ELSEVIER

Food Chemistry 71 (2000) 91–95

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidant activity of extracts from roasted wheat germ

Ulrich Krings [a], Yasser S. El-Saharty [b], Bader A. El-Zeany [b], Bettina Pabel [a], Ralf G. Berger [a,*]

[a] *Institut für Lebensmittelchemie, Zentrum Angewandte Chemie der Universität, Wunstorferstr. 14, D 30453 Hannover, Germany*
[b] *Faculty of Pharmacy, Cairo University, Kasrel-Aini St., ET- 11562 Cairo, Egypt*

Received 15 February 2000; accepted 3 May 2000

Abstract

Solvents extracts of roasted wheat germ, an actual waste stream of wheat processing, retarded the autoxidation of corn oil stored at 60°C. Best stabilisation of stripped corn oil was obtained with an ethanolic extract (antioxidative extract, AOE) from wheat germ that was roasted at 160°C for 20 min. Even high dosages of 20 and 40% AOE did not show prooxidative effects. Using accelerated ageing of commercial plant oils, and peroxide value, conjugated diene hydroperoxide concentration and α-tocopherol concentration as analytical indicators, a significant improvement of storability was demonstrated for each oil. Results indicate the presence of classes I and II antioxidants in ethanolic AOE of wheat germ.

Keywords: Antioxidants; Ethanolic extract; Wheat germ; Roasting

1. Introduction

Autoxidation of unsaturated fatty acids may lead to off-flavours, reduction of nutritional quality and even to formation of toxic products in lipidic foods. Its retardation by the use of synthetic antioxidants, such as 2,6-di-*tert*-butyl-p-hydroxytoluene (BHT), *tert*-butyl-4-hydroxyanisole (BHA), or 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin) is continuing practice in industrial food processing. However, recent trends ('all-natural', 'low-labelling') have stimulated the search for natural ingredients with antioxidative activity (Evans & Reynhout, 1992; Frankel, 1993; Rajalakshmi & Narasimhan, 1996).

Work was concentrated on vitamins C, E and carotenoids (Frankel, 1996; Miller & Rice-Evans, 1997), and on plant extracts containing non-nutritive antioxidants (Aeschbach & Rossi, 1996; Velioglu, Mazza, Gao & Oomah, 1998). Among the best-investigated sources of natural antioxidants are solvent extracts of tea, legume seeds and spices, which contain flavan glycosides, such as quercetin, kaempferol, myricetin, epigallocatechin gallate, epicatechin gallate, epigallocatechin and epicatechin, or phenolic diterpenes and phenolic acids, such as carnosol, carnosic acid and rosmarinic acid (Lee, Howard & Villalón, 1995; Lindberg Madsen, Sørensen, Skibsted & Bertelsen, 1998; Tian & White, 1994; Tsaliki, Lagouri & Doxastakis, 1999; Wanasundara & Shahidi, 1998; Zandi & Gordon, 1999). Cort (1974) has shown the enhanced efficiency of several solvent extracts as opposed to the use of the whole spice in an emulsion system. A novel approach for antioxidant extraction permitted the production of hydrosoluble, directly applicable antioxidant extracts by contacting the plant materials with a carrier at elevated temperature, followed by pressing on a piston press (Aeschbach and Rossi). Antioxidant-like effects of different cereals and cereal fractions in aqueous suspension were attributed to the protection of linoleic acid from oxidising catalyst by binding to soluble and nonsoluble fibres (Lehtinen and Laakso, 1997).

During previous work on key flavour and off-flavour compounds of fresh, over-stored, and roasted wheat germ, a remarkable stability of the flavour profile of the roasted germs was discovered (El-Saharty, Tawakkol, El-Zeany & Berger, 1997; El-Saharty, El-Zeany & Berger, 1998; El-Saharty, El-Zeany, Tawakkol & Berger, 1998). Follow-up work is presented that evaluated the

* Corresponding author. Tel.: +49-511-762-4582; fax: +49-511-762-4567.
E-mail address: rg.berger@mbox.lci.uni-hannover.de (R.G. Berger).

0308-8146/00/$ - see front matter
PII: S0308-8146(00)00148-5

presumed antioxidative activities and compared them with synthetic antioxidants commonly used in food.

