Technical

Effects of β-Carotene on Light Stability of Soybean Oil

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β-Carotene was added to soybean salad oils to study its effect in inhibiting flavor deterioration due to light exposure. Flavor evaluations indicated that (a) when oils treated with citric acid were exposed to light (7535 lux) for 8 to 16 hr, oils containing 5 to 10 ppm β-carotene showed improved flavor stability compared to oils containing 0 to 1 ppm β-carotene; and (b) when oils were not treated with citric acid, only oils containing 20 ppm β-carotene were more stable to light. Capillary gas chromatographic analysis showed that the addition of 1 to 20 ppm of β-carotene significantly decreased formation of 2-heptenal and 2,4-decadienal in the absence or presence of citric acid. Determination of peroxide values showed the same trends as gas chromatographic analyses of volatiles. In the presence of 15 and 20 ppm β-carotene, some off-flavors, as well as poor ratings for color quality, were reported by panelists. Therefore, flavor deterioration initiated by light can be inhibited effectively in soybean oil, without affecting color quality, by addition of β-carotene at concentrations from 5 to 10 ppm to oils treated with citric acid.

Light exposure has been recognized for a long time to cause deterioration of oils and fat-containing foods. Many studies have shown that vegetable oils are especially susceptible to photooxidation (1-4). Research at this laboratory showed that soybean oil and hydrogenated soybean oil bottled in amber glass had significantly better flavor scores than oils bottled in clear glass or plastic after only 4 hr of exposure to fluorescent light at 7535 lux (4). However, recognized practices to prevent light exposure, such as amber glass or opaque containers, are generally no longer used for economic, esthetic, or marketing reasons. Clear glass and plastic bottles have become popular for salad and cooking oils. Therefore, alternate methods are needed to protect oils against light deterioration.

One possibility to obtain light stability is the addition of β-carotene, which is one of the most widely used yellow colorants added to foods (5). Research on model systems has shown that β-carotene naturally inhibits singlet oxidation occurring during exposure to light in the presence of sensitizers such as chlorophyll (6-9). Sattar et al. (2) reported that β-carotene provided strong protective effects on light stability, as measured by peroxide value, of corn, coconut, rapeseed and soybean oils. These authors suggested that the carotene functioned as a built-in filter for light of short wavelengths. β-Carotene also showed a protective effect in butterfat up to the point when photobleaching decreased the pigment's concentration to 2 ppm or less. Kiritsakis and Dugan (10) reported that the presence of 4 or 6 ppm of β-carotene decreased peroxide formation in the early stages of light oxidation of olive oil. They concluded that carotenes either acted as singlet oxygen quenchers or were oxidized, thus sparing the oil until the carotenes were destroyed by oxidation.

Carlsson et al. (11) also reported that β-carotene is an efficient singlet oxygen quencher, which can provide protection at a level of ~0.01% by weight. They concluded that the inclusion of a nontoxic, effective singlet oxygen quencher in foods containing unsaturated oils could substantially improve their shelf life.

Previous work on the effect of β-carotene on the light stability of oil was based primarily on either peroxide value or conjugated diene measurements. However, we found no information in the literature on

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TABLE 1

Lovibond Tintometer Readings and Color Quality of Soybean Oil Treated with β-Carotene

β-Carotene (ppm)	Yellow (Y)	Red (R)	Quality rating[a]
0	5	0.2	Good
1	40	2.5	Good
5	40	5.0	Fair
10	50	6.0	Fair
15	70	8.0	Poor
20	70	9.0	Poor

[a]Sensory panel rating scale from excellent to bad.

