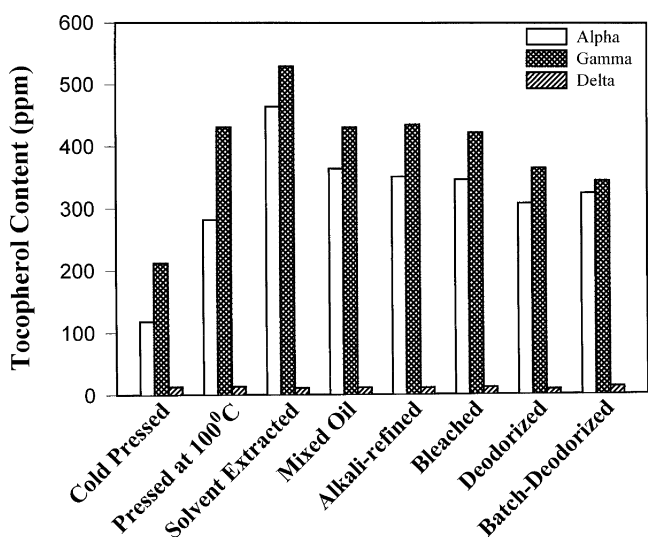


Figure 9: Changes of Tocopherols During Processing. Continuous Deodorization at 245°C and 3.5 mbar; Batch Deodorization at 180°C and 3 mbar for 6 Hours. Mixed oil - Mixture of Solvent Extracted and Pressed Oils. Adapted from Willner et al. (1997)

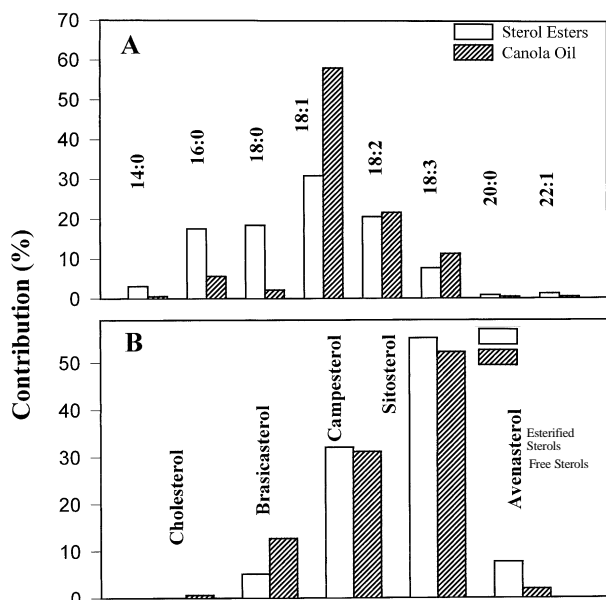


The lowest content of tocopherols was found in cold pressed canola oil. When temperature of pressing was increased, the amount of tocopherols doubled (Figure 9). Solvent extracted oils contain higher amounts of tocopherols than cold pressed oil, and similar amounts as oils from hot pressing. Refining decreased the tocopherol content of canola oil, and deodorization caused the removal of the largest portion of these compounds.

Sterols

Sterols are present in canola oil in equal amounts in two forms, free and esterified (Ackman, 1983; Evershed et al., 1987). The composition of fatty acids and sterols in the esterified sterol fraction of canola oil is presented in Figure 10.

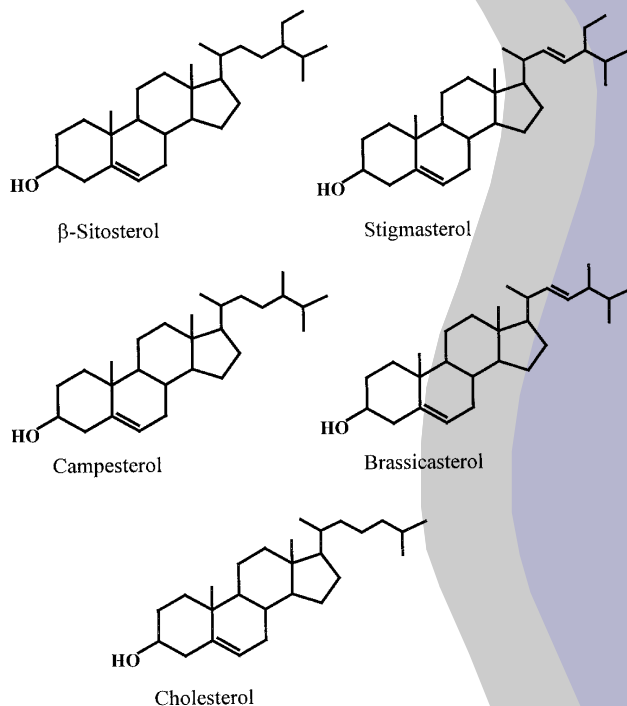
Figure 10: Composition of Esterified Sterols in Canola Oil. A - Fatty Acids in Esterified Sterol; B - Sterol Composition. Adapted from Gordon et al. (1997)



