ELSEVIER

PII: S0308-8146(96)00351-2

Food Chemistry, Vol. 60, No. 2, pp. 193–199, 1997

0308-8146/97 $17.00+0.00

Chemistry of free radicals in lipids

R. J. Hamilton,[a] C. Kalu,[a] E. Prisk,[a] F. B. Padley[b] & H. Pierce[b]

[a]*School of Chemical and Physical Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK*
[b]*Unilever Research, Colworth House, Bedford, UK*

A review of the chemistry of free radicals in lipids is presented. The keeping qualities of fish oil, sunflower oil and maize oil are studied with the peroxide value compared to their sensory evaluations. The levels of tocopherols in the three oils do not appear to account for their respective stabilities. Maize oil in the presence of fish oil remains unaffected by oxidation for lengthy periods. In attempting to obtain fractionation of the maize oil, a novel chromatographic medium was used which maintained the good keeping qualities of maize oil. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

Lipids

The technical definition of a lipid is any derivative of a fatty acid which covers a host of compounds but the bulk of lipid normally associated with the term is the triacylglycerol—an ester with three fatty acids attached to one alcohol—glycerol. Equally significant, though not from a bulk consideration, are the phospholipids which are so important in liposomes and other structural features. The ester groups in lipids can be hydrolysed to free fatty acids and, when this is achieved with the enzyme lipase, it is called lipolysis.

There are three families of fatty acids:

Oleic acid
ω-9 or n-9 family

Oleic acid itself is the most abundant fatty acid in nature but there are other acids with 14 and 16 carbon atoms with one double bond, nine carbon atoms in from the ω end.

Linoleic acid
ω-6 n-6

These acids have the first double bond, six carbon atoms from the methyl end; γ-linolenic and arachidonic acid are in this family.

Linolenic acid
ω-3 or n-3

α linolenic has the first double bond, three carbon atoms from the methyl end. (Gottenbos, 1985).

Free radicals

These can be defined as any chemical species having one or more unpaired electrons. Such a wide definition covers the hydrogen atom, transition metal atoms and the oxygen molecule itself which, with its two unpaired electrons, acts like a biradical.

One of the major areas in which carbon free radicals and oxygen free radicals are involved, is in the oxidation of lipids.

Autoxidation

This is the process in foods and bulk lipids which leads to rancidity (Hamilton, 1994). Rancidity is the spoiled off-flavour obtained by subjective organoleptic appraisal of food.

In autoxidation, the lipid is converted to an intermediate which subsequently will be converted to the derived lipid. In rancidity it is the derived lipid that gives the off-flavour whilst, in many of the analytical techniques used to follow oxidation, it is the intermediate which is monitored.

Lipid → Intermediate → Derived Lipid

The first steps in autoxidation are called initiation steps (Fig. 1). The mechanisms for this step have not been fully elucidated but they produce free radicals, e.g. both oxygen and carbon free radicals, e.g. peroxy $RO_2\cdot$, alkoxy $RO\cdot$ and alkyl $R\cdot$.

Having produced a free radical, it can react in equation 1 with oxygen in a very fast reaction with $K = 10^9$ l $mol^{-1}s^{-1}$. If the peroxy radical is formed it can attack

Initiation

$$RH + O_2 \longrightarrow R\cdot + RO_2\cdot + OH\cdot + H_2O$$

Propagation

$$R\cdot + O_2 \longrightarrow RO_2\cdot \quad (1)$$
$$RO_2\cdot + RH \longrightarrow ROOH + R\cdot \quad (2)$$

Branching

$$ROOH \longrightarrow RO\cdot + OH\cdot \quad (3)$$
$$2ROOH \longrightarrow ROO\cdot + RO\cdot + H_2O \quad (4)$$

Termination

$$2R\cdot \longrightarrow R{\cdot}R \quad (5)$$
$$R\cdot + RO_2\cdot \longrightarrow ROOH \quad (6)$$
$$RO_2\cdot + RO_2\cdot \longrightarrow ROOH + O_2 \quad (7)$$

Where R = Fatty Acid Radical

ROOH	=	Fatty Acid Hydroperoxide
$RO_2\cdot$	=	Peroxy radical
$RO\cdot$	=	Alkoxy radical

Fig. 1. Classical free radical autoxidation

another lipid molecule or the starting molecule to remove a hydrogen. In this way a hydroperoxide is formed, and another free radical produced. It is this hydroperoxide which is the intermediate mentioned earlier.