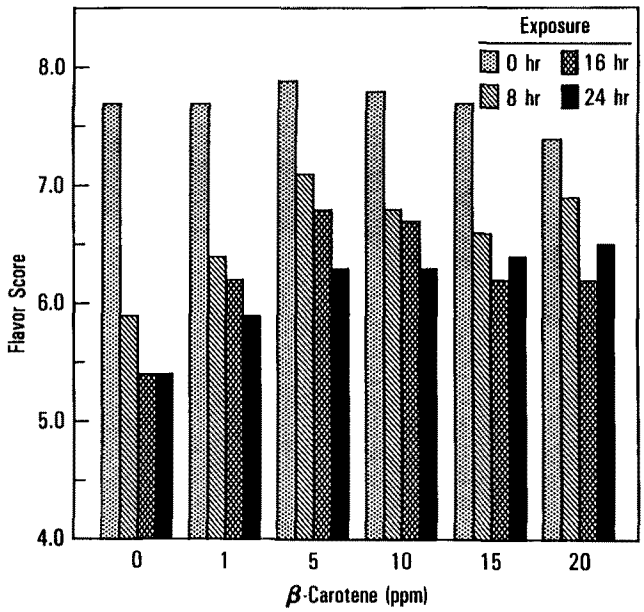

FIG. 1. Effect of β-carotene on flavor stability of light-exposed soybean oil containing citric acid.

the effects of β-carotene on flavor deterioration resulting from low levels of oxidation initiated by light exposure. Previous studies with oil or model systems (6,7,12,13) used impractically high levels of β-carotene, which would add unacceptable flavor and color characteristics to vegetable oils used for salads and cooking.

This paper presents studies to evaluate the effectiveness of low levels of β-carotene in improving flavor stability and color quality of soybean oil based on sensory analyses. Oxidative stability also was measured by capillary gas chromatographic analyses of volatiles and by peroxide values.

EXPERIMENTAL

Methods. A commercial, refined, bleached soybean oil was deodorized in the laboratory (14,15) and treated with citric acid and/or β-carotene. Citric acid (CA) (J.T Baker Chemical Co., Phillipsburg, New Jersey) was added to a portion of the oil at 100 ppm as a 20% aqueous solution on the cooling side of deodorization. β-Carotene (Sigma Chemical Co., St. Louis, Missouri) was added in a powder form to a portion of the 80 C finished oils at 20 ppm. Blends of this oil were made with oils without added β-carotene to obtain 1, 5, 10 and 15 ppm of β-carotene. The fatty acid composition of the soybean oil determined by GC analysis was: C16:0, 10.3%; C18:0, 4.4%; C18:1, 22.7%; C18:2, 54.1%, and C18:3, 8.5%. The oils were placed in 4-oz, narrow-mouthed clear glass bottles with screw-top closures, and exposed either to light (7535 lux = 700 ft candles) at 25 C for 4 to 24 hr, or stored in the dark at 60 C for 4 and 8 days. All samples were aged with air in the headspace (2/3 oil, 1/3 headspace). An automated light exposure apparatus containing fluorescent light bulbs was used to control the light intensity throughout the test (1,4). Light intensity was measured with a General Electric Light Meter (Type 214) (Cleveland, Ohio).

Frying tests were conducted on oils treated with citric acid and containing different levels of β-carotene. Procedures for frying bread cubes in oils heated for periods up to 17 hr have been described previously (16).

Sensory evaluation. A 15-member trained panel, experienced in analytical-descriptive sensory testing (17,18), evaluated the oils for overall odor and flavor intensity and individual odor and flavor characteristics. Previous publications from our laboratory present detailed information on these procedures (4,18-20). All tests were conducted under red fluorescent lights to mask color differences in the oils except during the evaluation of oil color quality, when only white fluorescent lighting was used. Methods for room odor evaluations (19) and fried bread quality (16) have been reported previously.

