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Effects of soybean oil and oxidized soybean oil on the stability of β-carotene

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Abstract

The effects of 1.0%, 2.5%, and 5.0% purified soybean oil and thermally oxidized soybean oil on the stability of 100 ppm β-carotene as a fat-soluble vitamin A and singlet oxygen quencher in isooctane have been studied. The samples were stored under 1000, 2000, or 4000 lx at 20 °C for 2 days and at 50 °C for 16 days in the dark. The β-carotene was determined by high-performance liquid chromatography. The centrifugation and filtration of vegetable mixture, during sample preparation for β-carotene analysis by HPLC, decreased the coefficient of variation from 4.13% to 1.02%. The purified soybean oil and thermally oxidized soybean oil stabilized β-carotene in isooctane under light and in the dark at $\alpha = 0.05$. The losses of β-carotene, with 1.0% purified oil, 1.0% thermally oxidized oil and without any oil during 48 h under light, were 11.2%, 80%, and 100%, respectively. 100 ppm TBHQ had a protective effect on the stability of β-carotene in isooctane at $\alpha = 0.05$. The β-carotene stability decreased as the light intensity increased from 1000 to 2000 or 4000 lx at $\alpha = 0.05$. The stability of vitamins in fruit and vegetable drinks enriched with fat-soluble vitamins and antioxidants during storage can be greatly improved by adding approximately 1.0% high quality non-oxidized soybean oil.

Keywords: Purified soybean oil; Thermally oxidized soybean oil; TBHQ; β-Carotene

1. Introduction

The fat-soluble vitamins, such as vitamin A, D, E, and K, are dissolved in the lipid fraction of foods. The degradation of fat-soluble vitamins generally parallels the oxidative degradation of unsaturated lipids (Belitz & Grosch, 1999). Factors that promote the oxidation of unsaturated lipids also enhance the degradation of fat-soluble vitamins.

β-Carotene, which is the most common carotenoid in foods, exhibits the highest vitamin A activity and is referred to as provitamin A (Reische, Lillard, & Eitenmiller, 2002). β-Carotene has been known as a powerful singlet oxygen scavenging antioxidant (Choe & Min, 2005; Jeevarajan & Kispert, 1996; Lee, Ozcelik, & Min, 2003). The effects of β-carotene in the reduction of cancer and cardiovascular diseases have been extensively investigated in clinical studies (Rice-Evans, Sampson, Bramley, & Holloway, 1997; Tavani & La Vecchia, 1999; Ziegler, 1989).

β-Carotene is susceptible to isomerization and photosensitization, thermal and chemical oxidations during processing and storage of foods (Gloria, Grulke, & Gray, 1993; Khachik et al., 1992). Oxidized β-carotene acts as a prooxidant in foods (Steenson & Min, 2000). The oxidative degradation of β-carotene can decrease the nutritional value of vitamin A and the activity of antioxidant. The oxidation of β-carotene causes loss of natural flavour and the decomposition of chromophores of foods, which makes the products less acceptable or unacceptable to consumers (Ager & Schroeder, 1993).

The stability of β-carotene in vegetable oil is improved by using more oxidatively stable oils in food applications (Goulson & Warthesen, 1999). The oxidation of oil forms dimerized or polymerized compounds with hydroxyl groups, carbonyl groups and *trans* double bonds. The thermally oxidized compounds show prooxidant effects on the

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oxidative stability of soybean oil (Yoon, Jung, & Min, 1988). Billek, Guhr, and Waibel (1978) reported that the oxidized compounds in the deep fat fried soybean oil after 64 h at 180 °C were 26.2%. The commercially available high quality soybean oil had 1.2% oxidized triglycerides (Yoon et al., 1988).

Some commercial fruit and vegetable beverages are enriched with fat-soluble vitamins, including β-carotene (USDA, 2005). It is extremely important to protect fat-soluble vitamins, such as β-carotene, in fruit and vegetable juices and beverages and to maintain the high quality of nutrition and flavour of products. Information on the stability protection of the fat-soluble vitamins in water based fruit and vegetable beverages are not available. The objectives of this study were: (1) to investigate the effects of purified soybean oil and thermally oxidized soybean oil on the stability of β-carotene as a model for fat-soluble vitamins and antioxidants; and (2) to study the effects of centrifugation and filtration of fruits and vegetables, during sample preparation for HPLC, on the reproducibility of β-carotene analysis.