Two of the major sterols (campesterol and sitosterol) are equally distributed in the esterified and free sterol fractions in canola oil (Figure 11B). Twice the amount of brassicasterol is found in the free sterols than in the esterified. The fatty acid distribution in the esterified sterol fraction differs from the fatty acid distribution of canola oil (Figure 11A). A higher palmitic and stearic acid content was observed in the esterified sterols.

The total amount of sterols in rapeseed and canola oils ranges from 0.53 to 0.97%. The composition of major sterols in common vegetable oils is presented in Table 12, and their structures are presented in Figure 12.

Figure 12: Structure of Phytosterols and Cholesterol



Brassicasterol is one of the major sterols present in rapeseed and canola, and is also unique to these oils. This sterol is often used to determine the presence of rapeseed or canola oils in other oils (Strocchi, 1987; Ackman, 1990). Sterols are also affected by processing. Significant portions (up to 40%) of sterols are removed from the oil during deodorization. Refining also causes removal and isomerization of these compounds (Kochar, 1983; Marchio et al., 1987).

Table 12: Proportions of Major Sterols in Selected Vegetable Oils^a (%)

Sterol	HEAR	CAN	LLCAN	HOCAN	HOLLCAN	SOY	SUN	Corn
Cholesterol	0.4	0.1	0.1	0.1	0.1	0.3	0.1	0.1
Brassicasterol	13.2	13.8	12.2	10.8	16.2	-	-	-
Campesterol	34.4	27.6	31.2	33.9	28.8	18.1	7.5	17.2
Stigmasterol	0.3	0.5	0.2	0.8	0.1	15.2	7.5	6.3
β-Sitosterol	47.9	52.3	51.3	48.7	50.9	54.1	58.2	60.3
Δ ⁵ -Avenasterol	2.1	1.9	1.9	1.8	2.1	2.5	4.0	10.5
Δ ⁷ -Avenasterol	1.6	1.1	1.1	1.9	0.8	2.0	4.0	1.1
Δ ⁷ -Stigmasterol	2.1	2.3	2.1	2.1	2.3	1.4	7.1	1.8
Total(mg/kg)	8810.0	6900.0	6326.0	7102.0	6892.3	4600.0	4100.0	9700.0
Esterified(mg/kg)	4356.8	4231.5	3987.6	4356.8	4156.2	576.4	2068.8	5654.8

^a Adapted from Ackman (1990), Strocchi (1987), Zambiasi (1997) and Gordon and Miller (1997)

The amount of total sterols in canola oil is about 50% higher than in soybean oil. Corn oil, which is produced from the corn seed embryo, contains the highest amount of sterols, or roughly two times that found in canola oil. The chemical structure of phytosterols is similar to that of cholesterol, so it is possible that these compounds are involved in oxida-

tive reactions (Figure 12). Recently, Przybylski and Eskin (1991) found plant sterol oxidation products formed during the storage of fried food products. Similar oxidation products have been found in soybean oil and wheat flour (Nourooz-Zadeh & Appelqvist, 1992). During the last few years more and more data is also being published showing the positive effect of plant sterols and their derivatives, particularly stanol esters, on human plasma cholesterol (Hendriks et al., 1999).

Pigments

Pigments present in canola and other oilseeds are important factors as they can impart undesirable colour to vegetable oils, promote oxidation in the presence of light, and inhibit catalysts used for hydrogenation.