Volatiles analyses by capillary gas chromatography. As a measure of oxidative stability, volatile compounds were determined by a direct injection technique with a Perkin-Elmer capillary gas chromatograph (GC), model 3920 (Oakbrook, Illinois). The chromatograph was equipped with a flame ionization detector and a narrow-bore fused silica capillary column (Durabond-5) (15 m × 0.25 mm i.d.) with 1.0 μ film (polydiphenyldimethylsiloxane) thickness (J. & W. Scientific, Inc., Rancho Cordova, California). For routine analyses, a 2-μl oil sample was injected directly onto a small plug of silanized glass wool placed inside the inlet liner of the injector. The sample was held in the 180 C inlet for 8 min with the capillary splitter closed; the splitter was then opened and column temperature programmed from 0 to 250 C at 4 C/min. Peaks were identified by matching gas chromatographic retention times with those of authentic compounds and confirmed by mass spectral (MS) analyses with a Finnegan 1020 GC/MS (Palo Alto, California). For routine quantitative analyses, only 2-heptenal and 2,4-decadienal peaks were monitored because they were the major components that showed the highest correlation with changes in light exposure times. However, selected chromato-

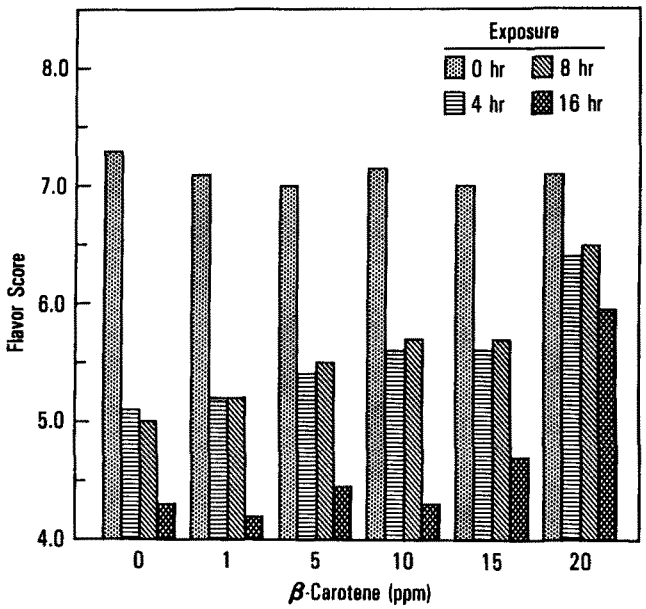

FIG. 2. Effect of β-carotene on flavor stability of light-exposed soybean oil containing no citric acid.

TABLE 2

Effect of β-Carotene on Flavor and Peroxide Development of Soybean Oil[a] Stored in the Dark

β-Carotene (ppm)	Flavor scores[b,c] (peroxide values)[d]		
	0 days[e]	4 days[e]	8 days[e]
0	7.7 (0)	6.5 (1.1)	6.2 (6.2)
1	7.7 (0)	6.7 (1.1)	6.1 (9.1)
5	7.9 (0)	6.9 (1.2)	6.7 (10.2)
10	7.8 (0)	6.3 (1.3)	6.0 (11.6)
15	7.5 (0)	6.3 (1.3)	6.0 (7.1)
20	7.4 (0)	5.8 (1.2)	5.9 (7.9)

[a]+100 ppm citric acid.
[b]Based on 1-10 scale (10 = bland; 1 = strong).
[c]Least significant difference = 0.8.
[d]me/kg.
[e]Storage time at 60 C.

grams were made to identify other peaks by using a temperature program starting at −75 C and increasing to 250 C at 4 C/min.

Peroxide value analyses. Oxidative stability was also evaluated by peroxide value analyses at the time of each sensory panel evaluation (21).

Spectrophotometric analyses. Absorbance at 454 nm was used to monitor the changes in β-carotene concentration after storage stability tests in light and dark, and after frying tests. Scans were obtained on a Varian Cary 219 spectrophotometer (Palo Alto, California) between 350 and 600 nm on oils diluted with spectrograde hexane (22) by using spectrograde hexane as a blank.

Lovibond measurements. Color of control and β-carotene treated oils was determined on a Lovibond AOCS Tintometer (Salisbury, England) with 5 1/4" tubes prior to light exposure, storage or heating.