2. Materials and Methods

2.1. Chemicals

Untreated wheat germ was kindly supplied by Bruno Zimmer, Obertal, Germany. Corn oil, sunflower oil and soybean oil were purchased from the local market, stripped corn (tocopherol-free, peroxide value < 5, conjugated diene hydroperoxide value < 12) from Acros Organics, Geel, Belgium. Ascorbyl palmitate was obtained from Merck, Darmstadt and BHA from Fluka, Neu-Ulm, both Germany. All solvents were p.a. grade and redistilled prior to use.

2.2. Roasting of wheat germ

Twenty-five-gram samples of wheat germ 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. Wheat germ samples were also heated at 140, 180 and 200°C for 20 min. The roasted samples were shock-cooled with liquid nitrogen and submitted to solvent extraction.

2.3. Solvent extraction of roasted wheat germ

Immediately after cooling, the roasted germ samples were stirred with 200 ml of solvents (diethyl ether, acetone, or 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 (data not shown). Alternatively, the roasted samples were Soxhlet extracted for 16 h. Prior to use, the extracts were filtered once more, if necessary. Hereafter the concentrates are called AOE (antioxidative extract). A concentration of 20% AOE, for example, means that the extract of 5 g roasted germ, solved in 4 ml solvent, was added to 25 g of oil.

2.4. Accelerated oxidation tests

Oxidation experiments at 50 or 60°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% w/w), BHA, *tert*-butyl-4-hydroxyanisole (0.02% w/w) and different amounts of solvent extracts, to 25 g of stripped corn oil or plant oils, respectively. A control sample (control) contained the same volume of the extraction solvent and was used as an analytical blank. Samples were stored at elevated temperatures without stirring in the dark (drying oven).

2.5. Chemical analysis

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

The peroxide value was determined iodometrically. Three to five grams of the oil was weighed into a 250 ml Erlenmeyer glass-stoppered conical flask and 50 ml of 3:2 (v/v) acetic acid:isooctane solution was added to dissolve the sample. Half a millilitre of saturated potassium iodide was then added and the solution was allowed to stand with occasional shaking for 1 min. After dilution with 75 ml of distilled water the solution was titrated against 0.1 N sodium thiosulfate.

α-Tocopherol was determined by HPLC (MD-910 multi wavelength detector, PU-980 pump, DG-980-50-3 line degasser, and LG-980-02 ternary gradient unit, Jasco, Japan) using a silica gel column, Nucleosil (200×4 mm, 5 μm particle size), Macherey and Nagel AG (Düren, Germany). The mobile phase was *n*-hexane: diethyl ether (95:5 v/v isocratic) at a flow rate of 1.5 ml min^{-1}. The injection volume was 20 μl and the detection was at 295 nm.

All data are means of two independent samples which were analysed in duplicate.

3. Results and discussion

Antioxidative activity of plant extracts can be screened by various accelerated storage methods and analytical tests. Which test is more likely to evaluate the true antioxidative potential depends on the chemical nature of the extract's constituents. The present study measures the progressing chemical events with five independent methods (Fig. 1a–c). Results obtained using headspace capillary gas chromatography of *n*-pentane and using the Rancimat apparatus gave equal trends and are, therefore, not shown.

Ethereal extracts of roasted wheat germ, originally prepared for flavour analysis, gave first indications for antioxidative properties. There was a slight stabilisation of genuine corn oil with acetone extracts of roasted wheat germ, but the beneficial effects were more pronounced with diethyl ether and ethanol extracts. The increases of peroxide value and conjugated diene concentration were delayed; so was the thermal decomposition of α-tocopherol (Fig. 1a–c).

As all of the three analytical methods appeared to give comparable trends, the following measurements were

largely restricted to the determination of conjugated diene hydroperoxide values. To avoid interference of naturally occurring lipoids (especially tocopherols) with the antioxidants added, stripped corn oil was used for stability tests. This ensured that antioxidative activities measured were indeed caused exclusively by the AOEs added.

