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Interactions between tocopherols, tocotrienols and carotenoids during autoxidation of mixed palm olein and fish oil

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ABSTRACT

Electron spin resonance (ESR) and spin trapping detection of radical formation showed that the oxidative stability of palm olein/fish oil mixtures increased with the amount of palm olein. Mixtures with red palm olein were less stable than were mixtures with yellow palm olein. Addition of ascorbyl palmitate and citric acid gave further reduction of radical formation, whereas no effect was observed by adding lecithins. Storage of palm olein/fish oil mixtures (4:1) at 30 °C confirmed that red palm olein mixtures were less stable than were yellow palm olein mixtures. Ascorbyl palmitate together with citric acid improved the stability in both cases. The concentrations of α-tocopherol and α-tocotrienol decreased during storage, whereas β-, γ-, and δ-tocotrienols were unaffected. Ascorbyl palmitate reduced the losses of α-tocopherol and α-tocotrienol. The rate of loss of carotenoids was independent of the presence of fish oil and, except for an initial fast drop, also of the presence of ascorbyl palmitate.

1. Introduction

Many clinical and epidemiological studies have shown important functional roles for n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) (C20:5) and docosahexaenoic acid (DHA) (C22:6), in infant brain development, protection against cancer and cardiovascular diseases, and more recently, also in protection against various mental illnesses, including depression, attention-deficit hyperactivity disorder and dementia (Riediger, Othman, Suh, & Moghadasian, 2009).

Fish oil is a good source of EPA and DHA. As a consequence, there is an interest in enriching food products with fish oil to increase the intake of the n-3 PUFA. However, necessary measures must be taken to stabilise the fish oil in food products since polyunsaturated fatty acids are highly susceptible to oxidation. A number of publications have reported the efficacy in delaying the peroxidation by using antioxidant systems based on α, or γ/δ, or δ-tocopherol concentrates, ascorbic acid (or ascorbyl palmitate), citric acid, and lecithin (Hamilton, Kalu, McNeill, Padley, & Pierce, 1998). The ability of these antioxidant systems to prevent formation of off-flavours is, however, limited (Hamilton et al., 1998; Olsen, Vogt, Saarem, Greibrokk, & Nillsson, 2005), while it has been reported that the dilution of fish oil with vegetable oil is effective in retarding development of fishy off-flavours in some products (Hamilton et al., 1998).

Palm olein, a relatively inexpensive edible oil, is widely consumed as a major lipid source in developing countries. Both red palm olein and yellow palm olein are rich in tocopherols and the chemically closely related tocotrienols (about 1 g/kg oil), which have been reported to protect palm olein from oxidation during deep fat frying of potato chips (Schroeder, Becker, & Skibsted, 2006). Red palm olein contains high amounts of carotenoids, including α- and β-carotene (pro-vitamin A), from which the red colour of red palm olein originates. Vitamin A deficiency is a serious problem in many developing countries, though yellow palm olein is often preferred since it has been regarded as a higher quality refined oil than red palm oil. Synergistic antioxidant effects have been demonstrated where tocopherols and tocotrienols regenerate carotenes which are the primary substrates for the lipid derived-radicals in red palm olein (Schroeder et al., 2006). Carotenoids are also able to efficiently inhibit light-induced lipid oxidation.

The combination of fish oil with red palm olein could give an edible oil with interesting nutritional properties, since it would be rich in valuable long chain n-3 PUFA, vitamin E and provitamin A carotenoids. The high levels of antioxidant compounds in the palm olein could furthermore provide efficient protection of the fish oil fatty acids that are highly susceptible to oxidation and thereby improve the storage stability.

In this study, the oxidation stability of fish oil mixed with red or yellow palm olein has been examined by electron spin resonance (ESR) spectroscopy and differential scanning calorimetry (DSC), in order to perform initial fast screening before a storage study.

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Concentrations of tocopherols, tocotrienols and carotenes, as well as of primary and secondary oxidation products, were monitored during the storage study, in order to examine their interactions during the oxidative processes in the mixed oils. The overall purpose of the combined studies was to evaluate whether a simple mixture of palm oleins and fish oil could provide a nutritionally valuable oil with, at the same time, a good protection against oxidation.

2. Materials and methods

2.1. Materials

All chemicals used were of analytical grade. N-tert-butyl-α-phenylnitrone (PBN, purity above 97%), and ascorbyl palmitate were purchased from Fluka Chemie Gmbh (Buchs, Germany). All other chemicals and solvents, including L-α-phosphatidylethanolamine (P8193, Sigma), and L-α-phosphatidylcholine (P3556, Sigma), were obtained from Sigma–Aldrich Laborchemikalien GmbH (Steinheim, Germany) or Merck (Darmstadt, Germany).

Neutralised, bleached, and deodorized yellow (iodine value (IV) = 65.0 g I_2/100 g) and red (IV = 64.9 g I_2/100 g) double-fractionated palm oleins were obtained from Unitata (Teluk Intan, Malaysia). Fish oil was a gift from TripleNine Fish Protein a.m.b.a. (Esbjerg, Denmark) and was purified by alumina column chromatography according to Fuster, Lampi, Hopia, and Kamal-Eldin (1998) before use.