RESULTS

Effect on oil color. β-Carotene is added to some processed fats and oils to produce a yellow color, e.g. in salad oil and shortening, 0.6-1.2 ppm; frying oil, 1.2-3.0 ppm and corn popping oil, 6-12 ppm (23). Analyses in our laboratory showed that β-carotene ranged from 35-60 ppm in commercial crude oils. In this study, we added different concentrations of β-carotene to soybean salad oil to measure its effectiveness in light stability tests. The upper level of 20 ppm was established by the deep orange color and distinctive flavor contributed by β-carotene to the oil. Lovibond Tintometer readings are presented in Table 1. As an index of color quality, our panel rated the oils under white fluorescent light on a scale ranging from excellent to bad. The color of the control oil and of the sample containing 1 ppm of β-carotene were rated as good quality. Additions of 5 and 10 ppm of β-carotene caused the oils to become medium yellow in color and decreased the ratings for color quality to fair. However, the addition of 15 and 20 ppm produced a medium yellow to orange oil color and lowered the ratings to poor quality.

Flavor and oxidative stability. The flavor stability of soybean oils containing citric acid and β-carotene was evaluated by our sensory panel after light exposure. Samples containing 0 to 15 ppm of β-carotene were of good quality, with initial flavor scores ranging from 7.7 to 7.9 (Fig. 1). The oil containing 20 ppm β-carotene had a lower flavor score (7.4), apparently because the flavor of β-carotene became detectable at this level. After exposure to fluorescent light for 8 hr, oils were scored between 7.1 (5 ppm β-carotene) and 5.9 (control). Scores decreased slightly after 16 hr of light exposure in oils containing 1 to 10 ppm of β-carotene. However, after 24 hr of light exposure, oils containing 15 and 20 ppm of β-carotene had slightly higher scores than samples exposed for 16 hr. β-Carotene provided a significant protective effect at levels of 5 ppm and above. Addition of even 1 ppm of β-carotene showed a protective effect over the control, but the difference in flavor scores was not significant.

Flavor scores for oils containing no citric acid decreased significantly in shorter light exposure times than for oils containing citric acid (Fig. 2). Initial scores ranged from 7.0 to 7.3 and indicated lower flavor quality than for oils containing citric acid (Fig. 1). After 4 hr exposure to fluorescent light, oils containing 0 to 15 ppm of β-carotene were rated significantly lower than the corresponding oils stored without light exposure. However, the addition of 20 ppm of β-carotene was effective in preventing significant light deterioration compared to the control. Increasing light exposure from 4 to 8 hr did not cause much change in flavor scores, but scores were significantly decreased after 16 hr of light exposure. Therefore, in

TABLE 3

Effect of β-Carotene on Peroxide Values[a] (me/kg) in Soybean Oils Exposed to Light

	Light exposure (hr)		
β-Carotene (ppm)	4	8	16
No citric acid			
0	0.8	1.3	3.0
1	0.7	1.0	2.0
5	0.5	0.6	1.8
10	0.4	1.0	2.2
15	0.5	1.1	1.4
20	0.3	1.1	2.1
+100 ppm citric acid			
0	—[b]	2.2	3.5
1	—	0.9	3.3
5	—	0.4	2.9
10	—	1.1	0.7
15	—	0.9	1.3
20	—	1.0	2.3

[a]Initial peroxide value was 0 in all samples.
[b]—, not tested.

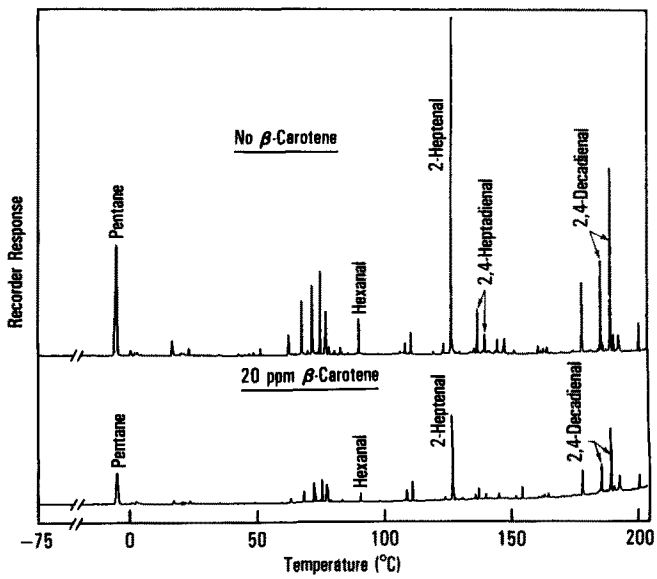

FIG. 3. Capillary gas chromatogram of volatiles from soybean oil containing citric acid after exposure to light for 24 hr.

