# HEALTH PROMOTING CONSTITUENTS IN PLANT DERIVED EDIBLE OILS

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## ABSTRACT

*Of all the edible oils, only that produced from olives has had its health attributes studied in detail. For maximum nutritional benefit, an edible oil should contain minimal levels of saturated fats, especially lauric and myristic acids and minimal levels of trans fatty acids. If the oils are not to be heated repeatedly and if they contain high levels of antioxidants, they should contain omega-3 and possibly omega-6 polyunsaturated fatty acids. The fatty acid profile should be dominated by monounsaturated fatty acids. Secondary products which act as antioxidants including polyphenols, proanthocyanidins, tocopherols and carotenoids increase the shelf-life of oils, reportedly reduce cardiovascular disease and provide some anticarcinogenic properties. More research is also required, but there is evidence that phytosterols and squalene are also beneficial components of edible oils. Selection and breeding can be used to increase the desirable components of edible oils. Geographic, culture and environmental factors can influence the properties of the oil produced by crops, and methods of processing can greatly reduce the levels of health promoting components. Despite the current anti-GMO sentiments, biotechnology should be used both in*

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*the development of plant cultivars which produce nutritional oils and in processing to maximize the desirable components.*

## INTRODUCTION

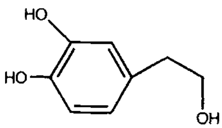
The high incidence of diet related disorders has led to worldwide interest in healthy foods. High fat intake, especially saturated fat, has been implicated in cardiovascular disorders including atherosclerosis (blocking of cardiac arteries), thrombosis (blood clotting), certain cancers and diabetes. On the other hand, there is increasing evidence that diets containing higher levels of certain lipid components are associated with reduced incidence of several diseases.

Edible oils are derived from a diverse range of plants and their origin is the primary determinant of composition. Nevertheless, the basic features of composition are the same in all edible oils. Thus, the major constituents (generally up to 98% by mass) in all oils are triacylglycerols. Numerous minor components comprise the remainder. The latter includes free fatty acids, partial acylglycerols, phenols, tocopherols, sterols, stanols, phospholipids, waxes, squalene and other hydrocarbons (Fig. 1). The precise nature of these minor constituents varies greatly among edible oils and both horticultural practices and processing can greatly affect the content of these in oils. Hence, the composition of commercial oils from the same source may differ greatly and there is a need to consider both the nature (e.g. olive, canola, sunflower oil) and source of an oil in deciding its potential benefits. Process-induced changes are of two fundamental types, simple reduction in the level of a component(s) or, alternatively, chemical modification of one or more components. For instance, refining reduces phytosterol contents in peanut and olive oils with the loss being greater in the latter (Awad *et al.* 2000). On the other hand, dimer triacylglycerols, normally not present in crude oils, have been identified in refined oils (Gertz and Klostermann 2000). The extent of such losses or modifications depends on processing conditions, particularly temperature. Olive oil presents an interesting case because extra virgin olive oil has not been exposed to elevated temperatures. Nevertheless, Owen *et al.* (2000) have identified the need to consider processing effects in relation to olive oils. Indeed, stigmasterol content of olive oils was affected by the extraction system, being highest in pressed oils (Koutsaftakis *et al.* 1999). The ratio of campesterol/stigmasterol was significantly higher in oils extracted by dual- and three-phase centrifugation.

Background information on edible fats and oils including health issues has recently appeared (Akoh and Min 1998). The following review examines the latest information on the relationship between components of edible oils and health, and shows that there is a great need for more research examining the healthy constituents of the wide range of edible oils now available. The issues

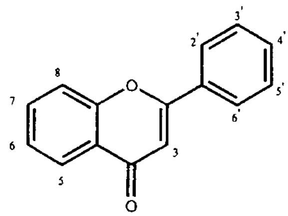
**Phenol**

Simple



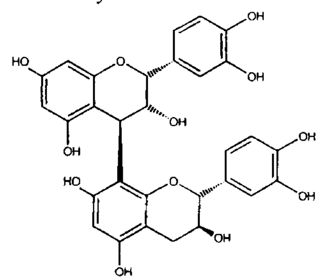

3,4 dihydroxphenylethanol
(hydroxytyrosol)

Flavone



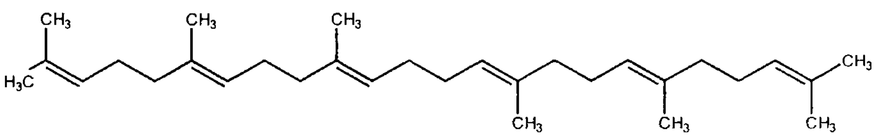

Apigenin 5 = 7 =4' = OH

Cyanidin

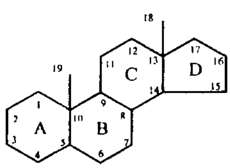



Procyanidin B-1

**Squalene**

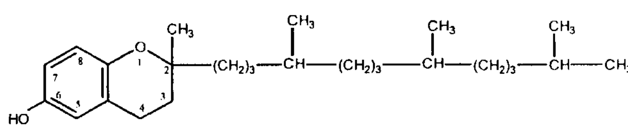



**Phytosterol**



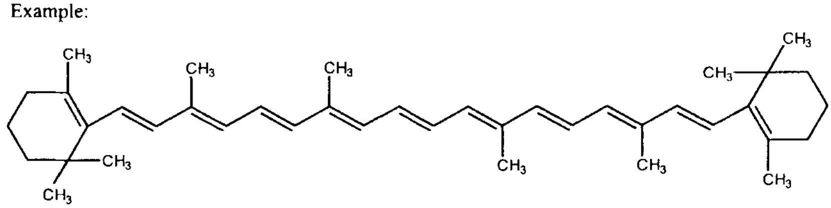

Perhydrocyclopentanophenanthrene
nucleus

**Tocopherols**




Tocol

**Carotenoid**
Example:




$\beta$-Carotene

FIG. 1. STRUCTURES OF PHENOLS, SQUALENE, PHYTOSTEROLS, TOCOPHEROLS AND CAROTENOIDS

of incidental additives such as pesticide residues (Ranalli *et al.* 2001) and authenticity or adulteration (Kamm *et al.* 2001) are outside the scope of the present review.

