

CHAPTER 2

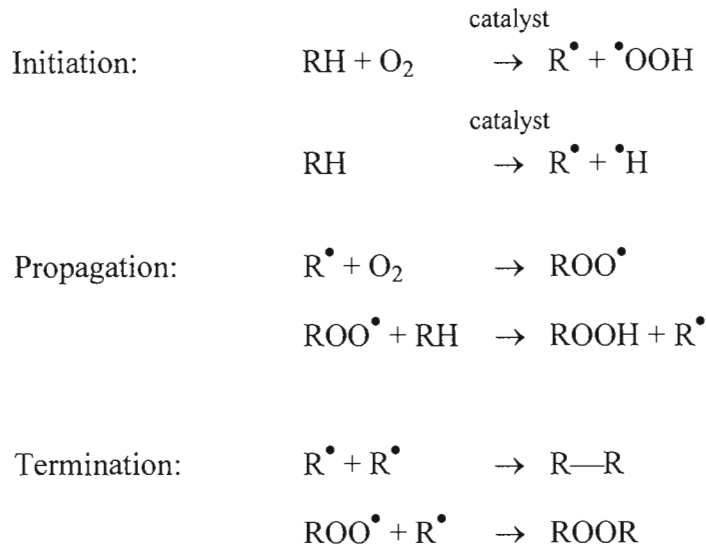
LITERATURE REVIEW

2.1 OXIDATION AND RANCIDITY OF FATS AND OILS

Lipid oxidation is one of the major reasons that foods deteriorate and is caused by the reaction of fats and oils with molecular oxygen leading to off-flavours that are generally called rancidity. Exposure to light, pro-oxidants and elevated temperature will accelerate the reaction. Rancidity is associated with characteristic off-flavour and odour of the oil. There are two major causes of rancidity. One occurs when oil reacts with oxygen and is called oxidative rancidity. The other cause of rancidity is by a combination of enzymes and moisture. Enzymes such as lipases liberates fatty acids from the triglyceride to form di- and/or monoglycerides and free fatty acids and such liberation of free fatty acids is called hydrolysis (Hamilton, 1994). According to Hamilton (1994) hydrolysis is also caused by chemical action that is prompted by factors such as heat or presence of water. (Hamilton, 1994). Rancidity caused by hydrolysis is called hydrolytic rancidity. Oxidation is concerned mainly with the unsaturated fatty acids. Oxidative rancidity is of special interest as it leads to the development of unfavourable off-flavours that can be detected early on in the development of rancidity (Przybylski and Eskin, 1995), more so than in the case of hydrolytic rancidity.

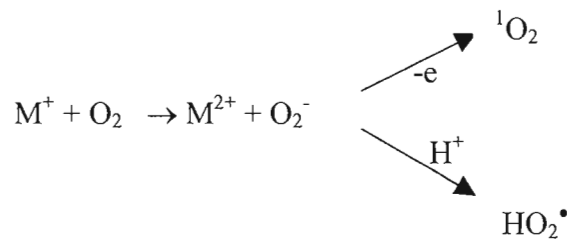
2.1.1 Primary and secondary oxidation products

Primary oxidation products are formed by the reaction of an alkyl radical, which is formed by reaction to light or heat (initiation), with oxygen to form a peroxy free radical. The peroxy free radical reacts with an unattacked unsaturated fatty acid to form a fat hydroperoxide and an alkyl free radical (propagation). This product is tasteless and odourless. The reaction continues until there is a depletion of oxygen or when a fatty radical reacts with a stable antioxidant radical or when two unstable radicals react (termination). This process, which involves three steps, namely initiation, propagation and termination, is called autoxidation (Labuza, 1971; Frankel, 1980):



where RH = unsaturated lipid, R^\bullet = lipid radical and RO_2^\bullet = lipid peroxy radical.

Initiation of oxidation by singlet oxygen is an important aspect of oil oxidation. The stable triplet oxygen is not very reactive and is unlikely to react directly with unsaturated fatty acids (Frankel, 1980; Nawar, 1985). Activation of oxygen can be induced by electronic excitation such as photosensitization, metals and natural pigments among others (Frankel, 1980; Nawar, 1985). The formation of singlet oxygen and peroxy radical by metal catalysis is shown (Frankel, 1980):



Both products formed are good chain initiators. It was found that singlet oxygen reacts about 1500 times faster than $^3\text{O}_2$ with unsaturated double bonds (Nawar, 1985). The reaction of singlet oxygen with unsaturated fatty acids proceeds by a different mechanism than normal autoxidation (Frankel, 1985). The singlet oxygen reacts directly with double bonds and thus produces different hydroperoxides and these intermediates lead to formation of other volatiles than normally found in autoxidation (Frankel, 1985; Przybylski and Eskin, 1995). It

is thus important to remember that the cause of oxidation has to be considered when attempting to explain volatiles detected.