the absence of citric acid, β-carotene provided a significant protective effect only at the 20 ppm level.

To determine if β-carotene had any effect without light exposure, we aged oils treated with citric acid and β-carotene at 60 C in a modified Schaal oven test (18,24). Panelists rated the control oil with no added β-carotene as not significantly different from any of the treated samples after 0, 4 and 8 days storage at 60 C in the dark (Table 2). Oils containing 20 ppm β-carotene were rated lower than some of the other oils after 4 and 8 days storage, apparently because of the distinctive flavor of this additive. Peroxide values of oils aged 4 days also showed no effects of β-carotene. However, after 8 days in the dark, oils containing β-carotene showed higher peroxide values than the control at all levels. This result suggests that free radical oxidation was promoted by β-carotene in the dark.

All oils with or without citric acid showed increasing peroxide development with increasing light exposure time (Table 3). The control oils without added β-carotene consistently had the highest peroxide values. However, no consistent pattern was evident for the effectiveness of various levels of β-carotene on peroxide development. Soybean oil containing 5 ppm β-carotene when exposed to light showed an increase in peroxide values similar to that reported by Sattar et al. (2) with butterfat containing 4 ppm added β-carotene.

Light stability of oils was also evaluated by capillary gas chromatographic analyses of volatiles. The gas chromatograms in Figure 3 show the volatile compounds formed in citric acid-treated soybean oil with and without 20 ppm β-carotene after light exposure for 24 hr. Major peaks identified included pentane, hexanal, 2-heptenal, 2,4-heptadienal and 2,4-decadienal. Qualitatively, few differences were observed between chromatograms of light-exposed oils in the presence or absence of added β-carotene. Quantitatively, however, significantly greater amounts of each volatile were found in the control sample than in the oil containing 20 ppm of β-carotene. With 1 ppm of added β-carotene, 2-heptenal and 2,4-decadienal were significantly decreased after 8 and 24 hr of light exposure in soybean oil containing citric acid (Fig. 4). The effect on these volatiles of β-carotene at the 5 and 10 ppm levels was not significant, but there was a significant effect when 20 ppm of β-carotene was used. Three times more 2-heptenal was formed in the control oil (28000 integrator counts) than in the oil containing 20 ppm β-carotene (9000 integrator counts) after 24 hr of light exposure. The ratio for integrator counts of 2,4-decadienal was 2.75 between control (33000) and the oil containing 20 ppm of β-carotene (12000). Integrator counts for 2-heptenal and 2,4-decadienal in the initial oil were 4000 and 8000, respectively. Thus, use of 20 ppm β-carotene resulted in only small increases in the levels of these volatiles in oils after 24 hr of light exposure.

In oils containing no citric acid, β-carotene was more effective in preventing formation of volatiles than in oils containing citric acid. Oils exposed to light for 8 hr, without added citric acid or β-carotene, produced 4 times more 2-heptenal (49000 integrator counts) and 6 times more 2,4-decadienal (129000 integrator counts) than oils containing 20 ppm β-carotene only.

Williams and Applewhite (25) analyzed deodorized soybean oil by gas chromatography (direct injection, 170 C injector) and reported 5.1 ppm of 2-heptenal after 5 weeks of light exposure compared to 0.7 ppm in the same oil aged in the dark for 5 weeks at 30 C. Our analyses also showed a 7-fold increase of 2-heptenal in citric acid-treated soybean oil with no added β-carotene after 24 hr of light exposure at 7535 lux.