**Triacylglycerols**

The difficulty of analyzing parent triacylglycerols meant that oils were traditionally characterized by their fatty acid profiles following hydrolysis and transesterification typically to fatty acid methyl esters (de Koning *et al.* 2001). This analysis results in a loss of information, as two oils having the same fatty acid profile may differ greatly in their physical and chemical properties. The triacylglycerols that constitute the bulk of an edible oil are not a single chemical entity; rather the oil contains a mixture of mixed triacylglycerols. Analytical procedures are now available for direct measurement of the intact triacylglycerols (Holcapek *et al.* 2001; Parcerisa *et al.* 2000) and for the regiospecific analysis of triacylglycerols (location of fatty acids on glycerol backbone) (Mottram *et al.* 2001) including optical resolution of asymmetric triacylglycerols (Iwasaki *et al.* 2001).

The distribution of fatty acids both between and within the triacylglycerols is selective rather than random. For example, six fatty acids are found in palm oil, but only 14 combinations are found in the triacylglycerols (Man *et al.* 1998). Thus, distribution of fatty acids in the palm oil triacylglycerols is nonrandom. Evidence of selective distribution is also seen in most vegetable oils where unsaturated fatty acids preferentially occupy the sn-2 position and saturated fatty acids are usually located in the sn-1 and sn-3 positions (Yoshida *et al.* 2001a, b). There is intense interest in sn-2 fatty acids because they are preferentially absorbed as the 2-monoacylglycerol and serve as the template for reesterification by intestinal cells to reform triacylglycerols (Bell *et al.* 1997). Monounsaturated fatty acids predominate in the sn-2 glycerol position in olive oils (Ajana *et al.* 1998) and the main constituents of both olive and hazelnut oils are 1,2,3-trioleylglycerol, 2,3-dioleyl-1-palmitoylglycerol, 2,3-dioleyl-1-linoleylglycerol and 2,3-dioleyl-1-stearoylglycerol (Parcerisa *et al.* 2000). The regiospecific distribution of fatty acids within the triacylglycerols and diacylglycerols of olive oil has been examined at different stages of drupe ripening (Cossignani *et al.* 2001). All triacylglycerols in which the 1 and 3 positions carry different acyl groups produce enantiomeric forms (optically asymmetric isomers). Thus, enantiomers will exist for at least some of the species identified in olive and hazelnut oils. The nutritional impact of enantiomeric species has not yet been researched in detail. Indeed, the more general issue of the nutritional effect of the regiospecific distribution of the acyl groups on the glycerol backbone has only been addressed more recently. This can partly be attributed to an increased use of interesterification of fats for human consumption (Becker *et al.* 2001). Structured triacylglycerols are currently developed by interesterifying a mixture of conventional fats and oils to achieve a specific fatty acid distribution.

Human and animal feeding studies have shown (Kubow 1996) effects of triacylglycerol stereospecific composition on fat absorption, plasma cholesterol, and plasma triacylglycerol concentrations and atherogenesis. A recent study (Abia *et al.* 2001) evaluated the effects of dietary intake of oils with equal amounts of oleic acid but with different compositions of minor fatty acids and triacylglycerol molecular species. The results indicate that oleic acid content may not be the main factor affecting triacylglycerol metabolism, but rather the minor fatty acids such as linoleic acid and the 2-positional distribution of saturated stearic and palmitic acids may be important determinants of postprandial lipemia (plasma lipid and lipoprotein levels after a meal) in normolipidemic humans. Nevertheless, data considering the regiospecificity of acyl substitution and the fate of lipids beyond the bloodstream (absorption, chylomicron formation, and deposition in adipose tissue and in different liver lipids) are rather scarce and animal model studies are needed.

## Fatty Acid Profiles

Most published literature on fatty acids focuses on their role in triacylglycerols. Free fatty acids (FFA) are a minor component of most edible oils and increase with deterioration of the product during storage or heating (Agar *et al.* 1999; Xu *et al.* 1999). While elevated levels of FFA in the bloodstream are associated with obesity in the upper body including excess visceral fat which in turn is associated with adverse health conditions including hypertension and premature coronary death (Jensen 1997), it is not known if dietary intake of FFA is deleterious to health.

There is accumulating evidence that diets with fat intake high in monounsaturated fatty acids such as in olive oil, and high in omega-3 polyunsaturated fatty acids from certain fish including salmon, tuna and mackerel help prevent heart disease (Ponte *et al.* 1997; Temple 1996; Simopoulos 1999). One of the major factors involved in cardiovascular disease is low density lipoprotein (LDL) cholesterol in the bloodstream. In contrast, high density lipoprotein (HDL) cholesterol is linked to a reduced risk of coronary heart disease (Trichopoulou 1998). Salmon oil, which contains the omega-3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids, lowers LDL cholesterol to a lesser or similar degree as the plant omega-6 fatty acid, linoleic acid (Harris *et al.* 1983). Salmon oil, however, dramatically lowers very low density lipoprotein (VLDL) cholesterol and triacylglycerol levels without altering HDL cholesterol, the form of cholesterol which has beneficial effects on the cardiovascular system (Guoping *et al.* 1999). Harris (1989), however, has shown that fish oils may increase LDL cholesterol in a diet if saturated fat intake is not reduced. Fish oils also lower FFA levels in the bloodstream (Dagnelie *et al.* 1994). Linolenic acid, the omega-3 fatty acid in plant oils, also reduces LDL cholesterol. Omega-3 fatty acids also reduce plasma triacylglycerols, another factor in cardiovascular disease (Hwang *et al.* 1994). A review by Connor (2000) and a recent book by Shahidi and Finley (2001) describe the beneficial effects of omega-3 fatty acids on heart disease and other diseases. There has, however, been inadequate research done comparing the relative benefits of fish oil omega-3 fatty acids and omega-3 and omega-6 fatty acids in plant derived oils. For example, a recent review has shown no clear effect of omega-3 polyunsaturated fatty acids on intestinal inflammatory diseases (Teitelbaum and Walker 2001).

