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Singlet oxygen autoxidation of vegetable oils: Evidences for lack of synergy between β-carotene and tocopherols

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ABSTRACT

The synergy between β-carotene and tocopherols – antioxidants protecting oils from oxidation, was analyzed in a model system. The model used stripped borage and evening primrose oils. A chlorophyll extract, β-carotene and one of the tocopherols were added together or separately to the oils. Oil oxidation was initiated by singlet oxygen that was produced by chlorophylls irradiated with the use of a xenon lamp equipped with the cut-off 600 nm filter. Experiments were carried out at two mole ratios of tocopherols to β-carotene, i.e. at 1:1 and 23:1. Analyses were performed using absorption and fluorescence spectra in the UV + Vis region. The results demonstrated an antagonistic action of the antioxidants. The protective effect of unsaturated fatty acids was significantly better in the case of β-carotene compared to the tocopherols. Furthermore, tocopherols were less effective in protecting the oils in the presence of β-carotene than without it.

1. Introduction

Vegetable oils contain a set of antioxidants that prevent them from deteriorating. Carotenoids (Cars) and tocopherols (TOHs) prevent autoxidation and photooxidation in oils. Photooxidation can take place with the participation of triplet $^3O_2$ (TO) or singlet oxygen $^1O_2$ (SO). The oxidation process involving TO is approx. 1450 times less efficient than with the participation of SO (Rawls & Santen, 1970). The reactions of lipids with TO and SO will produce dienes and trienes and with SO only also unconjugated hydroperoxides (Choe & Min, 2006a). Oxidation reactions with TO involve radical formation, while the SO reactions do not, SO can directly react with the double bonds of fatty acids (FA) and oxidize them without the formation of an alkyl radical. Oxidized dienes and trienes are formed this way in the process of initiation of the oxidation reaction (Choe & Min, 2006a; Rawls & Santen, 1970). Type I or II photosensitizer (Sen) must be present in oil in order to produce SO. Sen II is a molecule that produces an SO when irradiated. Natural type II Sens in oils include: chlorophylls (Chls) and pheophytins (Phe). The quenching of SO produced by them may be done chemically or physically. Chemical quenching involves reaction of SO with the quenching agent to produce an oxidized product. Physical quenching causes the return of SO to TO and can proceed via either energy transfer or charge transfer. Therefore, TO quenchers must either be able to donate electrons or to accept energy 22.5 kcal above ground state (Min & Boff, 2002). An example of the latter is β-Car that has a low triplet energy state and can therefore accept the energy from singlet oxygen (Lee & Min 1988), making the process thermodynamically possible. Different efficiency of β-Car and TOHs results from a different mechanism of action, as both compounds act differently under the same aerobic conditions. β-Car can act directly on the SO by transferring it from the singlet to the triplet state and indirectly by transferring Sen in excited singlet or triplet state to the ground singlet state (Foote & Denny, 1968; Frąckowiak & Smyk, 2007). Both routes will not change β-Car molecule as the process occurs by physical rather than chemical way. TOHs actions have been described by Foote, Chang, and

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Denny (1970). They found that the mechanism by which TOHs act in quenching SO involves charge transfer. The reaction involves an electron transfer from TOH to SO, forming a charge transfer complex. The transfer complex undergoes an intersystem crossing to ultimately form triplet oxygen and the starting TOH. Similarly, as in the case of β-Car this process also did not change the TOH molecule. Cars can also exert a pro-oxidative effect on lipids, sustaining the oxidation reaction. TOHs protect β-Car against pro-oxidative action in the process of lipid autoxidation (Heinonen, Haila, Lampi, & Piironen, 1997) as well as photooxidation (Haila & Heinonen, 1994; Terao, Yamauchi, Murakami, & Matsushita, 1980). Oxidation of lipids containing TOHs is slower in the presence of β-Car, suggesting a protective effect with respect to one another (Frankel, 1989; Haila, 1999; Krinsky & Yeum, 2003). The protection of carotenoids by TOHs in oils occurs even for small concentrations of TOH (Haila, 1999). TOHs are considered to be the best natural lipophilic antioxidants that can interact with Cars providing a better protective effect than when acting alone (Choe & Min, 1992). Synergistic interaction effect of Cars and TOHs in inhibiting lipid peroxidation has been reported in many earlier works (Haila, 1999; Li, Wu, Ma, Liu, & Liu, 1995; Palozza & Krinsky, 1991, 1992; Prieto, Murado, & Vázquez, 2014; Terao et al., 1980; Wrona, Korytowski, Różańska, Sarna, & Truscot, 2003). The synergistic interaction in those studies depended on concentrations of antioxidants. When the concentration of α-TOH was much lower than that of the β-Car, this effect was not observed (Handelman, van Kuik, Chaterjee, & Krinsky 1991). Synergistic interaction of these compounds with a mole ratio of 1:1 was confirmed by oxidizing oleic acid (Shibasaki-Kitakawa, Kato, Takahashi, & Yonemoto, 2004) and triacylglycerols in the rapeseed oil of a variety low in erucic acid, in the presence of β-Car and γ- and α-TOH (Haila & Heinonen, 1994; Haila, Lievonen, & Heinonen, 1996; Heinonen et al., 1997). Bohm, Edge, McGarvey, and Truscott (1998) showed the synergistic effects of both antioxidants only in the presence of vitamin C. The effect occurred for the following pairs of antioxidants: β-Car – vitamin C and α-TOH – vitamin C. However, it did not occur when all the molecules were present together. Liu, Shi, Ibarra, Kakuda, and Xue (2008) studied the same antioxidants plus lycopene. For the mixtures of three-antioxidants only the lycopene + vitamin E + vitamin C showed synergism. For four-antioxidants, the following combination, e.g. a mixture of lycopene, vitamin E, vitamin C, and β-Car, produced a synergistic effect. A similar effect of synergism between β-Car and vitamin C was observed by Szymula (2004) who studied microemulsions containing different amounts of propanol and water. However, it can be said that there is relatively little research carried out on the synergy between β-Car and TOHs in vegetable oils and no explicit statements have been formulated.