The reason why these changes are so destructive is that the hydroperoxide can break down to give two free radicals (either an alkoxide and a hydroxyl) or it can give a peroxy free radical, hydroxyl free radical and water. These branching steps lead to proliferation of free radicals which may go back to aid the propagation steps, i.e. the reaction becomes autocatalytic. The reactions can be terminated in a low oxygen environment by eqn 5 and in a high oxygen environment by eqns 6 and 7.

As the lipid is oxidised its concentration falls and the amount of hydroperoxide increases to a maximum—but it falls subsequently. So the measure of oxidation based purely on peroxide value can be confusing. The volatile products, which are the derived lipids, then rise slowly.

The type of derived lipid will depend on the starting lipid.

Methyl oleate

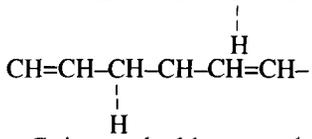
With methyl oleate, it was found that the double bond was not the weak point, i.e. the point of attack. Instead the attack occurred at the activated methylene group C_8. A free radical is formed by the breaking of the CH bond, i.e. a methylene hydrogen abstraction. Applying the concept of resonance, it is possible to write this with the free electron on C_{10}. (Fig. 2).

These free radicals can pick up oxygen with the production of two different hydroperoxides. All the reasoning concerning C_8 applies to C_{11}, and that gives C_{11} and C_9 hydroperoxides.

Indeed, there are eight different hydroperoxides possible, allowing for the different configurations of the double bond.

According to this scheme, there should be equal proportions of all four hydroperoxides, but instead, when Frankel studied these isomers, he found that the outer two were present in largest proportion for oleate (Frankel, 1985). The linoleate isomers are only formed at the 9 position and the 13 position (Fig. 3).

The bond dissociation energies for the C-H bond are 101 kcal mol^{-1} for CH_3–H, 98 kcal mol^{-1} for CH_3 CH, 77 kcal mol^{-1} for –CH–CH=CH and 65 kcal mol^{-1} for



C_8 is attacked because the bond dissociation energy is lowest at this point in the chain of linoleic acid and highest for the CH_3 at the end of a chain.

When the hydroperoxide breaks down to give the derived lipid aldehydes, alcohols, ketones and hydrocarbons are formed. These changes occur via free radicals (Fig. 4).

Short chain alkanes can be produced in this fashion with the result that ethane and pentane are used to monitor the autoxidation of lipids (Prior & Loliger, 1994; Kalu, 1995).

Oxidative damage can be prevented. The ease with which the hydroperoxide breaks down means that even ultraviolet radiation results in its decomposition. Thus,

Methyl oleate

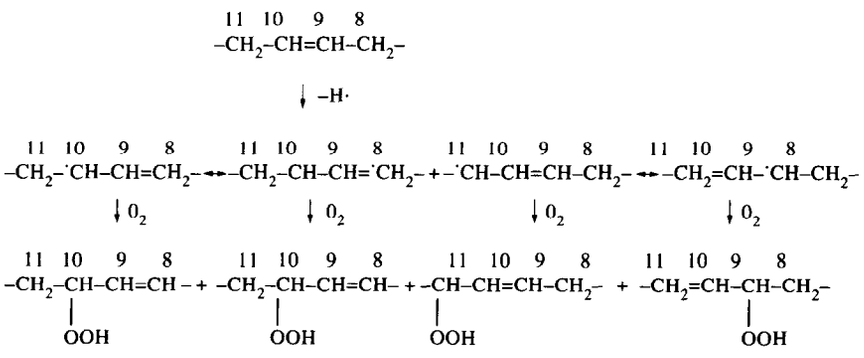


Fig. 2. 8, 9, 10 and 11 hydroperoxides both cis and trans.