A bleaching step is necessary during oil processing to remove chlorophyll-related pigments and other colour bodies. Changes in chlorophyll during canola oil processing are summarized in Table 13. During processing, chlorophyll completely decomposes to derivatives that are much harder to remove during bleaching. A bleaching test showed that pheophytin a and pyropheophytin a are more absorptive than their b isomers. Consequently, smaller amount of b isomers than a isomers are removed from the oil during bleaching. This necessitates the use of much higher amounts of bleaching activated earth in order to achieve similar removal of all chlorophyll derivatives (Suzuki and Nishioka, 1993).

Table 13: Chlorophyll Pigments in Canola Oil During Processing (ppm)^a

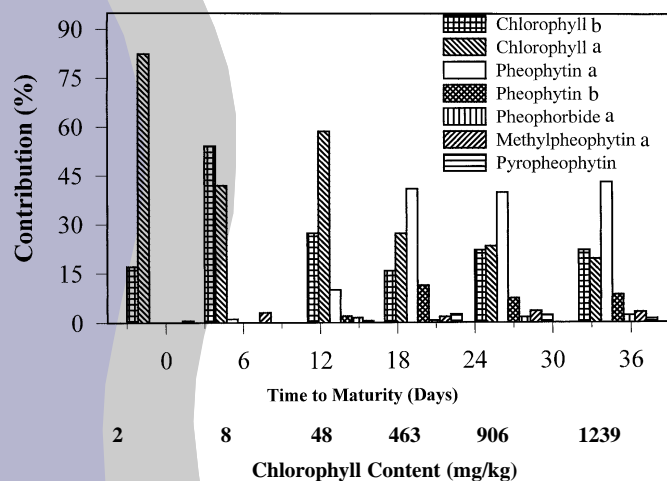
Oil After	Chlor a ^b	Pheo a ^b	Pheo b ^b	Pyro a ^b	Pyro b ^b
Expeller	6.27	4.48	1.79	5.37	0.67
Extraction	1.88	3.31	1.34	16.57	3.13
Expeller + Extraction	1.79	5.55	1.34	9.76	1.43
Degumming	0.27	7.16	1.07	9.40	1.84
Alkali Refining	0.22	6.27	1.12	9.13	1.79
Bleaching	-	0.56	0.32	0.21	0.25

^a Adapted from Suzuki and Nishioka (1993)

^b Chlor a - Chlorophyll a; Pheo a - Pheophytin a; Pheo b - Pheophytin b; Pyro a - Pyropheophytin a; Pyro b - Pyropheophytin b

The type and content of chlorophyll present is dependent on the maturity of the seed. In fully matured seed only 2 ppm of chlorophyll was observed, while in physiologically matured seed (35 days before maturity) 1239 ppm was found. Also at maturity only chlorophyll a and b were present while all possible isomers/derivatives were observed at other stages of maturation (Figure 12). These changes in the composition and content of chlorophylls can have a direct impact on the processing and quality of canola oil.

Figure 12: Changes in Composition and Content of Chlorophylls During Canola Seed Maturation. Adapted from Ward et al. (1994)



In addition to chlorophyll pigments, carotenoids were also found in canola oil. Crude canola oil carotenes are reported to be at a level of 95 ppm, and are composed of 90% xanthophylls and 10% carotenes (Hazuka and Drozdowski, 1987).

Saponification Value

Saponification value is defined as the weight of potassium hydroxide, in milligrams, needed to saponify one gram of fat. This parameter is inversely proportional to the molecular weight of the fat. Replacement of long chain fatty acids such as erucic acid (C22:1) in canola oil by eicosenoic (C18) fatty acids increased the saponification number from 168-181 to 188-192 due to the reduction in molecular weight.

Iodine Value

Iodine value (IV) is an empirical test indicating the degree of unsaturation of fat or oil. It is defined as the number of grams of iodine absorbed by 100 grams of fat. An iodine value of 97 - 108 was reported for rapeseed oil with 45 % erucic acid, as compared to 110 - 126 for canola oil (Ackman, 1983). The higher value for canola oil is due in part to the replacement of erucic acid with oleic acid, along with an increase in linoleic and linolenic acids. Iodine value can also be calculated from fatty acid composition as proposed by AOCS Method Cd 1c-85 where the content as a percentage is multiplied by the characteristic factor for each unsaturated fatty acid.