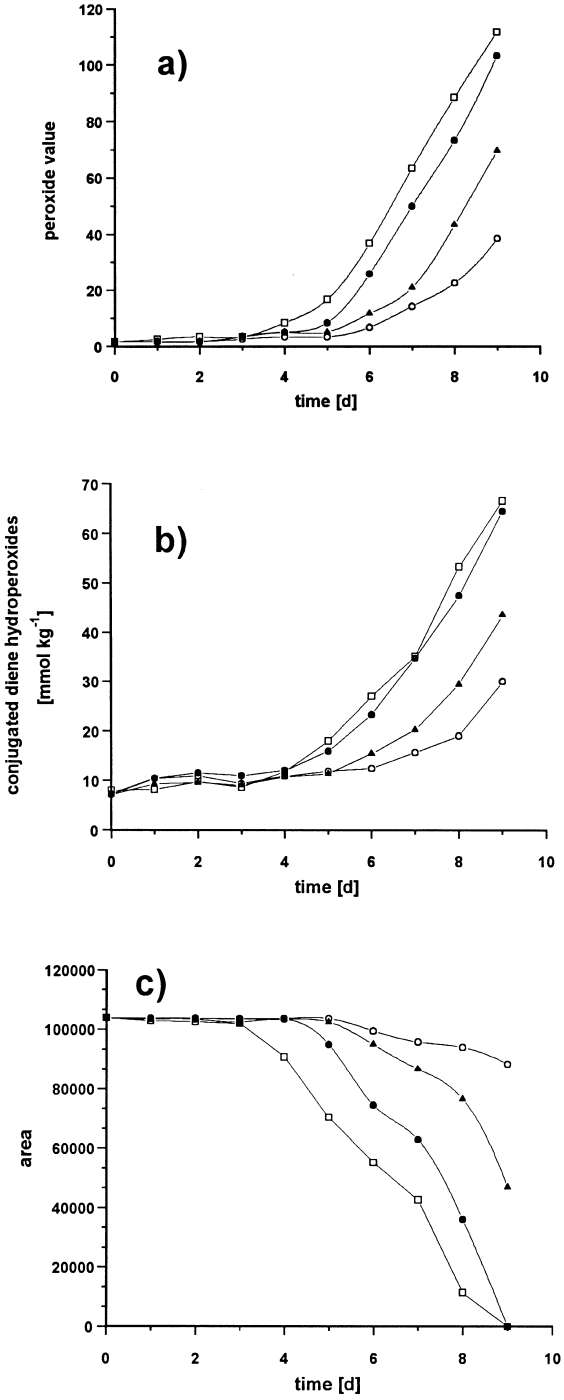

Fig. 1. Autoxidation of corn oil at 60°C after addition of extracts from roasted wheat germ obtained with different extraction solvents (20%). (a) Peroxide value; (b) conjugated diene hydroperoxides; (c) α-tocopherol content; □, control; ○, ethanol; ●, acetone; ▲, diethylether.

The impact of different conditions of preparation and dosage on the antioxidative activity of AOE is shown in Fig. 2a–c. The improvement gained using an exhaustive extraction procedure (Soxhlet) was marginal (Fig. 2a). Both roasting temperature and added dosage approximate to a maximum of antioxidative activity (Fig. 2b

