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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