Therefore, the aim of this work was to investigate, in a model system, the interaction of β-Car and α-, γ- and δ-TOH, protecting oils from photosensitized oxidation. The model system consisted of stripped evening primrose oil (EP) or borage oil (BO), with the addition of Chl and TOH and β-Car separately or TOH + β-Car together. SO served as an unsaturated fatty acid (UFA) oxidizing molecule that was formed in the oil samples as a result of Chl irradiation with red photons generated by the ozone-free xenon lamp and filtered through an absorption cut-off filter 600 nm.

2. Materials and methods

2.1. Materials

BO and EP seeds were purchased from Agropharm (Tuszyn, Poland). These seeds were first cold-pressed using a screw oil expeller featuring a cylindrical perforated strainer basket Komet laboratory CA 59 G (IBG Monfords Gmbh & Co., Mönchengla dbach, Germany) with temperatures ⩽ 40 °C. Mechanical impurities were removed from the pressed oil by centrifugation in a centrifuge type MLW T24D (Janetzki, Leipzig, Germany): 8940g, 10 min. Both oils were stripped according to a procedure given by Khan and Shahidi (2000). A chromatographic column (3.4 cm i.d. × 40 cm) was connected to a water-pump vacuum and packed sequentially with four adsorbents. The bottom layer consisted of activated silicic acid (40 g), followed by a mixture of Celite 545/activated charcoal (20 g; 1:2, w/w), a mixture of Celite 545/powdered sugar (80 g; 1:2, w/w), and activated silicic acid (40 g) as the top layer. All adsorbents were suspended in n-hexane. Oil (100 g) was diluted with an equal volume of n-hexane and passed through the chromatographic column. The solvent in the eluent (stripped oil) was evaporated under vacuum at 35 °C, and traces of the solvent were removed by flushing with nitrogen. FA composition obtained by GLC–FID was typical of these oils. The peroxide value (PV) was determined according to the AOCS Official Method Cd 8b-90 (1997). PV of these oils was determined at 2.2 ± 0.3 meqO_2 kg^{-1} for EP and at 3.9 ± 0.5 meqO_2 kg^{-1} for BO before stripping and after stripping PV was equal to: 1.3 ± 0.1 and 0.68 ± 0.16 meqO_2 kg^{-1} for EP and BO, respectively. The concentration of TOHs was established by HPLC. Chls and Cars were totally removed in stripped oils, their presence was under spectrometric detection limit. The Chls extract (from spinach) was obtained from Prof. Ryszard Amarowicz (Polish Academy of Sciences – branch in Olsztyn, Poland). TOHs: α, γ, δ and β-Car were purchased from Sigma–Aldrich (Poznań, Poland). All solvents and chemicals analytical grade for HPLC, GLC–FID and stripping procedure were purchased from Sigma–Aldrich (Poznań, Poland), Merck (Warsaw, Poland) and POCH (Gliwice, Poland).

2.2. Sample preparation

Samples were prepared in such a way as to obtain the same concentration of Chl in the oil samples. A stock solution was prepared by adding the Chl extract to the stripped oils. Chl extract has been added directly to oils without any solvent. Its concentration was 27.8 ppm and 35.8 ppm in the BO and EP oil, respectively. To one part of the stock solution β-Car was added in 2-fold higher concentration than the final concentration. To the second, third and fourth part of the stock solution α-, δ- and γ-TOHs were added also in 2-fold higher concentration than the final concentrations. Next, the stock solution with Chl was mixed with β-Car or particular TOH solution at 1:1 (w/w) ratio to obtain the final concentration. To obtain a sample with for example Chl + β-Car + α-TOH, the solution of β-Car + Chl and α-TOH + Chl were mixed in at 1:1 (w/w) ratio. In this way, the concentration of β-Car and TOHs was the same in the samples. The final concentration of β-Car was 62.5 ppm and that of α-TOH was about 1140 ppm, then the mole concentration ratio was equal to 1:23. The samples containing δ- and γ-TOH were prepared so as to obtain the same mole ratio to β-Car as in the case of α-TOH. In the second series, the final concentration of β-Car was 62.5 ppm and that of α-TOH was about 50 ppm (1:1 mole ratio). The concentration of δ- and γ-TOH was prepared as above. These ratios did not include concentrations of residual TOHs remaining in the oil after stripping. All samples were prepared by weighing the oil on a scale. The final mass of all samples was 15.00 ± 0.02 g. Samples for measurements of absorption and fluorescence spectra were prepared by diluting 350.0 ± 0.1 mg of oil in 10 mL of n-hexane, for the measurement of the absorption spectra in the range of 250–700 nm, and by 10-fold dilution of this solution for the measurement of the absorption spectra in the range 220–550 nm, and fluorescence spectra.