β-CAROTENE INDUCED OXIDATION OF HIGH OLEIC TRIACYLGLYCEROLS MODEL SYSTEM

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Abstract

High oleic triacylglycerols (TAGs) are the main components of some important edible oils like olive and rapeseed oils. During thermal oxidation triacylglycerols are degraded resulting in to the formation of unpleasant flavor and rancidity. In order to avoid rancidity, natural or synthetic antioxidants are used. These antioxidants increase the triacylglycerols resistance to the thermal oxidation. β-Carotene (300μg/g) was added to the high oleic triacylglycerols model system. The samples were oxidized in Rancimat at $110^{o}C$ and 20 L/h of air for 1 to 14 hours. The triacylglycerols were separated and analyzed using isopropanol-methanol as eluent by reversed phase HPLC-ESI-MS. β-Carotene was quantified using standard calibration using HPTLC method. Results suggest that β-carotene as well all TAGs were stable till 6 hours of thermal treatment. At higher exposure time of temperature, both β-carotene and triacylglycerols starts degrading. Among the oxidation products of triacylglycerols, hydroperoxides, bishydroperoxides, epoxides and diepoxides were identified. We found that β-carotene plays antioxidants role at lower temperatures and pro-oxidant at higher temperatures in thermal oxidation.

Key words

β-Carotene, triolein, triacylglycerols oxidation, oxidation products.

INTRODUCTION

Triacylglycerols (TAGs) are the integral part of fats and oils. Oxidation of triacylglycerols is one of the main sources of rancidity and off flavor in edible oils. During cooking triacylglycerols are oxidized to form oxygen containing species like hydroperoxides, peroxides, epoxides etc. The formation of such oxidized species depends on many factors like the degree of unsaturation, temperature of the medium, presence of antioxidants, contaminants

and presence or absence of oxygen. The nonvolatile TAG degradation and oxidation products present in the frying oil, are ingested together with the food and are potentially harmful [1].

β-Carotene is one of the most important oil soluble pigments. It is usually used as additive and colorant in food products like margarine, buttery oils, bakery shortening, fried potatoes, and popcorn oil [2]. β-Carotene is present in very small quantity in nearly all vegetable oils, including corn, groundnut, soybean, rapeseed, linseed, olive, barley, sunflower, cottonseed, sea buckthorn, palm, and soybean oils [3, 4]. β-Carotene has been shown to protect lipids from free radical auto-oxidation by reacting with peroxyl radicals, thus inhibiting propagation and promoting termination of the oxidation chain reaction [5]. Steensen & Min [6] found that during auto-oxidation of soybean oil held in the dark, thermal β-carotene degradation products act as pro-oxidants, while thermally degraded lycopene showed antioxidant activity in similar soybean oil systems. In addition, they found that β-carotene and lycopene degradation products exposed to singlet oxygen oxidation under light neither increase nor decrease the oxidative stability of their respective soybean oil samples. Because of the importance of β-carotene in industry as food colorant and a source of vitamin A, the thermally induced degradation and the resulting loss of color and properties like provitamin A and antioxidant activity, during processing is of great concern to the food manufacturers. We have studied the effect of β-carotene on the thermal stability of model high oleic triacylglycerols.

MATERIALS AND METHODS

High oleic triacylglycerols mixture were from Sigma Aldrich (Germany). All other chemicals and reagents were of ACS grade from Sigma Aldrich USA. The β-carotene fortified TAGs were oxidized in the Rancimat at 110°C and 20 L/h of air for 1 to 14 hours. β-Carotene was measured using our recently reported HPTLC method [7]. The TAGs samples (20mg±0.5mg) were dissolved in 2mL of acetone and HPLC solvent and directly injected in to the HPLC. An Agilent HP 1100 HPLC system coupled to ESI-MS (Agilent, Waldbronn, Germany) was used. The analytes were eluted using an isocratic solvent system consists of 18% isopropanol in methanol (0.1% acetic acid) with ammonium acetate (0.05%). The ESI-MS spectra were obtained at *m/z* range of 200 to 1000.

