Food Chemistry 123 (2010) 622–627

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of α-tocopherol, β-carotene and ascorbyl palmitate on oxidative stability of butter oil triacylglycerols

Ihsan Karabulut *

Department of Food Engineering, Inonu University, 44280 Malatya, Turkey

ARTICLE INFO

Article history:
Received 24 August 2009
Received in revised form 22 March 2010
Accepted 29 April 2010

Keywords:
Butter
Triacylglycerol
α-Tocopherol
β-Carotene
Ascorbyl palmitate
Oxidation
Peroxide value
p-Anisidine value

ABSTRACT

Butter oil triacylglycerols (BO-TAGs), free of antioxidants, including β-carotene, were obtained via sequential treatments with activated carbon (AC) adsorption and alumina column chromatography. α-Tocopherol, β-carotene and ascorbyl palmitate (AP) were added to BO-TAGs, individually, or in different combinations. An accelerated oven-oxidation test was carried out at 60 °C to determine the most effective dosages of the antioxidants. Among the antioxidants evaluated, α-tocopherol was found to be the most effective, at the concentration of 50 μg/g. To determine the possible synergism between the antioxidants, binary or ternary combinations of α-tocopherol, β-carotene and AP were added to BO-TAGs at concentrations of 50, 5, and 50 μg/g, respectively. Ternary combinations of these antioxidants were significantly better in retarding oxidation than were binary blends of α-tocopherol with β-carotene or AP. However, a prooxidant effect was observed, especially when β-carotene and AP were used individually or in binary combination.

1. Introduction

Fats and oils are important functional components of foods. They contribute to flavour, odour, colour and texture. However, lipid oxidation occurs during storage or processing and leads to depletion in the quality of food products, resulting in shortening of shelf life, reducing their nutritional quality (Frankel, 1991; St. Angelo, 1996). When lipids are exposed to environmental factors, such as air, light and temperature, autoxidation reactions start to produce undesirable flavours, rancid odours, discoloration and other forms of deterioration. The primary autoxidation products are hydroperoxides, which have no taste or flavour, while their degradation products, called secondary oxidation products, do have detectable tastes and flavours (Choe & Min, 2006).

α-Tocopherol is a primary antioxidant, functioning by terminating free-radical chain reactions, by donating hydrogen or electrons to free radicals and converting them to more stable products (Frankel, 1998). β-Carotene is an oil-soluble and natural pigment of many oils, such as palm, olive and butter oils. In addition to its use as a food colouring agent, β-carotene also has a strong antioxidant activity. It has been shown to protect lipids from autoxidation by reacting with peroxyl radicals, thereby inhibiting propagation and promoting termination of the oxidation chain reaction (Britton, 1995). β-Carotene retards potentially harmful oxidative reactions by trapping free radicals and quenching singlet oxygen (Yanishlieva, Aitzemüller, & Raneva, 1998). Ascorbic acid is a secondary antioxidant that can be broadly classified as an oxygen scavenger/singlet oxygen quencher; it reacts with free oxygen and removes it in a closed system (Perricone et al., 1999). Ascorbyl palmitate (AP) is a synthetically-derived fat/oil-soluble ester of ascorbic acid. It was reported that AP is not only a synergist, with α-tocopherol, but also a radical-type inhibitor of lipid autoxidation (Beddows, Jagait, & Kelly, 2001; Marinova & Yanishlieva, 1992).

Triacylglycerols (TAGs) of edible oils are usually used to evaluate the anti- and/or prooxidant activities of synthetic and natural antioxidants. In order to determine the effects of antioxidants in oils, TAGs free of antioxidants have been produced and used in some model oxidation studies (Fuster, Lampi, Hopia, & Kamal-Eldin, 1998; Haila, Lievonen, & Heinonen, 1996; Isnardy, Wagner, & Elmafda, 2003; Lampi & Piironen, 1998; Lampi, Piironen, Hopia, & Koivistoinen, 1997).