2. Materials and methods

2.1. Materials

Refined soybean oil was obtained from Karlshams Inc. (Columbus, OH). β-Carotene and tertiary butylhydroquinone (TBHQ) were obtained from Sigma Chemical Co. (St. Louis, MO) and UOP (Des Plaines, IL), respectively. HPLC grade isooctane and isopropyl alcohol were used. The vegetable mixture, which consisted of green beans, carrots, broccoli and lettuce, was provided by T.E. Webb (Department of Medical Biochemistry, the Ohio State University, Columbus, OH).

2.2. Purified soybean oil and thermally oxidized oil preparation

Refined soybean oil was purified by passing through a 4.4×55 cm glass column packed with 100 mesh silicic acid (Mallinckrodt, Paris, KN), 30 g of a 2:1 mixture of activated charcoal (J.T. Baker Chemical Co., Phillipsburg, NJ) and Celite (Sargent Welch Co., Cleveland, OH), 120 g of a 2:1 mixture of powdered sugar and Celite, and 100 g activated silicic acid (Jung & Min, 1991; Lee & Min, 1990).

Purified soybean oil (100 g) was thermally oxidized in a 250 ml beaker in an air-force oven (Blue M, Blueisland, IL) at 180 °C for 96 h. The thermally oxidized compounds in the thermally oxidized soybean oil were isolated by passing through a 2×30 cm silicic acid column (Yoon et al., 1988). The thermally oxidized compounds retained on the column were first washed with 200 ml of hexane to elute the residual unoxidized purified soybean oil and then eluted with 500 ml methanol. The methanol-eluted compounds were referred to as thermally oxidized compounds, and methanol was removed by rotary vacuum evaporator at 40 °C.

2.3. Sample preparation

The 100 ppm β-carotene in isooctane solvent was prepared and used as stock solution. The β-carotene solution samples having 0.0%, 1.0%, 2.5%, and 5.0% purified soybean oil and 0.0%, 0.1%, 1.0%, 2.5%, and 5.0% thermally oxidized soybean oil were prepared in duplicate. The 100 ppm TBHQ was added to isooctane containing 100 ppm β-carotene to study the effect of TBHQ on the stability of β-carotene.

Eight milliliters of samples were transferred into 10 ml serum bottles and then sealed, air-tight, with Teflon rubber septa and aluminium cap for storage study under light and in the dark.

2.4. Light and temperature storages

Sample bottles were stored in a light box (Choe & Min, 1992) for 2 days at 20 °C and analyzed every 12 h. The intensities of fluorescent light for the light box were 1000, 2000, or 4000 lx. Sample bottles were studied in the dark by placing them in an air-forced oven at 50 °C for 16 days and contents were analyzed every 2 days.

2.5. Determination of peroxide value and nonvolatile oxidized compounds in oils

The peroxide value, acid value, phosphorus content, *trans* fatty acids and conjugated dienes in soybean oils were determined in duplicate by the Official Method of the American Oil Chemists' Society (AOCS, 1998). Total oxidized compounds of refined vegetable oil were analyzed by liquid chromatography using silicic acid as stationary phase as reported by Billek et al. (1978). To characterize the thermally oxidized compounds and oils, the 10 mg sample which was smeared onto sodium chloride discs was analyzed by the Beckman Acculab 2 Infrared Spectrometer. Tocopherols in purified soybean oil were analyzed using HPLC by the method of Carpenter (1979).

2.6. Determination of β-carotene by HPLC

The 0.5 g vegetable mixture or fruit mixture was prepared in a 50 ml centrifuge tube in five replicates. 3.5 ml of distilled water was added to the centrifuge tube. The tube was vortexed for 40 s and then 25 ml methanol was added. The tube was vortexed for 30 s. After standing for 20 min at room temperature, 10 ml isooctane was added and the tube was vortexed for 45 s. 5.0 ml distilled water then was added and vortexed for 20 s. The sample was centrifuged for 10 min at 2000 rpm. The centrifuged isooctane layer was filtrated through a 0.45 mm syringe filter into a 2 ml sample vial. All samples were always covered with aluminium foil and all preparation steps were done under

diffuse and low intensity lighting to prevent photoisomerization of β-carotene.