To measure if β-carotene is effective in protecting frying oils, the room odor intensity of heated soybean oil was evaluated to monitor thermal stability. No significant differences were noted between oil containing 20 ppm β-carotene and the control (Table 4). In the initial room odor test, after 1 hr of heating and frying in the oil at 190 C, the sample with β-carotene had a slight fruity odor. After heating and frying in the oil at 190 C, no effects of β-carotene were detected in overall room odor intensity. The lack of protective effect of β-carotene in heated oils can be attributed to its thermal destruction established by spectrophotometric analysis (see below).

The stability of food fried in soybean oil treated with

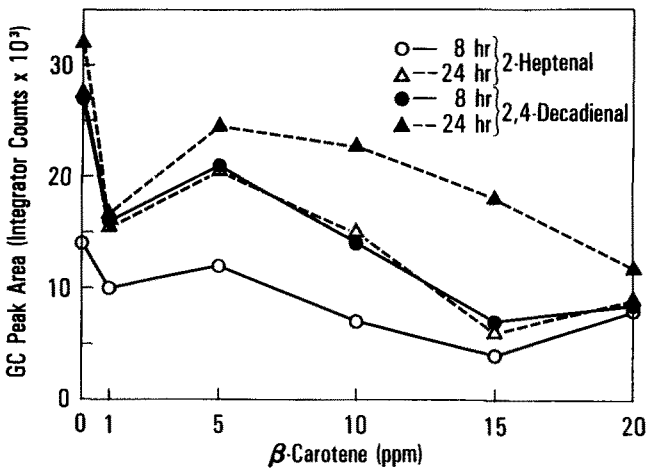

FIG. 4. Effect of β-carotene on gas chromatographic analysis of 2-heptenal and 2,4-decadienal in citric acid-treated soybean oil exposed to light for 8 and 24 hr.

TABLE 4

Effect of β-Carotene Level in Soybean Cooking Oil[a] on Room Odor Intensity

Heating at 190 C (hrs)	Room odor intensity scores[b]		
	0 ppm[c]	20 ppm[c]	Significance
1	4.6	5.1	N.S.[d]
2	4.7	4.8	N.S.
17	5.0	5.1	N.S.
19	5.1	4.9	N.S.

[a]+100 ppm citric acid.
[b]Based on a 0-10 intensity scale; 10 = strong, 0 = none.
[c]Added β-carotene.
[d]N.S., no significant difference at 95% confidence level.

TABLE 5

Effect of β-Carotene Level in Soybean Cooking Oil on Flavor Quality of Fried Bread

Heating at 190 C (hrs)	Storage of fried bread	Flavor quality scores[a]		
		0 ppm[b]	20 ppm[b]	Significance
1	Initial	7.7	7.5	N.S.[c]
	4 days at 60 C	6.6	6.9	N.S.
17	10 hr fluorescent light exposure	7.4	7.1	N.S.
	Initial	7.7	7.8	N.S.
	4 days at 60 C	4.5	4.5	N.S.
	10 hr fluorescent light exposure	6.5	6.7	N.S.

[a]Based on a 1-10 quality scale, 10 = excellent, 1 = bad.
[b]Added β-carotene.
[c]N.S., no significant difference at 95% confidence level.

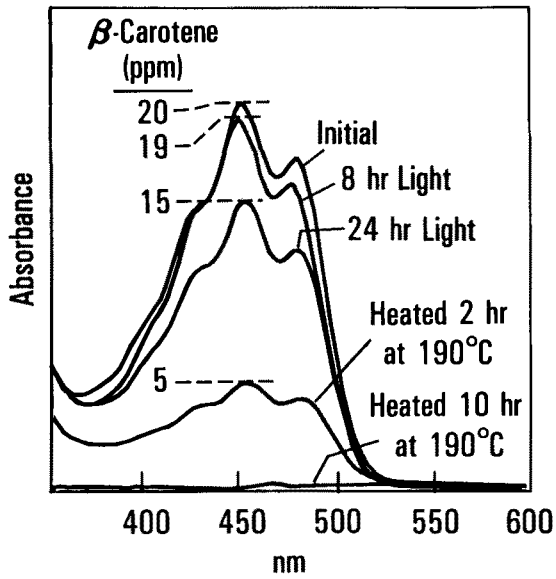

FIG. 5. Spectrophotometric analyses of β-carotene in oils exposed to light or heated at 190 C.

