ELSEVIER

Food Chemistry 72 (2001) 223–229

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidant activity of some roasted foods

U. Krings, R.G. Berger *

Institut für Lebensmittelchemie, Zentrum Angewandte Chemie der Universität, Wunstorferstr. 14, D 30453 Hannover, Germany

Received 5 April 2000; received in revised form 19 July 2000; accepted 19 July 2000

Abstract

The antioxidative potential of ethanolic extracts from roasted wheat germ was compared with extracts from other roasted food. Among the cereal products, roasted wheat germ and roasted press cake from wheat germ processing yielded the most efficient extracts when added to stripped maize oil under accelerated-oxidation conditions. Ground and roasted hazelnut and sweet almond, respectively, showed comparable protective effects in stripped maize oil. In the same model system, an ethanolic extract of commercial coffee suppressed lipid oxidation almost completely. Radical scavenging effects of some extracts were analysed using the bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The results were not in full agreement with those obtained in the accelerated oxidation experiments using stripped maize oil.

1. Introduction

In living tissues, labile acyl lipid constituents, such as unsaturated fatty acids, are sufficiently stable. Natural antioxidants and enzymes effectively prevent premature lipid oxidation. Once isolated from animal or plant materials and used in processed food, lipids autoxidise readily. As a result, organoleptic and nutritional quality are reduced, and even toxic products may be formed. The retardation of autoxidation is therefore a key to high product quality. Because most consumers prefer natural food additives over synthetic ones, natural antioxidants are of increasing importance.

Autoxidation is a complex process, but model studies have revealed that the rate of autoxidation is affected by fatty acid composition, degree of unsaturation, the presence and activity of pro- and antioxidants, partial pressure of oxygen, the surface exposed to oxygen (dispersed systems) and the storage conditions (light, temperature, moisture content Belitz & Grosch, 1999; Madhavi, Deshpande & Salunkhe, 1996). Antioxidants inactivate reactive radicals at the initial steps of autoxidation, thus avoiding the propagation of the radical chain reaction. Quantitative analysis of antioxidant power strongly depends on conditions; hence kinetics and endpoint of oxidation should be carefully considered (Frankel, 1993). Methods commonly evaluate the capability of an antioxidant, indirectly, by measuring the amount of lipid degradation products generated during the storage of a standard lipid. More recently, rapid tests based on radical scavenging have gained popularity. The decreasing absorptivity of a stable radical is measured, after the single occupied orbital is filled up with an electron provided by the antioxidant (Morelle, Lauzanne & Rothfuss, 1998; Re, Pellegrini, Pannala, Yang & Rice-Evans, 1999; Yen & Wu, 1999).

Previous work on the stabilisation of stripped (tocopherol-free) maize oil and of commercial plant oils under accelerated test conditions demonstrated the strong antioxidative activity of ethanolic extracts (AOE) of roasted wheat germ (Berger, El-Saharty, & Krings, 1999; Krings, El-Saharty, El-Zeany, Pabel & Berger, 2000). Peroxide value, conjugated diene hydroperoxide concentration, and α-tocopherol concentration were used as analytical markers. In the present work, the AOE of roasted wheat germ was compared with extracts from other roasted foods using an indirect method, the measurement of the concentration of conjugated diene hydroperoxides, and a direct method, scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals.

2. Materials and methods

2.1. Chemicals

Untreated wheat germ and wheat germ press cake (obtained after the first cold pressing) were kindly supplied by Bruno Zimmer, Obertal, Germany, maize germ by Wesermühlen, Hameln, Germany, and barley germ from Beck & Co, Bremen, Germany; coffee (Melitta

* Corresponding author. Fax: +49-511-762-4547.
E-mail address: rg.berger@mbox.1ci.uni-hannover.de (R.G. Berger).

0308-8146/01/$ - see front matter
PII: S0308-8146(00)00226-0

Cafe, Auslese mild), hazelnuts and sweet almonds (both without shell and peel) were purchased from the local market; stripped maize oil (tocopherol-free, peroxide value <5, and conjugated diene hydroperoxide value <12) were from Acros Organics, Geel, Belgium. Ascorbyl palmitate was obtained from Merck, Darmstadt, Germany. α-Tocopherol and (DPPH) were purchased from Sigma Aldrich, Deisenhofen, Germany. All solvents were p.a. grade and redistilled prior to use.