Chemical Stability

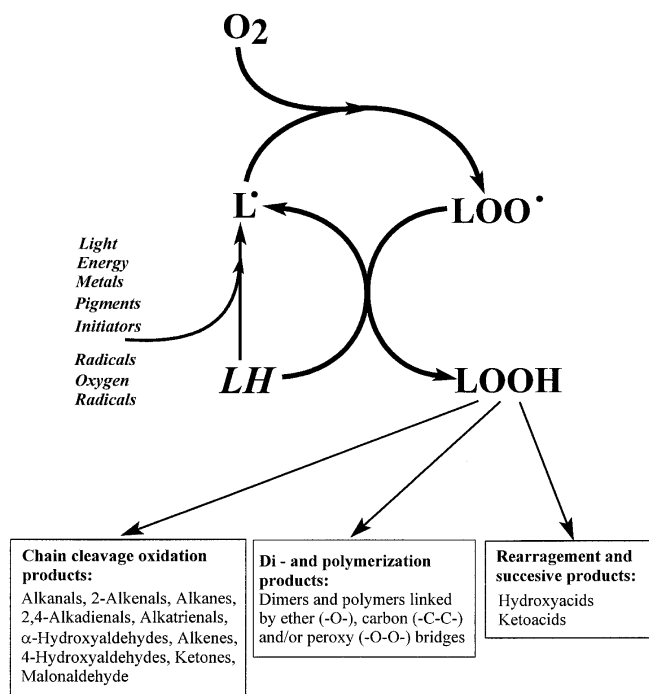
The stability of canola oil is limited mostly by the presence of linolenic acid, chlorophyll and its decomposition products, and other minor components with high chemical reactivity such as trace amount of fatty acids with more than three double bonds (Chapman et al., 1994). The presence of 7 to 11% of linolenic acid in the glycerides of canola oil places it in a similar category to soybean oil with respect to flavour and storage stability. The deterioration of flavour as the result of auto - and photooxidation of unsaturated fatty acids in oils and fats is referred to as oxidative rancidity.

Oxidative Rancidity

Oxidation of unsaturated lipids produces components that behave as catalysts for this process, making it autocatalytic. Generally, oxidation occurs when oxygen is present in an oil or in the head-space above the oil. Solubility of oxygen in oil is about three to five times greater than in water. The amount of oxygen present in oil, dissolved during manipulation, is sufficient to oxidize the oil to a peroxide value of around 10 (Przybylski and Eskin, 1988; Labuza, 1971). The rate of oxidation of fats and oils is affected by many factors, including oxygen partial pressure, exposure to oxygen, the degree of unsaturation of fatty acids, the presence of light, temperature, and the presence of antioxidants and prooxidants such as copper, iron and pigments. Oil stability was best when iron and copper contents were below 0.1 and 0.02 ppm, respectively (Smouse, 1994).

The phenomenon of flavour reversion is defined as the return to the flavour an oil had prior to deodorization. It does not appear to be related only to the oxidation of linolenic acid, as was originally thought (Smouse, 1994). This characteristic is distinct from rancidity, which is defined as the overall flavour defect noted when an oil first becomes oxidized. The mechanism of autoxidation is outlined in Figure 13.

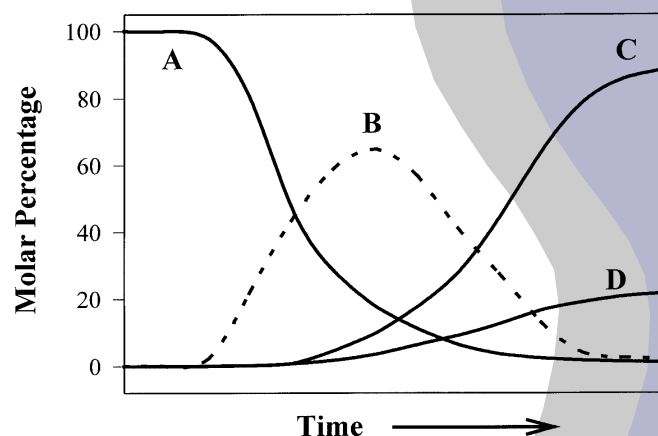
Figure 13: Schematic of Radical and Photooxidation of Fatty Acids. Adapted from Chan (1987)



Initiation of this process comes about with the abstraction of a hydrogen atom adjacent to the double bond by an excited molecule of catalyser or pigment which received energy from light or heat to form a free radical. The free radical then combines with oxygen to form a peroxy radical, which abstracts a hydrogen from another unsaturated fatty acid to form a hydroperoxy radical. The hydroperoxy radical reacts with another fatty acid molecule to form hydroperoxide and another free radical. This free radical catalyses the reaction causing an autocatalytic effect.

