420, 442, 471	80	402, 424, 450	Green
Violeoxanthin	(414),[a] 436, 464	Ethanol	(415),[a] 436, 464	86	384, 406, 435	Blue
13-*cis*-violaxanthin	410, 432, 458	Ethanol	—[d]	57	374, 398, 424	Blue
Violaxanthin	418, 440, 468	Ethanol	417, 440, 469	59	378, 402, 430	Blue
Sinesiaxanthin	370, 392, 416	Ethanol	372, 392, 416	70	352, 372, 396	Green

[a] Values in parentheses represent shoulders on spectra curves.
[b] Reported values of absorption spectra are from four references by Davies (1976), Bauernfeind (1981), Khachik *et al.* (1986), and Chen and Chen (1993).
[c] Determined by HPLC.
[d] Data not available.

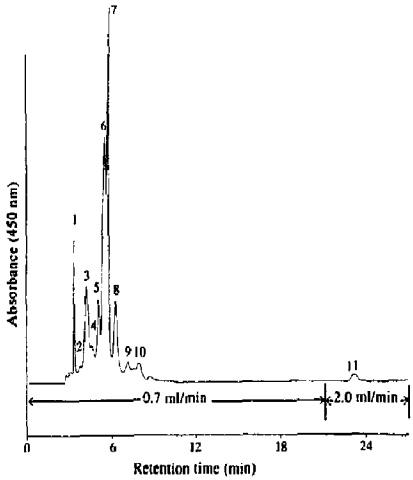

Fig. 1. HPLC chromatogram of carotenoids from Taiwanese orange peels by employing a solvent system of acetonitrile–methanol–ethyl acetate (80:10:10). Peaks: 1 = sinesiaxanthin, 2 = neoxanthin, 3 = violaxanthin, 4 = 13-*cis*-violaxanthin, 5 = violaxanthin epimer, 6 = violeoxanthin, 7 = violeoxanthin epimer I, 8 = violeoxanthin epimer II, 9 = lutein epoxide, 10 = lutein, 11 = β-cryptoxanthin.

colour change from yellow to green, blue, blue, blue and green, respectively. The probable purity of β-cryptoxanthin, lutein 5,6-epoxide, violeoxanthin, 13-*cis*-violaxanthin, violaxanthin and sinesiaxanthin, as determined by HPLC, was 84, 80, 86, 57, 59 and 70%, respectively. Only the major separated bands, consisting of violeoxanthin and TOPE, were collected and added to the soybean oil to determine the antioxidant effectiveness.

Figure 1 shows the HPLC chromatogram of carotenoids present in Taiwanese orange peels. A total of 11 peaks were resolved and identified as sinesiaxanthin, neoxanthin, violaxanthin, 13-*cis*-violaxanthin, violeoxanthin, violeoxanthin epimer I, violeoxanthin epimer II, lutein epoxide, lutein and β-cryptoxanthin. An epoxide test showed that all pigments but lutein and β-cryptoxanthin contained an epoxide group (Table 2). The assignment of 13-*cis*-violaxanthin was based on the presence of a strong peak in the UV region as well as exclusion of sterically hindered isomers such as 7-*cis*, 7'-*cis*, 11-*cis*, and 11'-*cis* (Khachik *et al.*, 1986; Chen & Chen, 1993). In contrast, the assignment of 9-*cis*-violaxanthin was based on the absence of a strong peak in the UV region as well as exclusion of sterically-hindered isomers as described above. Compared to violaxanthin, 9-*cis*- and 13-*cis*-violaxanthin resulted in hypsochromic shifts of 4 and 8 nm, respectively (Table 2). It was also found by HPLC that violeoxanthin epimer I was present in largest amount (38·8%) followed by violeoxanthin (19·3%), violaxanthin (11·8%), violaxanthin epimer (7·9%), neoxanthin (6·0%), violeoxanthin epimer II (5·7%), 13-*cis*-violaxanthin (4·5%), sinesiaxanthin (4·3%), β-cryptoxanthin (1·3%), lutein epoxide (0·3%) and lutein (0·2%).