2.2. Defatted wheat germ

Germ was extracted using *n*-hexane in a Soxhlet extractor for 16 h. *n*-Hexane was removed by distillation.

2.3. Roasting of wheat germ, wheat germ press cake, maize germ, barley germ, hazelnut and sweet almond

The nuts were cut into pieces of the same size as wheat germ prior to roasting. Twenty-five-gram samples were filled into centrifuge tubes (34 mm i.d.×100 mm), sealed with screw caps (Teflon septum), and heated at 160°C (oven temperature) for 20 min in a drying oven. The roasted samples were shock-cooled with liquid nitrogen and submitted to ethanolic extraction. Coffee, an already roasted product, was submitted directly to extraction without any pre-treatment.

2.4. Ethanolic extraction of roasted samples

Immediately after cooling, the roasted germ samples and the coffee were stirred with 200 ml of ethanol for 16 h in glass-stoppered 300 ml Erlenmeyer flasks. After filtration, the extracts were concentrated to 20 ml under vacuum at 35°C and stored for 24 h or longer at −20°C in the dark; no loss of activity was observed (Krings et al., 2000). Such a concentrate was called an AOE. A concentration of 20% AOE implies that the extract of 5 g roasted sample, dissolved in 4 ml solvent, had been added to 25 g stripped maize oil.

2.5. Accelerated oxidation tests

Oxidation experiments at 50°C were performed in open glass beakers (8.6 cm i.d.). The samples were prepared by the addition of different antioxidants, ascorbyl palmitate (0.02% wet wt.) or 4 ml of the different ethanolic extracts (20%) to 25 g of stripped maize oil. One sample (control) contained the same volume of ethanol and was used as an analytical blank. Samples were stored at elevated temperatures without stirring in the dark (drying oven).

2.6. Chemical analysis

Oxidative stability of stripped maize oil was evaluated by analysing samples every 24 h. For diene hydroperoxides, an absorptivity of 26,000 $cm^2\ mol^{-1}$ (λ_{max} 234 nm) for linoleate hydroperoxide was used (Chan & Levett, 1977). After 2 min of stirring, weighed oil samples were dissolved in 5 ml iso-octane, diluted to suitable concentration with iso-octane and mixed; the absorbance was measured immediately using a UV/VIS double beam spectrophotometer (Lambda 12, Perkin Elmer, Überlingen, Germany).

2.7. Radical-scavenging effect of ethanolic extracts on DPPH-radicals

The effect of ethanolic extracts on DPPH-radicals was estimated according to Hatano, Kagawa, Yasuhara and Okuda (1988) with some modifications. Different amounts of the ethanolic extracts and of α-tocopherol were added to 1 ml of a solution of DPPH radicals in ethanol and made up with ethanol to a final volume of 3 ml (final concentration of DPPH was 0.1 mM). The mixture was shaken vigorously and allowed to stand for 30 min; the absorbance of the resulting solution was measured at 517 nm.

The concentration of total phenols in ethanolic extracts was determined by using the Folin–Ciocalteau reagent (Gutfinger, 1981) and external calibration with caffeic acid.

All data are based on the means of two independent samples each of which had been analysed in duplicate.

3. Results and discussion

Prior work on solvent extracts of roasted wheat germ demonstrated strong antioxidative activity. The stability of stripped maize oil was improved with elevated roasting temperatures, indicating the involvement of Maillard-type antioxidants (Krings et al., 2000). A comparison of ethanolic extracts obtained from several roasted cereals (the most abundant in Western Europe) with ascorbyl palmitate is shown in Fig. 1. During the first 10 days of storage, ascorbyl palmitate (0.02%, wet wt.) showed nearly the same protecting ability as the AOE of roasted wheat germ, but then, a dramatic increase in conjugated diene hydroperoxides occurred. A higher storage temperature (60°C) resulted in a prooxidative effect (Krings et al., 2000).