RESULTS AND DISCUSSION

Triacylglycerols mixture was found stable till 6 hours of thermal oxidation in the Rancimat at 110°C. We found that the degradation of β-carotene was also stable till 6 hours.

When the samples were oxidized for 8 hours, a significant degradation of β-carotene were observed, there was no carotene at 10 hours of treatment. In our previous experiments with sunflower oil fortified with β-carotene (100 μg/g), β-carotene was found significantly degraded in the first hour of thermal treatment [7] and a significant (P < 0.05) degradation was observed after 3 h. So the faster degradation of β-carotene can be attributed to presence of other pro-oxidants in sunflower oil. While in this TAGs model system the stability of β-carotene at higher temperature for longer time can also be because of the absence of any other pro-oxidants and also to the high stability of oleic acid in TAGs. Triacylglycerols was stable till 8 hours of thermal treatment at $110^{\circ}C$ in the Rancimat. At 8 hours of treatments some minor peaks were observed in the ESI-MS chromatogram. Figure 1 showed structures of a few identified oxidation products. We found that the percent peak areas of peak 2 and peak 3 (Figure 2) were smaller at 12 hours of treatment, while their quantity increased after 14 hours of treatment. The stability of OOO and OOS were the same in the presence or absence of β-carotene at 12 hours of oxidation. The percent peak area of triolein epoxide (peak 7) increased significantly at 14 hours.

Figure 1. Chemical Structures of the oxidation products of TAGs identified from ESI-MS spectra. (A) 1-oleoyl-2,3-dilinolein bishydroperoxide (*peak 1*), (B) 1,3-dioleoyl-2-linolein epoxide (*peak 5*), (C) triolein epoxy diepoxide (at 12h of oxidation).
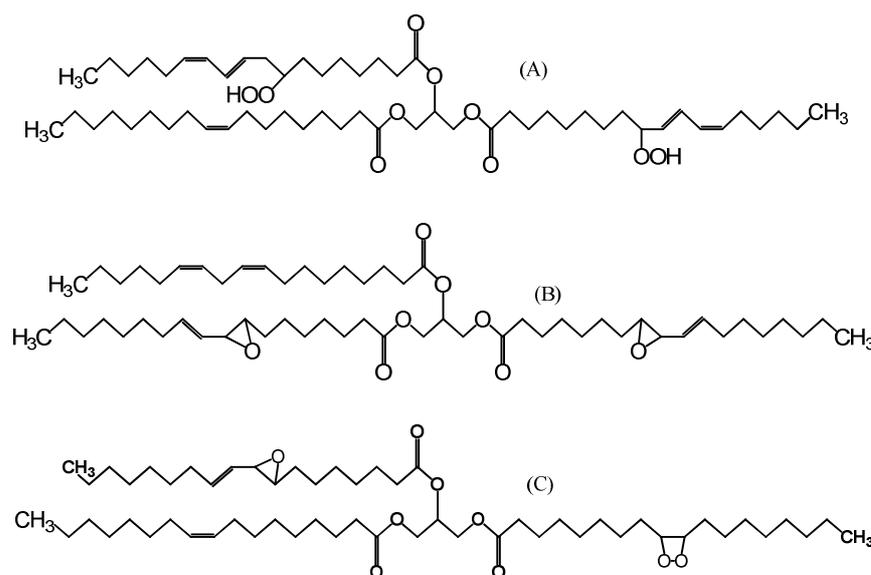

Figure 2 shows the comparison of un-oxidized control TAGs mixture having the composition of LLL (peak 9) , OLL (peak 10), OLO (peak 12), POO (peak 13), OOO (peak 14), OOS (peak 15) and AOO (peak 15) respectively, with the oxidized samples in the presence and absence of β-carotene. There was a significant changes in the chemistry of TAGs mixture after 10 hours of oxidation. At 14 hours, OOO and OOS showed more resistant to oxidation in the presence of β-carotene, while LLL (peak 9), OLL (peak 10), and OLO (peak 12) were completely degraded or oxidized. A total of 9 new peaks arises in the ESI-MS chromatograms of oxidized TAGs in the presence and absence of β-carotene (Figure 2). These were identified as 1-

