ELSEVIER

PII: S0308-8146(96)00067-2

Food Chemistry, Vol. 57, No. 1, pp. 51–55, 1996

0308-8146/96 $15.00+0.00

Antioxidants in lipid foods and their impact on food quality

Edwin N. Frankel

Department of Food Science and Technology, University of California, Davis, CA 95616, USA

This paper reviews the antioxidant properties of tocopherols and ascorbic acid in edible oils, and the impact of interfacial phenomena on their activities in emulsions and the effects of edible oil processing. Tocopherols are the most important natural antioxidants found in vegetable oil-derived foods. These antioxidants can interrupt lipid autoxidation by interfering with either the chain propagation or the decomposition processes. α-Tocopherol at high concentrations inhibits hydroperoxide decomposition but promotes hydroperoxide formation. The effect of antioxidants in inhibiting hydroperoxide decomposition may thus be critical in preserving food quality by reducing rancidity due to aldehyde formation. Ascorbic acid can regenerate α-tocopherol, inactivate metal initiators and reduce hydroperoxides. The activity of natural antioxidants is greatly affected by complex interfacial phenomena in emulsions and multi-component foods. In an oil-in-water emulsion system, the lipophilic antioxidants α-tocopherol and ascorbyl palmitate are more effective than in bulk oil, while the opposite trend is found for the hydrophilic antioxidants Trolox and ascorbic acid. The methodology to evaluate natural antioxidants must be carefully interpreted depending on whether oxidation is carried out in bulk oils or in emulsions, and what method is used to measure lipid oxidation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The development of rancidity in edible oils is a serious problem in some sectors of the food industry because of increasing emphasis on the use of polyunsaturated vegetable and fish oils, discontinuing the use of synthetic antioxidants and fortification of cereal foods with iron. Oxidation of lipids not only produces rancid odors and flavors, but can decrease the nutritional quality and safety by the formation of secondary products in foods after cooking and processing.

Effective control methods against lipid oxidation include processing by minimizing the loss of natural tocopherols, by eliminating metal contamination and by using antioxidants. In the last 15–20 years, special attention has been given to the use of natural antioxidants because of the worldwide trend to avoid or minimize the use of synthetic food additives. The interest in natural antioxidants continues to grow because they are presumed to be safe since they occur in foods and have been used for centuries, and the question of safety of synthetic compounds can thus be avoided. Furthermore, evidence is accumulating that natural antioxidants in foods may have clear benefits because they have anticarcinogenic effects and inhibit biologically harmful oxidation reactions in the body (Simic & Karel, 1980).

Much research has been carried out to better understand the basic processes of oxidation of polyunsaturated fatty acids, antioxidant action and the effects of decomposition products of lipid oxidation. In our research we recently found that interfacial phenomena are keys to a better understanding of antioxidant action in heterogeneous foods and biological systems. To learn about the real effects of antioxidants, it is important to obtain specific chemical information about what products of lipid oxidation are inhibited. Several specific assays are needed to elucidate how lipid oxidation products act in the complex multi-step mechanism of lipid oxidative deterioration in foods.

This paper reviews the stabilizing effects of tocopherols and ascorbic acid and esters in edible oils, and the effects of oil processing on their antioxidant properties. New work is reviewed on the effect of complex interfacial phenomena affecting the activity of natural antioxidants in emulsions and multi-component foods. The methodology to evaluate natural antioxidants must be carefully interpreted depending on whether oxidation is carried out in bulk oils or in emulsions, and what analytical method is used to determine extent and end-point of oxidation.

α-TOCOPHEROL ACTION

Polyunsaturated lipids (LH) can form alkyl radicals ($L^{\cdot}$) when they become oxidized in the presence of an initiator ($X^{\cdot}$), generally an alkoxy radical ($LO^{\cdot}$) produced by decomposition of hydroperoxides in the presence of trace metals (eqn 1). These alkyl radicals react very rapidly with oxygen to form peroxyl radicals ($LOO^{\cdot}$) (eqn 2), which react with more lipids to produce hydroperoxides (LOOH) (eqn 3). α-Tocopherol inhibits this free radical oxidation by reacting with the peroxyl radicals to stop chain propagation (eqn 4), and with the alkoxyl radicals to inhibit the decomposition of the hydroperoxides and decrease the formation of aldehydes (eqn 5). Thus, α-tocopherol behaves as a chain-breaking antioxidant by competing with the substrate (LH) for the chain-carrying peroxyl radicals, normally present in the highest concentration in the system (eqn 3):