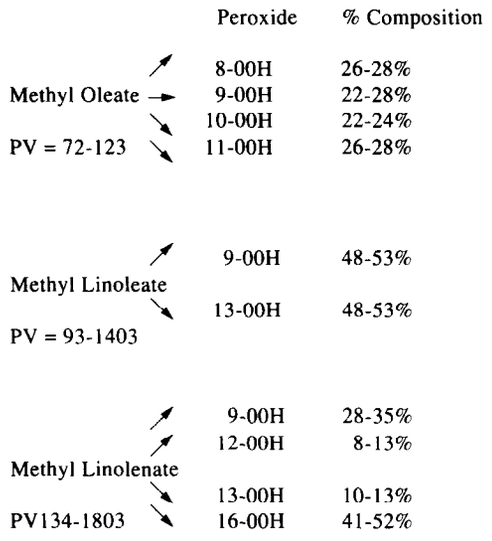


Fig. 3. Proportions of hydroperoxides when oxidised to stated peroxide values.

ultraviolet stabilisers can be used to absorb the radiation in preference to the hydroperoxide. So preventive antioxidants have been used in foodstuffs. Ultraviolet stabilisers include phenylsalicylate, carbon black and hydroxybenzophenone.

Metal atoms are also capable of breaking down the hydroperoxide.

The conversion of Fe^{2+} to Fe^{3+} produces an alkoxide free radical but the Fe^{3+} is also capable of decomposing the hydroperoxide, giving a peroxy free radical and itself being reduced to Fe^{2+}. This cycle of reactions results in large quantities of derived lipid.

Addition of metal chelating agents such as citric acid, ethylene diamine, phosphoric acid or ascorbic acid can take the metal out of solution in the lipid.

Peroxide decomposers, eg. catalase and glutathione, produce no free radical products, so they can be thought of as preventive antioxidants.

$$ROOH \xrightarrow{2H} ROH + H_2O$$

$$R_3COOH \xrightarrow{[H^+]} R_2CO + ROH$$

Chain breaking antioxidants are also used.

Chain breaking antioxidants

$$ROO\cdot + ArOH \longrightarrow ROOH + ArO\cdot$$

$$ROO\cdot + ArO \longrightarrow \text{Non-radical products}$$

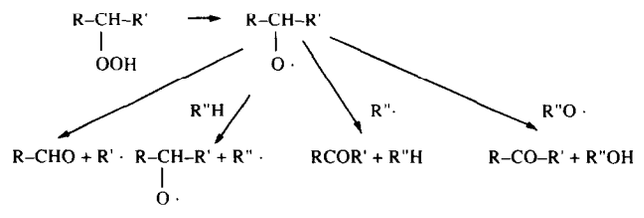


Fig. 4. Derived lipid.

The purpose of these compounds is to take the free radicals out of the reaction to prevent them from being involved in the propagation step. The antioxidant molecule ArOH is converted to a free radical ArO· which does not aid in the propagation reactions. In foodstuffs, BHA or BHT can be used but more frequently 'natural' antioxidants such as the tocopherols are preferred. The tocopherols have a long aliphatic side chain which gives the molecule its lipid solubility.

Table 1 relates the efficacy of the tocopherol antioxidants and standards with regard to their ability to provide the hydrogen for ROO·.

Simpler molecules are less effective but it can be seen that 4-methyl phenols are less reactive than 4 methoxy (Ingold, 1961).

The chain transfer is retarded when the phenoxyl oxygen is sterically protected by the 2 alkyl group and by the electron donating 4-methoxy group.