Effect of various pigments on the oxidation stability of soybean oil

Figure 2 shows the effect of various pigments (50 ppm) on the peroxide formation of soybean oil under light storage. By comparing the antioxidant effectiveness of each pigment, it can be found that at concentrations of 50 ppm, lutein resulted in the lowest peroxide formation during the initial illumination period, followed by β-carotene, α-carotene, TOPE and violeoxanthin. The low peroxide value is probably due to an antioxidant action or decomposition of hydroperoxide. In this study the formation and decomposition of hydroperoxide

Table 2. Identification data of carotenoids in Taiwanese orange peels by HPLC analysis

Peak no.	Pigments	Visible spectra			Epoxide test hypsochromic shift
		In-line[a]	Max. reported[b]	Solvent	
1.	Sinesiaxanthin	372, 393, 416	372, 392, 416	Ethanol	353, 374, 398
2.	Neoxanthin	419, 440, 466	415, 438, 467	Ethanol	401, 422, 447
3.	Violaxanthin	414, 440, 469	417, 440, 469	Ethanol	376, 402, 430
4.	13-*cis*-violaxanthin	(408),[c] 432, 459	—[e]	Ethanol	372, 395, 421
5.	Violaxanthin epimer[d]	414, 440, 469	417, 440, 469	Ethanol	378, 402, 432
6.	Violeoxanthin	(412),[c] 436, 464	(415),[c] 436, 464	Ethanol	(380),[c] 404, 430
7.	Violeoxanthin epimer I[d]	(412),[c] 436, 464	(415),[c] 436, 464	Ethanol	(378),[c] 401, 428
8.	Violeoxanthin epimer II[d]	(412),[c] 436, 464	(415),[c] 436, 464	Ethanol	(378),[c] 401, 428
9.	Lutein epoxide	419, 442, 470	420, 442, 471	Ethanol	401, 424, 451
10.	Lutein	424, 445, 474	423, 445, 474	Ethanol	—
11.	β-Cryptoxanthin	(429),[c] 451, 475	(428),[c] 449, 473	Ethanol	—

[a] Eluent ($CH_3CN:CH_3OH:C_2H_5COOC_2H_5$ = 80:10:10) was used as the solvent.
[b] Reported values of absorption spectra are from four references by Davies (1976), Bauernfeind (1981), Khachik *et al.* (1986) and Chen and Chen (1993).
[c] Values in parentheses represent shoulders on spectra curves.
[d] Tentatively identified.
[e] Data not available.

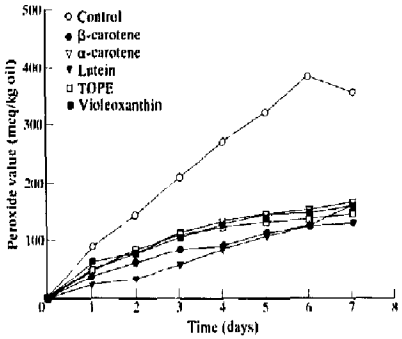

Fig. 2. Effects of various pigments (50 ppm) on the peroxide formation of soybean oil under light storage.

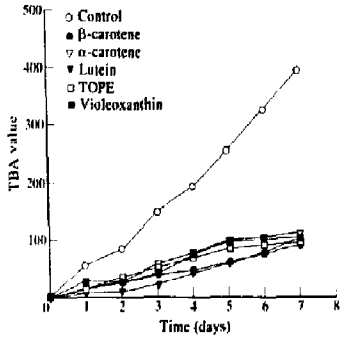

Fig. 3. Effects of various pigments (50 ppm) on the TBA value of soybean oil under light storage.