The primary reaction products are hydroperoxides, which decompose readily to form a range of secondary oxidation products (Figure 13). These include different carbonyl compounds such as unsaturated aldehydes, which possess strong disagreeable flavours and odours and have very low threshold levels (Hall and Andersson, 1983). The susceptibility of individual fatty acids to oxidation is dependent on their degree of unsaturation. Thus, the rate of oxidation of linolenic acid is 25 times higher than that of oleic acid and twice as fast as that of linoleic acid (Labuza, 1971). Oxidation products are formed according to the progress of this process (Figure 14). Primary products such as hydroperoxides appear first, along with the disappearance of unsaturated fatty acids. Secondary products, including non-volatile and volatile compounds, are produced as the result of primary product decomposition, and their presence can be detected after a certain period of time. The amount of hydroperoxides decreases with time as unsaturated fatty acids become oxidized and the decomposition process starts to be a dominating factor.

Figure 14: Formation of Oxidation Products from Unsaturated Fatty Acids. A - Unsaturated Fatty Acids; B - Hydroperoxides; C - Non-volatile Products; D - Volatile Compounds (Off-flavour, Rancid). Adapted from Chan (1987)



Photooxidation

Light is an important factor that affects the flavour stability of vegetable oils such as canola and soybean which contain polyunsaturated fatty acids and traces of pigments or pigment derivatives. The degradation of oils and fats due to light exposure is primarily a photo-catalyzed oxidation. During photooxidation, singlet oxygen is generated by the transformation of energy from a light to a sensitizer. Singlet oxygen is an extremely reactive species of oxygen, and reacts with double bonds of unsaturated fatty acids to form peroxides or free radicals. Typical photosensitizers are chlorophylls and their decomposition products formed during maturation of seed and processing, heme compounds and polycyclic aromatic hydrocarbons (Pokorny, 1987). It has been found that chlorophyll degradation products are more active as photosensitizers than chlorophyll itself (Smouse, 1994). Another important factor in photooxidation is the colour of light or its wavelength. It has been established that shorter wavelengths of light, such as UV and blue, have more detrimental effects than longer wavelengths (Sattar et al., 1976).

Exposure to light even for a short period of time initiates lipid oxidation, and each additional exposure further accelerates the deterioration of lipids (Chan, 1987). The presence of fluorescent light in many supermarkets, transmitting wavelengths between 350-750 nm, necessitates suitable packaging of oils to ensure that containers are impervious to low wavelength light. Tokarska et al. (1986) found light to be a critical factor in the development of off-flavours in canola oil and they strongly recommended that it be packaged in amber containers.

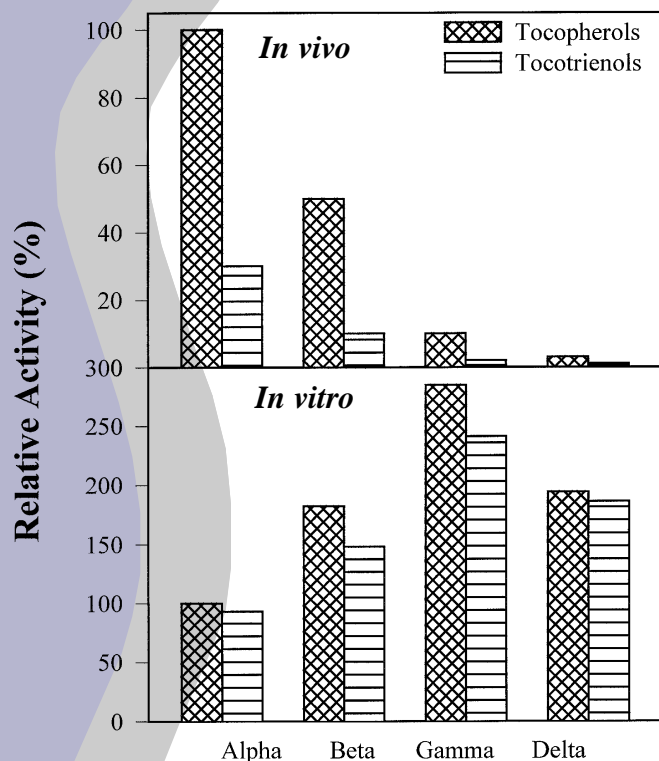
Antioxidants

The role of antioxidants in retarding rancidity is well established, although the efficacy of some of them has recently been questioned. The best antioxidants are the natural components of canola oil such as tocopherols, in particular its γ -isomer (Figure 9). This isomer is present in processed canola oil in an amount twice higher than the alpha isomer (Table 11).