Secondary oxidation products are formed when the hydroperoxides decompose to secondary oxidation products as a result of heating, radiation, or the presence of heavy metals (Cu, Fe) and other radical initiating agents. The secondary oxidation products are formed by either peroxide scission alone or simultaneous peroxide and chain scission. Chain scission leads to short-chain volatiles such as aldehydes, ketones, alcohols and acids, which cause the characteristic off-flavours and odours of rancid fats and oils. (Hoffman, 1989). The addition of antioxidants can retard autoxidation, as they are free radical scavengers and by interrupting the chain reaction prevent or slow down the propagation of oxidation. Synthetic antioxidants used are phenols such as BHA, BHT, TBHQ and propyl gallate (Hamilton, 1994). Tocopherols (Vitamin E) naturally present in oil act as natural antioxidants.

2.1.2 Factors influencing oxidative stability

It is important to be aware of the factors that influence oxidative stability to ensure the longest shelf-life possible for oil.

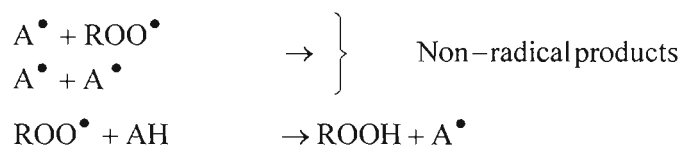
2.1.2.1 Fatty acid composition

The fatty acid composition gives important information regarding the stability of oil. Unsaturated fatty acids such as oleic- (C18:1), linoleic- (C18:2) and linolenic acid (18:3) are easier targets for oxidation (Frank, Geil and Freaso, 1982; Lomanno and Nawar, 1982). Linoleic acid has been studied extensively and it has been found to be 10-100 times more susceptible to oxidation than monoene or saturated fatty acids (Selke, Rohwedder and Dutton, 1980). The degree of unsaturation affects the oil stability as seen in the comparison of stored regular and low-linolenic canola oils (12.5 % and 2.5 % of 18:3 respectively) (Malcolmson, Vaisey-Genser, Przybylski, Ryland, Eskin and Armstrong, 1996). The low-linolenic canola oil had a longer shelf-life than the regular canola oil. Oxidative stability of maize oils with increased total saturated fatty acid composition was evaluated (Shen, Duvick, White and Pollak, 1999). Maize lines with elevated saturated fatty acids (15-17 % compared to 13 % in traditional maize oil) have been developed. The maize oils with elevated saturated fatty acids were more stable than the traditional maize oil. Similar results were found when oxidative stability of soybean oils with increased palmitate (C16:0) and reduced linolenate

(C18:3) content were evaluated (Shen, Fehr, Johnson and White, 1997). Increasing 16:0 and/or reducing 18:3 lead to more oxidative stable soybean oils as measured by PV. The positioning of the unsaturated fatty acids on the triglyceride also plays a role in lipid oxidative stability. The increased concentration of unsaturated linoleic acid on the carbon-2 position of the triglyceride instead of the carbon-1 and carbon-3 positions has a detrimental effect on the oxidative stability of oils (Neff and El-Agaimy, 1996).

2.1.2.2 Antioxidants

Antioxidants retard the onset of oxidation, thereby extending the shelf-life of fats and oils and food products, but cannot prevent it. It is the same for synthetic antioxidants such as BHA, BHT, TBHQ and natural antioxidants such as tocopherols. Antioxidants can act either as primary chain breaking antioxidants, or as secondary preventative antioxidants (Gordon, 1990). Most of the common food antioxidants (AH) act as chain breakers by donating hydrogen atoms to the lipid radicals formed during initiation, thereby halting or slowing down the propagation of oxidation as discussed earlier (Hamilton, 1994):



The free radical A^{\bullet} does not participate in propagation steps as it is stabilised by resonance (Hamilton, 1994).

Secondary antioxidants reduce the rate of chain initiation by various mechanisms such as scavenging oxygen, decompose hydroperoxides to non-radical species, binding to metal ions, absorb ultraviolet (UV) radiation or deactivate singlet oxygen (Gordon, 1990).

The requirements for an ideal antioxidant is that it is safe in use, does not impart odour, flavour or colour to the product, must be readily incorporated in the product, be effective at low concentrations, should survive processing procedures, cooking and frying and be economic to use (Coppen, 1994). Oils and food products from plant origin generally contain sufficient tocopherols for good stability (Giese, 1996). Tocopherols are monophenolic antioxidants consisting of eight naturally occurring homologues, namely α -, β -, γ - and δ -

tocopherol, characterised by a saturated side chain consisting of three isoprenoid units and their corresponding unsaturated tocotrienols (α -, β -, γ - and δ -) (Eitenmiller, 1997). The homologues have different antioxidant activities (Hoffman, 1989). Standard vitamin E activity (100%) is ascribed to α -tocopherol and the other tocopherols (β -, γ - and δ -) have less vitamin E activity than the α -homologue. The vitamin E activity refers to the biological activity *in vivo* and is not related to the antioxidant activities of the different homologues. In a study of the antioxidant activities of α - and γ -tocopherols in the oxidation of rapeseed oil triglycerides, it was found that at low levels ($\leq 50 \mu\text{g/g}$), α -tocopherol was a more stable and effective antioxidant than γ -tocopherol. However, at higher α -tocopherol levels ($>100 \mu\text{g/g}$), γ -tocopherol was a more effective antioxidant than α -tocopherol in terms of increased formation of hydroperoxides and increased consumption of the tocopherol (Lampi, Kataja, Kamal-Eldin and Vieno, 1999).