The β-carotene content was analyzed by HPLC, consisting of a HP 1050 series Pumping System, a HP 1050 Series Variable Wavelength Detector and a HP 2296 Series II Integrator (Hewlett–Packard Co., Wilmington, DE). An ESI CN column (3 μm, 150 mm × 60 Å) was packed with cyanopropyl bonded silica (ES Industries, Berlin, NJ). The injection volume was 20 μl. The flow rate of the mobile phase (0.15% isopropyl alcohol in isooctane) was 1.0 ml/min at 900 psi. The absorbance of sample was measured at 436 nm.

2.7. Statistical analysis

The reproducibility of HPLC analysis to β-carotene was determined by coefficient of variation. Data were analyzed by one-way analysis of variance (ANOVA) and Tukey's multiple comparison method at $\alpha = 0.01$ or $\alpha = 0.05$. All statistical analyses were conducted with the Statistical Analysis System (SAS Inst., Cary, NC).

3. Results and discussion

3.1. Purified soybean oil and thermally oxidized soybean oil

The soybean oil eluted from column chromatography was designated as purified soybean oil. The original refined soybean oil had 1.2% oxidized compounds according to silicic acid chromatography. The purified soybean oil was colourless, tasteless, and odourless. The free fatty acids, phospholipids, conjugated dienes, tocopherols, carotenoids and oxidized compounds were not detected in the purified soybean oil. The purified soybean oil had a peroxide value of 0 and was made up of triacylglycerols.

The contents of oxidized compounds in the purified soybean oil and thermally oxidized soybean oil were 0% and 31%, respectively. The thermally oxidized soybean oil was viscous and dark yellowish-red. The infrared spectroscopy and chemical analyses showed that the thermally oxidized compounds were very rich in hydroxyl groups according to the absorption at 2.9 μm, which is due to the intermolecular hydrogen bonding of hydroxyl groups. The absorption band at 10.3 μm of thermally oxidized oil suggested the presence of *trans* double bonds. The absorption at 5.8 μm, due to the carbonyl group stretching vibration of thermally oxidized oil, was larger than that of either soybean oil or purified soybean oil. The increased peak size of 5.8 μm absorption suggested that additional carbonyl groups were formed during thermal oxidation. The thermally oxidized soybean oil was made up of 31% oxidized polar compounds and 69% triacylglycerols. The oxidized polar compounds were cyclic or noncyclic carbon-to-carbon linked dimers, noncyclic hydroxyl dimers, dimers and trimers joined through carbon-to-carbon linkages, and trimers joined through carbon-to-oxygen linkages (Paulose & Chang, 1973).

3.2. Determination of β-carotene by HPLC

The HPLC analyses of β-carotene in isooctane model system, vegetable mixture, and fruit mixture are shown in Table 1. The coefficients of variation for β-carotene analyses in isooctane, vegetable, and fruit mixture were 0.95%, 1.02% and 1.78%, respectively, which are considered as excellent. The coefficients of variation of β-carotene in vegetable and fruit mixture in the preliminary study were 4.13% and 5.02%, respectively. The centrifugation and filtration through a 0.45 mm syringe filter of vegetable mixture, during sample preparation for β-carotene HPLC analysis, decreased the coefficient of variation from 4.13% to 1.02%. Bushway (1986) reported that the coefficients of variation for β-carotene analyses by HPLC in fruits and vegetables ranged from 2.79% to 7.26%, depending on the types of fruits and vegetables. The β-carotene analyses in fruit mixture ranged from 0.72 to 0.75 ppm for five replicates. The sensitivity of HPLC analysis for β-carotene content was as low as 0.01 ppm in fruit mixture. The retention time of β-carotene was only 2.2 min. The correlation coefficient between the HPLC response and the concentration of β-carotene was 0.99 in isooctane, vegetable or fruit mixture. The HPLC analysis for the β-carotene in isooctane, vegetable or fruit mixture was very reproducible, very sensitive and simple. This sensitive and high reproducible method could be used to determine β-carotene in fruit or vegetable drinks enriched with β-carotene as a fat-soluble vitamin and antioxidant.

