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Comparing antioxidative food additives and secondary plant products – use of different assays

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Abstract

Twelve food additives and six secondary plant products were analysed on their antioxidant activity by using three different test systems (Trolox equivalent antioxidant capacity, photochemiluminescence, ferric reducing antioxidant power). The results differed depending on the assay. All the food additives showed antioxidant activities comparable to the calibration substance Trolox. In contrast, the secondary plant products had an up to 16 times higher antioxidant potential. This might present a good reason for the food industry to use natural antioxidants instead of synthetic ones to get storage stability for processed food items – which, according to recent surveys, is in the interest of consumers.

Keywords: Food additives; Antioxidant activity; Trolox equivalent antioxidant capacity; Photochemiluminescence; Ferric reducing antioxidant power

1. Introduction

Lipid peroxidation is one reaction in food products which leads to off-flavour and other quality losses (e.g. changes in colour and texture) with large economical relevance (Kanner, 1994). To stabilize products, the food industry uses food additives with antioxidant activity. Typical antioxidants are butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) as well as ascorbic acid and their derivatives. During the last decade, the consumer asked more and more for natural antioxidants instead of synthetic ones. Secondary plant products, widely known for their health promoting effects, have then been evaluated also for their use as food ingredients with stabilizing effects (Britt, Gomaa, Gray, & Booren, 1998; Madsen & Bertelsen, 1995; Madsen, Andersen, Christiansen, Brockhoff, & Bertelsen, 1996). Now, a variety of analytical methods exists to determine the antioxidant activity in all kind of matrices. The use of more than one assay has been strongly recommended. An overview on most of these assays determining the antioxidant potential is given by Böhm and Schlesier (2004).

The aim of the present investigation was, on the one hand, to compare different regularly used food additives regarding their antioxidant activity by using more than one assay system. There are only scarce investigations on natural antioxidants which are asked for by the consumer more and more. Thus, the results of the food additives were then compared to those of some exemplarily selected, prominent secondary plant products, which were also tested by using

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the same assays, to check their antioxidative potential.

2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical grade. Special reagents were ABTS (2,2′-azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid)) (Sigma NO. A 1888, Sigma–Aldrich, Taufkirchen, Germany), Myoglobin (Sigma NO. M 1882), ACL kit (ACL, integral antioxidant capacity of lipophilic substances) (Analytik Jena AG NO. 400.803, Analytik Jena AG, Jena, Germany), ACW kit (ACW, integral antioxidant capacity of water soluble substances) (Analytik Jena AG NO. 400.801), TPTZ (2,4,6-tripyridyl-*s*-triazine) (Sigma NO. T 1253), Trolox® ((*S*)-(−)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Aldrich NO. 39192-1, Sigma–Aldrich, Taufkirchen, Germany). As samples, the following compounds were analysed on their antioxidant activity: (a) food additives: ascorbic acid (⩾99%, Merck NO. 500074, Merck, Darmstadt, Germany), isoascorbic acid (⩾99%, Fluka NO. 58320, Sigma–Aldrich, Taufkirchen, Germany), calcium ascorbate (⩾99%, Fluka NO. 11138), sodium ascorbate (⩾99%, Fluka NO. 11140), BHA (⩾98%, Fluka NO. 20021), BHT (⩾99%, Sigma NO. B 1378), propyl gallate (Sigma NO. P-3130), octyl gallate (⩾99%, Fluka NO. 48700), α-tocopherol (⩾95%, Calbiochem NO. 613424, Merck, Darmstadt, Germany), β-tocopherol (⩾95%, Calbiochem NO. 613424), γ-tocopherol (⩾ 95%, Calbiochem NO. 613424), δ-tocopherol (⩾95%, Calbiochem NO. 613424); (b) secondary plant products: caffeic acid (⩾99%, Sigma NO. C 0625), (±)-catechin (Sigma NO. C 1788), eugenol (⩾99%, Riedel-de Haën NO. 35995, Sigma–Aldrich, Taufkirchen, Germany), gallic acid (⩾98%, Fluka NO. 48630), quercetin (Extrasynthese NO. 1135 S, Extrasynthese, Genay, France) as one of the most investigated polyphenols, and rosmarinic acid (⩾97%, Fluka NO. 44699). For the hydrophilic compounds aqueous solutions (1 mmol/L) were prepared and used undiluted or diluted up to 1:50 for the measurements. The tocopherols were dissolved at concentrations of approx. 2.5 mmol/L in *n*-hexane and diluted 1:20–1:200 for analysis. The other lipophilic substances were dissolved at concentrations of around 1 mmol/L in ethanol and diluted up to 1:50 for the determinations.

2.2. Equipment

Measurements were done in disposable cuvettes or microplates or reaction tubes using a spectrophotometer model Uvidec-610 (Jasco, Groß-Umstadt, Germany), a microplate reader model anthos ht2 (Anthos, Krefeld, Germany) and a Photochem® (Analytik Jena AG, Jena, Germany). The following methods were used as originally described, only in some cases slightly modified: Trolox equivalent antioxidant capacity (TEAC) assay (Böhm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002; Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993; Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996), photochemiluminescence (PCL) assay (Popov & Lewin, 1999), Ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996; Bub et al., 2000).

2.3. TEAC assay with ABTS and Metmyoglobin (=TEAC I) (Miller et al. 1993)

Antioxidant activity was analysed by using the TEAC assay. This test is based on the oxidation of ABTS in the presence of H_2O_2 and metmyoglobin to the radical cation $ABTS^{\bullet+}$ (blue–green colour), which is photometrically measured at 734 nm. Dependent on the concentration of radical trapping substances, oxidation is delayed. All solutions were prepared in phosphate buffered saline (PBS), pH 7.4. Stock solutions of antioxidants were diluted with distilled water. Absorbance was recorded continuously. After formation of the radical cation $ABTS^{\bullet+}$, an increase of absorbance was registered. The antioxidant potential of the substances was checked by measuring the lag phase.

2.4. TEAC with MnO_2 (=TEAC II) (Böhm et al., 2002; Miller et al., 1996)

Antioxidant activity was determined following a procedure similar to that of Miller, Sampson et al. (1996) slightly modified (Böhm et al., 2002). The $ABTS^{\bullet+}$ radical cation was generated by filtering a solution of ABTS (in PBS) through manganese dioxide powder. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2-μm syringe filter. This solution was diluted in 5 mM PBS pH 7.4, adjusted to an absorbance of 0.700 at 734 nm and pre-incubated at room temperature prior to use for 2 h.

One millilitre of the $ABTS^{\bullet+}$ solution and 200 μL of the solution of antioxidants were vortexed for 30 s in reaction tubes, which were then centrifuged for 60 s at 10,000 rpm. The absorbance (734 nm) of the lower phase was taken exactly 2 min after initiation of mixing. Solvent blanks were run in each assay. The antioxidant activity of the substances was calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\%\text{antioxidant activity} = [(E\ (ABTS^{\bullet+}) - E\ (\text{Standard}))/E\ (ABTS^{\bullet+})] * 100 \quad (E = \text{extinction}).$$