Tocopherols also act as pro-oxidants depending on the concentration of α - tocopherol present (Jung and Min, 1990). In a study on maize oil stripped of natural antioxidants the effects of individual tocopherols and tocopherol mixtures on oxidative stability were evaluated (Huang, Frankel and German, 1995). They found that α -tocopherol was more effective at 100 ppm than γ - and δ -tocopherols. However, in contrast to α - and γ -tocopherols, δ -tocopherol showed antioxidant activity at 2000 ppm and below, whereas α - and γ - acted as pro-oxidants at high concentrations. According to Yoshida, Kajimoto and Emura, (1993) the optimum concentration of tocopherols required to increase oxidative stability were 100 ppm α -, 150-200 ppm β - or γ - and 500 ppm for δ - tocopherol, respectively. They found that the antioxidant effect decreased in the order $\alpha > \beta \cong \gamma > \delta$ which means that α -tocopherol was consumed first, followed by β - or γ - tocopherol and δ - tocopherol was consumed more slowly. δ -Tocopherol is the most potent antioxidant of the homologues (Hoffman, 1989). The stability of the tocopherol homologues were also studied in oat products where it was found that α -tocopherol and α -tocotrienol degraded faster than the γ -tocopherol, β -tocopherol and β -tocotrienol homologues (Peterson, 1995).

When evaluating the role of tocopherols as antioxidants it is important to take into account oxidation conditions that influence their role as inhibitors of lipid oxidation such as temperature, availability of oxygen, the chemical nature and physical state of the lipid or

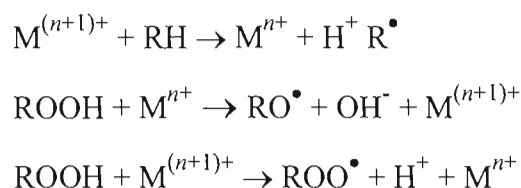
product and the concentration of tocopherols (Lampi *et al.*, 1999). Processing of oils through refining, bleaching and deodorising, decomposes the tocopherols and a large proportion is removed: 18% in olive oil, 25% in soybean and rapeseed oils, 32% in maize oil, 36% in cottonseed oil, 37% in sunflower oil and 40% in peanut oil (Frankel, 1996). However, according to Frankel (1996) the tocopherol levels left in rapeseed, sunflower, cottonseed, soybean and maize oils may be sufficient to protect the oil against oxidation under ambient conditions.

Recently, other natural antioxidants are receiving more attention as synthetic antioxidants are thought to have detrimental toxicological effects (Matemu, 1998). Matemu (1998) investigated the antioxidant effect of black or green tea extracts originating from Southern Africa in bulk sunflower oil since tea polyphenols possess excellent antioxidant activity. South African green and black, clonal and seedling crude tea extracts had lower antioxidant activities than pure TBHQ and epigallocatechin gallate. However, the extracts displayed better inhibition to oxidation compared to the control sample.

Synthetic antioxidants such as TBHQ are added to fats and oils as protection during the processing from crude to deodorised oil as they are effective in the prevention of secondary oxidation products as measured by the anisidine value and also have a stabilising effect on tocopherols (O'Brien, 1998). O'Brien (1998) also stated that TBHQ is removed during the processing and additional TBHQ must be added to the final deodorised oil for protection of the finished oil. TBHQ is also found to be very effective in stabilising crude oils during storage, which is very useful for countries such as Malaysia that has long shipping distances and consequently long storage times until oils reach their destinations (Coppen, 1994).

2.1.2.3 Pro-oxidants

Pro-oxidants in oil have a detrimental affect on oil stability. Metals act as pro-oxidants by electron transfer whereby they liberate radicals from fatty acids or hydroperoxides as in the following reactions (Gordon, 1990):



Two of the more active metals to induce oxidation are copper and iron of which copper is the most pro-oxidative (Garrido, Frías, Díaz and Hardisson, 1994). Addition of 0.07 mg/kg copper and 70 mg/kg copper to oil stored for 35 days at 40°C led to a double and 70 fold increase respectively in the volatile oxidation product, hexenal, compared to the sample without added copper (Andersson and Lingnert, 1998). In contrast, a study done on the thermogravimetric degradation rate, iron and tin had a greater influence on oil oxidation since the degradation rate increases whereas for copper and lead no important changes in the degradation rate was observed (Paz and Molero, 2000). Metals in oils often exceed the allowed concentration as can be seen in the study done on Spanish edible oils which revealed that 18.3 % of the oils tested exceeded the level of copper and 2.8 % exceeded the level of iron permitted in the oil as allowed by the FAO/WHO (Garrido *et al*, 1994). Smouse (1995) recommends copper levels of less than 0.02 mg/kg and iron levels of less than 0.1 mg/kg for good oxidative stability in refined, bleached and deodorised oil. The presence of metals is due to possible contamination with machinery and equipment during processing or could have been present in the seed from the start. High amounts of chlorophyll compounds (more than 50 µg/kg) also has a pro-oxidant effect (Smouse, 1995). The levels in oil are reduced during degumming, refining and mostly during the bleaching step.