Table 3. Effects of carotenoids (50 ppm) on the TBA value of soybean oil under light storage at 30 ± 2°C

Carotenoid	Number of analysis	Day							
		0	1	2	3	4	5	6	7
Control	1	3·2	58·7	84·3	147·6	195·2	255·5	325·4	395·6
	2	2·8	57·4	85·7	150·2	194·2	257·1	324·3	397·2
	3	4·2	52·5	81·1	147·4	191·1	251·2	319·9	390·7
	Mean	3·40	56·20	83·70	148·40	193·50	254·60	323·20	394·50
	SD	0·72	3·27	2·36	1·56	2·14	3·05	2·91	3·39
	CV (%)	21·21	5·82	2·82	1·05	1·10	1·20	0·90	0·86
β-Carotene	1	3·2	8·4	25·2	40·7	50·2	67·4	78·8	95·2
	2	2·8	9·8	22·6	41·3	49·1	62·8	77·2	92·8
	3	4·2	9·4	20·6	32·8	46·2	59·2	73·2	88·5
	Mean	3·40	9·20	22·80	38·27	48·50	63·13	76·40	92·17
	SD	0·72	0·72	2·31	4·74	2·07	4·11	2·88	3·39
	CV (%)	21·21	7·84	10·12	12·40	4·26	6·51	3·78	3·68
α-Carotene	1	3·2	12·4	28·1	57·2	79·2	101·8	107·4	116·9
	2	2·8	15·1	27·2	56·4	76·2	100·2	102·8	112·4
	3	4·2	13·1	25·4	61·6	74·1	92·6	101·5	107·4
	Mean	3·40	13·53	26·90	58·40	76·50	98·20	103·90	112·23
	SD	0·72	1·40	1·37	2·80	2·56	4·92	3·10	4·75
	CV (%)	21·21	10·35	5·11	4·79	3·35	5·01	2·98	4·23
Lutein	1	3·2	4·8	7·8	25·8	47·8	58·4	85·2	107·6
	2	2·8	4·9	8·6	22·9	44·1	63·6	79·4	110·4
	3	4·2	5·8	8·2	18·6	38·6	55·7	75·7	108·4
	Mean	3·40	5·17	8·20	22·43	43·50	59·23	84·43	108·80
	SD	0·72	0·55	0·40	3·62	4·63	4·02	12·06	1·44
	CV (%)	21·21	10·66	4·88	16·15	10·64	6·78	14·29	1·33
TOPE	1	3·2	13·8	37·8	57·6	73·1	88·2	93·2	101·4
	2	2·8	12·8	32·9	56·8	69·2	82·7	91·2	98·2
	3	4·2	10·6	29·8	51·5	66·6	79·4	88·4	96·6
	Mean	3·40	12·40	33·50	57·30	69·63	83·43	90·93	98·73
	SD	0·72	1·64	4·03	0·44	3·27	4·45	2·41	2·44
	CV (%)	21·21	13·20	12·04	0·79	4·70	5·33	2·65	2·48
Violeoxanthin	1	3·2	28·2	31·4	45·8	74·2	98·4	99·2	108·6
	2	2·8	26·2	29·1	41·2	75·8	96·2	103·4	104·9
	3	4·2	21·3	24·2	39·8	69·6	92·3	96·6	102·4
	Mean	3·40	25·23	28·23	42·27	73·20	95·63	99·73	105·30
	SD	0·72	3·55	3·68	3·14	3·22	3·09	3·43	3·12
	CV (%)	21·21	14·07	13·03	7·43	4·40	3·23	3·44	2·96

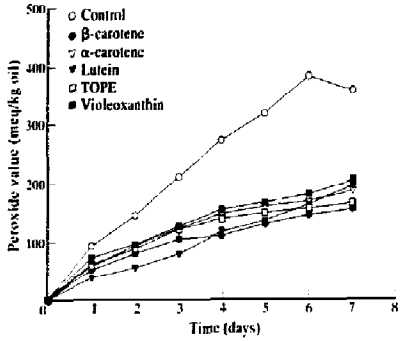

Fig. 4. Effects of various pigments (25 ppm) on the peroxide formation of soybean oil under light storage.