3.3. Effects of purified soybean oil and thermally oxidized soybean oil on the stability of β-carotene under light

The effects of 1.0%, 2.5%, or 5.0% purified soybean oil and 1.0%, 2.5%, or 5.0% thermally oxidized oil on the stability of 100 ppm β-carotene under 1000 lx at 20 °C for 48 h are shown in Fig. 1. The β-carotene content decreased as the storage period increased. The β-carotene in the control sample without soybean oil was destroyed by more than 60% in 12 h and completely destroyed in 48 h under light.

The effects of 0.0%, 1.0%, 2.5%, and 5.0% purified soybean oil on the β-carotene during 24 h under 1000 lx

Table 1
Coefficient of variation (CV) and standard deviation (SD) for determination of β-carotene in isooctane model system, vegetable mixture and fruit mixture

Replicate	β-Carotene content (ppm)		
	Isooctane model system	Vegetable mixture	Fruit mixture
1	60.3	92.4	0.73
2	60.7	90.7	0.72
3	61.2	90.4	0.75
4	60.6	91.2	0.74
5	61.8	90.0	0.72
Mean	60.9	90.9	0.732
SD	0.58	0.93	0.013
CV (%)	0.95	1.02	1.78

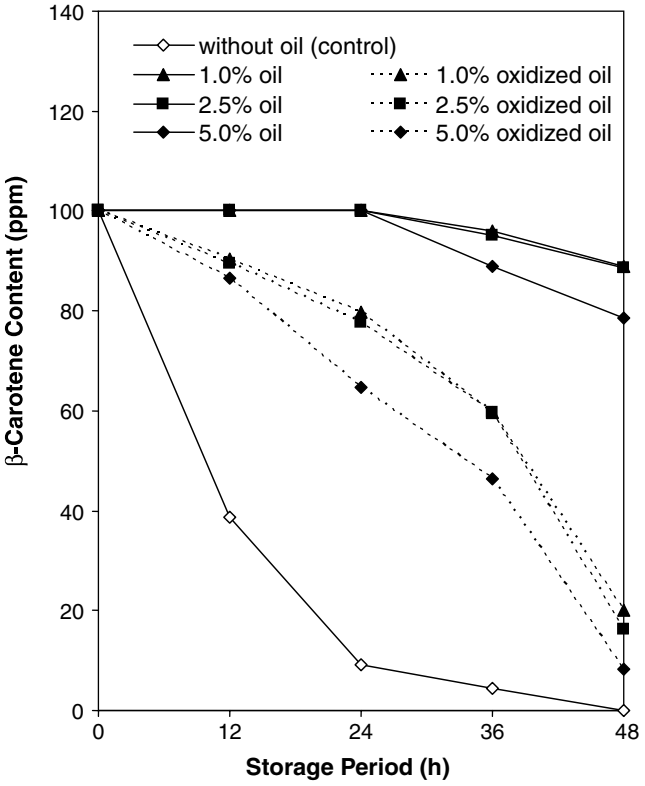

Fig. 1. Effects of purified soybean oil and thermally oxidized soybean oil on the stability of β-carotene in isooctane under 1000 lx at 20 °C.

showed that the sample without soybean oil had 9.2% β-carotene and the sample with 1.0%, 2.5%, or 5.0% oil had 100% (Fig. 1). The peroxide value of purified soybean oil did not increase during the first 24 h of storage and started to increase rapidly after the 24 h of storage (data not shown). The oxidation induction time of purified soybean oil under light was considered to be 24 h. The β-carotene in the sample with 1.0%, 2.5%, or 5.0% purified soybean oil was not degraded during the first 24 h of storage when the purified soybean oil was not oxidized (Fig. 1). The losses of β-carotene in the sample with 1.0%, 2.5%, or 5.0% purified soybean oil from 24 to 48 h of storage were 11.2%, 11.4% or 21.4%, respectively. The oxidized content formed in the 5.0% purified soybean oil during the storage from 24 to 48 h was assumed to be 5 times that in the 1.0% purified soybean oil. The amount of oxidized compounds formed from the purified oil was proportional to the amount of purified soybean oil when purified soybean oil content was less than 10%. The loss of β-carotene increased from 24 to 48 h as the newly formed oxidized content from the purified soybean oil increased. The oxidized soybean oil is a prooxidant (Yoon et al., 1988).