$$LH + X^{\cdot} \rightarrow L^{\cdot} + XH \tag{1}$$

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \tag{2}$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \tag{3}$$

$$LOO^{\cdot} + AH \rightarrow LOOH + A^{\cdot} \tag{4}$$

$$LO^{\cdot} + AH \rightarrow LOH + A^{\cdot} \tag{5}$$

The tocopherol radical can form non-radical products, including dimers, stable peroxides, alkyl or unsaturated derivatives, whereby the antioxidant is regenerated.

Tocopherol mixtures are generally regarded as poor antioxidants in edible oils, especially those containing polyunsaturated fatty acids. With soybean oil containing 1500 μg/g mixed tocopherols, we found that the oxidative stability is markedly increased by removing a portion of the natural tocopherols with carbon-black (Frankel *et al.*, 1959*a*). The oxidative stability of soybean oil was optimum at concentrations between 400 and 600 μg/g. This observation has now been confirmed in studies with soybean oil–tocopherol mixtures added to stripped corn oil (Huang *et al.*, 1995).

The relative antioxidant activity of different tocopherol homologs has received much attention in the literature. On the basis of induction period measurements of oxidation of methyl linoleate, lard, cottonseed, and linseed esters, γ- and δ-tocopherols have been reported to be relatively more effective than β- and α-tocopherols (Lea, 1960; Lea & Ward, 1959). On the basis of initial rates of oxidation of styrene in chlorobenzene solution in the presence of azo-bis(isobutyronitrile) as chain initiator, the relative activities of tocopherol isomers increased in the same order as their vitamin E acivities: $\alpha > \beta > \gamma > \delta$ (Burton & Ingold, 1981).

The temperature of oxidation had a marked effect on the relative activities of tocopherol homologs in linseed esters high in linolenate but not in cottonseed esters in which linoleate was the main unsaturated fatty acid (Lea & Ward, 1959). The discrepancies in the literature concerning the relative antioxidant activities of tocopherol homologs can be attributed to the wide differences in unsaturated substrates tested, the level of oxidation used in the tests and the method used to analyze oxidation.

α-Tocopherol can act as an antioxidant or pro-oxidant depending on the test system, the concentration, the oxidation time and the method used to follow oxidation. On the basis of hydroperoxide formation (measured by conjugated diene formation) in bulk corn oil, α-tocopherol exhibited optimum antioxidant activity at lower concentration (100 μg/g) than in the corresponding oil-in-water emulsion (250–500 μg/g). However, on the basis of hydroperoxide decomposition (measured by hexanal formation), the antioxidant activity of α-tocopherol increased with concentration and oxidation time in both bulk and emulsified oil (Huang *et al.*, 1994).

The effect of different lipid substrates has a significant impact on the activity of different antioxidants according to their hydrophilic or lipophilic character. We recently compared the antioxidant activities of α-tocopherol and its carboxylic acid analog Trolox in different lipid substrates (Huang *et al.*, 1996). In bulk methyl linoleate and corn oil triglycerides, Trolox was a better antioxidant than α-tocopherol, but the opposite trend was observed in the corresponding oil-in-water emulsions. On the other hand, Trolox was a better antioxidant than α-tocopherol in both bulk and emulsified linoleic acid. Linoleic acid constitutes a unique substrate because of the formation of mixed micelles. For this reason, linoleic acid is not an appropriate lipid to test antioxidants since their behavior in this substrate would be significantly different than that in foods composed mainly of triglycerides. Yet linoleic acid is commonly used to evaluate natural antioxidants (Frankel, 1993).

ASCORBIC ACID ACTION

Ascorbic acid is noted for its complex multi-functional effects. Depending on conditions ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or as an oxygen scavenger. In aqueous systems containing metals, ascorbic acid can act as a pro-oxidant by reducing the metals, which become more active catalysts of oxidation in their lower valence state (Uri, 1961). In the absence of added metals, ascorbic acid is an effective antioxidant at high concentrations (Cort, 1982). In non-aqueous media, ascorbic acid and esters are not good antioxidants (Porter, 1980).