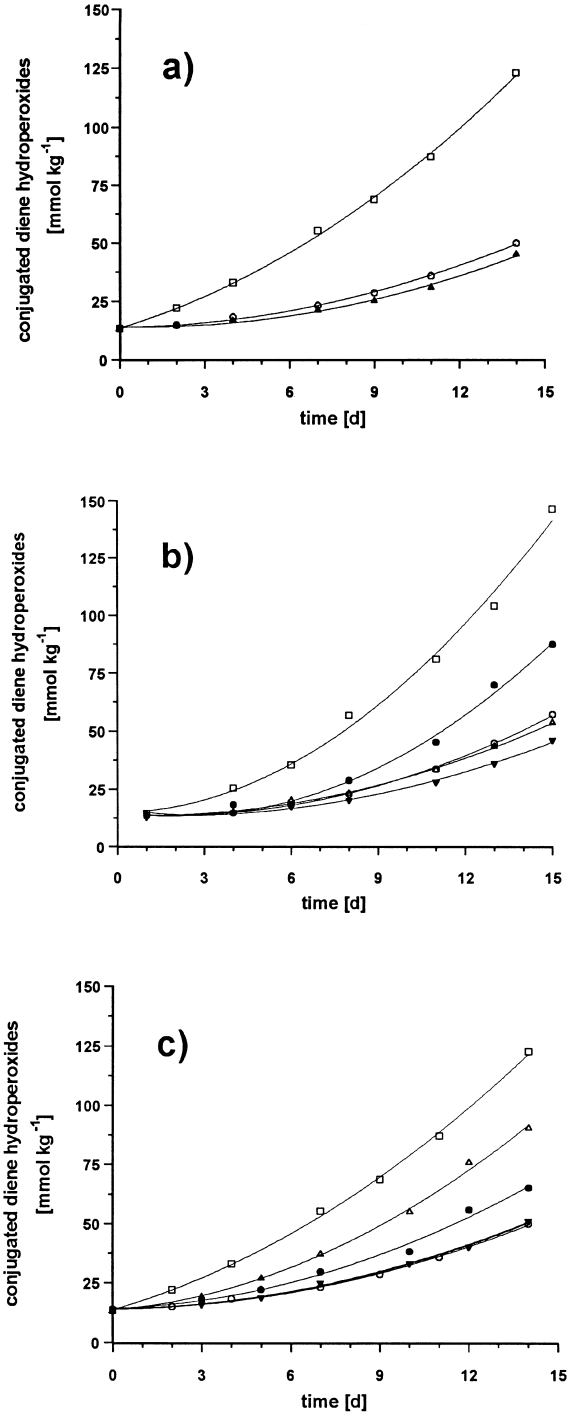

Fig. 2. Autoxidation of stripped corn oil at 50°C after addition of AOE (a,b = 20%). (a) Effect of extraction method; □, control; ○, EtOH; ▲, Soxhlet-EtOH. (b) Effect of roasting temperature; □, control; ●, 140°C; ○, 160°C; △, 180°C; ▼, 200°C. (c) Effect of concentration of AOE; □, control; △, 5%; ●, 10%; ▼, 20%; ○, 40%.

and c). The increased activity obtained at elevated roasting temperatures indicated that at least a part of the antioxidative principle was generated by Maillard type reactions of wheat germ constituents. Several natural antioxidants were reported to occur in wheat germ, especially in the lipid fraction (per 100 g: about 30 g available carbohydrates, 18 g raw fibre, 2.2 g free amino acids, 30 mg tocopherols, lipoic acid, phospholipids, and phenols), but most of them are expected to be degraded at elevated temperature (Azizah, Nik Ruslawati & Swee Tee, 1999); hence, the genuine stock of antioxidative compounds should be greatly reduced by roasting. On the other hand, Amadori compounds (Chuyen, Ijichi, Umetsu & Moteki, 1998), reductones (Eichner, 1981; Singhara, Macku & Shibamoto, 1998), amino reductones (Kurata & Otsuka, 1998; Pischetsrieder, Schoetter & Severin, 1998) and Maillard-type polymers (Tressl, Wondruk, Kersten, Krüger & Kewicki, 1998), with antioxidative activity, are accumulated during the Maillard reaction. An antioxidative effect of browning products of phospholipids was also reported (Husain, Terao & Matsushita, 1986).

With increased processing temperatures, an increased generation of toxic IQ compounds is to be noted in food (Milic, Djilas & Canadanovic-Brunet, 1993). As the full antioxidative power of wheat germ extracts is already attained at mild roasting conditions (160°C, 20 min), an adverse effect on human consumers is highly unlikely (Berger, Saharty & Krings, 1999).

To assess its relative antioxidative activity, the ethanolic AOE was compared to commonly used antioxidants, such as BHA and ascorbyl palmitate (Fig. 3). Using accelerated oxidation conditions at 60°C in the dark and a common dosage of BHA and ascorbyl palmitate, the ethanolic AOE of roasted wheat germ showed the best protection of stripped corn oil. Ascorbyl palmitate was prooxidative under these conditions.

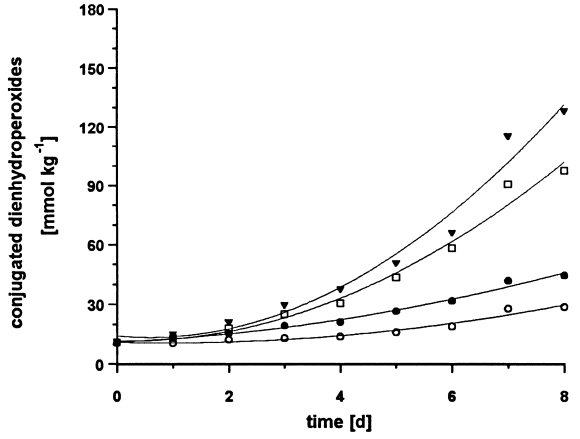

Fig. 3. Autoxidation of stripped corn oil at 60°C after addition of different antioxidants: □, control; ●, BHA 0.02% (w/w); ▲, ascorbyl palmitate 0.02% (w/w); ○, AOE (20%).