Polyunsaturated fatty acids may be more effective than monounsaturated fats in lowering total blood serum cholesterol without affecting HDL cholesterol (Howard *et al.* 1995). This controversial result needs further corroboration. Linoleic acid and other polyunsaturated fats while reducing LDL cholesterol, probably facilitate LDL cholesterol oxidation which may be the most critical factor in the development of atherosclerosis (Mensink *et al.* 1998). As expected, diets rich in monounsaturated oleic acid reduce the susceptibility of LDL to oxidation when compared with diets rich in polyunsaturates (Svegliati *et al.* 1999). In addition, Mata *et al.* (1992) concluded that monounsaturated fatty acids increase beneficial HDL cholesterol to a greater extent than polyunsaturates. Canola, olive oil, soybean oil, sunflower oil and safflower oil have all been shown to reduce total blood serum cholesterol and LDL cholesterol if they substitute for fats high in saturated fatty acids (Denke and Grundy 1991; McDonald 1993).

Intake of saturated fats such as palmitic acid (Watts *et al.* 1996), lauric acid and myristic acid (Chong and Ng 1991) may be harmful to health because they raise both total cholesterol and LDL cholesterol in the bloodstream. Both lauric acid and myristic acid, which together constitute two thirds of the fatty acids in coconut and palm kernel oils and also occur in dairy products, both have a stronger effect than palmitic acid in raising both total blood serum cholesterol and LDL cholesterol (Hayes and Khosla 1992; Zock *et al.* 1994; Temme *et al.* 1996). A number of animal and human studies indicate that the ratio of myristic acid to linoleic acid is important in regulating total blood cholesterol; myristic acid increases it while linoleic acid decreases it (Pronczuk *et al.* 1994). Stearic acid, although a saturated fat, does not raise serum cholesterol, however, a controversial study by Watts *et al.* (1996) showed that stearic acid and the trans fatty acid, elaidic acid, increased coronary disease independent of plasma cholesterol levels. In contrast, there is evidence that stearic acid lowers LDL cholesterol (Grande *et al.* 1970; Bonanome and Grundy 1988; Denke and Grundy 1991).

The impact of saturated fats on LDL cholesterol thus appears to be inversely related to the number of carbon atoms with lauric acid (C12) > myristic acid (C14) > palmitic acid (C16) > stearic acid (C18). More research is required, as serum LDL cholesterol is only one of the several factors involved in the development of cardiovascular disease.

Table 1 shows that fatty acid profiles vary enormously among different oil crops. Coconut and palm oils are high in saturated fatty acids whereas soybean, corn, olive, sesame, linola, sunflower, grapeseed, safflower and canola oils are low in saturated fatty acids. Plant based edible oils also vary in the content of polyunsaturated fatty acids, with safflower, sunflower, grapeseed, corn, soybean, linola (altered flax oil), sesame, cottonseed, and peanut oils having high levels. Monounsaturated and polyunsaturated fatty acids are the predominant fatty acids in hazelnut oil although there are significant varietal differences (Parcerisa *et al.* 2000). Most of the polyunsaturated fatty acid is linoleic acid (an omega-6), and only soybean and canola have significant levels of linolenic acid (an omega-3). Olive and canola oils alone have high levels of monounsaturated fatty acids. The fatty acid content of olive drupes changes rapidly during fruit development with palmitic and linolenic acids declining while oleic and linoleic acids increase during fruit ripening (Ayton *et al.* 2001; Ajana *et al.* 1998). The fatty acid profile of olive oil will depend then on the maturity of the fruit. There is thus much scope for altering fatty acid profiles by blending various plant derived oils, and it is clear that healthy oil blends could be developed (Carola 1974; Klahorst 1998).

## Trans Fatty Acids

Trans fatty acid intake is correlated with increased risk of cardiovascular disease because it increases total cholesterol and LDL cholesterol and reduces beneficial HDL cholesterol (Mensink and Katan 1990; Judd *et al.* 1994; Khosla and Hayes 1996; Nelson 1998; Lichtenstein 2000). The role of trans isomers of fatty acids in causing cardiovascular disease has however been questioned (Sambaiah and Lokesh 1999), and they may have a lesser effect than saturated fatty acids (Judd *et al.* 1994).

Trans fatty acid isomers form when oils are heated. Each heating episode increases the levels of trans fatty acids (Biernat and Grajeta 1998). Heat stability of oils, including the propensity to form trans fatty acids, depends on the fatty acid profile and antioxidant content. Oils with high levels of polyunsaturated fatty acids are prone to form trans fatty acids. A number of studies have shown that linolenic acid is 13-14 times more prone to isomerization than linoleic acid (Wolfe 1992). The type and level of antioxidants in an oil also determine the degree of trans fatty acid formation, and will be discussed in the section on antioxidants. A commercial product, Good-Fry(R) has been developed (Kochhar

TABLE 1.

PERCENTAGE OF TOTAL FATTY ACID CONTENT IN OILS, AND A MIXTURE OF 2 PARTS OLIVE AND 1 PART GRAPESEED OILS. IN THIS TABLE, THE ABSENCE OF DATA FOR SOME OF THE FATTY ACIDS GENERALLY INDICATES THAT THERE IS NO INFORMATION IN THE SCIENTIFIC LITERATURE

| Fatty Acid | Olive[1] | Grape[2] | Olive/ Grape 2:1 | Canola | Low linolenic canola | Sun- flower | Saf- flower | Soy- bean | Linola | Sesame | Cotton- seed | Palm[3] | Coconut | Avocado[4] | Apricot[5] | Almond[6] | Peanut |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Myristic[s] 12:0 | | | | | | | | | | | | | 18 | | | | |
| Lauric[s] 14:0 | | | | | | | | | | | | | 49 | | | | |
| Palmitic[s] 16:0 | 12 (7-18)* | 8 (6-9) | 12 | 4 | 7 | 11 | 9 | 15 | 11 | 13 | 26 | 42 | 26 | 17-27 | 8 | 4-13 | 19 |
| Stearic[s] 18:0 | 2 | 4-10 | 2.5 | 3 | ? | | | | | | | | | | | 0.4-10 | |
| Palmitoleic[m] 16:1 | | 4 | | | | | | | | | | | | 8 | | 0.4-2 | |
| Oleic[m] 18:1ω9 | 76 (69-82) | 17 (13-21) | 56 | 63 | 69 | 23 | 14 | 23 | 18 | 45 | 16 | 40 | 6 | 43-65 | 65-71 | 43-81 | 45 |
| Linoleic[p] 18:2ω6 | 10 (4-13) | 73 (62-78) | 31 | 20 | 18 | 66 | 77 | 54 | 69 | 41 | 58 | 6 | 2 | 10-24 | 12-26 | 12-44 | 34 |
| Linolenic[p] 18:3ω3 | 0.7 (0.4-4.6) | 0 | 0.5 | 10 | 3 | 0 | 0 | 8 | 2 | 1 | 0 | 12 | 20 | | | 0.1-1.3 | |