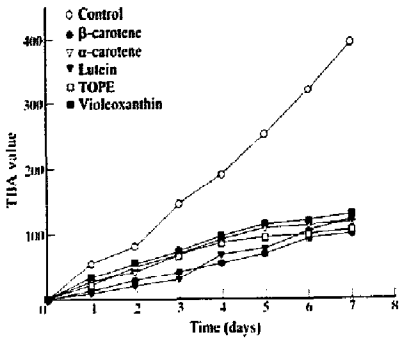

Fig. 5. Effects of various pigments (25 ppm) on the TBA value of soybean oil under light storage.

Table 4. Effects of carotenoids (25 ppm) on the TBA value of soybean oil under light storage at 30 ± 2°C

Carotenoid	Number of analysis	Day							
		0	1	2	3	4	5	6	7
Control	1	3·2	58·7	84·3	147·6	195·2	255·5	325·4	395·6
	2	2·8	57·4	85·7	150·2	194·2	257·1	324·3	397·2
	3	4·2	52·5	81·1	147·4	191·1	251·2	319·9	390·7
	Mean	3·40	56·20	83·70	148·40	193·50	254·60	323·20	394·50
	SD	0·72	3·27	2·36	1·56	2·14	3·05	2·91	3·39
	CV (%)	21·21	5·82	2·82	1·05	1·10	1·20	0·90	0·86
β-Carotene	1	3·2	13·2	32·4	45·3	54·4	70·6	98·4	108·8
	2	2·8	16·6	30·6	40·5	56·4	71·4	97·2	105·6
	3	4·2	13·4	24·9	38·4	62·6	76·7	93·8	99·2
	Mean	3·40	14·40	29·30	41·40	57·67	72·90	96·47	104·53
	SD	0·72	1·91	3·92	3·53	4·05	3·32	2·39	4·89
	CV (%)	21·21	13·25	13·36	8·54	7·03	4·55	2·47	4·68
α-Carotene	1	3·2	24·4	46·2	72·2	93·4	115·3	116·5	122·8
	2	2·8	27·8	48·8	71·7	99·4	114·2	117·6	123·4
	3	4·2	24·6	47·6	69·1	92·6	110·8	114·6	117·9
	Mean	3·40	25·60	47·53	70·80	95·13	113·43	116·23	121·37
	SD	0·72	1·91	1·30	1·57	3·72	2·35	1·52	3·02
	CV (%)	21·21	7·45	2·74	2·22	3·91	2·07	1·31	2·49
Lutein	1	3·2	6·8	20·2	35·2	66·4	85·2	109·2	128·4
	2	2·8	7·2	19·2	28·2	71·4	78·8	110·4	129·2
	3	4·2	11·2	16·4	27·8	69·8	78·4	104·1	121·8
	Mean	3·40	8·40	18·60	30·40	69·20	80·80	107·90	126·47
	SD	0·72	2·43	1·97	4·16	2·55	3·82	3·35	4·06
	CV (%)	21·21	28·97	10·59	13·69	3·69	4·72	3·10	3·21
TOPE	1	3·2	22·2	54·6	74·1	88·8	103·2	104·4	112·9
	2	2·8	23·4	53·5	75·6	93·6	97·6	105·8	111·6
	3	4·2	18·3	49·5	65·4	88·2	94·7	99·1	112·9
	Mean	3·40	21·30	52·43	71·70	90·20	98·50	103·10	109·77
	SD	0·72	2·67	2·85	5·51	2·96	4·32	3·53	4·35
	CV (%)	21·21	12·52	5·44	7·68	3·28	4·39	3·43	3·96
Violeoxanthin	1	3·2	30·8	57·2	76·4	103·2	119·4	126·3	134·8
	2	2·8	35·6	59·6	74·6	100·1	118·7	125·6	133·8
	3	4·2	36·2	52·4	70·7	96·2	113·5	119·2	128·8
	Mean	3·40	34·20	56·40	73·90	99·83	117·20	123·70	132·47
	SD	0·72	2·96	3·67	2·91	3·51	3·22	3·91	3·21
	CV (%)	21·21	8·65	6·50	3·94	3·51	2·75	3·16	2·43