2.1.2.4 Oxygen availability

The availability of oxygen is an important rate-determining factor as oxidation cannot take place without oxygen (Berger, 1994). The rate of lipid oxidation measured by hexanal formation increased with increasing concentrations of oxygen (1.2 %, 4.5 %, 10.0 %, 15.4 %) in a closed system (Koelsch, Downes and Labuza, 1991). It is also well known that samples with a high surface area in contact with air oxidise more rapidly (Gordon, Mursi and Rossell, 1994). This is clearly illustrated in a study with extracted crude sunflower oil stored under three different storage conditions; in a capped flask, open flask and capped flask under nitrogen atmosphere (Crapiste, Bredvan and Carelli, 1999). There was little difference in the oxidation rate between the open and capped flask, which indicates that oxidation rate

depends on the relation between oil surface area exposed to air and sample volume, whereas the capped flask under nitrogen showed very little oxidative activity. Oxygen can be replaced by utilising a protective gas practice such as nitrogen blanketing that will protect oil in storage tanks, during bulk transport and when packaged against oxidation (O'Brien, 1998).

2.1.2.5 Temperature

Temperature also has a big influence on shelf-life, as the rate of reaction of oxygen with fats roughly doubles for every 10°C increase in temperature (Rossell, 1992; Berger, 1994). In a storage trial done by Crapiste *et al* (1999) the PV and AV increased faster with higher storage temperatures of 30°C, 47°C and 67°C. The difference between storage at 50°C and 60°C of shortening blends is also clear as the PV at 60°C increases much more rapidly than at 50°C (Berger, 1994). Sensory tests confirmed the results.

2.1.2.6 Light

Light has a promoting influence on oil oxidation through photo-oxidation (Hamilton, 1994). The mechanism of oxidation by photosensitisation proceeds differently than normal free radical oxidation, as discussed previously. Photosensitised oxidation involves activation of substrate, which subsequently reacts with unsaturated fatty acids, for example sensitised-riboflavin that reacts with fatty acid double bonds (Frankel, 1985). As discussed by Frankel (1985), another mechanism of photosensitised oxidation is by singlet oxygen.

Selecting the most suitable type of packaging material for oils makes quite a difference in the shelf-life. The rate of oxidation is slower in brown than in clear glass bottles (Tekin, Kaya and Öner, 1995). Refined sunflower oil remains stable for two years when stored in high-density polyethylene bottles and sealed tins without developing pronounced off-flavours and odours. (Semwal and Arya, 1992). The influence of different packaging materials on lipid oxidation in potato crisps exposed to fluorescent light was examined and it was found that visible light with wavelengths longer than 380 nm could lead to oxidation of the lipids in the crisps (Lennersten and Lingnert, 1998).

2.1.3 Effects of rancidity on the food use of fats and oils

Flavour deterioration is the most common concern regarding the use of rancid fats and oils but the deterioration of colour and texture attributes as well as nutritional implications such as loss of nutritional value and formation of possible toxic oxidation products are also very important effects (Haumann, 1993).

Rancidity in fats and oils has a characteristic, unpalatable off-flavour and odour in oils, which can be picked up easily by subjective sensory appraisal (Hamilton, 1994). Secondary oxidation products such as short-chain aldehydes cause the typical off-flavour, which depending on their structure and the amounts formed, lead to odours such as beany, grassy, painty, fishy, tallowy or plain rancidity (Hoffman, 1989). The characteristic odours and flavours from volatiles formed from secondary oxidation products are given in Table 1. The threshold value is the minimum concentration of a volatile that can be picked up by 50% of evaluators. This is very important as a certain component may be present in small concentrations but contributes significantly to the flavour (Przybylski and Eskin, 1995).

Table 1: Characteristics of individual volatiles (Malcolmson *et al*, 1996).

Volatile	Reported odour threshold in oil (mg/kg)	Reported odour descriptors
Hydrocarbons		
Pentane	340	-
Hexane	-	-
Saturates		
Butanal	0.025	-
Pentanal	0.070	Painty, herbal
Hexanal	0.120	Fatty, green, fruity, cut grass, herbal, rancid, painty, crushed weeds
Heptanal	0.055	Weeds, green, sour, sweaty, herbal, painty, rancid
Octanal	1.50	Lime, grassy, citrus, sharp, heavy, candle-like, crushed weeds
Nonanal	1.00	Green, soapy, rubbery, beany
Decanal	-	Fruity, candle-like
Monounsaturates		
Propenal	-	-
2-Pentenal	1.00	-
3-Hexenal	0.003	Green, apple-like
2-Heptenal	1.50	-
2-Nonenal	0.15	Green, fatty, tallowy
2-Decenal	2.10	Metallic
Polyunsaturates		
2,4-Hexadienal	-	-
2,4-Heptadienal	0.04	Fatty, nutty
2,4-Octadienal	2.40	-
2,4-Decadienal	0.135	Waxy, fatty, green
-	no value was reported	

Sensory evaluation is generally considered to be the most reliable indicator of rancidity and measurement of flavour quality of plant oils (Warner and Frankel, 1985). In a study on canola oil stored and characterised for consumer acceptability a characteristic of rancidity was the detection of a painty odour and taste (Malcolmson *et al.*, 1996). This characteristic flavour is generally accepted as detection of rancidity. It is not only the degradation of the unsaturated fatty acids present that contributes to the off-flavours and odours but also some components of the unsaponifiable matter (Meara, 1980).