* range where known; s, saturated; m, monounsaturated; p, polyunsaturated; totals of some columns add up to more than 100% because of use of multiple references

[1] *Sedgley 2000*

[2] Mehran and Filsoof 1974; Badolato *et al.* 1987; Schirra *et al.* 1993; Abou Rayan *et al.* 1998

[3] Palm kernel oil has a fatty acid profile similar to coconut oil

[4] Szpiz *et al.* 1987; Martinez *et al.* 1988

[5] Beyer and Melton 1990; Sherin *et al.* 1993

[6] von Aitzetmuller and Ihrig 1988

2000) that incorporates a variety of natural antioxidants from sesame seed oil and rice bran oil. Deodorization of canola, soybean, sunflower, peanut and olive oils causes a build up in trans-isomeric fatty acids and reduces total tocopherol content (Ferrari 1996; Schone *et al.* 1998; Grob *et al.* 1996; Henon *et al.* 1999).

**Antioxidants**

An increasing body of literature implicates antioxidants in a wide range of health promoting benefits including reducing some forms of cancer and cardiovascular disease. A large number of antioxidants occur in edible oils derived from plants (Table 2). They include polyphenols (also flavonoids such as proanthocyanidins), carotenoids, tocopherols and phytosterols (Fig. 1). The most widespread and biologically active of the tocopherols (Parcerisa *et al.* 2000) is $\alpha$-tocopherol, (5,7,8-trimethyltocol). Other common tocopherols are $\beta$-tocopherol (5,8-dimethyltocol), $\gamma$-tocopherol (7,8-dimethyltocol) and $\delta$-tocopherol (8-methyltocol). The structurally related tocotrienols are less widespread but occur in large quantities in wheat germ oil, corn oil and palm oil. The only difference between the two series is that the trienols have a long side chain at carbon-2 which consists of 3 isoprene units instead of the saturated side chain in the tocol series. The major carotenoids found in vegetable oils have an all trans-configuration as shown in Fig. 1.

Antioxidants may prevent oxidation of polyunsaturates in the blood stream and thus protect LDL cholesterol from oxidation, a key factor in the development of atherosclerotic lesions (Mensink *et al.* 1998). Although both linoleic acid, and omega-3 polyunsaturated fatty acids from fish oils may lower LDL cholesterol, they also make it more prone to oxidation. Addition of vitamin E to oils rich in omega-3 polyunsaturated fatty acids may reduce the oxidation of LDL (Mensink *et al.* 1998). Carotenoids have antioxidant properties and the high levels in red palm oil have a potent dietary anticarcinogenic effect (Elson 1992). Virgin olive oil also contains carotenoids and tocopherols, the content of which varies with cultivar and method of processing (Cimato *et al.* 1996). Antioxidant activity in virgin olive oil inhibits LDL cholesterol oxidation *in vitro* (Fito *et al.* 2000).

Olive oil has also been reported to lower blood pressure, but not through its high oleic acid content (Perona and Ruiz-Gutierrez 1998). This could be due to the presence of antioxidants. Olive oil has higher polyphenol content than macadamia, avocado, sesame, canola, soy, grapeseed, sunflower, walnut, peanut, and almond oils (Colquhoun *et al.* 1996), although this depends on processing and other factors such as cultivar and environmental factors. Olive oil is the only vegetable oil obtained from whole fruit and thus many of the

TABLE 2.
ANTIOXIDANT PROPERTIES OF EDIBLE OILS

| **Antioxidant/ oxidation resistance** | **Olive[1]** | **Grape** | **Almond** | **Canola[2]** | **Soybean[2]** | **Corn[3]** | **Sunflower[2]** | **Macadamia** | **Cottonseed[3]** |
|---|---|---|---|---|---|---|---|---|---|
| Total sterols | 245-2000 | | | 881 | 440 | 1980 | 495 | | 3970 |
| Cholesterol (mg $kg^{-1}$) | 0-2.1 | | | | | 11.7 | | | 11 |
| Campesterol (mg $kg^{-1}$) | 42-98 | | | 2940 | 790 | 1230 | 371 | | 313 |
| Campestanol (mg $kg^{-1}$) | | | | | | 89.8 | | | |
| Stigmasterol (mg $kg^{-1}$) | 7-22 | | | 354 | 660 | 462 | 396 | | 39.9 |
| Sitosterol (mg $kg^{-1}$) | 1162-2082 | | | 4229 | 2376 | 4540 | 2871 | | 3430 |
| Sitostanol (mg $kg^{-1}$) | | | | | | 236 | | | 21.6 |
| Avenasterol (mg $kg^{-1}$) | 150 | | | | 88 | 228; 49.3 | 150 | | 85.2; 11.3 |
| Brassicasterol | 0 | | | 1144 | 0 | | 0 | | |
| Squalene (mg $kg^{-1}$) | 30-90 | | | | | | | | |
| Total polyphenols (mg $kg^{-1}$) | 10-1700 | 2 | 0 | 4 | 4 | 0 | 0 | 9 | |
| Tyrosol (mg $kg^{-1}$) | 2-20 | | | | | | | | |
| Hydroxytyrosol (mg $kg^{-1}$) | 1-15 | | | | | | | | |
| o-coumaric acid ($\mu g\ kg^{-1}$) | 40-1000 | | | | | | | | |
| Cinnamic acid ($\mu g\ kg^{-1}$) | 50-1500 | | | | | | | | |
| Sinapic acid ($\mu g\ kg^{-1}$) | 10-400 | | | | | | | | |
| p-coumaric acid ($\mu g\ kg^{-1}$) | 30-300 | | | | | | | | |
| Siringic acid ($\mu g\ kg^{-1}$) | 70-400 | | | | | | | | |