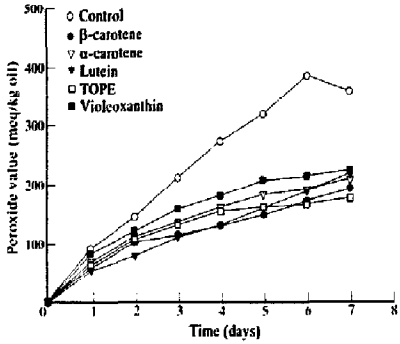

Fig. 6. Effects of various pigments (10 ppm) on the peroxide formation of soybean oil under light storage.

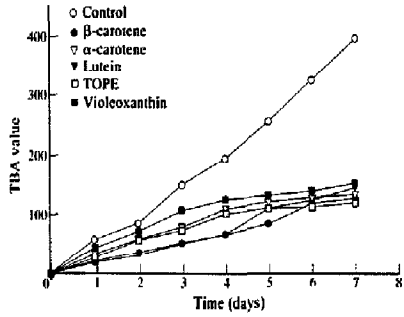

Fig. 7. Effects of various pigments (10 ppm) on the TBA value of soybean oil under light storage.

Table 5. Effects of carotenoids (10 ppm) on the TBA value of soybean oil under light storage at 30 ± 2°C

Carotenoid	Number of analysis	Day							
		0	1	2	3	4	5	6	7
Control	1	3·2	58·7	84·3	147·6	195·2	255·5	325·4	395·6
	2	2·8	57·4	85·7	150·2	194·2	257·1	324·3	397·2
	3	4·2	52·5	81·1	147·4	191·1	251·2	319·9	390·7
	Mean	3·40	56·20	83·70	148·40	193·50	254·60	323·20	394·50
	SD	0·72	3·27	2·36	1·56	2·14	3·05	2·91	3·39
	CV (%)	21·21	5·82	2·82	1·05	1·10	1·20	0·90	0·86
β-Carotene	1	3·2	20·7	38·4	55·6	71·2	88·4	117·6	128·1
	2	2·8	23·6	35·9	54·1	69·3	89·9	121·1	131·1
	3	4·2	17·2	31·9	49·9	65·3	83·9	117·7	125·7
	Mean	3·40	20·50	35·40	53·20	68·60	87·40	118·80	128·30
	SD	0·72	3·20	3·28	2·95	3·01	3·12	1·99	2·70
	CV (%)	21·21	15·63	9·26	5·55	4·39	3·57	1·67	2·11
α-Carotene	1	3·2	28·1	65·2	82·3	114·9	125·2	126·5	137·1
	2	2·8	32·4	58·9	78·1	108·2	121·4	127·4	139·9
	3	4·2	31·9	56·8	75·1	107·8	117·9	132·2	133·5
	Mean	3·40	30·80	60·30	78·50	110·30	121·50	128·70	136·50
	SD	0·72	2·35	4·37	3·62	3·99	3·65	3·06	2·75
	CV (%)	21·21	7·64	7·25	4·61	3·62	3·00	2·38	2·01
Lutein	1	3·2	16·1	32·5	54·5	67·7	115·2	128·3	149·2
	2	2·8	15·9	33·2	50·8	68·2	109·4	127·6	144·7
	3	4·2	20·8	28·6	48·4	64·3	106·1	121·9	142·4
	Mean	3·40	17·60	31·43	51·23	66·73	110·23	125·93	145·43
	SD	0·72	2·77	2·48	3·07	2·12	4·61	3·51	3·46
	CV (%)	21·21	15·76	7·89	6·00	3·18	4·18	2·79	2·38
TOPE	1	3·2	26·7	58·4	74·4	107·6	111·2	117·8	123·6
	2	2·8	28·6	61·4	75·8	103·9	108·6	114·4	121·2
	3	4·2	20·9	57·9	78·2	98·2	108·2	110·8	116·2
	Mean	3·40	25·40	59·23	76·13	103·23	109·33	114·33	120·33
	SD	0·72	4·01	1·89	1·92	4·74	1·63	3·50	3·78
	CV (%)	21·21	15·79	3·20	2·52	4·59	1·49	3·06	3·14
Violeoxanthin	1	3·2	42·8	72·8	109·4	127·6	134·6	141·2	157·9
	2	2·8	45·6	73·2	108·6	126·2	130·8	140·4	153·8
	3	4·2	42·6	68·4	105·8	122·4	128·2	137·2	147·8
	Mean	3·40	43·67	71·47	107·93	125·40	131·20	139·60	153·17
	SD	0·72	1·68	2·66	1·89	2·69	3·22	2·12	5·08
	CV (%)	21·21	3·84	3·73	1·75	2·15	2·45	1·52	3·32