Tocopherols are recognized as very effective natural antioxidants, but the isomers have varying antioxidant activity (Figure 16). The antioxidative activity of tocopherol and tocotrienol isomers is structure dependent. If a phenolic compound contains electron-releasing substituents in position *ortho* and/or *para* to the hydroxy group this increases the electron density of the active center. This combination

facilitates the hemolytic fission of the hydroxyl bond and makes tocopherol a good hydrogen donor, thus improving reactivity with peroxy radicals. α -tocopherol has methyl groups substituted at all positions, making it a very potent hydrogen donor and by structure the most potent antioxidant among all the tocopherol isomers (Figure 9). α -tocopherol has the highest biological activity (Figure 16). In food systems, antioxidant activity decreased in the following order: $\gamma > \delta > \beta > \alpha$ (Figure 15).

Figure 15: Biological (*In vivo*) and Antioxidant (*In vitro*) Activity of Tocopherols and Tocotrienols. Adapted from Kamal-Eldin and Appelquist (1996)



Tocopherols are excellent antioxidants, about 250 times more effective than BHT (Burton and Ingold, 1989). These compounds seem to be the most efficient lipid antioxidants provided by nature. Lipid peroxy radicals react with tocopherols several magnitudes faster than with other lipids. Consequently, a single molecule of tocopherol can protect about $10^3 - 10^6$ molecules of polyunsaturated fatty acids. This effectiveness explains why the ratio of tocopherol to PUFA in cells, usually 1:500, is sufficient to provide protection (Patterson, 1981).

Tocopherols are also good singlet oxygen quenchers, but are less efficient than carotenoids. A single molecule of tocopherol can quench up to 120 molecules of singlet oxygen (Bowry and Stocker, 1993). Plastochromanol-8, a derivative of γ -tocotrienol but with a longer side chain, was detected in canola oil (Figure 9; Table 11). During evaluation of the antioxidant activity of tocopherols it was established that plastochromanol-8 and α -tocopherol have similar effectiveness in the oil (Zambiazzi, 1997).

The most commonly used synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ). These phenolic compounds react with free radicals to form relatively stable antioxidant free radicals that further degrade to produce quinones, thereby terminating the chain reaction of autoxidation. Hawrysh et al. (1988) found that BHT/BHA was not effective in promoting canola oil stability, while TBHQ substantially improved the stability of canola oil at levels as low as 100 ppm. Recently, TBHQ was approved in Canada as an antioxidant

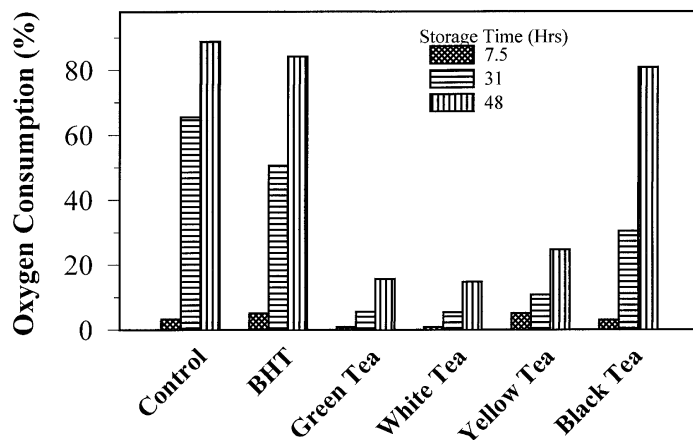
for vegetable oils. Hawrysh et al. (1992) showed that a mixture of PG and ascorbyl palmitate (AP) was also effective in the retardation of canola oil deterioration. Citric acid and its salts are used as chelating agents to deactivate metal catalysers present in the oils. Warner et al. (1989) reported that the use of citric acid did improve canola oil stability.