When we removed the tocopherols almost completely from soybean oil by carbon-black treatment, added α-tocopherol or BHT were less effective than citric or ascorbic acids (Frankel *et al.*, 1959*a*) (Fig. 1). The mixtures of α-tocopherol and citric acid gave the same induction period as citric acid alone. The mixture of BHT and citric acid was as effective as the mixture of α-tocopherol and citric acid. Therefore, in soybean oil

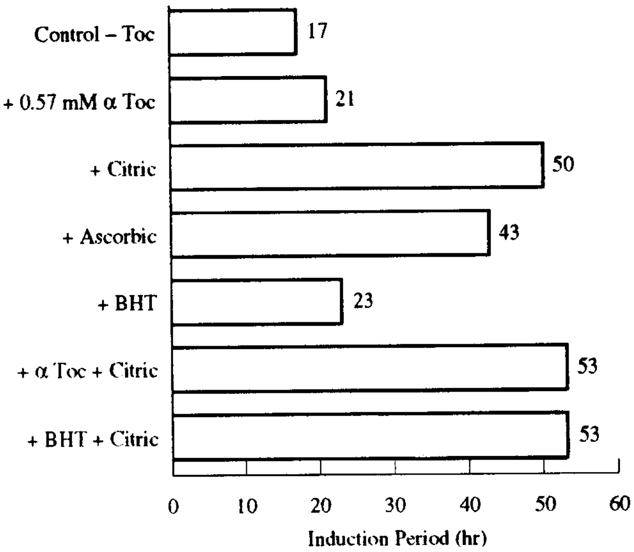

Fig. 1. Effect of tocopherols and antioxidants on oxidative stability of soybean oil at 60°C (Frankel *et al.*, 1959*a*). Control-Toc is carbon-treated soybean oil containing 45 μg/g tocopherols; 0.57 mM/kg oil equivalent to 0.01% ascorbic acid.

the metal chelation effect by citric acid may be more important than the antioxidant effects of α-tocopherol or BHT.

Cort (1974, 1982) also obtained different antioxidant trends under different oxidation conditions. In soybean oil oxidized at 45°C, ascorbic acid was less effective than ascorbyl palmitate, which was in turn more effective than BHA and BHT. TBHQ was the most active of the antioxidants tested. At 98°C the reverse trend was observed and ascorbic acid was more active than both ascorbyl palmitate and TBHQ.

The mixture of α-tocopherol and ascorbic acid exhibits a strong synergistic effect which is well recognized (Uri, 1961; Tappel *et al.*, 1961). Evidence based on pulse radiolysis (Packer *et al.*, 1979) and electron spin resonance studies (Bascetta *et al.*, 1983; Niki *et al.*, 1984; Löliger *et al.*, 1986) supports a redox mechanism involving reduction of the tocopheroxyl radical intermediate by ascorbic acid to regenerate α-tocopherol. By this synergistic mechanism tocopherols and ascorbic acid can mutually reinforce one another by regenerating the oxidized form of the other. Another mechanism for synergism involves the metal inactivating effect of ascorbic acid. Tocopherols are readily decomposed in the presence of metals and their activity is significantly improved by adding a metal inactivator (Frankel *et al.*, 1959*b*).

The lipophilic derivative ascorbyl palmitate is also known for its synergistic activity with natural tocopherols in vegetable oils where it is more active than BHT and BHA (Cort, 1974). The mixtures of α-tocopherol, ascorbic acid or ascorbyl palmitate and phospholipids are also known for their good synergistic activities (Bourgeois, 1981; Löliger, 1989*a*).

The multiple effects of ascorbic acid and ascorbyl palmitate include: (a) hydrogen donation to regenerate the stable antioxidant radical; (b) metal inactivation to reduce the rate of initiation by metals; (c) hydroperoxide reduction to produce stable alcohols by non-radical processes; and (d) oxygen scavenging.