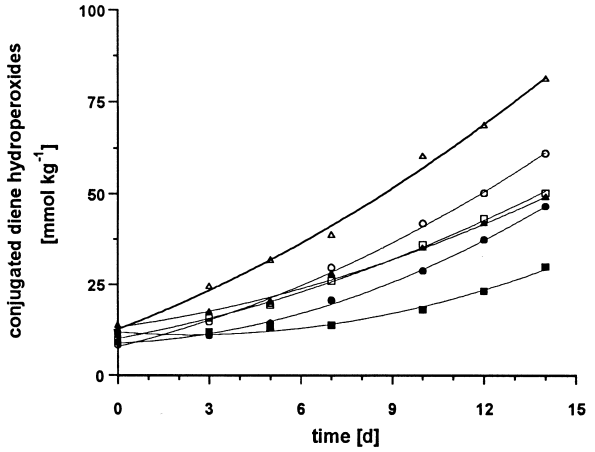

Fig. 4. Autoxidation of vegetable oils at 50°C with and without addition of 20% AOE: △, corn oil; ▲, corn oil + AOE; ○, sunflower oil; ●, sunflower oil + AOE; □, soybean oil; ■, soybean oil + AOE.

The order of activity and ranking of different antioxidants depends strongly on whether they are tested at high or low temperatures. Phenolic antioxidants in natural extracts, for example, decompose at elevated temperatures (Frankel, 1993).

Refined commercial plant oils with high levels of unsaturated fatty acids were stressed with and without addition of ethanolic AOE of roasted wheat germ (Fig. 4). Due to their genuine tocopherol content, these oils offer self-defence against autoxidation. However, the addition of AOE strongly improved the stability of all of the plant oils. Autoxidation was slowest with soybean oil, the one which shows the highest content of total tocopherols, followed by sunflower oil which is lowest in total tocopherols among the three oils, but possesses the highest concentration of α-tocopherol; corn oil was the least stable under all conditions.

4. Conclusions

The ethanolic AOE possessed strong antioxidative properties when applied to genuine or stripped plant oils. The protection of genuine α-tocopherol in corn oil indicates the presence of class II antioxidants which are able to protect or to regenerate α-tocopherol (class I antioxidant). The stabilisation of stripped corn oil which is free from any genuine antioxidant proves the occurrence of class I antioxidants which were generated during the roasting process. This agrees with the extended stabilisation of tocopherol-containing vegetable oils. As ethanol was the best extraction solvent, the main antioxidative components are supposed to possess more polar characteristics. Extended analytical work, to reveal the chemical identity of the antioxidant principle(s), is under way.

Acknowledgements

The authors are grateful to the Fonds der Chemischen Industrie, Frankfurt, and to Karin Schwarz for her assistance, and to the Faculty of Pharmacy, Cairo University, for granting an academic leave to Y.S.E.

References

Aeschbach, R., & Rossi, P. (1996). Novel approach for antioxidant extraction. In (1996). Oils–fats–lipids Proceedings of the World Congress of the International Society of Fat Research, Vol. 2, (pp. 331–332). Bridgwater, UK: G. P. J. Barnes & Associates.

Azizah, A. H., Nik Ruslawati, N. M., & Swee Tee, T. (1999). Extraction and characterisation of antioxidant from cocoa by-products. *Food Chemistry, 64*, 199–202.

Berger, R. G., Saharty, Y. S., & Krings, U. (1999). Mittel zur 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.

Chuyen, N. V., Ijichi, K., Umetsu, H., & Moteki, K. (1998). Antioxidative properties of products from amino acids or peptides in the reaction with glucose. In F. Shahidi, C.-T. Ho, & V. van Chuyen, *Process-induced chemical changes in food* (pp. 201–212). New York and London: Plenum Press.

Cort, W. M. (1974). Hemoglobin peroxidation test screens antioxidants. *Food Technology, 28*, 60–66.