β-Carotene is a difficult molecule to classify under preventive or chain breaking types. It is thought that it can trap free radicals and the mechanism is believed to be related to what happens with other non-phenolic compounds.

So with carotene the single electron can be delocalised over the many double bonds.

Photo oxidation

It is known that light causes the acceleration of oxidation but direct photo oxidation is unlikely because the unsaturated fatty acid cannot absorb light energy of wavelength < 220 nm. Oxygen behaves like a biradical and the orbital picture of oxygen shows that there are two ways in which electrons can be inserted. The triplet state has two unpaired electrons whilst singlet oxygen has electrons paired and is not a free radical. The fact that it has a vacant orbital means that it is looking for electrons, i.e. electrophilic, so it can react with the double bonds of unsaturated fatty acids.

For these oxidations to occur, sensitiser molecules which can be dyes such as rose bengal, etc, or pigments (chlorophyll and porphyrin) need to be present (Miskoski *et al.*, 1994). These molecules can absorb light energy. In so doing they are converted into an excited

Table 1. Rates of reactions of antioxidants with peroxy radicals

Compound	Relative rate of reaction with peroxy free radical
α-Tocopherol	235
β-Tocopherol	166
γ-Tocopherol	159
δ-Tocopherol	65
Pentamethylhydroxy coumarin	24
Tetramethyl methoxyphenol	21
Pentamethylphenol	6
2,6-di-t-Bu-4 methoxyphenol	8
2,6-di-t-Bu-4-methylphenol	1

singlet state (^{1}Sens*). This sensitiser molecule can emit fluorescent light and return to the ground state or it can react by a process called Intersystem Crossing to form 3Sens*. This triplet sensitiser can react by two mechanisms.

The sensitiser reacts with the lipid molecule, forming a complex.

Type I Mechanism

$$^3\text{Sens}^* + \text{RH} \longrightarrow \text{R}\cdot + \text{SensH}$$
$$\downarrow {}^3\text{O}_2$$
$$\text{ROOH}$$

This complex can react with $^3O_2$ to give free radical and singlet sensitiser.

Again a free radical is formed. The hydroperoxides have similar structures to those formed by autoxidation.

Note: chain breaking antioxidants may interfere with Type I photo-oxidation mechanisms but not with Type II.

Type II Mechanism

$$^3\text{Sens}^* + {}^3\text{O}_2 \longrightarrow {}^1\text{O}_2 \longrightarrow \text{ROOH}$$

$$^3\text{Sens}^* \xrightarrow{^3O_2} O_2^- + \text{Sens}^*$$

In this case ^{3}Sens* reacts with $^3O_2$ to give singlet oxygen which can attach to unsaturated compounds directly. The reaction is called the ene reaction and involves interaction of the orbitals by an orbital symmetry approach.

In a simple way it can be shown that the 10 and 9-hydroperoxides result from oleic acid so that the hydroperoxides are different from those in autoxidation. In this case no free radicals are involved, so radical scavengers do not interfere with this reaction. This reaction can be inhibited by using a molecule that reacts faster with singlet oxygen than does lipid, e.g. carotene acts as a quencher for singlet oxygen (Jung & Min, 1991).

Cholesterol behaves differently toward photosensitised oxidation compared to autoxidation.

By the free radical mechanism the 7α and 7β hydroperoxide are formed which is expected when the methylene adjacent to the double bond is attacked.

In photo oxidation, the oxygen adds across the double bond to give the 3β-hydroxycholest-6-en-5-hydroperoxide.

Lipoxygenase

Glycerides, glycolipids and phospholipids are hydrolysed by lipases to free fatty acids. Lipoxygenase then reacts with free acid much more readily than with bound lipid and produces a hydroperoxide.

Hydroperoxide lyase can then convert this molecule to aldehydes, ketols, divinyl ether fatty acids, epoxy hydroxy fatty acids and trihydroxy fatty acids.

Lipoxygenases (LOX) are both regio and stereo-specific enzymes.