EFFECT OF PROCESSING

Tocopherols are decomposed during vegetable oil processing but a large proportion are left in the finished oils. Mixed tocopherols in refined, bleached and deodorized (RBD) soybean oil range between 550 and 1000 μg/g (Sherwin, 1978). The loss of α-tocopherol in soybean oil was 4.3% after refining, 15% after bleaching and 20–51% after deodorization (Kanematsu *et al.*, 1983*b*). This loss of tocopherols does not reduce the oxidative stability of the RBD soybean oil because its final tocopherol content, ranging between 700 and 1000 μg/g, is still above the optimum range of 400–600 μg/g for antioxidant activity (Frankel *et al.*, 1959*a*). The losses of tocopherols reported in other refined vegetable oils were 18% in olive oil, 25% in soybean and rapeseed oils, 32% in corn oil, 36% in cottonseed oil, 37% in sunflower oil and 40% in peanut oil (Kanematsu *et al.*, 1983*a*). The tocopherol levels in rapeseed, sunflower, cottonseed, soybean and corn oils, ranging from 640 to 1170 μg/g, may be adequate to protect the oils against oxidation under ambient conditions. However, the levels of tocopherols left in the olive oil (110 μg/g) and the peanut oil (200 μg/g) may not be sufficient for optimum oxidative stability, but the level for best antioxidant protection has not been established for these oils.

The oxidative stability of virgin olive oils containing high levels of natural phenolic compounds was significantly decreased by their high initial peroxide values compared with an RBD olive oil, which contained much lower concentrations of phenolic compounds and peroxide value (Satué *et al.*, 1995). Phenolic compounds extracted from virgin olive oils increased the oxidative stability when added to the RBD olive oil. α-Tocopherol behaved as a pro-oxidant at concentrations higher than 250 μg/g, on the basis of peroxide value, but was very effective in inhibiting hexanal formation in RBD olive oil. Whether or not determination of hexanal formation is a better index of rancidity than peroxide value remains to be established in vegetable oils of known storage history.

ACTION OF ANTIOXIDANTS

The published data comparing the antioxidant activities of plant extracts and spices are difficult to evaluate because of the diverse testing methods used and the questionable conditions of oxidation (Frankel, 1993). Natural antioxidants exhibit complex interfacial affinities between air–oil and oil–water interfaces that significantly affect their relative activities in different lipid systems. Porter (1980, 1983) and Porter *et al.* (1989)

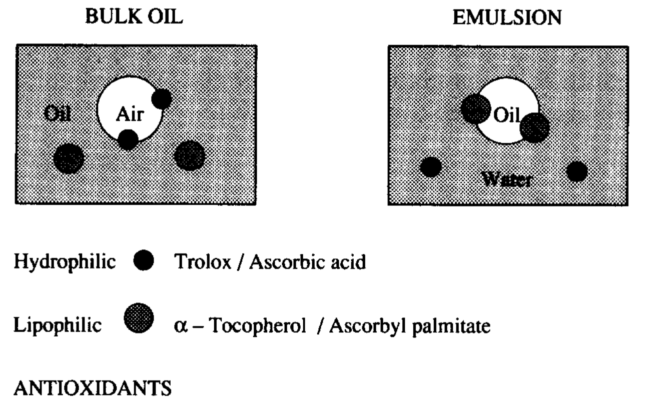

Fig. 2. Interfacial phenomena to explain the action of antioxidants in bulk oil and oil-in-water emulsion systems (Frankel *et al.*, 1994).

observed that in food systems of low surface-to-volume ratio (e.g. bulk vegetable oils) polar antioxidants are more effective than non-polar lipophilic antioxidants. In contrast, in foods of high surface-to-volume ratio (e.g. emulsified oils) lipophilic antioxidants are strongly favored. Trolox proved to be superior to α-tocopherol under a wide range of conditions and test systems (Cort *et al.*, 1975).