The effects of 1.0%, 2.5%, or 5.0% thermally oxidized soybean oils on the β-carotene under 1000 lx are shown in Fig. 1. The thermally oxidized soybean oil had 31% oxidized polar compounds and 69% nonoxidized triacylglycerols. The initial oxidized compounds in the 1.0%, 2.5%, and 5.0% thermally oxidized oils were 0.31%, 0.78%, and 1.55%, respectively (Table 2). The amounts of β-carotene in the sample with 1.0%, 2.5%, or 5.0% thermally oxidized oil during 24 h were 79.8%, 77.8% or 64.8%, respectively, when the control sample with no oil had only 9.2% of β-carotene. The added 1.0%, 2.5%, or 5.0% thermally oxidized oil had a significant effect on the protection of β-carotene during the storage at $\alpha = 0.05$. Since the oxidized polar compounds in the 1.0%, 2.5%, or 5.0% thermally oxidized oil are prooxidants (Yoon et al., 1988), the protective effect of thermally oxidized oil on the β-carotene might be due to the nonoxidized soybean oil in the thermally oxidized oil, which is 69%, initially. The less protective effect of 5.0% thermally oxidized soybean oil than 1.0% or 2.5% is most likely due to the higher amounts of the oxidized prooxidant polar compounds in the 5.0% thermally oxidized soybean oil.

Comparison of the protective effects of purified soybean oil and thermally oxidized soybean oil on the stability of β-carotene showed that the purified soybean oil had a more significant protective effect than had the thermally oxidized oil at $\alpha = 0.01$ (Fig. 1). The difference between the purified soybean oil and thermally oxidized soybean oil is the absence or presence of oxidized polar compounds. Yoon et al. (1988) also reported that the oxidized compounds had prooxidant effects on the oxidative stability of purified soybean oil. The rapid destruction of β-carotene with thermally oxidized oil during storage from 24 to 48 h might be due to combination of the initial oxidized polar compounds and the rapidly formed new oxidized compounds from nonoxidized soybean oil and β-carotene. The initial oxidized compounds in the thermally oxidized oil catalyze the oxidation of oil and β-carotene. The oxidized β-carotene formed during the storage is a prooxidant (Steenson & Min, 2000). The rapid destruction of β-carotene in the sample during storage from 24 to 48 h is due to the autocatalytic oxidation of β-carotene by the oxidized compounds (Steenson & Min, 2000). The nonoxidized oil in the 1.0%, 2.5%, or 5.0% thermally oxidized oil seems to protect the β-carotene by coating or dissolving the 100 ppm β-carotene, which minimizes the contact of β-carotene with oxygen in the sample. To protect the fat-soluble vitamins and antioxidants, such as β-carotene, in fruit and vegetable juices and beverages during storage, it seems to be important to add approximately 1.0% of high quality nonoxidized oil.

3.4. Effects of light intensity and TBHQ on the stability of β-carotene at room temperature

The effects of 1000, 2000, or 4000 lx and 100 ppm TBHQ on the β-carotene stability in isooctane at 20 °C are shown in Fig. 2. As the storage light intensity increased from 1000 to 2000 or 4000 lx, the β-carotene stability decreased at ($\alpha = 0.05$) as was expected (Fig. 2). The losses of β-carotene in isooctane after 12 h were 50.9%, 64.1% or 70.7% under 1000, 2000, or 4000 lx, respectively. However, the losses

Table 2
Effects of 1.0%, 2.5%, and 5.0% purified soybean oil and thermally oxidized soybean oil on the content of β-carotene in isooctane under 1000 lx at 20 °C during 48 h

