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Antioxidant activities of tocopherols/tocotrienols and lipophilic antioxidant capacity of wheat, vegetable oils, milk and milk cream by using photochemiluminescence

Jasmin Karmowski [a], Victoria Hintze [a], Josephine Kschonsek [a], Margrit Killenberg [b], Volker Böhm [a,*]

[a] Institute of Nutrition, Friedrich Schiller University Jena, Dornburger Straße 25-29, D-07743 Jena, Germany
[b] Analytik Jena AG, Jena, Germany

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ABSTRACT

The purpose of this study was to measure the antioxidant activity (AOA) of tocopherols and tocotrienols by using photochemiluminescence (PCL). This method enables to detect total lipophilic antioxidants. The AOA of all vitamin E isomers depended on number and position of methyl groups in the chroman ring. Correlation between the AOA and the redox potential and the biological activity of the tocochromanols was observed. The second aim was to analyse different kinds of wheat, vegetable oils, milk and milk cream on their antioxidant capacity (AOC) by using PCL and α-TEAC. The contents of vitamin E and carotenoids were analysed by HPLC. Correlations between the sum of carotenoids and vitamin E and the AOC were detected. Based on high vitamin E contents, the oils had the highest and in contrast, the product macaroni showed the lowest AOC. A concentration-dependent effect was observed in both assays, PCL and α-TEAC.

1. Introduction

The term vitamin E refers to a group of eight fat-soluble vitamins which can be divided in four tocopherols (T) and four tocotrienols (T3) (Abu-Shahin, Stone, Ramsauer, & Krishnan, 2013; Colombo, 2010; Vardi, Levy, & Levy, 2013). Tocopherols have a phytyl chain, while tocotrienols have an unsaturated side chain with double bonds at positions 3′, 7′, and 11′. These two groups of vitamin E have four isomers each, designated as α-, β-, γ- and δ-, which differ in number and position of methyl groups in the chroman ring (Kamal-Eldin & Appelqvist, 1996). Vitamin E is only synthesized by plants and cyanobacteria, and therefore essential for human and animal nutrition (Müller, Theile, & Böhm, 2010; Schneider, 2005). Good sources of vitamin E for the human diet are vegetable oils. α-Tocopherol is predominant in olive-, wheat germ-, and sunflower oils. γ-Tocopherol is the major vitamin E compound in soya-, corn-, rapeseed-, and linseed-oils. δ-Tocopherol and δ-tocotrienol are found in very low concentrations. Other sources are almonds, cashews, hazelnuts, peanuts, walnuts and macadamia nuts (Elmadfa & Wagner, 1997; Kornsteiner, Wagner, & Elmadfa, 2006). Vitamin E has the ability to prevent chronic diseases, cardiovascular diseases, atherosclerosis and cancer (Brigelius-Flohé & Traber, 1999). Epidemiological studies have reported that high vitamin E intakes are correlated with a reduced risk of cardiovascular diseases (Brigelius-Flohé & Traber, 1999).

Prior, Wu, and Schaich (2005) gave an overview on different methods to determine the antioxidant capacity and described

Abbreviations: AOA, antioxidant activity; AOC, antioxidant capacity; DPPH, 2,2′ diphenylpicrylhydrazyl scavenging activity; FRAP, ferric reducing antioxidant power; HPLC, high performance liquid chromatography; CL, chemiluminescence oxygen radical-scavenging activity assay; ORAC, oxygen reducing antioxidant capacity; PCL, photochemiluminescence; T, tocopherols; T3, tocotrienols; TE, trolox equivalents; α-TE, α-tocopherol equivalents; α-TEAC, α-tocopherol equivalent antioxidant capacity.

* Corresponding author. Tel.: +49 3641 949633; fax: +49 3641 949702.
E-mail address: volker.boehm@uni-jena.de (V. Böhm).

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besides other tests the PCL-assay. The PCL-assay can be used for the determination of hydrophilic and lipophilic antioxidants using the PHOTOCHEM® instrument, commercialised by Analytik Jena AG (Jena, Germany). Kits provided by the manufacturer have to be used (Prior et al., 2005).

The purpose of this study was to measure the lipophilic antioxidant activities of tocopherols and tocotrienols by a photochemiluminescence system (PCL) using a cuvette apparatus, which use an optical excitation (365 nm) of a photosensitive compound with UV-light, thus activating a chemical reaction resulting in production of photo energy to be measured. The relationship between the AOA and the chemical structure of tocopherols and tocotrienols will be discussed. Based on the lipophilic character, tocopherols/tocotrienols are generally resorbed in the human organism from vegetable oils. Therefore, our second intention was to determine the antioxidant capacity of various vegetables oils, wheat products as well as milk and milk cream. Furthermore, the contents of vitamin E and carotenoids in all food products were analysed by using HPLC.

2. Materials and methods

2.1. Chemicals

Carbonate buffer, polar-aprotic mixture, photosensitizer and all other chemicals were of analytical grade. HPLC grade water (18 MΩ) was prepared using a Millipore Milli-Q purification system (Millipore GmbH, Schwalbach, Germany). DMSO was obtained from Roth (Karlsruhe, Germany) and MTBE (methyl tert.-butyl ether) was bought from Sauerbrey (Reinhardshagen, Germany). All tocopherols were purchased from Calbiochem (Darmstadt, Germany) and all tocotrienols from Davos Life Science (Singapore). 2,2-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was obtained from Sigma–Aldrich (Taufkirchen, Germany).