In our research we recently found that interfacial phenomena are keys to a better understanding of antioxidant action in heterogeneous foods and biological systems. Thus, the lipophilic antioxidants α-tocopherol and ascorbyl palmitate were more effective in an oil-in-water emulsion system than in bulk oil, while the opposite trend was found for the hydrophilic antioxidants Trolox and ascorbic acid (Frankel *et al.*, 1994). Mixtures of α-tocopherol and ascorbic acid were more active in bulk oil, while mixtures of α-tocopherol and ascorbyl palmitate were more efficient in emulsion systems. Differences observed in the efficiency of antioxidants were explained by their affinities toward the air–oil interfaces in bulk oil and the oil–water interfaces in emulsions (Fig. 2). In the bulk corn oil system the hydrophilic antioxidants are oriented in the air–oil interface and become more protective against oxidation than the lipophilic antioxidants which remain in solution in the oil. In contrast, in the oil-in-water emulsion system, lipophilic antioxidants are sufficiently surface active to be oriented in the oil–water interface to better protect oil against oxidation. By moving to the water phase the hydrophilic antioxidants become too diluted to adequately protect the oil in the oil–water interface. According to this mechanism, hydroperoxide formation and decomposition in emulsion systems are dependent on the effective concentrations of antioxidants in the oil and the water phases, and the interface.

Rosemary extracts provide a major source of commercial natural antioxidants used in foods (Löliger, 1989*b*). In a recent study, significant differences in antioxidant activities were found for a commercial rosemary extract and its components, carnosic acid, carnosol and rosmarinic acid, when they were compared in bulk corn oil stripped of natural tocopherols and in the corresponding corn oil-in-water emulsions (Frankel *et al.*, 1996). In bulk corn oil, the rosemary extract, carnosic acid, rosmarinic acid and α-tocopherol were significantly more active than carnosol. In contrast, in corn oil-in-water emulsion, the rosemary extract, carnosic acid, carnosol and α-tocopherol were more active than rosmarinic acid. These differences in antioxidant action can be explained by the same interfacial phenomenon as observed with α-tocopherol and Trolox (Frankel *et al.*, 1994). Thus, polar hydrophilic rosemary compounds may be less active in the emulsion system because they partition into the water phase and become less protective than in the bulk oil system.

REFERENCES

Bascetta, E., Gunstone, F. & Walton, J. C. (1983). Electron spin resonance study of the role of vitamin E and vitamin C in the inhibition of fatty acid oxidation in a model membrane. *Chem. Phys. Lipids*, **33**, 207–210.

Bourgeois, C. F. (1981). Propriétés antoxygènes des tocophérols et du palmitate d'ascorbyle dans les matières grasses. *Rev. Fr. Corps Gras*, **28**, 353–356.

Burton, G. W. & Ingold, K. U. (1981). Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants *in vitro*. *J. Am. Chem. Soc.*, **103**, 6472–6477.

Cort, W. M. (1974). Antioxidant activity of tocopherols, ascorbyl palmitate and ascorbic acid and their mode of action. *J. Am. Oil Chem. Soc.*, **51**, 321–325.

Cort, W. M. (1982). Antioxidant properties of ascorbic acid in foods. *Adv. Chem. Ser.*, **200**, 533–550.

Cort, W. M., Scott, J. W., Araujo, M., Mergens, W. J., Cannalonga, M. A., Osadca, M., Harley, H., Parrish, D. R. & Pool, W. R. (1975). Antioxidant activity of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. *J. Am. Oil Chem. Soc.*, **52**, 174–178.

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

Frankel, E. N., Cooney, P. M., Moser, H. A., Cowan, J. C. & Evans, C. D. (1959). Effect of antioxidants and metal inactivators in tocopherol-free soybean oil. *Fette Seifen, Anstrichm.*, **10**, 1036–1039.

Frankel, E. N., Evans, C. D. & Cooney, P. M. (1959). Tocopherol oxidation in fats. Natural fats. *J. Agric. Food Chem.*, **7**, 438–441.

Frankel, E. N., Huang, S.-W., Aeschbach, R. & Prior, E. (1996). Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol and rosmarinic acid in bulk oil and oil-in-water emulsion. *J. Agric. Food Chem.*, **44**, 131–135.

Frankel, E. N., Huang, S.-W., Kanner, J. & German, J. B. (1994). Interfacial phenomena in the evaluation of antioxidants: bulk oils *versus* emulsions. *J. Agric. Food Chem.*, **42**, 1054–1059.

Huang, S.-W., Frankel, E. N. & German, J. B. (1994). Antioxidant activity of α- and γ-tocopherols in bulk oils and in oil-in-water emulsions. *J. Agric. Food Chem.*, **42**, 2108–2114.