2.2. ESR spectroscopy

Radical formation in lipids was determined by the spin trapping method, as described previously (Velasco, Andersen, & Skibsted, 2004). PBN (1 mg/g) was added to samples before the start of incubation at 60 °C. ESR spectra were obtained on a Jeol Jes-FR-30 ESR spectrometer (JEOL Ltd., Tokyo, Japan). The parameters were as follows: microwave power, 4 mW; sweep width, 5.0 mT; sweep time, 2 min; modulation width, 0.125 mT; time constant, 0.3 s. The radical intensity was defined as the peak-to-peak amplitude of the first line of the PBN-radical adducts signal and the signal of the Mn(II) marker attached to the cavity of the spectrometer.

2.3. Differential scanning calorimetry (DSC) analysis

Induction periods were determined by a DSC method on a DSC 1 Star^e system (Mettler Toledo, Schwerzenbahn, Switzerland) under isothermal conditions (100 °C), with an air flow of 60 ml min^{-1}, as described previously (Velasco et al., 2004).

2.4. Preparation of oil samples and storage study

Ascorbyl palmitate, citric acid, and (or) lecithin were dissolved in methanol, and the solvent was removed with a stream of nitrogen. The residue was dissolved in palm olein/fish oil to obtain samples with specified concentrations of antioxidants. Sample aliquots (4 g) in air-permeable 10 ml polypropylene tubes (Z12-1828, Merck, Darmstadt, Germany) were stored in the dark at 30 °C for 21 days. Hydroperoxides, TBARS, tocopherols and tocotrienols, and carotenes were determined regularly during the storage period.

2.5. Determination of lipid hydroperoxides

The method of Adamsen, Hansen, Møller, and Skibsted (2003) was followed with minor modifications. Lipid hydroperoxide levels were determined as the peroxide values (POV). Briefly, 10 mg of oil were dissolved in 2 ml of butanol:methanol solution (1:1 v/v); 2 ml 0.13 M ammonium thiocyanate were added to the sample and mixed, followed by addition of 2 ml of 7.7 mM ammonium sulphate; after exactly 2 min, the absorbance of the solution was measured at 510 nm, using a UV–Visible scanning spectrophotometer (UV 1201, Shimadzu, Japan). Hydroperoxide concentrations were determined using a standard curve prepared with iron powder.

2.6. Determination of thiobarbituric acid-reactive substances (TBARS)

The method of MacDonald and Hultin (1987) was followed with minor modifications. TBARS were determined by mixing 15 mg of oil, 2 ml of Mili-Q water, and 2 ml of TBA reagent (15% (w/v) trichloroacetic acid and 0.375% (w/v) thiobarbituric acid in 0.25 M HCl) in test tubes, followed by heating in a boiling water bath for 15 min. The test tubes were cooled to room temperature for 10 min. Then the absorbance was measured at 532 nm. Concentrations of TBARS were determined using standard curves prepared with 0.1 to 24 μM malonic dialdehyde-bis(diethylacetal) (TEP).

2.7. Determination of tocopherol and tocotrienols

Tocopherols and tocotrienols were measured by HPLC, as described previously (Schroeder et al., 2006) on a silica-based Supercosil LC-NH2 column (Supelcosil^{TM} LC-NH_2, 25 × 4.6 mm, 5 μm; Sigma–Aldrich Co.) with the eluting solvent of heptane/ethylacetate (88:15 v/v) at a flow rate of 1.0 ml/min. The eluent was monitored fluorometrically at an excitation wavelength of 295 nm and detection at the emission wavelength 330 nm, using an Agilent fluorescence detector G 1321A. 50 mg of oil sample were dissolved in 5 ml of heptane with BHT, of which 20 μl were injected into the HPLC. Linear standard curves ($r^2 > 0.9997$) were made with the individual tocopherols and tocotrienols dissolved in hexane, using concentrations between 0.25 and 10 mg/l.

2.8. Determination of carotenoids

Carotenoids were determined according to the PORIM test method (PORIM, 1995). Oil samples were dissolved in hexane; the absorbance was measured at 446 nm. The carotenoid content of oil samples was expressed as mg beta-carotene/kg oil (molar absorption coefficient was 1.40×10^5 l mol^{-1} cm^{-1}).

2.9. Statistical analysis

All experiments were performed in duplicate. Significant differences between samples were calculated by comparison of means using the Origin® 7 software. Significance was declared at $p < 0.05$. Statistical analyses were performed using Excel (Microsoft).

3. Results and discussion

3.1. Radical formation in palm olein/fish oil mixtures

The oxidative stabilities of fish oil and palm olein mixtures were tested by using the spin trapping technique and ESR detection, since this method is fast and can be carried out at moderately elevated temperatures (Andersen, Velasco, & Skibsted, 2005; Falch, Velasco, Aursand, & Andersen, 2005). The method determines the tendency to radical formation, and thereby provides information about the relative importance of pro- and antioxidative components. As expected, the radical formation in oils increased with increasing levels of fish oil (0%, 20%–50%; Fig. 1). The relative