oleoyl-2,3-dilinolein bishydroperoxide (peak 1), triolein bishydroperoxide (peak 2), triolein bisepoxide (peak 3), 1,2-dioleoyl-3-stearin diepoxide (peak4), 1,3-dioleoyl-2-linolein epoxide (peak 5), triolein hydroperoxide (peak 6), triolein epoxide (peak 7) and 1,2-dioleoyl-3-stearin epoxide (peak 11) respectively. Neff and Byrdwell [8] also found similar oxidation products for the auto-oxidation of standard TAGs using APCI-MS. Interestingly the fragmentation mechanism proposed in their work is similar to our fragmentation mechanism with certain exceptions. This method was found more faster and simple than reported earlier.
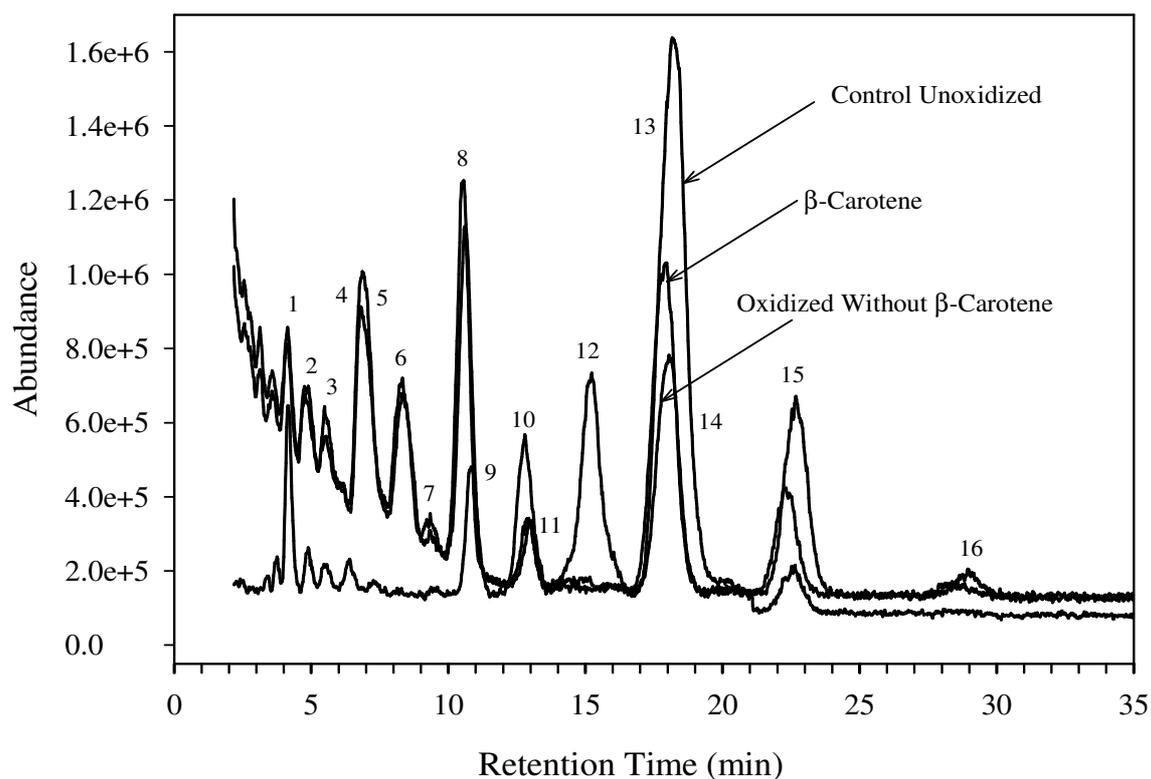
Figure 2. A comparison of TIC ESI-MS chromatograms of control un-oxidized sample with the samples in the presence and absence of β-carotene. The new peaks (1-8 and 10) are the initial oxidation products of Triacylglycerols.

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