The gross chemical composition of the cereal grains examined is very similar. Greatest variation exists in the non-saponifiable portion of the lipid fraction and in the lipid content itself. The total carotenoid, tocopherol and phospholipid contents are highest in wheat grain and in the germ oil, respectively, whereas the fat content of maize grain is twice as high as that of wheat and barley grain (Souci, Fachmann & Kraut, 1999). Compounds in the non-saponifiable fraction of plants have been reported to be natural antioxidants, but most of them

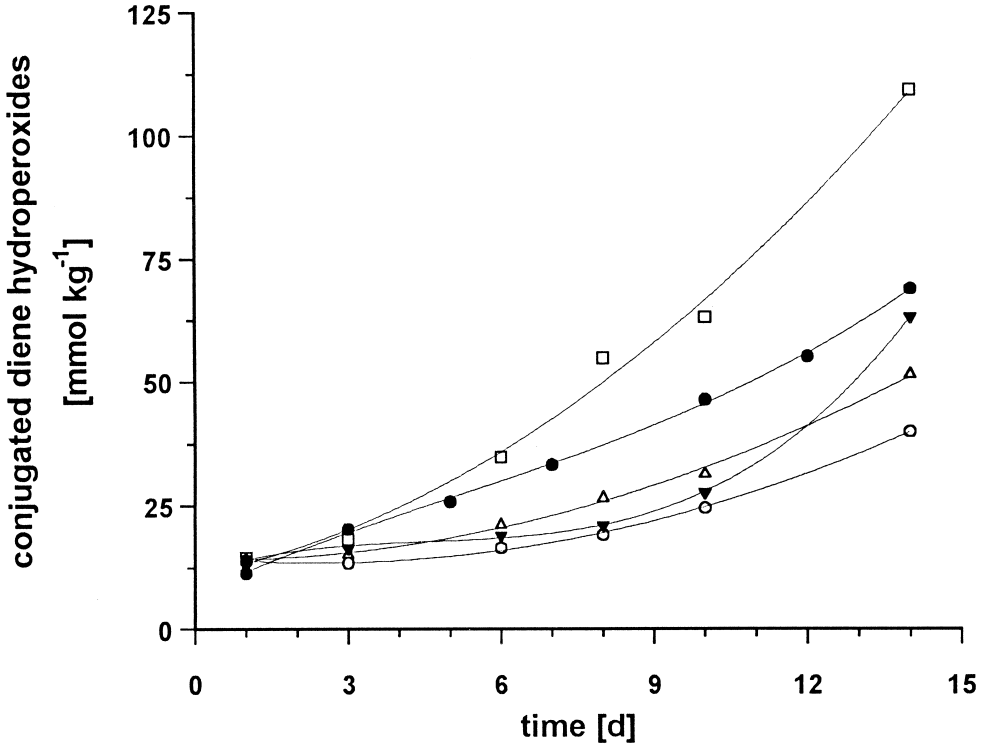

Fig. 1. Autoxidation of stripped maize oil at 50 °C after addition of antioxidant extracts (20%) from different roasted cereals and ascorbyl palmitate (0.02% wet wt. △, maize germ; ○, wheat germ; ●, barley germ; ▼ ascorbyl palmitate; □ control (4 ml ethanol).

are degraded at elevated temperature (Azizah, Ruslawati & Swee Tee, 1999; Frankel, 1993). To investigate the influence of lipid content and lipid constituents, samples of defatted wheat germ and the residue of cold-pressed wheat germ oil (wheat germ press cake) were roasted under the same conditions and compared with roasted wheat germ (Fig. 2). The AOE of lipid-free wheat germ showed the worst result, whereas the press cake was even slightly better than the unprocessed wheat germ. Up to now, the press cake is regarded as a waste material which is disposed of in animal feed. Wheat germ oil is particularly high in tocopherols. However, even if a complete recovery by the ethanolic extraction is assumed, this would not nearly explain the superior antioxidative quality of roasted wheat germ (see later).