Components of mixed antioxidant systems can contribute to the inhibition of oxidation, with the resulting antioxidant activity reflecting either additive or synergistic effects of the components. Due to uncompleted application of stripping, cooperative actions of β-carotene, naturally present in the oil with added antioxidants, have been neglected in reported studies (Lampi & Piironen, 1998; Lampi et al., 1997). Therefore, the aim of this study was to compare the effectiveness of α-tocopherol, β-carotene and AP, individually,

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* Tel.: +90 4223410010; fax: +90 4223410046.
E-mail address: ikarabulut@inonu.edu.tr

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doi:10.1016/j.foodchem.2010.04.080

or in combination, on oxidative state of butter oil triacylglycerols (BO-TAGs) under accelerated oxidation conditions. Primary oxidation products were measured by peroxide value (PV) and secondary oxidation products were measured by p-anisidine value (AV).

2. Materials and methods

2.1. Reagents

Butter was purchased from local markets. Organic solvents and neutral aluminium oxide were purchased from Merck (Darmstadt, Germany). β-Carotene (Type I, 95%), p-anisidine and AP were purchased from Sigma–Aldrich (Steinheim, Germany). Activated carbon (AC) was prepared from waste apricots in our laboratory, as described in a previous report (Basar, 2006).

2.2. Methods

2.2.1. Preparation of BO-TAGs

BO was produced by melting of butter at 55 °C, and filtering through a plain filter paper coated with anhydrous sodium sulphate at the same temperature. BO was kept at −18 °C, until used in subsequent procedures. Fatty acid composition (mol%) of BO was determined by a GC technique as follows: C4: 1.8, C6: 2.2, C8: 1.2, C10: 2.9, C12: 3.6, C13: 0.1, C14: 12.1, C14:1: 1.4, C15: 1.4, C15:1: 0.5, C16: 35.4, C16:1: 2.2, C17: 0.6, C17:1: 0.4, C18: 9.0, C18:1: 24.9, C18:2: 1.9, C18:3: 0.3, and C20:1: 0.2. Tocopherol and β-carotene contents of BO were determined by normal phase HPLC with a Phenomenex Luna Silica column (4.6 mm i.d. × 250 mm, 5 μm). Separation was based on isocratic elution with *n*-hexane (99%) and *iso*-propanol (1%) at 292 nm (Karabulut, Topcu, Yorulmaz, Tekin, & Ozay, 2005). Tocopherol and β-carotene contents were determined as follows: α-tocopherol, 22.1 μg/g; β-tocopherol, 0.5 μg/g; δ-tocopherol, 2.6 μg/g; γ-tocopherol, 1.4 μg/g; β-carotene, 3.7 μg/g.

To obtain pure BO-TAGs, β-carotene and tocopherols were removed from BO by the same procedure as described in a previous report (Karabulut, Topcu, Akmil-Basar, Onal, & Lampi, 2008). To remove β-carotene, BO (60 g) was mixed with AC (6 g) in a flask (100 ml) connected to a rotary evaporator at 60 °C for 300 min. Thereafter, BO was stripped to remove pro- and antioxidants to obtain purified BO-TAGs, applying the method of Lampi, Dimberg, and Kamal-Eldin (1999) with the following modifications. A double-jacketed glass chromatography column (300 × 9 × 28 mm i.d.) was packaged with a slurry of 150 g neutral aluminium oxide (activated at 100 °C for 16 h and 220 °C for 8 h) and conditioned with 250 ml of *n*-hexane. The mixture, containing AC and BO, obtained from an adsorption experiment, was suspended in an equal volume of *n*-hexane and loaded onto the column. The column, which was maintained at 37 °C by circulating water from a water bath, was eluted with 150 ml of *n*-hexane. In order to prevent light-induced oxidation of the purified BO-TAGs, the column and the collecting flask were wrapped with aluminium foil. The solvent was removed with a rotary evaporator at 37 °C under vacuum. The BO-TAGs were then dried under nitrogen and stored at −18 °C for further experiments. It was determined that the BO-TAGs contained no tocopherols or β-carotene.