TABLE 2. Continued

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Vanillic acid ($\mu$g kg$^{-1}$) | 20-450 | | | | | | |
| Caffeic acid ($\mu$g kg$^{-1}$) | 5-100 | | | | | | |
| $\alpha$-tocopherol (mg kg$^{-1}$) | 90-140 | | | 70-200 | 55-90 | 130-180 | 608 |
| $\gamma$-tocopherol (mg kg$^{-1}$) | 7 | 3 | | 180-613 | 435-740 | 400-750 | 11 |
| $\gamma$-tocotrienol | | | | | | | |
| $\delta$-avenasterol | | | | | | | |
| Vitamin A | | | | | | | |
| Carotenoids (mg kg$^{-1}$) | 20-50 | | | | | | |
| Proanthocyanidins | | | | | | | |
| ORAC value[4] | ? | | | | | | |
| Peroxide value | 3-28 | | | | | | |
| Oxidative resistance (h at 120C) | 2-11 | | | | | | |

[1] Alessandri *et al.* 1997; Ackman 1983; Colquhoun *et al.* 1996
[2] Ackman 1990
[3] Reina *et al.* 1999
[4] Oxygen Radical Absorbance Capacity (Prior and Cao 2000)

compounds are transferred to the fluid during processing (Galli and Visioli 1999). Most other vegetable oils are extracted from seeds and contain little or no carotenoids or flavonoids (Trichopolou 1998). Processing reduces polyphenol content of oils, and virgin olive oils have higher polyphenol content than more highly processed oils (Catalano and Caponio 1996; Colquhoun *et al.* 1996; Owen *et al.* 2000). Shukla *et al.* (1997) after reviewing the literature concluded that processing removes large amounts of antioxidants from canola, olive, sesame and soybean oils. There is at least a threefold variation in total polyphenol content among olive cultivars (Cimato *et al.* 1996). There are a large number of polyphenols in virgin olive oil including hydroxytyrosol and oleuropein which have been shown to be very strong free radical scavengers, and thus may contribute to the lower incidence of cardiovascular disease and cancer which are associated with Mediterranean diets (Manna *et al.* 1997; Visioli *et al.* 1998; Visioli and Galli 1998; Coni *et al.* 2000). Simple phenols, secoiridoids and lignans in extra virgin olive oil represent 47% of its total phenols (Owen *et al.* 2000). Lignans are major antioxidants in olive oil and have been shown to inhibit skin, breast, colon and lung cancer cell growth (Owen *et al.* 2000). Polyphenols in olive oil have been shown to reduce oxidation of LDL cholesterol which reduces the formation of atheroscleric lesions which are a major factor in cardiovascular disease (Aviram 1996; Wiseman *et al.* 1996).

Grapeseed oil is very resistant to oxidation and is thus a beneficial additive to other plant oils (Kang *et al.* 1998). The major tocopherol of grapeseeds is $\gamma$-tocotrienol (Oomah *et al.* 1998), however, proanthocyanidins in grapeseed oil have been promoted as the significant antioxidants with Bagchi *et al.* (1998) demonstrating that these have much greater antioxidant properties than vitamin E, vitamin C and $\beta$-carotene. Oils with high $\gamma$-tocopherol and oleic acid content such as high oleic canola and high oleic soybean oils are more resistant to polymerization during frying (Lampi and Kamal-Eldin 1998). Contrary to expectation, these authors found that high oleic and high $\alpha$-tocopherol oils such as high oleic sunflower oil are not as resistant. Abdalla (1999) has shown that squalene, $\delta$-avenasterol and tocopherols increased the oxidative stability of frying oils. The increased resistance to oxidation provided by antioxidants is complex because repeated heating or frying of oils also reduces tocopherols and other antioxidants (Lampi and Kamal-Eldin 1998). Moreover, phytosterols may act as prooxidants or antioxidants depending on their chemical structures (Boskou 1998).

Peroxide value, which is a measure of the formation of hydroperoxides and other oxidized substances, increases as oils oxidize after heating or storage (Gordon and Kourimska 1995). In a survey of 31 olive cultivars, Pandolfi *et al.* (1994) also showed that the peroxide value of olive oils was not negatively correlated with linoleic acid content as expected, presumably antioxidant components override the susceptibility to oxidation of linoleic acid. These authors further showed that oxidative resistance was only partly related to total polyphenol content. Aparicio *et al.* (1999), however, showed that the antioxidant properties of olive oils was mainly due to phenolic and orthodiphenolic compounds (51% contribution), and the composition of fatty acids (24% contribution). $\alpha$-Tocopherol, carotenoids and chlorophylls had a minor effect and $\beta$- and $\gamma$-tocopherols had negligible effect. Fito *et al.* (2000) also showed that phenolic compounds in virgin olive oil have a stronger protecting effect on the oxidation of LDL cholesterol than $\alpha$-tocopherol. The antioxidants, $\alpha$-, $\gamma$- and $\delta$-tocopherol at concentrations of 100-500 ppm increased the oxidative stability of soybean oil (Jung and Min 1990). Different antioxidants may have additive or synergistic effects in oils (Hawrysh 1990; Blekas *et al.* 1995). Recently, a measure known as oxygen radical absorbance capacity (ORAC) has been developed to quantify the antioxidant capacity of foods (Prior and Cao 2000). There are, however, no published results of this measure on edible oils.