The AOE of roasted wheat germ was compared with extracts from foods which are typically roasted prior to consumption (Fig. 3). Coffee was chosen as a low-fat (~13%) and high-temperature (200–250°C) roasted food, whereas hazelnuts (~62%) and sweet almond (~54%) represented high-fat foods. All extracts showed significant antioxidative properties. Nearly identical effects were observed for AOEs of wheat germ, hazelnut and sweet almond. Coffee extract prevented autoxidation of stripped maize oil almost completely, as indicated by the stable level of the conjugated diene hydroperoxides. Methylpyrrole, hydroxymethylfurfural and maltol, typical Maillard reaction intermediates, were identified as the most potent antioxidants in subfractions of dichloromethane extracts of brewed coffee. The strong radical scavenging properties of the three molecules were assumed to be the source of the main antioxidative activity, and a hypothetical mechanism of the radical-scavenging reaction has been proposed (Singhara, Macku & Shibamoto, 1998).

Evaluation of antioxidative activities using accelerated-oxidation conditions with daily sampling and analysis is a tedious procedure. Recently-developed rapid methods use the bleaching of stable spin traps such as N-centred DPPH or 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals. Such assays are based on the correlation of the extent of the scavenging by hydrogen or electron donation of a pre-formed free radical with the antioxidant activity (Morelle et al., 1998; Re, 1999). The radical-scavenging activities of AOE of coffee, wheat germ and wheat germ press cake were in agreement with the antioxidative activities measured in accelerated-oxidation experiments (Fig. 4). Even low concentrations of the AOE of coffee possessed high scavenging activity. Again, the press cake of wheat showed a slightly higher activity than wheat germ itself. AOEs of hazelnut, sweet almond, and wheat germ, about equally potent in stabilising stripped maize oil (Fig. 3), showed entirely different DPPH-scavenging activity: the hazelnut extract was significantly more active than wheat germ and close to coffee, while the sweet almond extract showed poor radical-scavenging

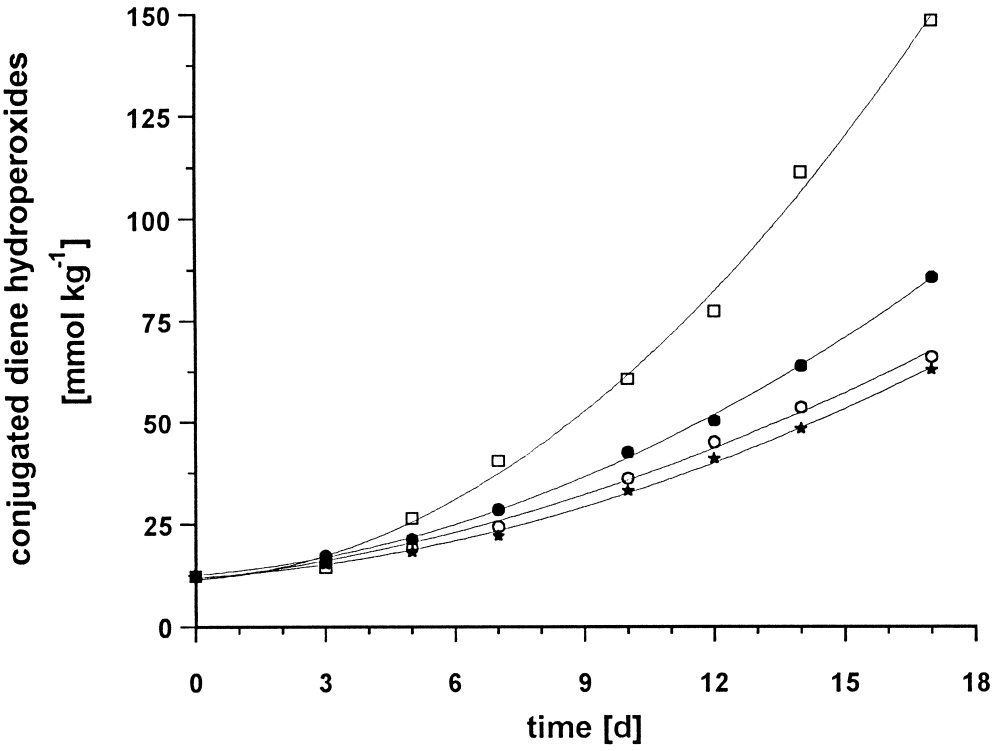

Fig. 2. Autoxidation of stripped maize oil at 50°C after addition of antioxidant extracts (20%) from ○, roasted wheat germ, ●, defatted roasted wheat germ, ⋆, roasted wheat germ press cake; and □, control (4 ml ethanol).