Soyabean gives 4% of the 9 hydroperoxide but 96% of attack is at the 13 position (regio specificity). (Table 2).

Of the 2 hydrogens at the 13 and the 9 positions which are attacked by soya bean LOX, stereo specificity is found, i.e. 3% 13R and 97% 13S, whereas it is 50:50% at 9S and 9R. If lipoxygenase is isolated from wheat a different type of pattern is formed. The proportions are 15% at the 13 position and 85% at 9, whilst the 13S:13R ratio is 66:33. In addition, at the 9 position 2.5% is R and 97.5% is S.

Superoxide

Superoxide $O_2^-\odot$ can be generated in a number of ways:

$$O_2 + \text{Fe(II)} \longrightarrow \cdot O_2^- + \text{Fe(III)}$$

Some complexes of iron, e.g. haem, generate superoxide,

$$O_2 + \text{HaemII} \longrightarrow \cdot O_2^- + \text{HaemIII}$$

Reducing agents such as ascorbate contribute to the redox cycle.

$$\text{FeIII} + \text{AsH}^- \longrightarrow \text{Fe(II)} + \cdot\text{A}^- + \text{H}^+$$

Xanthine oxidase can also generate the superoxide radical.

$$\text{X} + O_2 \longrightarrow \text{urate} + nO_2^- + (n-1)H_2O_2$$

Superoxides react with many molecules but their rates are low (Hippeli & Elstner, 1991).

So the rate of oxidation by peroxy radical with the Fatty Acids is given in Table 3 but it reacts even faster with BHT, BHA and tocopherols. It can be seen that rates of reaction with $\cdot O_2^-$ are low.

Table 2. Regiospecific and stereospecific effects of lipoxygenases

Source of Lipoxygenase	pH	Regiospecific (R)	Stereospecific (S)	
		% at position 13:9	% at position 13S:13R	% as at position 9S:9R
Soyabean LOX	9.0	96:4	97:3	50:50
Wheat LOX	6.8	15:85	66:33	97.5:2.5

But if superoxide reacts as its conjugate acid $\cdot O_2H$, the oxidative reactivities are greatly enhanced, whilst the rates of reaction with $\cdot OH$ are all in range 10^8 to 10^{10}. (Simic *et al.*, 1992)

As part of an on going study into ways of protecting fish oils to permit their use in food products, the effects of mixing vegetable oils (maize and sunflower) with fish oils have been examined. Fish oil in the presence of maize oil kept significantly better than when kept alone. In order to study this phenomenon attempts were made to fractionate the vegetable and marine oils. The present paper indicates some of the difficulties experienced and a possible solution.

MATERIALS AND METHODS

Materials

Neutral aluminium oxide, analytical grade, silica kiesel gel H 60, (230–400 mesh), citric acid, magnesium turnings and iodine were purchased from BDH. Light petroleum (40–60°), chloroform, diethyl ether and absolute methanol, were redistilled before use and stored with a nitrogen headspace. Spectrosol grade stock metal ion solutions and 4-methylpentan-2-one were purchased from BDH.

Sunflower oil, maize oil and fish oil were specially refined by treating with soda ash.

Methods

Tocopherol stripping

A slurry of 60 g of alumina in 250 ml of light petroleum (40–60°) was poured into a 2 litre separating funnel with a glass wool plug in the outlet. After the solvent had been allowed to flow out of the separating funnel, 30 g of sunflower oil dissolved in 60 ml of light petroleum was poured onto the alumina. The sunflower oil was then eluted from the alumina by allowing a further 120 ml of light petroleum to percolate through the separating funnel. The solvent was removed under vacuum. The glassware was covered with a black cloth to reduce the risk of photolytic reactions.

Column chromatography on silica/citric acid columns

A plug of 3.5 cm depth of citric acid was introduced into a 2 litre separating funnel with a glass wool plug in the outlet. A slurry of 60 g of silica gel in 250 ml of light petroleum (40–60°) was introduced above the citric acid. Thirty g of maize oil in 60 ml of light petroleum was placed at the top of the silica gel and subsequently eluted with light petroleum: diethyl ether (95:5).