## Plant Stanols and Sterols

Phytosterols occur widely in plants and may be considered as derivatives of a fused, reduced ring system, perhydrocyclopentanophenanthrene (Fig. 1), comprising three fused cyclohexane rings (A, B, and C) in the nonlinear phenanthrene arrangement plus a terminal cyclopentane ring (D). Specifically, the stanols and sterols contain a hydroxyl group at carbon-3 of ring A and a branched aliphatic chain of eight or more carbons at carbon-17. The two groups are distinguished by the degree of unsaturation at carbon-5 (stanols being saturated and sterols unsaturated). The commonly consumed plant sterols are sitosterol, stigmasterol and campesterol which are predominantly supplied by vegetable oils (Piironen *et al.* 2000). Vegetable and plant oils are also a rich source of sterol esters.

Phytosterols inhibit cholesterol absorption in humans and can lower total blood serum cholesterol and LDL cholesterol by 10-15% (Jones *et al.* 1997, 1999; Hendriks *et al.* 1999; Moghadasian and Frohlich 1999; Sierksma *et al.* 1999; Williams *et al.* 1999). The major phytosterol in grapeseed oil and oil from avocados is $\beta$-sitosterol (Miric *et al.* 1992; Martinez *et al.* 1988), and olive oil contains a number of phytosterols. The principal sterols in both olive and hazelnut oils are $\beta$-sitosterol and $\delta$(5)-avenasterol (Ajana *et al.* 1998; Parcerisa *et al.* 2000) with campesterol and stigmasterol as minor sterol compounds. Obtusifoliol, which is a major sterol in olive oil, is unique to this oil. The phytosterol levels in unrefined peanut oil are higher than those in unrefined olive oil (Awad *et al.* 2000) although caution must be exercised in such comparisons as phytosterol levels in olive oil are significantly affected by fruit maturity and processing practices (Koutsaftakis *et al.* 1999; Gutierrez *et al.* 2000). High levels of phytosterols in corn oil are a major factor in its LDL cholesterol lowering properties (Howell *et al.* 1998). Oat oil contains a range of phytosterols including δ(5)-avenasterol (Maatta *et al.* 1999).

For ease of intake from processed oils and margarines, plant sterols and stanols can be converted to fat-soluble esters (Miettinen and Gylling 1999). Stanols occur in low amounts in oils and are equally effective in lowering plasma cholesterol and, unlike the sterols are not readily found in plasma (Piironen *et al.* 2000). There is evidence that campestanol is more effective than sitostanol in reducing cholesterol absorption and lowering LDL cholesterol (Gylling and Miettinen 1999), although soybean sterol esters (sitosterol, campesterol and stigmasterol) are effective in lowering total blood serum cholesterol and LDL cholesterol (Pelletier *et al.* 1995; Weststrate and Meijer 1998; Jones and Ntanios 1998).

## Squalene

Squalene is a hexaisoprenoid or triterpene widely found in plant and animal tissue and is a precursor of sterols. Olive oil appears to have a cancer protective effect and this may be the result of its squalene content (Rao *et al.* 1998; Smith *et al.* 1998). There is some evidence that squalene reduces colon cancer (Rao *et al.* 1998; Kelly 1999) and skin cancer (Owen *et al.* 2000). There are, however, reports that high levels of squalene intake increases total serum cholesterol and harmful LDL cholesterol (Miettinen and Vanhanen 1994), although other workers have shown that lower intakes of squalene had no effect on serum cholesterol (Strandberg *et al.* 1990). Squalene is present in olive oil and some seed oils. Some researchers have shown that squalene levels in olive oil vary from 30 to 70 mg $kg^{-1}$ (Cimato *et al.* 1996; Koutsaftakis *et al.* 2000). In contrast, a squalene content 100 times higher has been determined by other workers; 4200-4700 mg $kg^{-1}$ in extra virgin and 3400 mg $kg^{-1}$ in refined virgin oil (Alessandri *et al.* 1997; Owen *et al.* 2000), and between 1700 and 2500 mg $kg^{-1}$ in a range of oils (El Antari *et al.* 2000). The huge variation in squalene contents of olive oils needs further investigation because of the serious inconsistencies in the published data. Oils other than those from olives have low squalene contents (Owen *et al.* 2000).

## Other Components

There are many other compounds found in vegetable oils and some warrant mention here. Erythrodiol, a triterpene diol, is not usually found in vegetable oils other than olive oil (Blanch *et al.* 1998) but was identified in cottonseed oil in small but measurable amounts (Reina *et al.* 1999). Waxes (also termed wax esters) are fatty acid esters of any alcohol other than glycerol. Common examples found in vegetable oils include stearyl behenate (C40) and lauryl arachidate (C32) (Herrman *et al.* 1999; Reiter *et al.* 1999; Botha *et al.* 2000). Waxes comprise the bulk of jojoba oil which is poorly digested (VanBoven *et al.* 1997) and suggested as a low-energy substitute for conventional oils. Gas chromatograms of the wax fraction of edible oils often contain unexplained peaks (Botha *et al.* 2000; Henon *et al.* 2001). These waxes were recently identified (Henon *et al.* 2001) in vegetable oils as monounsaturated waxes, esters of long-chain saturated fatty acids, and a monounsaturated alcohol, mainly eicosenoic alcohol. Such waxes were absent in corn or rice bran oils.

A number of compounds are formed in oils as a result of oxidative changes. Oxidation proceeds continuously in the presence of suitable substrates until a blocking defence mechanism occurs. Lipid oxidation proceeds (Adegoke *et al.* 1998) via three different pathways: (1) nonenzymatic free radical mediated chain reaction, (2) nonenzymatic, nonradical photooxidation and (3) enzymatic reaction. An example of route (2) is the stoichiometric oxidation of oleic acid by singlet oxygen (Tanielian and Mechin 1994; Lercker *et al.* 1998) to produce two allylic hydroperoxides via addition of oxygen at either end of the double bond. The singlet oxygen is produced by sensitizers such as myoglobin or chlorophyll. High chlorophyll levels increase the rate of tocopherol decomposition and formation of polymers in heated rapeseed oil (Boskou 1998). Pathway (3) involves the action of lipoxygenases on various substrates; particularly linoleic and linolenic acids (Ranalli *et al.* 2001). The lipoxygenases are activated following cell damage. Regardless of pathway, a range of unstable hydroperoxides is formed and decomposition of these primary products of lipid oxidation generates a complex mixture including epoxides, ketones (e.g. butanones, pentanones, octanones), hydrocarbons, and saturated and unsaturated aldehydes such as hexanal (Halliwell and Gutteridge 1999). An oil is perceived as rancid when the level of these secondary oxidation products reaches a critical value. However, the same compounds formed via the lipoxygenase pathway (Ranalli *et al.* 2001) are essential to the flavor of olive oil and many lipoidal foods such as oats. The distinction is between the level of the compounds in the oil or food (Antolovich *et al.* unpublished).