One of the major uses for oils is for frying of food. There are two types of frying namely deep frying (e.g. potato chips) and shallow frying (e.g. patties). Deep frying is especially of concern as the oil is reused and can be held at high temperatures for long times which is why a high stability oil is preferred for snack foods requiring a long shelf-life (Du Plessis, Van Twisk and Parsons, 1999). The degradation of the frying oil produces harmful compounds, which are absorbed by the product and for this reason the discard point of the frying oil is very important (Paul and Mittal, 1997; Du Plessis *et al.*, 1999).

Claims of nutritional implications of the consumption of oxidised fats and oils are varied. According to Sanders (1994) the symptoms of rancid fat toxicity are diarrhoea, poor growth rate, myopathy (replacement of healthy muscle with scar tissue), hepatomegaly (enlarged liver), steatitis or yellow fat disease, haemolytic anaemia and secondary deficiencies of vitamins A and E. Evidence exists that dietary oxidation products are involved in arterial injury, arteriosclerotic plaque formation and thrombosis/spasm which are potentially dangerous (Haumann, 1993). According to the publication by Haumann (1993), cyclic monomers of fatty acids formed during the frying process have been shown to have toxicological effects at levels as low as 0.01 % of the diet in rats. Unfortunately, no reference to toxic levels of oxidation products had been given although it was stated that it was not always possible to detect oxidised compounds at low levels but there may be reasons for concern at these low levels. It was generally thought that people would be better off if they did not consume oxidised foods.

2.2 ASSESSING THE QUALITY OF FATS AND OILS

Buyers and users of oil are faced with the problem of assessing the quality of the oils that they want to use in their product or sell. They need to determine if the oil is acceptable when received and if it is acceptable, for how long will it remain so (Hudson and Gordon, 1994). Both the suppliers of the raw product, as well as buyers, should universally accept the analytical methods used in the industry to determine the quality and stability of oils (O'Brien, 1998). The methods can be divided into: a) quick tests which are easily done as routine in a laboratory or factory, b) more specialised tests which require special equipment or are more time consuming and c) accelerated stability tests that give an indication of the long term stability of an oil by accelerating the normal oxidation process.

2.2.1 Quick tests

* Free fatty acids

The FFA determination measures the amount of hydrolytic activity that has occurred in the oil. The percentage free fatty acids present in the oil are measured by the equivalent amount of sodium hydroxide needed to neutralise the FFA (Sonntag, 1979b). In the calculation of the FFA percentage, the assumption is made that the average molecular weight of the fatty acids is that of oleic acid (Sonntag, 1979b). Hydrolytic rancidity is generally caused by a combination of catalyst (such as enzymes or soap residues remaining after refining) and moisture (Rossell, 1994). In a storage trial with crude sunflower oil at 30°C it was found that the FFA remained mainly constant over 98 days, indicating absence of hydrolytic alteration (Crapiste *et al*, 1999). Only at high temperatures (67°C) and higher air-to-oil ratios was a definite increase in FFA apparent. Under normal storage temperatures not enough energy is supplied to break the ester linkages to form FFAs and glycerol, as hydrolysis requires hydrothermal energy (Tekin *et al*, 1995).

The amount of FFA present is of importance, not only because it indicates hydrolytic activity, but also because FFA has a pro-oxidant effect, the intensity of which is related to FFA concentration (Frega, Mozzon and Lercker, 1999). According to the Codex Alimentarius Commission (1999) the recommended maximum % FFA (as oleic acid) limits for refined plant oils is 0.3 %, for cold pressed and virgin oil 2.0 % and for virgin palm oils 5 %.

* Peroxide value

The PV measures the hydroperoxides formed during the initial stages of oxidative rancidity of fats and oils (Hahm and Min, 1995; O'Brien, 1998). The method generally used is based on an iodometric titration with standardised sodium thiosulphate which measures the iodine liberated from potassium iodide by the peroxides present in the oil (Rossell, 1994). The PV is expressed in terms of milli-equivalents of oxygen per 1 kg of fat (meq/kg). The requirements according to the Codex Alimentarius Commission (1999) are that the PV of refined oils should be not more than 10 meq/kg and for cold pressed and virgin oils not more than 15 meq/kg. These requirements changed from the previous Codex Alimentarius Commission (1997) which stated that the PV for refined oils should be up to 5 meq/kg and for cold pressed and virgin oils up to 10 meq/kg. These values are very lenient and the values according to Robards, Kerr and Patsalides (1988) might be more useful when assessing oil for stability. They state that PV of >7.5 can indicate sufficient breakdown to aldehydes, which will produce rancid flavour in chips. In addition, Frankel (1993b) mentioned that flavour and quality deterioration might already occur in soybean oil at PVs less than 10. The acceptable PV level seems to depend on the oil type and the intended use for the oil.