	Soybean oil	Oxidized oil[a] (%)	Nonoxidized oil[a] (%)	β-Carotene content (ppm)		
				0 h	24 h	48 h
1.0%	Purified oil	0	1.00	100	100	88.8
	Thermally oxidized oil	0.31	0.69	100	79.8	20.0
2.5%	Purified oil	0	2.50	100	100	88.6
	Thermally oxidized oil	0.78	1.72	100	77.8	16.2
5.0%	Purified oil	0	5.00	100	100	78.6
	Thermally oxidized oil	1.55	3.45	100	64.8	8.4

[a] Oxidized oil content and nonoxidized oil content in purified oil or thermally oxidized oil.

of β-carotene in isooctane containing 100 ppm TBHQ after 12 h were 15.3%, 23.6% and 30.7% under 1000, 2000, or 4000 lx, respectively. TBHQ had a significant effect on the stability of β-carotene in isooctane at $\alpha = 0.05$. TBHQ is one of the most effective synthetic antioxidants commonly used to minimize the oxidation of soybean oil. Min and Wen (1983) reported that TBHQ was a better antioxidant in soybean oil than were BHA or BHT.

3.5. Effects of purified soybean oil and thermally oxidized soybean oil on the stability of β-carotene in the dark at 50 °C

The effects of 1.0%, 2.5%, or 5.0% purified soybean oil and thermally oxidized soybean oil on the 100 ppm β-carotene in the dark at 50 °C are shown in Fig. 3. The β-carotene in the sample without soybean oil was decreased from 100 to 7.8 ppm after 2 days and it completely disappeared after 4 days.

The loss of β-carotene in the sample with 1.0%, 2.5%, or 5.0% of purified soybean oil during 10 days of storage was 0% (Fig. 3). The losses of β-carotene in the sample without any purified soybean oil, with 1.0%, 2.5%, or 5.0% purified soybean oil during the 16 days of storage were 100%, 0%, 0%, or 7.4%, respectively. The loss of β-carotene in the sample with 5.0% purified oil during from 10 to 16 days of storage was from 0% to 7.4%. The peroxide value of the purified soybean oil during the first 10 days did not increase and it started to increase after 10 days of storage (data not shown). The oxidation induction time of purified soybean oil at 50 °C in the dark was considered to be 10 days. The amounts of newly formed oxidized polar compounds in the 5.0% purified oil formed from 10 to 16 days

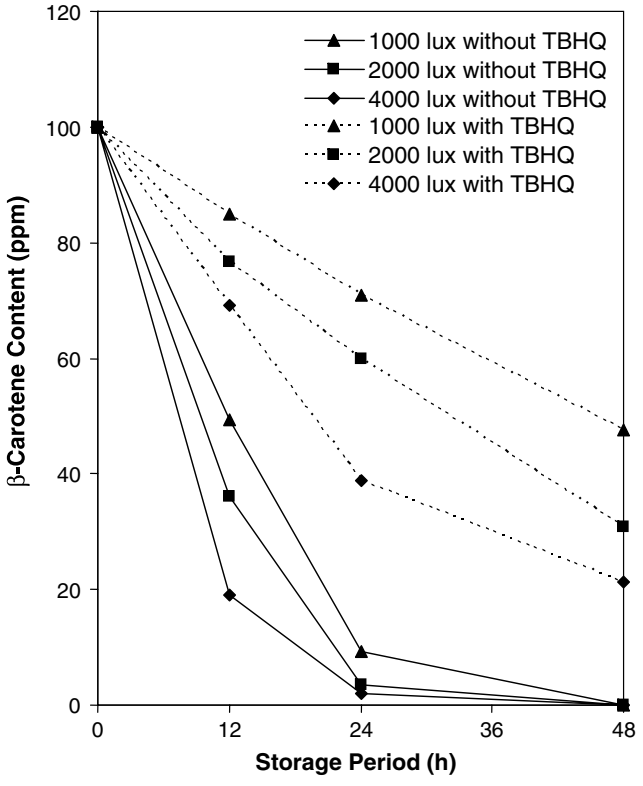

Fig. 2. Effects of 1000, 2000, or 4000 lx and 100 ppm TBHQ on the stability of β-carotene in isooctane at 20 °C.