Huang, S.-W., Frankel, E. N. & German, J. B. (1995). Effects of individual tocopherols and tocopherol mixtures on the oxidative stability of corn oil triglycerides. *J. Agric. Food Chem.*, **43**, 2345–2350.

Huang, S.-W., Hopia, A., Frankel, E. N. & German, J. B. (1996). Antioxidant activity of α-tocopherol and trolox in different lipid substrates: bulk oils *vs* oil-in-water emulsions. *J. Agric. Food Chem.*, **44**, 444–452.

Kanematsu, H., Aoyama, M., Maruyama, T., Niiya, I., Tsukamoto, M., Tokairin, S. & Matsumoto, T. (1983). Studies on the improvement of antioxidant effect of tocopherols. I. Addition levels of tocopherols and oxidative stability of edible fats. *J. Jap. Oil Chem. Soc.*, **32**, 475–479.

Kanematsu, H., Ushigusa, T., Maruyama, T., Niiya, I., Fumoto, D., Toyoda, T., Kawaguchi, Y. & Matsumoto, T. (1983). Comparison of tocopherol contents in crude and refined edible vegetable oils and fats by high performance liquid chromatography. *J. Jap. Oil Chem. Soc.*, **32**, 122–126.

Lea, C. H. (1960). On the antioxidant activities of the tocopherols. II. Influence of the substrate, temperature and level of oxidation. *J. Sci. Food Agric.*, **11**, 212–218.

Lea, C. H. & Ward, R. J. (1959). Relative antioxidant activities of the seven tocopherols. *J. Sci. Food Agric.*, **10**, 537–548.

Löliger, J., Lambelet, P., Savoy, M.-C. & Duvet, F. (1986). Radical exchange reactions between autoxidizing lipids, vitamin E and vitamin C in binary lipid/water systems. *Fette Seifen Anstr-Mittel*, **88**, 584–587.

Löliger, J. (1989*a*). European patent 0 326 829 A2.

Löliger, J. (1989*b*). Natural antioxidants. In *Rancidity in Foods*, eds J. C. Allen & R. J. Hamilton. Applied Science, London, pp. 89–107.

Niki, E., Saito, T., Kawakami, R. & Kamiya, Y. (1984). Inhibition of oxidation of methyl linoleate in solution by vitamin E and vitamin C. *J. Biol. Chem.*, **259**, 4177–4182.

Packer, J. E., Slater, T. F. & Wilson, R. L. (1979). Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature*, **278**, 737–738.

Porter, W. L. (1980). Recent trends in food applications of antioxidants. In *Autoxidation in Food and Biological Systems*, eds M. G. Simic & M. Karel. Plenum Press, New York, pp. 295–365.

Porter, W. L. (1983). Paradoxical behavior of antioxidants in food and biological systems. In *Antioxidants: Chemical, Physiological, Nutritional and Toxicological Aspects*, ed. G. M. Williams. Princeton Scientific, Princeton, New Jersey, pp. 93–122.

Porter, W. L., Black, E. D. & Drolet, A. M. (1989). Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. *J. Agric. Food Chem.*, **37**, 615–624.

Satué, M. T., Huang, S.-W. & Frankel, E. N. (1995). Effect of natural antioxidants in virgin olive oil on oxidative stability of refined, bleached, deodorized olive oil. *J. Am. Oil Chem. Soc.*, **72**, 1131–1137.

Sherwin, E. R. (1978). Oxidation and antioxidants in fat and oil processing. *J. Am. Oil Chem. Soc.*, **55**, 809–814.

Simic, M. G. & Karel, M. (1980). *Autoxidation in Foods and Biological Systems*. Plenum Press, New York.

Tappel, A. L., Brown, W. D., Zalkin, H. & Maier, V. P. (1961). Unsaturated lipid peroxidation catalyzed by hematin compounds and its inhibition by vitamin E. *J. Am. Oil Chem. Soc.*, **38**, 5–9.

Uri, N. (1961). Physico-chemical aspects of autoxidation. In *Autoxidation and Antioxidants*, Vol. I, ed. W. O. Lundberg. John Wiley & Sons, New York, pp. 55–106.