According to Rossell (1994) freshly refined oil should have a PV of less than 1 meq/kg, whereas oils that have been stored for some time after refining could have PVs up to 10 meq/kg before undue off-flavours are detected. PV has shown good correlation with flavour scores but it has to be kept in mind that the PV is limited to the initial stages of oxidation, as it reaches a peak value and then oxidises to secondary oxidation products (Hahm and Min, 1995; O'Brien, 1998). This means that high PVs usually give poor flavour scores. However, a low PV is not necessarily an indication of good flavour scores. In a study on refined sunflower oil stored in tins and high-density polyethylene bottles for two years at room temperature, the PV increased to 8.5 and 22.6 respectively without any detection of off-flavours (Semwal and Arya, 1992). Yousuf Ali Khan, Lakshminarayana, Azeemoddin, Atchyuta Ramayya and Thirumala Rao (1979) found that in raw sunflower oil that had been stored at ambient room temperature, off-odours only became detectable once the PV had reached levels of above 25 meq/kg. According to Shen, Moizuddin, Wilson, Duvick, White and Pollack (2001) PV correlates well with traditional sensory evaluation as well as with electronic nose analysis (AromaScan) but unfortunately no PV was given to relate to the onset of rancidity as found by sensory evaluation. Frustratingly, similar good correlations

between PV, pentane values, oxygen absorption values and average flavour scores were found by Fioriti, Kanuk and Sims (1974) but the authors did not give actual PVs to relate to sensory evaluation.

It has to be kept in mind that PV only gives an, sometimes misleading, indication of the current state of oxidation of an oil sample and does not indicate the potential to oxidise.

* Conjugated diene and triene value

When polyunsaturated fatty acids are oxidised to form hydroperoxides, a shift in the position of the double bond occurs and they become conjugated. The extent of double bond displacement is directly related to the degree of peroxidation that has occurred in the oil and thus the amount of oxidation of the oil (Rossell, 1994; White, 1995). The conjugated diene value (CV) is expressed as a percentage of conjugated dienoic acid in the oil and is an indication of primary oxidation (White, 1995). The conjugated acids absorb ultra violet (UV) light with a maximum between 232 and 234 nm. CV value is a simple method whereby the oil is dissolved in a volumetric flask in a solvent such as iso-octane, read on a spectrophotometer at 232 nm against the solvent as blank and calculated in percentage (White, 1995). According to White (1995) the CV values of oxidised oils range between 0 and 6 %, depending on the type of oil. The CV accumulates to a certain percentage in the oil and then plateaus as the dienes break down further to other oxidation products. Trienes are frequently formed at this stage. The secondary oxidation products, particularly di-ketones and conjugated trienes, can also be measured by UV absorbance at 268 nm, although it has to be kept in mind that the absorption of various compounds overlap in this range (Rossell, 1994).

CV values, among other measurements, were used to determine the storage stability of potato chips fried in modified canola oils (Petukhov, Malcolmson, Przybylski and Armstrong, 1999). The chips were fried in regular, hydrogenated, low-linolenic and high-oleic canola oils, packaged and stored at 60°C for 0, 1, 2, 4, 8 and 16 days, at which times the fat was extracted and analysed. The CV showed an increase with storage time. The regular canola oil was the least stable, as CV increased more than the other oils. The hydrogenated canola oil had the lowest level of CVs. These values were paralleled by the PVs, polar components, FFAs and total volatiles. Noor and Augustin (1984) used the combination of conjugated diene and triene values, along with PV, AV, FFA and IV, as criteria to compare the stability

of banana chips fried in palm-olein containing different antioxidants. The primary oxidation products measured by PV and CV increased as well as the secondary oxidation products measured by AV and conjugated trienes. However, the AV and conjugated trienes did show different trends, which might be attributed to different secondary oxidation products measured by the two tests. Similarly Jung, Bock, Back, Lee and Kim (1997) used both conjugated diene and triene values, along with PV, colour, tocopherols and fatty acid composition to assess oxidative stabilities of soybean oils that were roasted at different temperatures and they found that both conjugated diene and triene values increased with an increase in roasting temperature. The effect of chemical and enzymatic randomisation of plant oils on their oxidative stability was measured by CV values (Tautorus and McCurdy, 1990). The CV value of the oils stored at 28°C gave a clear indication that both maize and soybean oils in a randomised state had higher amounts of conjugated fatty acids than the native oils. In a comparative study of the oxidative stability of one canola and six soybean oils of various altered fatty acid compositions, CV values were used amongst others for comparison (Liu and White, 1992). Oils were stored at 60°C for 15 days and it was found that CV values showed a very slow increase over the time period. At day 15 the CV values of the six soybean oils generally followed in the order that the higher the polyunsaturated fatty acid (PUFA) level, the higher the CV values. Canola oil with high linolenic acid (C18:3) content differed from the soybean oils in that the rate of CV increase, from day 0 to day 7, was different. After 7 days the rate of change became the same. According to Liu and White (1992) the CV did not correlate with the C18:3 contents of the oils or with the total PUFA levels, whereas the PV and flavour scores after 15 days of storage at 60°C were highly correlated with the initial C18:3 contents of oils.

The CV value is useful because of the simplicity and speed of the method, although it should be taken into account when assessing oils for rancidity based on the CV value, that dienes are also found in lipid alcohols derived from peroxides and in certain non-oxidised fatty-acids, and that hydroperoxides from monounsaturated fatty acids such as oleic acid do not possess a conjugated diene group (Prior and Löliger, 1994). Similarly, interfering substances might also be taken for conjugated trienes in some circumstances (Jung *et al.*, 1997).