Atomic absorption spectrometry

A niobium solution (1000 mg/ml) was prepared by weighing niobium metal (0·100 g) into a polyethylene beaker and 5 ml concentrated hydrofluoric acid was added. Concentrated nitric acid was added dropwise until the metal just dissolved and this solution was diluted to 100 ml in water and stored in a polyethylene bottle. Working standards contained 0·0, 0·1, 0·2 and 0·5 $\mu g\ ml^{-1}$ of copper and 0·0, 0·2, 0·5 and 1·0 $\mu g\ ml^{-1}$ of iron.

Samples of oil and standards were warmed in an oven at 50°C. In the determination of iron, the niobium solution was injected into the furnace first to coat the graphite tube. Twenty μl of the highest concentration working standard was injected into the graphite furnace and the temperature varied according to the programme.

	Temperature °C	Ramp time (sec)	Hold time (sec)	Internal flow (ml/min)
Step 1	110	5·0	25·0	300·0
Step 2	1100	40.0	40.0	300.0
Step 3	2700	0.0	5.0	0.0
Step 4	2800	1.0	3.0	300.0

For copper the wavelength was set at 324·7 nm whilst for iron the value is 372·0 nm.

Table 3. Rates of reaction of fatty acids

Fatty acid	Reaction rate constants ROO·	O_2H·	O_2
18:0	10^{-3}	LOW	LOW
18:1	0.1–1.0	LOW	LOW
18:2	60	1.2×10^3	LOW
18:3	120	1.7×10^3	LOW
20:4	180	3.0×10^3	LOW
BHT	10^4	2.4×10^3	n.m.
BHA	2.6×10^6	n.m.	n.m.
Toc	5.7×10^6	2.6×10^5	6
Asc	2.2×10^6	n.m.	5.0×10^4

BHT: Butylated hydroxy toluene
BHA: Butylated hydroxanisole
Toc: Tocopherols
Asc: Ascorbate.

Table 4. Peroxide values of oils

	PV/meq			
Week	0	1	2	3
Fish	0.1	0.4	2.2	4.3
Sunflower	1.1	0.9	1.3	1.5
Maize	0.1	0.3	0.4	0.6
Alumina-treated oils				
Fish	1.0	56.8	118.7	172.1
Sunflower[a]	2.2	16.5	46.3	60.7
Maize[a]	2.3	18.5	27.3	55.8

[a]Chromatography under nitrogen.

Tocopherol determination

The oil was saponified and the tocopherols were determined on the unsaponifiable material which was injected onto the column (10 cm×0·49 cm i.d.) packed with Nucleosil $C_{18,5}$. The tocopherols were eluted with methanol at a flow rate of 1·0 ml min^{-1} and detected by fluorimeter set at excitation 295 nm and emission 340 nm. Every four injections, a standard of α-tocopherol was injected (Carpenter, 1979).

RESULTS AND DISCUSSION

Fish oil is notorious for its susceptibility towards oxidation as can be seen in Table 4 where the peroxide value (PV) of fish oil has risen from 0.1 to 4.3 meq over the period of three weeks. By contrast, the PVs for sunflower and maize oils remain below 1·5 m Eq over this period. In addition to monitoring the degree of oxidation by PVs, sensory evaluations by way of olfactory assessments for fish oil and maize oil were performed (Table 5). It can be seen that, by week 2, strong drying odours, paint and varnish notes were observed in fish oil whereas maize oil showed no perceptible odour at week 3 and indeed no rancid odour was perceived during the six week period. The chemical and sensory data were well correlated. (Pohle *et al.*, 1964).