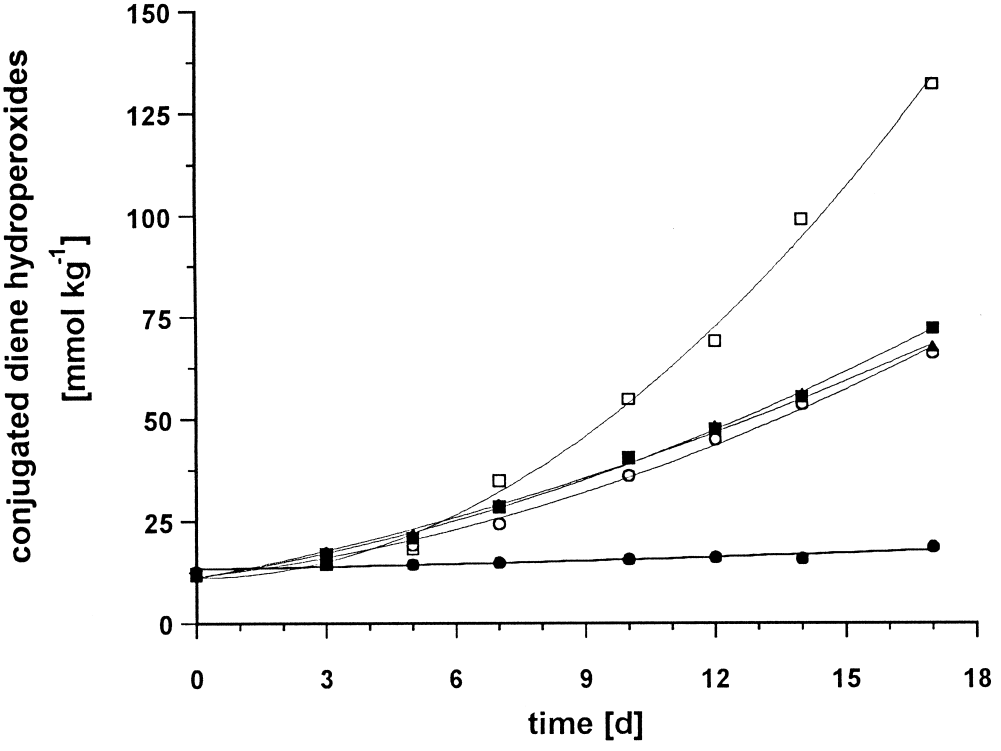

Fig. 3. Autoxidation of stripped maize oil at 50°C after addition of antioxidant extracts (20%) ○, from roasted wheat germ; ●, coffee; ■, roasted hazelnut; ▲, roasted sweet almond; and □, control (4 ml ethanol).