Oxidation is not restricted to the triacylglycerols and fatty acids. Oxidation products of the main phytosterols, $\beta$-sitosterol and stigmasterol, are hydrocarbons (3,5-diene and 3,5,22-triene), mono-, di- and triunsaturated ketosteroids (4-en-3-one, 3,5-dien-7-one, 3,5,22-trien-7-one), 5,6-epoxy derivatives, 3,7-diols and pregnane derivatives (Boskou 1998). Quantification of the steradienes, e.g. 3,5-stigmastadiene and other steroidal hydrocarbons is a valid tool for the recognition of refining (in particular bleaching) of edible oils (Toschi *et al.* 1996).

Finally, the use of enzyme aids in olive processing (Ranalli *et al.* 2001) means that residues will ultimately find their way into the oil. These residues are said to be harmless to consumer health but nutritional data on the diverse compounds discussed here are generally unavailable.

## Breeding and Selection

There is considerable interest in breeding and selecting of plants that produce oils with healthier fatty acid profiles. In the USA, 'Soyola' is available which is processed from a variety of soybean with low linolenic acid content and which will produce less harmful trans fatty acids at high temperatures (Burton unpublished). Low linolenic acid varieties of canola have also been selected (Hu *et al.* 1995; Xu *et al.* 1999).

Sunflower lines have been bred with less than 6% total palmitic and stearic acids (Heaton *et al.* 1992), and soybean with 4% palmitic acid has been produced by mutagenesis (Fehr *et al.* 1991). Palm oil, which has high levels of saturated fat, is being developed with higher monounsaturated fat content (Jalani *et al.* 1997).

A number of publications show that the levels of saturated fatty acids vary greatly between different cultivars of olive trees (Averna *et al.* 1972; Talantikite and Ait 1988; Surinder and Sharma 1991; Alessandri *et al.* 1994; Tous and Romero 1994; Cimato *et al.* 1996; Guinda *et al.* 1996; Mincione *et al.* 1996; Alessandri 1997; Spangenberg *et al.* 1998; Vlahov *et al.* 1999). In most studies where a large range of cultivars were tested, the cultivars with the highest levels contain twice as much palmitic acid as the cultivars with the lowest levels. Olive oil low in palmitic acid and high in oleic acid is generally of higher quality (Baldini *et al.* 1996).

## Environmental, Regional and Cultural Practice Effects

Variation in the composition under different environmental, regional and cultural regions has been studied in many oils. It is likely however that most oil crops will have oil composition altered by environmental factors. For example, in soybean, low temperatures during seed development increase linoleic and linolenic acids contents (Heppard *et al.* 1995). Much work would be needed to quantify environmental effects on oils from the wide range of oil crops in cultivation.

Tous and Romero (1994) and Colakoglu (1972) have shown that quality and fatty acid profiles in some olive cultivars varied considerably between locations in Spain and Turkey, respectively. There are also a number of papers showing that the fatty acid profile of mono-varietal olive oils varies with regions (Alessandri 1997; Spangenberg *et al.* 1998). Whether these effects are temperature related or due to other climatic and cultural factors is unknown. Barone *et al.* (1994) have shown that palmitic acid, linoleic acid and polyphenol content of olive oils increase with reduced crop load. Palmitic acid content of olives also decreases as the fruits mature (Frega *et al.* 1991; Parlati *et al.* 1994), although Tombesi *et al.* (1994) found the opposite trend, and Frega *et al.* (1991) found little differences in fatty acid profiles with time of harvest and locality. Ranalli and Morelli (1999) and Gutierrez *et al.* (1999) have shown that in olive cultivars, the level of phenols, carotenoids and tocopherols falls as olives mature with a resulting fall in oil quality. This shows that it is possible to alter oil quality in olives by planting in different regions and by manipulating the trees.

## Biotechnology and Genetic Engineering

Biotechnology has already led to the development of oilseeds with altered fatty acid profiles. Since the gene for stearoyl-ACP desaturase, which converts stearic acid to oleic acid, has already been cloned from olives and *Thunbergia elata* (Haralampidis *et al.* 1998; Cahoon *et al.* 1997), there is an opportunity to genetically engineer olives and other plants for reduced saturated fat levels, especially by using different promoters to give over-expression. Levels of C16:0 (palmitic acid) and C18:1 (oleic acid) fatty acids are controlled by the enzymes acyl-acyl carrier protein (ACP) thioesterase and $\beta$-keto acyl ACP synthase II, and specificity or activity of these enzymes could be modified by genetic engineering. An ACP thioesterase isolated from mangosteen has been introduced into *Brassica napus* to increase its stearic acid content (Facciotti *et al.* 1999). Artificially constructed ACP desaturases have been developed using mutagenesis which can produce novel monounsaturated fatty acids from palmitic and stearic acids (Cahoon *et al.* 1997).

Increased expression of $\delta$-9 desaturase enzymes in oilseeds will reduce saturated fatty acid content, and suppression of plant desaturases such as omega-6 desaturase in soybeans and $\delta$-9 desaturase in rapeseed favors oils which are less prone to trans isomer formation because of decreased polyunsaturated fatty acids and increased stearic acid, respectively (Kinney 1996).

The palmitic acid content of olives could also be reduced by switching off the gene for palmitoyl ACP thioesterase by antisense technology. Stearic acid has also been increased in canola by suppression of 18:0-ACP desaturase using antisense molecular techniques (Voelker 1995).

Mutagenesis could be used to increase monounsaturated fatty acid content by modifying ACP desaturase activities (Cahoon *et al.* 1997). The above approaches could lead to production of healthy edible oils by reducing their palmitic acid content. Varieties of rapeseed and soybean with altered oil profiles resulting from genetic modification have been approved for cultivation in the USA. However, the market acceptance of 'healthy' oils from genetically modified plants could be a long way off.