may proceed simultaneously as indicated by the steady increase of TBA value during illumination (Fig. 3). With illumination time increased to four days and above, the antioxidant effectiveness of lutein gradually decreased, probably because of its degradation after prolonged exposure to light. Instead, β-carotene showed the maximum oxidation stability towards soybean oil. The control group showed the highest peroxide formation after illumination for 6 days, and then began to decline, indicating that the degradation rate of hydroperoxide was faster than the formation rate after prolonged illumination. For violeoxanthin and TOPE, the antioxidant abilities were quite similar, mainly because the latter constitutes about 64% violeoxanthin. Compared to α-carotene, β-carotene showed a higher antioxidant ability, probably because the latter contains more conjugated carbon-carbon double bonds than the former. Lee and Min (1990) also demonstrated that the antioxidant effectiveness of carotenoids increased as the number of the conjugated double bonds of carotenoids increased. Figure 4 shows the effect of various pigments (25 ppm) on the peroxide formation of soybean oil under light storage at 30°C. This result was similar to that in Figure 2 with the exception that the peroxide value formed was higher, indicating that a higher concentration (50 ppm) of pigment was necessary to achieve good oxidation stability of soybean oil during illumination. Figure 5 shows the effect of each pigment (25 ppm) on the TBA value of soybean oil under light storage at 30°C. The result was also similar to that in Fig. 3 with the exception that the TBA value formed was higher, indicating that a higher concentration of pigment can result in better oxidation stability of soybean oil. Figures 6 and 7 show the effect of various pigments (10 ppm) on the peroxide and TBA values of soybean oil during illumination, respectively. Both showed the same trend as described above, indicating that a low concentration (10 ppm) of pigment can be degraded faster during prolonged illumination and thus its antioxidant ability towards soybean oil was greatly reduced. Variations in TBA values are shown in Tables 3, 4 and 5.

From the above discussions, it can be found that all carotenoid pigments investigated possessed antioxidant ability, and the ability increased with increasing concentration. Under the same illumination time it was found that the highest β-carotene concentration (50 ppm) resulted in the lowest peroxide and TBA values, followed by 25, 10 and 0 ppm. This result was similar to that reported by Fakourelis *et al.* (1987), who found that β-carotene minimized lipid oxidation of olive oil under light storage by its light-filtering effect. The antioxidant effectiveness of the other pigments, when added to soybean oil at 0, 10, 25 and 50 ppm, was the same as β-carotene under the same illumination time.

By comparing the antioxidant ability of each pigment, lutein possessed the highest antioxidant effectiveness during the initial illumination period. However, its antioxidant ability gradually decreased and reached a plateau as illumination time increased to 7 days. β-Carotene had a higher antioxidant ability than α-carotene. The antioxidant abilities of violeoxanthin and TOPE were quite similar, probably because the latter constitutes about 64% violeoxanthin.

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