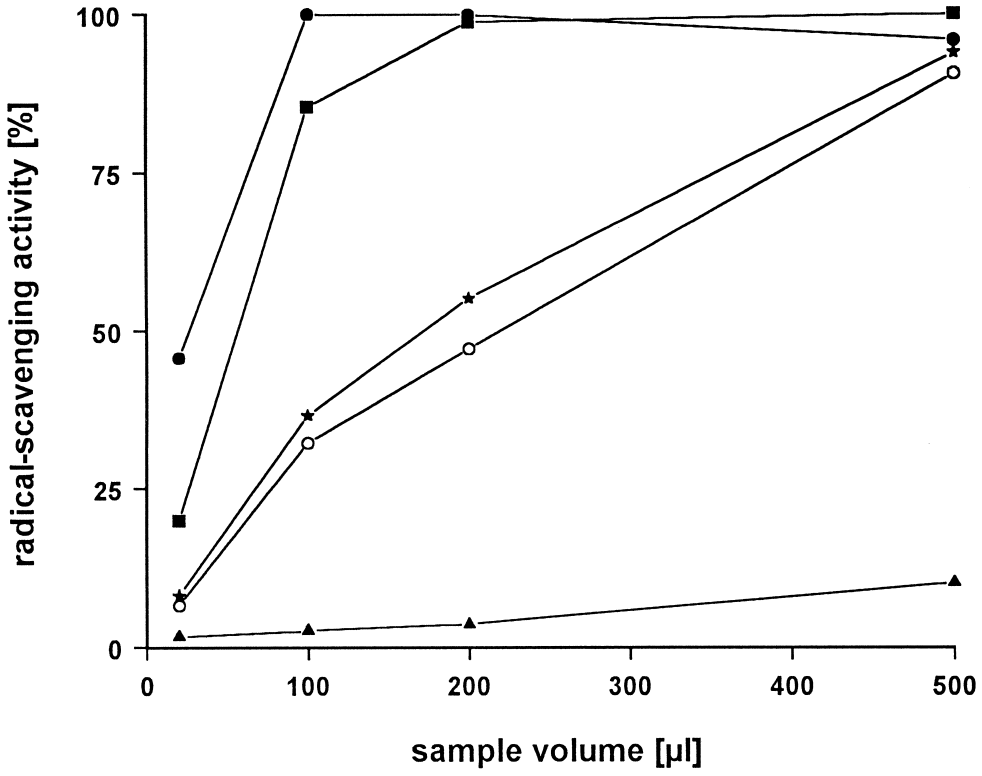

Fig. 4. Scavenging activities on 1,1-diphenyl-2picrylhydrazyl radical of antioxidant extracts (20%) from ○, roasted wheat germ; ●, coffee; ■ roasted hazelnut; ▲, roasted sweet almond; and ⋆, roasted wheat germ press cake.

activities (Fig. 4). The antiradical properties of roasted hazelnut and almond correlated with the total phenols of the extracts (Table 1). A highly significant relationship between total phenols and antioxidant activity was found for methanolic extracts of fruits, vegetables and grain products (Velioglu, Mazza, Gao & Oomah, 1998). However, the phenolic content of barley was decreased by 62 to 85% by malting (usually at 80°C; Goupy, Hugues, Boivin & Amiot, 1999). Apparently, the loss of phenols in wheat germ during roasting was over-compensated by the generation of Maillard-type antioxidants.

The antioxidant capacity of ethanolic AOE of roasted wheat germ can be expressed as α-tocopherol equivalents, using the linear part of the calibration curve (Fig. 5). The equivalent of 43 mg α-tocopherol in 20 ml ethanolic extract (obtained from 25 g roasted wheat germ) far exceeded the natural tocopherol content of wheat germ (31 mg 100 g^{-1}, Souci et al., 1999). Even if all tocopherols were to survive the roasting process and were then to be completely extracted by the ethanol, they would explain less than 20% of the total antioxidative activity observed.

Table 1
Total phenols in ethanolic extracts from roasted foods (25 g 20 ml^{-1})

Antioxidative extract of	µg ml^{-1}
Wheat germ	22.2
Wheat germ press cake	23.3
Coffee	82.3
Sweet almond	18.7
Hazelnut	45.5

Bleaching of DPPH with α-tocopherol was linear up to a concentration of 10 mM, where the stoichiometric factor of α-tocopherol to DPPH was 0.5:1 (Fig. 5.). The bleaching percentage was somewhat above the stoichiometric factor calculated for this radical-scavenging reaction. The phenomenon of stoichiometric factors greater than calculated was observed with DPPH scavenging experiments using catechols and catechins as antioxidants (Suzuki, Mori, Nanjo & Hara, 1999). Polymers of caffeic acid inhibited the DPPH radical more strongly than caffeic acid (Chen, Yokozawa & Chung, 1999). The bleaching of DPPH and ABTS by the same concentration of several radical scavengers was compared, and it was found that resinous exudates from *Heliotropium* species were able to scavenge ABTS radicals much more easily (factors ABTS / DPPH up to 37) than DPPH radicals (Lissi, Modak, Torres, Escobar & Urzua, 1999). Study of the reaction kinetics and reaction products of 4-methylcatechol and (+)-catechin showed that there were two stages: first a rapid reaction and then a slow one. The deviating stoichiometric factors may be due to steric hindrance of the hydrogen- or electron-donating group. The non-linear relations