β-carotene was also evaluated by determining the flavor quality of fried bread cubes after their storage and light exposure. The addition of 20 ppm of β-carotene did not improve the flavor stability of bread fried in either fresh oil (1 hr) or in oil heated 17 hr (Table 5). The complete loss of β-carotene by 10 hr of heating, established by spectrophotometric analysis, accounted for the lack of protection observed.

Spectrophotometric analyses. Carotenoids in foods bleach when they are exposed to oxygen and light (26). Studies have demonstrated the loss of β-carotene in milk fat and fatty acid ester solutions exposed to light (2,6). To measure the change in β-carotene content in light-exposed soybean oil, absorbance of the pigment was monitored spectrophotometrically at 454 nm. Decreases in β-carotene in soybean oil containing citric acid were observed after exposure to fluorescent light (Fig. 5). Quantitative analyses showed that β-carotene in the oil decreased from 20 ppm to 19 ppm after 8 hr and to 15 ppm after 24 hr of light exposure. Therefore, β-carotene was only partially photobleached under our conditions of light exposure, but oil quality deteriorated even in the presence of residual β-carotene.

Spectrophotometric analyses of aliquots of oil taken from the deep-fat fryers revealed a rapid disappearance of β-carotene (Fig. 5). After only 2 hr of heating and frying in the oil, the concentration of β-carotene decreased from 20 ppm to 5 ppm. After 10 hr, no β-carotene was detected. These data agree with previous results showing rapid disappearance of β-carotene in Nigerian palm oil after heating at high temperatures (27). Our spectrophotometric analyses showing the rapid loss of β-carotene account for its lack of impact on room odor intensity or fried food quality (Table 5).

DISCUSSION

The present study showed that β-carotene at low levels (5-20 ppm) had a significant effect in protecting soybean oil against light deterioration. These results are in agreement with previous work from this laboratory (7) showing lower peroxide development when soybean oil esters containing 1000 ppm β-carotene were exposed to light. Because this protective effect was not observed in the dark, β-carotene was regarded as an effective singlet oxygen quencher. The high concentration of β-carotene used in that study apparently was required with distilled soybean esters because they were not protected by tocopherols and citric acid. The quenching effect of β-carotene would be expected to increase with its concentration. However, in the present work, the addition of β-carotene at levels exceeding 20 ppm was found to be impractical because its distinctive flavor and deep color adversely affected flavor and color quality of the oil.

There is evidence that soybean oil or esters contain sensitizers even after refining or distillation (7,29). No photosensitized oxidation was observed when soybean esters were treated with carbon black to remove sensitizers (7,12). The singlet oxygen quenching effect of β-carotene is complicated by its susceptibility to free radical autoxidation. When soybean oil was chromatographed through Florisil, β-carotene acted as a prooxidant because the tocopherols were removed (12). However, in the presence of δ-tocopherol, β-carotene behaved synergistically in preventing oxidation. Apparently, tocopherols protect the β-carotene from free radical autoxidation. Our present finding that β-carotene provides light stability at concentrations below 20 ppm can be attributed to the protective effect of the natural tocopherols in the soybean oil. In our storage tests in the dark, however, β-carotene apparently was not sufficiently protected by tocopherols, and its free radical oxidation may account for the increased peroxide values observed (Table 2). These results agree with previous work with butter showing an early inhibitory effect of β-carotene, which then behaved as a prooxidant (28). Therefore, β-carotene is an effective

inhibitor of photooxidation of soybean oil only when its oxidation is prevented by tocopherols. Further studies are needed to determine how natural tocopherols in soybean oil interact with added β-carotene to optimize its photoprotective effect.

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