## Designing a Healthy Edible Oil

Defining a precise composition of the ideal 'healthy' oil to be used for cooking and other purposes is difficult as the nature of epidemiological and trial studies prevents precise modelling of the effects of constituents. Nevertheless, some general principles can be established. Firstly, fatty acid content should be dominated by monounsaturates such as oleic acid. Recent work has, however, shown that polyunsaturated fatty acids can also improve blood cholesterol profiles, although their susceptibility to oxidation (*in vitro*) could accelerate atherosclerosis *in vivo*, a major factor in cardiovascular disease. The polyunsaturated linolenic acid is also not heat stable and undergoes trans isomerization when heated. On the best available evidence, linolenic acid levels should be kept to a minimum in a healthy oil if it is to be subjected to repeated heating. As discussed earlier, the saturated fatty acid stearic acid may actually reduce LDL cholesterol.

The fatty acid profile of edible oils should therefore have 80-90% monounsaturates such as oleic acid with the remainder mostly the polyunsaturated linoleic acid. While both linoleic and linolenic acids are essential fatty acids in human nutrition, studies in the US recommend that healthy oils should contain 4 to 10 times as much linoleic acid as linolenic acid (Dupont *et al.* 1989). Linolenic acid should also be kept to a minimum unless accompanied with antioxidants. Many epidemiological and trial studies have shown that high intake of the saturated fatty acids, myristic, lauric and palmitic acids, results in elevated blood serum levels of total cholesterol and harmful LDL cholesterol. Myristic, lauric, and palmitic acids should thus be kept to a minimum. Canola oil, especially with low linolenic acid, has a fatty acid profile approaching this (Table 1). Olive oil and blends with grapeseed oil as a minor component also mimic this fatty acid profile (Table 2).

Most published work shows detrimental effects of trans fatty acids on human health, and healthy oils should contain minimal amounts of trans fats. This has important implications for the deodorizing and other heat treatments of oils. Deodorizing is an important part of the refining process of most oils to be sold as 'light'. Highly refined 'light' edible oils which contain linolenic acid may also have significant levels of trans fatty acids. 'Light' oils may also have undergone bleaching prior to deodorizing which reduces antioxidant levels.

It is clear that healthy oils need to contain antioxidants for both anticancer properties and to increase oxidative stability of the oil and its fatty acids both *in vitro* and *in vivo*. Both $\alpha$- and $\gamma$-tocopherols and other vitamin E homologs should be significant constituents of a healthy oil, especially if it contains significant levels of polyunsaturated fatty acids. Both olive and grapeseed oils contain significant levels of antioxidants, a number of which have stronger antioxidant activity than $\alpha$-tocopherol. Polyphenols, tocopherols, flavonoids such as proanthocyanidins and carotenoids have antioxidant properties, and because they have been implicated in both reducing certain cancers and lowering harmful LDL cholesterol, they should be constituents of a healthy edible oil. Squalene could be a constituent of a healthy edible oil because of its putative anticancer properties. Table 3 summarizes the known beneficial effects of various components of edible oils, and there are still many gaps in our knowledge.

TABLE 3.
HEALTH ATTRIBUTES OF CONSTITUENTS OF EDIBLE OILS

| Constituent | Cardiovascular Benefit | Anti-cancer Benefit |
|---|---|---|
| Monounsaturated fatty acids | + | ? |
| Polyunsaturated fatty acids | +/- | ? |
| Squalene | ? | + |
| Phytosterols | + | ? |
| Polyphenols | + | + |
| Tocopherols | + | + |
| Carotenoids | ? | + |
| Proanthocyanidins | ? | + |

## Future Research Directions

More work is required to clarify the relationship between fatty acid types, other oil components and the development of cardiovascular disease and various cancers. There is also a need to establish the dietary effects of position of different fatty acids on the glycerol backbone of triacylglycerols. Despite the immense number of publications describing analyses of edible oils there is no single study which, under defined conditions, compares both fatty acid profiles and the levels of key antioxidants and other health promoting substances in the major edible oils. As Table 2 shows there is not a great deal of reliable quantitative data on the antioxidant profiles of edible oils and how this is altered during processing. A standard method of measuring the oxidative resistance of oils is needed, and the Rancimat, ORAC and other methods need to be compared. Even though there has been a great deal of research on polyunsaturated fatty acids in oils, more research is needed to determine what are 'healthy levels' in oils, and in particular what associated level of antioxidants is required to offset oxidative vulnerability. Only extensive analytical research has been done in olive oil. The International Olive Oil Council has set standards for analyses and labelling of virgin olive oils but there is no internationally accepted set of protocols for analyzing and labelling of 'healthy' oils. In particular, detailed standards for antioxidant and polyphenol content need to be established. The chemical composition labelling of edible oils is generally inadequate. For example, listing sodium, potassium and cholesterol levels which are usually at negligible levels is not useful. The term 'light' is undefined and should not be used unless referring to the color or some other defined parameters. Edible oils promoted as 'healthy' should have a complete fatty acid composition, not simply list the percent saturated, polyunsaturated and monounsaturated fat. All health promoting substances including phenols, phytosterols, tocopherols, carotenoids, flavonoids such as proanthocyanidins and other antioxidants should be quantified. Work also needs to be done to more accurately screen the chemical profiles of blends of olive and grapeseed oils from different cultivars, which on the available evidence, have the healthiest attributes. Work is also required on the effect of region and cultural practices including time of harvest (crop maturity) on the chemical composition of edible oils of all major oil crops. There is little quantitative data on the relative benefits of different sterols in oils. There is also little quantitative data on which of the large number of antioxidants found in plant-derived oils have the greatest benefits to health. However, existing literature shows that edible oils with added phytosterol and stanol esters could also be developed. More research is needed on processing to maximize health promoting components of edible oils, especially antioxidants.

Since the chemical composition of oils produced from different cultivars varies widely, selection of olive and grape varieties with low levels of palmitic and stearic acids, and high levels of key antioxidants should be a priority for research as this would have great marketing advantage.

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