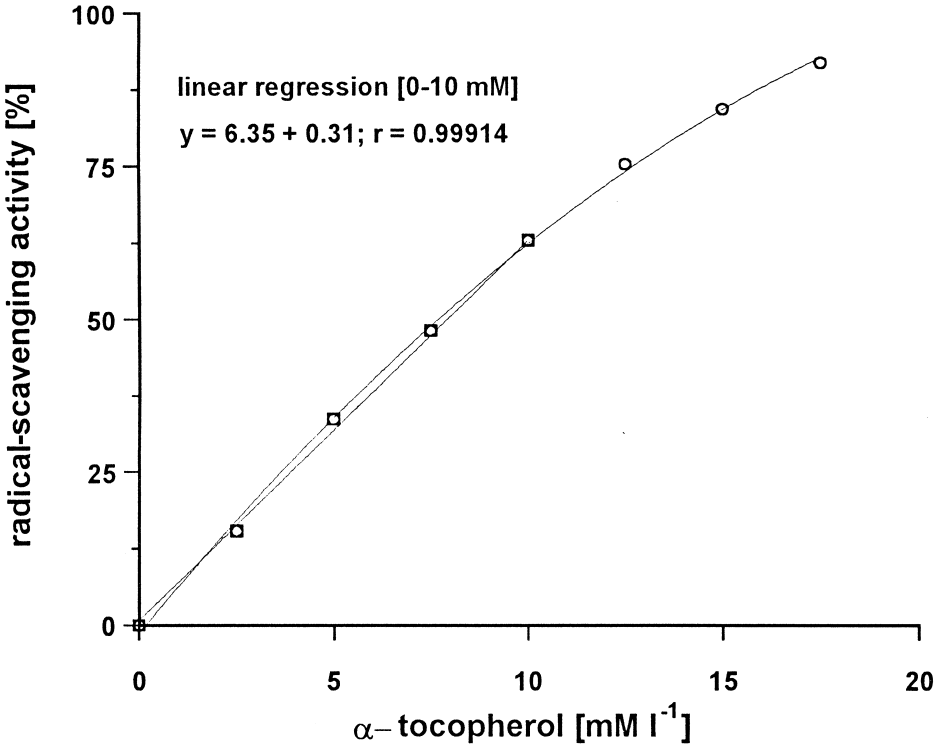

Fig. 5. Concentration dependent scavenging activity of α-tocopherol on 1,1-diphenyl-2-picrylhydrazyl-radical.

between radical-scavenger concentrations and colour, and different scavenging kinetics were taken into account by the so called 'antiradical efficiency' (AE = 1/EC50 × TEC50; EC50 = amount of sample necessary to decrease, by 50%, the initial DPPH concentration, and TEC50 = time needed to reach the steady state at EC50 concentration) which was introduced by Sánchez-Moreno (Sánchez-Moreno, Larrauri & Saura-Calixto, 1999).

Radical scavengers have attracted special interest in nutrition and medicine, because they may protect human cells from free radicals, thereby preventing diseases, including cancer (Nakayama, Yamada, Osawa & Kawakishi, 1993). Even though it is still not known whether these compounds are active against free radicals after being absorbed and metabolised by hepatic cells, radical-scavenging tests have gained acceptance for the rapid analysis of different materials. The present findings underline, however, that radical-scavenging activity and stabilisation of food lipids need not be strictly correlated.

4. Conclusions

In comparing ethanolic extracts of roasted barley, maize, and wheat germ, the last of those prevented autoxidation of stripped maize oil most effectively. Maillard reaction products, in addition to polar portions of wheat germ lipids, are thought to be the active principles. Extracts of some other roasted foods also showed antioxidative effects, but radical-scavenging and lipid stabilisation were not strictly correlated. Ethanolic AOE of coffee surpassed the other AOEs in both tests, but, in view of its high price and distinct sensory attributes, does not offer a feasible alternative. Wheat germ and wheat germ press cake are by-products of wheat processing; therefore, their ethanolic extracts are an economic source of natural antioxidants. Further work is in progress to elucidate the identity of relevant antioxidative compounds and precursors in ethanolic AOEs of wheat germ.