The highly unsaturated fatty acids in fish oil compared to vegetable oils have been studied by means of their head space volatiles (Frankel, 1993; Kalu, 1995). It is often suggested that the stability of the vegetable oils, such as maize and sunflower, is due to the presence of tocopherols. It can be seen in Table 6 that fish oil has only 115 ppm of α-tocopherol, with 10 ppm of the mixture of β and γ tocopherols. By contrast, maize oil contains 192 ppm of α-tocopherol, 1390 ppm of β and γ tocopherols and 80 ppm of δ-tocoperol. Sunflower oil also has substantial quantities of these natural antioxidants, *viz* 775 ppm of α-tocopherol, 75 ppm of β and γ-tocopherols but no δ-tocopherol (Carpenter, 1979).

The oxidative stability of the oil may depend as much on the pro-oxidant components, e.g. metals, as it does on the antioxidants. The levels of copper and iron in the oils were determined by atomic absorption spectrometry. The original sample of fish oil has 0·25 ppm of iron compared to 0·06 ppm in maize oil and less than 0·05 ppm in sunflower oil. The levels of copper are no higher than 0·01 ppm in any of the oils.

In order to consider the effects of these variables, the tocopherols were stripped from the oils by treatment of each of the oils with alumina. It can be seen in Table 6 that such stripping reduces the α-tocopherol to 0 ppm for fish oil and 46 ppm of the mixed β and γ tocopherols in maize. These treated oils were very easily oxidised. Thus the PV for fish reached 56.8 after one week, at which stage maize oil has a PV of 16.5 (Table 4).

Table 5. Sensory evaluations of oils (odour profiles)

Week	Descriptors—fish oil	Descriptors—maize oil
Week 0	Mild CLO, bland overall	Bland initially, slight corn, fried odour
Week 1	Strong CLO, slight acidic, sharp green after-note, slight varnish	No perceptible odour
Week 2	Mild CLO, strong 'drying' odours, painty, varnish over-tone, acidic green	Similar to Week 1
Week 3	Similar to Week 2	Mild sweetcorn odour
Week 4	Strong drying oil, sharp acidic—like vinegar	Similar to Week 3
Week 5	Varnish (polymerised oil to cap)	Egg—Raw
Week 6	Acidic—vinegar, strong paint (burning, pungent, lachrymatory)	Slight sweetcorn, raw egg, pastry

Table 6. Tocopherol and metal levels (ppm)

	α-T	β/δ-T	δ-T	Cu	Fe
Fish	115	10	0	<0.01	0.25
Fish[a]	0	0	0	0.02	0.08
Fish[b]	19	5	0	0.03	0.06
Maize	192	1390	80	0.01	0.06
Maize[a]	0	46	5	0.01	<0.05
Maize[b]	175	1056	34	0.6	0.01
Sunflower	755	75	0	<0.01	<0.05
Sunflower[a]	20	16	0	0.01	<0.05
Sunflower[b]	455	38	0	0.02	0.04

[a]Alumina-treated oils
[b]Silica-treated oils
Eluted on absorbent media with light petroleum.

Table 7. Peroxide values for maize oil eluted on alumina and silica columns

	PV/meq					
Week	0	1	2	3	4	5
MZc alumina	2.3	18.5	27.3	55.8	>100.0	>170.0
MZc silica	0.3	2.1	9.8	16.7	22.0	29.0
MZ[a]	<0.1	0.3	0.4	0.6	0.6	1.2

[a]Unchromatographed maize oil.

Table 8. Peroxide values for maize oil eluted from silica/citric acid columns[a]

	PV/meq			
Week	0	4	8	12
MZc	0.4	16.8	33.0	36.0
MZc + CA	1.0	1.3	1.7	6.7

[a]MZc eluted from a silica column in light petrol/diethyl ether; MZc+CA eluted from a silica column containing a bed of citric acid.

In an attempt to obtain a fraction from maize oil which had antioxidant properties, the maize oil was chromatographed on silica gel. This procedure is normally a milder treatment and, indeed, Table 7 shows that the maize oil after treatment with silica gel has a PV of 2.1 meq after one week compared to 18.5 meq after alumina treatment.