The efficiency of antioxidants in inhibiting the development of heated room odors and delaying changes in heated canola oils has been examined. The use of antioxidants in the frying fat did not contribute to stability of the fried products (McMullen, 1988). Normand (1998) has shown that naturally occurring tocopherols need to be present at adequate levels in order to benefit frying oil stability.

Recent trends to find alternatives to synthetic food additives have caused the search for natural antioxidants to be intensified. Consumers may prefer natural food additives to synthetic compounds because they occur naturally in foods that have been consumed for centuries. Most natural antioxidants of plant origin are phenolic in nature.

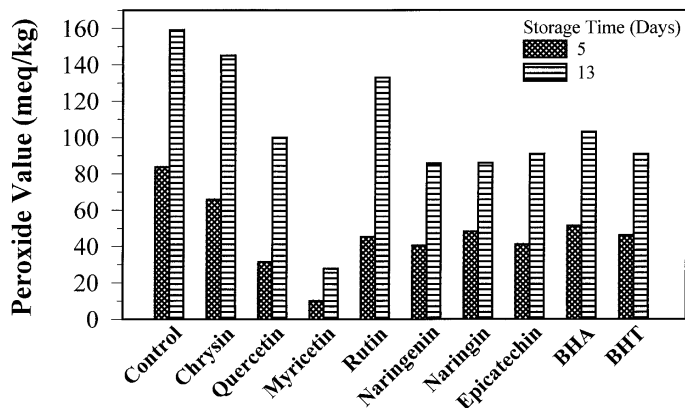
For years tea has been perceived as a source of antioxidants due to the relatively high content of phenolic compounds. Chen et al. (1996) tested the efficiency of ethanolic tea extracts on the oxidative stability of canola oil. Selected results are presented in Figure 16. Extracts from green, white and yellow tea were the most efficient in protecting from oxidation, while black tea extract was ineffective.

Figure 16: Antioxidant Activity of Tea Extracts During Storage of Canola Oil at 100°C. Adapted from Chen et al. (1996)



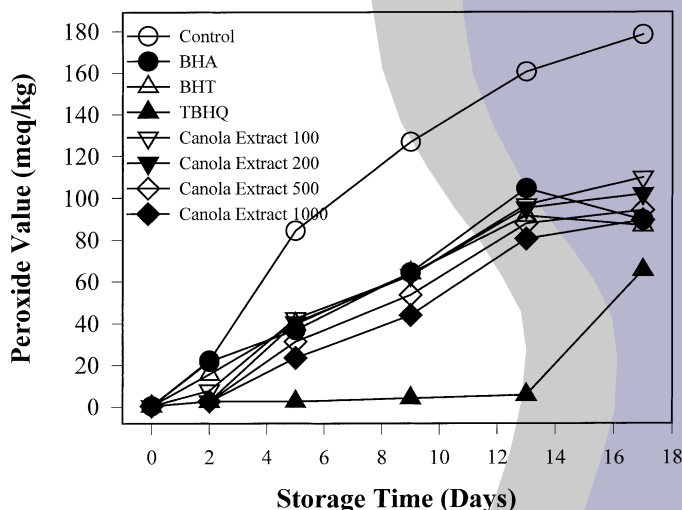
Wanasundara and Shahidi (1994) evaluated the effectiveness of flavonoids as antioxidants, and found that some of these natural components were effective in protecting canola oil (Figure 17). Flavonoids are a plant phenolic, and may be found in a variety of plant materials. In particular, quercetin and myricetin were more efficient than BHA/BHT.

Figure 17: Antioxidant Activity of Flavonoids During Storage of Canola Oil at 65°C. Adapted from Wanasundara and Shahidi (1994)



Wanasundara and Shahidi (1994) also examined the effectiveness of ethanolic extracts produced from canola meal. When added at a level of 100 ppm, these extracts effectively prevented the oxidation of canola oil (Figure 18). The extracts were as effective as BHT and BHA, although they showed lower antioxidative activity than TBHQ. The authors also stated that the canola meal extracts did not impart colour and odour to canola oil. Therefore, these and other extracts produced from plant origin material are a potential source of natural food antioxidants.

Figure 18: Antioxidant Activity of Canola Meal Extracts During Storage of Canola Oil at 65°C. Adapted from Wanasundara and Shahidi (1994)



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