Acknowledgements

The authors are grateful to the Fonds der Chemischen Industrie, Frankfurt, and to H. Deeken for financial and technical support.

References

Azizah, A. H., Ruslawati, N. M. N., & Swee Tee, T. (1999). Extraction and characterization of antioxidant from cocoa by-products. *Food Chemistry, 64*(1), 99–202.

Belitz, H. D., & Grosch, W. (1999). *Food Chemistry* 2nd ed. Berlin-Heidelberg-New York-Tokyo: Springer-Verlag.

Berger, R.G., El-Saharty, Y.S.I. & Krings, U. (1999). Mittel zu Stabilisierung von Lebensmitteln und kosmetischen Mitteln sowie Verfahren zu dessen Herstellung. PCT patent application, PCT/DE99/02258.

Chan, H. W. S., & Levett, G. (1977). Autoxidation of methyl linoleate. Separation and analysis of isomeric mixtures of methyl linoleate hydroperoxides and methyl hydroxylinoleates. *Lipids, 12*, 99–104.

Chen, C. P., Yokozawa, T., & Chung, H. Y. (1999). Inhibitor effect of caffeic acid analogs isolated from *Salviae miltiorrhizae radix* against 1,1-diphenyl-2-picrylhydrazil radical. *Experimental Toxicology and Pathology, 51*, 59–63.

Frankel, E. N. (1993). In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends in Food Science & Technology, 4*, 220–225.

Goupy, P., Hugues, M., Boivin, P., & Amiot, M. J. (1999). Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *Journal of the Sciecne of Food & Agriculture, 79*, 1625–1634.

Gutfinger, T. (1981). Polyphenols in olive oils. *Journal of the American Oil Chemists Society, 58*, 966–968.

Hatono, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effect. *Chemistry & Pharmacology Bulletin, 36*, 1090–1097.

Krings, U., El-Saharty, Y. S. I., El-Zeany, B. A., Pabel, B., & Berger, R. G. (2000). Antioxidant activity of extracts from roasted wheat germ. *Food Chemistry*, 91–95.

Lissi, E. A., Modak, B., Torres, R., Escobar, J., & Urzua, A. (1999). Total antioxidant potential of resinous exudates from *Heliotropium* species, and a comparison of ABTS and DPPH methods. *Free Radical Research, 30*, 471–477.

Madhavi, D. L., Deshpande, S. S., & Salunkhe, D. K. (1996). *Food antioxidants*. New York: Marcel Dekker Inc.

Morelle, J., Lauzanne, E. & Rothfuss, J., (1998). Method for measuring the antioxidant activity of food products and for producing extracts with quantified antioxidant activity. WO 9857168 A1.

Nakayama, T., Yamada, M., Osawa, T., & Kawakishi, S. (1993). Suppression of active oxygen-induced cytotoxicity by flavanoids. *Biochemistry & Pharmacology, 45*, 265–267.

Re, R., Pellegrini, N., Pannala, A., Yang, M., & Rice-Evans, C. A. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*, 1231–1237.

Sánchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1999). Free radical scavenging capacity of selected red, rosé and white wines. *Journal of the Science of Food & Agricultural, 79*, 1301–1304.

Singhara, A., Macku, C., & Shibamoto, T. (1998). Antioxidative activity of brewed coffee extracts. In *Functional foods for disease prevention II: medicinal plants and other foods* (Vol. 702, pp. 101–109). Washington, DC: American Chemical Society.

Souci, S. W., Fachmann, H. J., & Kraut, A. (1999). *Food composition and nutrition tables*. Stuttgart: Medpharm Scientific Publ.

Suzuki, M., Mori, M., Nanjo, F., & Hara, Y. (1999). Antioxidant mechanisms of catechins on DPPH radical. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu, 40*, 631–636.

Velioglu, Y.S., Mazza, G., Gao, L., & Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural & Food Chemistry, 46*, 4113–4117.

Yen, G-C., & Wu, J-Y. (1999). Atioxidant and radical scavenging properties of extracts from *Ganoderma tsugae*. *Food Chemistry, 65*, 375–379.