2.2.2 Specialised tests

* Tocopherols

The amount and ratio of different homologues of the natural tocopherols present in the oil affects the rate of lipid oxidation (Bramley, Elmadfa, Kafatos, Kelly, Manios, Roxborough, Schuch, Sheehy and Wagner, 2000). Tocopherols are measured by various means but mainly by high performance liquid chromatography (HPLC) using either reverse phase or normal phase columns. They are detected by either UV absorbance (between 280 nm and 297 nm) or fluorescence detection (excitation at between 290 and 296 nm and emission at between 325 and 340 nm) (Van Niekerk, 1975; Kochhar and Rossell, 1990).

According to Bramley *et al* (2000), the ratio of tocopherol and tocotrienol distribution is significant as shown by the OSI induction time of high oleic soybean oil, which is much longer than that of high oleic sunflower oil, even though their fatty acid compositions are similar. Soybean oil is high in γ - and δ - tocopherols, whereas the sunflower oil is high in α -tocopherol. Semwal, Narashima Murthy, Sharma and Arya (1996) also found with sunflower oil, the oil sample with the highest natural tocopherol concentration had a much slower oxidation rate, compared to the oil sample with the lowest natural tocopherol concentration, which oxidised the fastest of the five samples. Also, the rate of disappearance of tocopherols in sunflower oil during storage was much faster in the oil sample with initial low levels of tocopherols, than in the oil sample with initial high levels of tocopherols.

The natural tocopherol content can thus be used as an indication of the stability and quality of oil. Unfavourable treatment of oilseeds, oil bearing fruit or oil such as exposure to sun, high temperatures and presence of oxygen, would lead to low levels of tocopherols (Bramley *et al*, 2000).

* Anisidine value

The AV measures the secondary oxidation products. Aldehydes are one of the products of secondary oxidation and are the main contributors to off-odours. The AV procedure uses the reaction of α - and β -aldehydes (primarily 2-alkenals) with *p*-anisidine reagent in the presence of acetic acid (Robards *et al*, 1988; White, 1995). The resulting Schiff base compound leads to the formation of a yellowish colour that is measured at 350 nm. The molar absorbance increases by a factor of four to five if the aldehyde has a double bond conjugated to the carbonyl double bond, thus AV measures mainly 2-alkenals (White, 1995; O'Brien, 1998).

This means that the AV is comparable only within each oil type as the absorption maximum for each oil differs as well as the intensity of absorption of the complexes. The initial AV varies among oil types. Generally, well-refined oils have AV between 1.0 and 10.0 mmol/kg sample (White, 1995). Oils with high levels of polyunsaturated fatty acids have more potential for the formation of 2-alkenals and their AV might be higher than 10.0 even when fresh.

O'Brien (1998) claims that the AV can only be useful as indicator of the past history of the oil (quality of crude oils and efficiency of processing procedures), but that it is not suitable for the detection of fat oxidation. AV is not mentioned in an article by Frankel (1993b) in which he discusses and compares different methods to evaluate natural antioxidants and oxidative stability in food lipids. Crapiste *et al* (1999) used AV as one of the parameters to determine the effects of temperature and oxygen concentration on oxidative deterioration during storage of crude sunflower oils. According to their research, AV remained practically constant during the initial stages of oxidation but increased rapidly following the decomposition of peroxides. This would indicate that the AV could be a useful parameter to study oxidative deterioration. Lampi *et al* (1999) found that AV could not be compared between rapeseed and butter oil triglycerides to characterise oxidation, although it was found to be a useful indicator of secondary oxidation for each individual oil.

* Fatty acid composition and iodine value

The fatty acid composition of an oil determines its stability to a great degree, as discussed earlier. A high degree of unsaturation results in susceptibility to attack by oxygen or other instigators of radical formation. Iodine value (IV) measures the unsaturation in oils and can therefore be calculated from the fatty acid composition (O'Brien, 1998). According to O'Brien (1998) each unsaturated fatty acid has a constant value with higher constant values attributed to most unsaturated fatty acids. The IV is calculated by multiplying the percentage of each unsaturated fatty acid present with its constant value and addition of the results.

Oil unsaturation is measured by gas chromatographic determination of the fatty acid pattern (Christie, 1980). A titration method, the Wijs procedure (O'Brien, 1998) can also be applied to measure total unsaturation.

IV is useful for polyunsaturated oils, whereby the decrease in the proportion unsaturated fatty acids to total or saturated fatty acids is measured. No mention of the use of a decrease in polyunsaturated fatty acids over storage was found in the literature. It would have been ideal to use it in the study by Frankel (1993a), in which the thermal decomposition and oxidative stability of oxidized fish oils were compared with oxidized plant oils.

* Volatile compounds

The volatile components are directly responsible for off-flavours in oils and fat. For that reason, it is very important to determine the presence and to identify the volatiles. A wide range of volatile components is present and for different oils the range varies (Przybylski and Eskin, 1995). The main carbonyl compounds formed by oxidation of oleic and linoleic acids are pentanal, hexanal, propan-2-one and pentan-2-one (Robards *et al*, 1988). The characteristic volatile formation is dependent on the oil type and the volatiles for each oil type should be assessed separately. In a method for analysing oil volatiles using multiple headspace extraction (MHE) twelve volatiles were identified in plant oils, namely: propanal, pentane, pentanol, hexanal, 2-pentenol, 3-hexenal, 2-heptenal, octen-3-ol, 2,4-heptadienal, nonanal and 2,4-decadienal (Snyder and Mounts, 1990; Przybylski and Eskin, 1995).