2.2.2. Oxidation

At the first stage of the oxidation experiments, 5.0 g aliquots of liquid BO, BO-TAGs (control), and BO-TAGs containing antioxidants alone (different concentrations of α-tocopherol, β-carotene and AP) were placed in 2.4 cm (i.d.) vials (15 ml) and oxidised in an oven set at 60 °C ± 1. To prepare BO-TAGs spiked with antioxidants, aliquots of solutions of α-tocopherol in acetone, β-carotene in chloroform and AP in acetone, at known concentrations, were added to the BO-TAGs. The solvents were evaporated under a stream of nitrogen. Concentrations of α-tocopherol (10, 25 and 50 μg/g), β-carotene (5, 10, 25 and 50 μg/g) and AP (5, 50, 100 and 200 μg/g) were selected, based on the results of numerous preliminary studies in which concentrations of the antioxidants were evaluated. Two vials, one for each sample, were withdrawn at definite time intervals (2, 4, 6, 8, 16 and 32 days for BO and BO-TAG + α-tocopherol mixtures; 2, 4, 6 and 8 days for BO-TAG, BO-TAG + β-carotene and BO-TAG + AP mixtures) to assess the oxidation status. Flow charts, showing the experimental design, including oxidation times and antioxidant dosages, are summarised in Fig. 1. After determination of effective dosages that provide the lowest PV and AV, binary or ternary mixtures of antioxidants, at effective dosages with BO-TAGs, were subjected to oxidation under the same conditions.

2.2.3. Measurements of oxidation status

Primary oxidation products (hydroperoxides) were determined by PV measurements. Approximately 1 ± 0.1 g of oil was weighed and subjected to iodometric determination of PV according to Official Method Cd 8-53 (AOCS, 1997). Formation of secondary oxidation products was measured by AV according to Official Method Cd 18-90 (AOCS, 1997). Duplicate measurements were conducted for each vial.

2.2.4. Statistics

SPSS version 9.0 was used to perform statistical calculations. Significant differences in the means of PVs and AVs between BO and BO-TAG, with and without antioxidants, were determined by using a least significant difference test and analysis of variance procedure ($p < 0.05$).

3. Results and discussion

BO-TAGs, free of antioxidants, including tocopherols and β-carotene, partial glycerides and free fatty acids, were obtained *via* sequential treatments, using AC adsorption and alumina column chromatography. After this treatment, no detectable levels of PV and AV were found in BO-TAGs. When the fatty acid composition of the BO-TAG is taken into account, unsaturated fatty acids, including oleic, linoleic and linolenic acids, were destroyable molecules in the BO-TAGs. Thus, BO-TAGs began to oxidise at the beginning of oxidation. Compared to the PV of the BO, significantly higher values were observed ($p < 0.05$) for BO-TAGs throughout the oxidation experiment, as shown in Fig. 2. Similarly, Lampi and Piironen (1998) have reported that PV of the BO-TAGs reached 32 meq/kg at the 16th day of oxidation, monitored at 40 °C. As shown in Fig. 2, the increasing trend observed for AV results is very similar to that obtained in PV experiments. Fast oxidation of BO-TAGs has to be a consequence of the instability of the hydroperoxides. As hydroperoxides decomposed, new reactive species were formed which catalysed oxidation (Lampi & Piironen, 1999). Both PV and AV indicated that the BO had a resistance to oxidation due to its own (naturally occurring) antioxidants. While the induction period of BO was between 16 and 32 days, there was no apparent induction period for BO-TAGs, as shown in Fig. 2.

Fig. 3 shows the effects of α-tocopherol, β-carotene and AP, individually, on oxidation, monitored by PV and AV under the same conditions as with BO and BO-TAGs, with the exception of oxidation times. There was a significant difference ($p < 0.05$) between PV and AV of three antioxidants when they were added to BO-TAGs at the same levels. Compared to β-carotene and AP, a higher oxidation time is needed to reach an induction period for α-tocopherol. As shown in Fig. 3A, among the antioxidants tested, α-tocopherol