Eichner, K. (1981). Antioxidative effect of maillard reaction intermediates. *Progress in Food Nutrition Science, 5*, 441–451.

El-Saharty, Y. S., El-Zeany, B. A., & Berger, R. G. (1998b). Analysis of odour active compounds of roasted wheat germ. *Advances in Food Science (CMTL), 20*, 198–202.

El-Saharty, Y. S., El-Zeany, B. A., Tawakkol, M. S., & Berger, R. G. (1998a). Volatile flavour and key off-flavour compounds of overstored wheat germ. *Advances in Food Science (CMTL), 20*, 53–58.

El-Saharty, Y. S., Tawakkol, M. S., El-Zeany, B. A., & Berger, R. G. (1997). Odour active compounds of fresh wheat germ. *Advances in Food Science (CMTL), 19*, 90–94.

Evans, R. J., & Reynhout, G. S. (1992). Alternatives to synthetic antioxidants. In G. Charalambous, *Food science and human nutrition* (pp. 27–42). Amsterdam: Elsevier Science Publisher.

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

Frankel, E. N. (1996). Antioxidants in lipid foods and their impact on food quality. *Food Chemistry, 57*, 51–55.

Husain, S. R., Terao, J., & Matsushita, S. (1986). Effect of browing products of phospholipids on autoxidation of methyl linoleate. *Journal of the American Oil Chemists' Society, 63*, 457–460.

Kurata, T., Otsuka, Y. (1998). Amino-reductones: formation mechanisms and structural characteristics. In F. Shahidi, C.-T. Ho, & V. van Chuyen, *Process-induced chemical changes in food* (pp. 201–212). New York and London: Plenum Press.

Lee, Y., Howard, R. L., & Villalón, B. (1995). Flavanoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. *Journal of Food Science, 60*, 473–476.

Lehtinen, P., & Laakso, S. (1997). Antioxidative-like effect of different cereals and cereals fractions in aqueous suspension. *Journal of Agricultural Food Chemistry, 45*, 4606–4611.

Lindberg Madsen, H., Sørensen, B, Skibsted, L. H., & Bertelsen, G. (1998). The antioxidative activity of summer savory (*Satureja hortensis* (L.) and rosemary (*Rosmarinus officinalis* L.) in dressing stored exposed to light or in darkness.. *Food Chemistry, 63*, 173–180.

Milic, B. L., Djilas, S. M., & Canadanovic-Brunet, J. M. (1993). Synthesis of some heterocyclic amino imidazoazarenes. *Food Chemistry, 46*, 273–276.

Miller, N. J., & Rice-Evans, C. A. (1997). The relative contribution of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chemistry, 60*, 331–337.

Pischetsrieder, M., Schoetter, C., & Severin, T. (1998). Formation of an aminoreductone during the Maillard reactrion of lactose with N-acetyllysine or proteins. *Journal of Agricultural and Food Chemistry, 46*, 928–931.

Rajalakshimi, D., & Narasimhan, S. (1996). Food antioxidants: Sources and methods of evaluation. In D. L. Madhavi, S. S. Deshpande, & D. K. Salunkhe, *Food antioxidants* (pp. 65–158). New York: Marcel Dekker.

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 (pp. 101–109). Washington: American Chemical Society.

Tian, L. L., & White, P. J. (1994). Antioxidant activity of oat extract in soybean and cottonseed oils. *Journal of the American Oil Chemists' Society, 71*, 1079–1086.

Tressl, R., Wondrak, G., Kersten, E., Kriiger, R. P., & Rewicki, D. (1998). Identification of maillard type polymers with antioxidative activity. In T. Shibamoto, J. Terao, & T. Osawa, *Functional foods for disease prevention* (pp. 209–221). Washington: American Chemical Society.

Tsaliki, E., Lagouri, V., & Doxastakis, G. (1999). Evaluation of the antioxidant activity of lupin seed flour and derivatives (*Lupinus albus* ssp *Graceus*). *Food Chemistry, 65*, 71–75.

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 and Food Chemistry, 46*, 4113.

Wanasundara, U. N., & Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chemistry, 63*, 335–342.

Zandi, P., & Gordon, M. H. (1999). Antioxidant activity of extracts from old tea leaves. *Food Chemistry, 64*, 285–288.