This silica treatment did not leave the maize oil in as good a condition as the unchromatographed oil (Table 7). Studying the metal levels (Table 6) suggested that copper ions had increased in level from 0·01 ppm to 0·6 ppm for maize and from < 0·01 to 0·03 in fish oil.

In the subsequent studies of fractions from maize oil, it was recognised that this degree of contamination by metal ions would alter the antioxidative properties of the fractions. To ensure that this contamination was minimised, a novel chromatographic medium was devised. A chromatographic column was prepared with silica gel as the major component of the system, and a short length (4 cm) of citric acid was introduced into the system. The quality of maize oil which was eluted from this novel column was much improved. This citric acid-treated maize oil remains unaffected by oxidation until week 8. It is probable that the silica gel and/or the alumina chromatography had removed the citric acid from the maize oil. Citric acid is normally added to a refined oil after the deodorisation step where it acts as a preventive antioxidant. By use of the novel chromatographic column, citric acid has been re-introduced to the maize oil where it is capable of removing copper or iron from their active pro-oxidant state.

This unusual chromatographic medium will permit the fractionation of maize oil without contaminating the fractions, and will enable an examination of the fractions from maize oil to determine which provides maize oil with its antioxidant properties (Nieto *et al.*, 1993).

ACKNOWLEDGEMENTS

C. K. thanks Liverpool John Moores University for a research studentship and Unilever for additional support.

REFERENCES

Carpenter, A. P. (1979). Determination of tocopherols in vegetable oils. *J. Amer. Oil Chem. Soc.*, **56**, 668–670.

Frankel, E. N. (1985). *Progress Lipid Res.*, **23**, 197.

Gottenbos, J. J. (1985). In *The role of fats in human nutrition*, ed. F. B. Padley and J. Podmore, Ellis Horwood, Chichester.

Hamilton, R. J. (1994). In *Rancidity in foods' 3rd edn*, ed. J. C. Allen & R. J. Hamilton. Chapman & Hall, London, 1–22.

Hippeli, S. & Elstner, E. F. (1991). In *Oxidative stress*, ed. H. Sies. Academic Press, New York.

Ingold, K. V. (1961). *Chem. Rev.*, **61**, 563.

Jung, M. Y. & Min, D. B. (1991). Effects of quenching mechanisms of carotenoids on the photosensitised oxidation of soyabean oil. *J. Amer. Oil Chem. Soc.*, **68**, 653.

Kalu, C. U. U. (1995). Ph.D. Thesis, Liverpool John Moores University, UK.

Min, D. B., Lee, S. H. & Lee, E. C. (1989). In Flavor chemistry of lipid foods, ed. D. B. Min & T. H. Smouse. *Amer. Oil Chem. Soc.*, Champaign, IL.

Miskoski, S., Nicotra, V., Saavedra, E., Balsaretti, V. & Garcia, N. A. (1994). *Fat Sci. Technol.*, **96**, 77.

Nieto, S., Garrido, A. & Sanheuza, J. (1993). Flavanoids as stabilizers of fish oil. *J. Amer. Oil Chem. Soc.*, **70**(8), 773.

Pohle, W. D., Gregory, R. L. & van Gilssen, B. (1964). Relationship of peroxide value and thiobarbituric acid value to development of undesirable flavour characteristics in fats. *J. Amer. Oil Chem. Soc.*, **41**, 649–650.

Prior, E. & Loliger, J. (1994). In *Rancidity in foods*, 3rd edn ed. J. C. Allen & R. J. Hamilton. Chapman and Hall, London, 104–127.

Simic, M. G., Jovanovic, S. V., Niki, E. (1992). In Lipid oxidation in food, ed. A. J. St. Angelo. *Amer. Chem. Soc.*, Ser 500, New York.

Simic, M. G. (1991). *J. Environ. Sci. Health*, **C9**, 113–153.