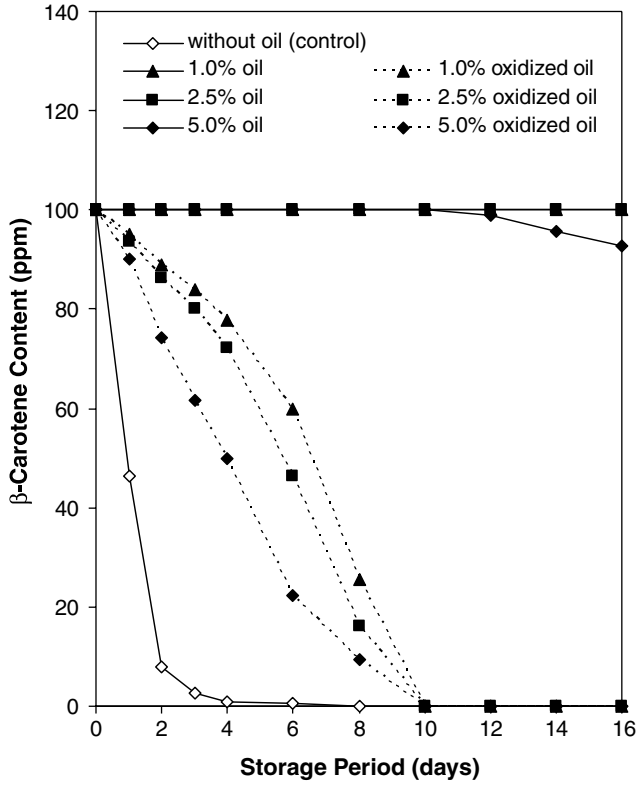

Fig. 3. Effects of purified soybean oil and thermally oxidized soybean oil on the stability of β-carotene in isooctane in the dark at 50 °C.

of storage would be 5 times that of the 1.0% purified oil. The newly formed oxidized compounds are prooxidants.

The effects of 1.0%, 2.5%, or 5.0% thermally oxidized soybean oil on the 100 ppm β-carotene in the dark at 50 °C showed that thermally oxidized soybean oil had a protective effect on the β-carotene at $\alpha = 0.05$ (Fig. 3). The thermally oxidized soybean oil is made of 31% oxidized polar compounds and 69% nonoxidized soybean oil. The oxidized polar compounds are prooxidants. Therefore, the protective effects on 1.0%, 2.5%, or 5.0% thermally oxidized oil must be due to the nonoxidized oil. The losses of β-carotene in isooctane with 1.0%, 2.5%, or 5.0% thermally oxidized oil after 6 days of storage were 40.2%, 53.6%, or 77.8%, respectively, and 100% after 10 days of storage. The 1.0% or 2.5% thermally oxidized oil (on the stability of β-carotene) had a better effect than had the 5.0% at $\alpha = 0.05$. The oxidized polar compounds in the initial 1.0%, 2.5%, or 5.0% thermally oxidized oil were 0.31%, 0.78%, or 1.55%, respectively. The higher the concentration of oxidized compounds in the sample with 5.0% thermally oxidized oil the higher were the losses of β-carotene.

The β-carotene in the sample without purified or thermally oxidized oil was almost completely destroyed after 4 days of storage. The β-carotene in the sample with 1.0%, 2.5%, or 5.0% thermally oxidized oil was also completely destroyed within 10 days. It seems to be extremely important to use approximately 1.0% of high quality nonoxidized oil to dissolve and protect β-carotene in fruit or vegetable juices and drinks enriched with fat soluble vitamins and antioxidants during storage.

4. Conclusions

A sensitive, reproducible and simple HPLC method for the determination of β-carotene in foods was developed. The stability of β-carotene in an isooctane model system without soybean oil decreased rapidly under light or in the dark. The addition of 1.0% purified soybean oil increased the stability of β-carotene at $\alpha = 0.01$. The oxidized compounds formed in the purified soybean oil during storage and in the thermally oxidized oil acted as prooxidants. The 100 ppm TBHQ stabilized β-carotene in isooctane under light storage.

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