2.2. Materials

Peanut oil, maize corn oil, sesame oil, sunflower oil, walnut oil as well as milk and milk cream were bought in a local supermarket and stored in the fridge at 4 °C until analysis. Different kinds of corn were provided by the Max Rubner-Institut in Detmold, Germany, and stored in darkness, under dry conditions. Durum macaroni was also bought in a local supermarket and stored together with the wheat samples at room temperature.

Stock solution of each tocopherol/tocotrienol standard was prepared in ethanol and stored at −30 °C until analysis. The concentrations of these stock solutions were determined spectrophotometrically at their specific absorption maxima (Werner & Böhm, 2011).

2.3. Extraction procedures

All operations were processed under subdued light conditions. All vegetables oils were weighted in a volumetric flask and diluted directly with *n*-hexane. Milk and milk cream were extracted with diethyl ether in falcon tubes. Ethanol was added after a centrifugation (1 min, 0.2 rcf). The upper phase was collected in a volumetric flask and the extraction was repeated five times. The samples were stored at −30 °C until measurement. All wheat samples were weighed into an Erlenmeyer flask each and after the addition of 5 mL water and extraction solvent methanol/tetrahydrofuran (1/1, v/v), each sample was homogenised using an ultra turrax (type T25, IKA-Werke, Staufen, Germany) for 30 s on ice. The extract was filtered under vacuum through filter paper No. 390 (Filtrak, Niederschlag, Germany) on a Büchner funnel with 200 mg magnesium hydroxide carbonate. This extraction was repeated until colourlessness. The combined extracts were rotary-evaporated under reduced pressure at 30 °C. The dried residues were dissolved in 5 mL (volumetric flask) of ethanol using an ultrasonic bath. After centrifugation (5 min, 18,407 rcf), the samples were used for PCL and HPLC measurement.

2.4. Determination of lipophilic antioxidant capacities

2.4.1. PCL

Tocopherol (T), tocotrienol (T3) stock solutions and food extracts were dried under nitrogen. The T/T3 concentrations were prepared to 25, 50 and 75 μmol/L in ethanol and the food extracts were diluted in different concentrations in MtBE/DMSO (1/4, v/v). The AOA and AOC are denoted as α-tocopherol equivalents (α-TE), thus, α-tocopherol was used for a seven-point-calibration (2.5–125 μmol/L). A polar-aprotic mixture, carbonate buffer, photosensitizer and sample were pipetted in a quartz glass cuvette (Hellma GmbH, Müllheim, Germany). The measurement was done in a cuvette apparatus (Analytik Jena AG, Jena, Germany). The lipophilic antioxidant capacity is expressed in μmol α-tocopherol equivalents per 100 g (μmol α-TE/100 g).

2.4.2. α-TEAC

The procedure to measure the α-tocopherol equivalent antioxidant capacity was modified from Müller, Fröhlich, and Böhm (2011). The measurement was done by using a V-530 spectrophotometer (Jasco, Gross-Umstadt, Germany) at 734 nm accordingly (Müller et al., 2011). The method is based on the decolourisation of the ABTS radical cation to determine the antioxidant potential of samples. 100 μL sample solution were mixed with 1000 μL ABTS radical cation working solution. The mixture was shaken for 30 s in reaction tubes and transferred completely in half-micro cuvettes. After centrifugation (30 s, 200 rcf) and exactly 2 min after initiation of mixing, the absorbance was measured at 734 nm. The lipophilic antioxidant capacity is expressed in μmol α-tocopherol equivalents per 100 g (μmol α-TE/100 g).

2.5. HPLC analysis

The analysis of tocopherols and tocotrienols was performed by an isocratic (1.5 ml/min, *n*-hexane/MtBE, 98/2, v/m) HPLC procedure (Merck Hitachi, Darmstadt, Germany) with a fluorescence detector (excitation: 292 nm, emission: 330 nm) and using a normal-phase Eurospher 100 Diol column (250 × 4.0 mm, 7 μm, Knauer, Berlin, Germany) at 35 °C according to Franke, Fröhlich, Werner, Böhm, and Schöne (2010). The contents of carotenoids were analysed by using a RP-HPLC with diode array detection at 450 nm (Merck Hitachi, Darmstadt, Germany) and a Develosil RP-Aqueous (250 × 4.6 mm, 5 μm) C_{30}-column (Phenomenex, Aschaffenburg, Germany) at 13 °C. As mobile phase (1.0 mL/min) the following gradient procedure consisting of MeOH (solvent A) and MTBE (solvent B) was used: initial conditions 90% solvent A and 10% solvent B; 40 min linear gradient to 50% solvent B; 2 min linear gradient to 60% solvent B, 40% solvent A and 60% solvent B for 23 min; 5 min linear gradient to 10% solvent B; and 90% solvent A and 10% solvent B for 5 min (Bauerfeind, Hintze, Kschonsek, Killenberg, & Böhm, 2014).

2.6. Statistical analysis

All experiments were done in triplicate. Results are expressed as mean ± standard deviation. The data were analysed with ANOVA using SPSS statistical software (Version 22.0; SPSS, Chicago, USA). The Student–Newman–Keuls procedure was used to compare the