Przybylski and Eskin (1995) describe the methods used to analyse volatiles and the problems surrounding the analyses. Volatiles are very soluble in lipids and are usually present in very low concentrations. This makes it a difficult task to analyse the volatiles concurrent with problems such as the oil viscosity, oil affinity for volatiles, contamination and interference by additives and solvents. Volatile analysis is done mainly by GC although in some cases HPLC has been used. The methods most widely used are static headspace, dynamic headspace and direct GC. Static headspace is based on transferring a certain volume of the equilibrated gasses from the area above the sample in a closed container, which is injected into the GC column where it separates and is quantified. Dynamic headspace involves trapping the volatiles from the heated sample onto a porous adsorbent, which are then transferred into a GC column by thermal desorption or solvent extraction/desorption. Direct GC is a technique whereby the volatiles are injected directly into a packed GC column by either placing the sample in an injector insert positioned in the injection port of the GC or the sample is injected directly into an injection port where the glass wool plug is placed. A third version of

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direct GC is by transferring the volatiles from the sample to an external unit with temperature control before being injected directly into the GC column.

Volatile analysis has been used extensively to determine oil quality and during storage trials of oils. Morales, Rios and Aparicio (1997) studied the changes in volatile composition of virgin olive oil during oxidation. They found that the volatiles correlated well with sensory evaluation but not with the peroxide value. The maximum limit for the peroxide value of 20 was too high as the sensory panel, surprisingly, rejected the oil when the PV was <4, which is generally regarded as an acceptable PV.

Olive oil is unique in that initially it has a great amount of volatiles that contribute to the characteristic flavour of virgin olive oil. Care has to be taken in the choice of volatiles to be used as oxidation indicators as some volatiles such as hexanal, which is normally used in plant oils as indicator, is already present in the original oil (Morales *et al.*, 1997).

Volatile compound analysis has been used as a tool to investigate what effect hydrogenation and additives have on the formation of total and individual volatiles in a study by Snyder, Frankel and Warner (1986). Oxidative stability of soybean oils was determined by volatile analysis after storage at 60°C for eight days. Hydrogenation decreased the formation of volatiles during storage. This is in accordance with earlier work where it was found that an increase in saturates decreases the amount of volatiles formed from oxidation of unsaturated fatty acids (Selke, Rohwedder and Dutton, 1977; Selke *et al.*, 1980). It is thus of importance to be informed of the composition of the oil and its treatments before assessing the oil purely on volatile formation.

Warner and Frankel (1985) used the determination of induction periods, based on volatile formation, to predict the flavour stability of soybean oil. The induction periods for formation of individual and total volatiles were used. They found a high correlation between volatiles formed and sensory score compared with a low correlation between PV and sensory score.

There are a variety of methods used for volatile analysis and different volatile markers are used in the assessment of oil oxidation. This could influence the conclusions made from the analysis. The concentration of volatiles are also expressed in different units from recorder

response (Robards *et al*, 1988), parts per million (ppm) (Grün, Barbeau and Crowther, 1996; Koelsch *et al*, 1991), volatile GC peak area $\times 10^{-3}$ and 100 (Snyder *et al*, 1986; Frankel, 1993a), nmol/ml (Frankel and Tappel, 1991) to integrator count $\times 10^5$ (Warner and Frankel, 1985). This makes comparisons and interpretations difficult.

* Sensory evaluation

Sensory evaluation relies on humans to assess the acceptability and sensory properties of a product. No instrument can replicate or replace the human response, and sensory evaluation is therefore of importance in a quality assessment system for food products (Malcolmson, 1995). Malcolmson (1995) further states that sensory evaluation is the application of knowledge and skills of various scientific disciplines among them food science, psychology, physiology, mathematics and statistics. Sensory evaluation of oils is limited mainly to the senses of taste and smell (Warner, 1995). The different types of sensory panels are represented in Table 2. Two general types are consumer and analytical. Consumer panels are important for market research to evaluate likes and dislikes. They are more likely used for fat-containing products. Analytical panels are used more often to evaluate oils when subtle flavours are looked for or to determine off-flavours in oils (Warner, 1995). The type of sensory panel selected depends on the information required. Setting up a sensory panel is a time consuming and costly endeavour.

Table 2: Types and characteristics of sensory panels (Warner, 1995).

Types	Characteristics
Analytical	Trained testers Normal sensory acuity 5-20 members
Difference Triangle, Duo-trio, ranking, Paired comparison	Measures differences between samples
Descriptive Scalar scoring, Descriptive analysis	Measures intensity or quality
Consumer	Measures acceptance, preference, or like-dislike response 50+ untrained testers

Instrumental or chemical analyses such as GC volatiles, PV and others can be correlated with sensory data (Warner, 1995). Odumosu *et al* (1979) did a comparison study of chemical and sensory methods to evaluate thermally oxidised groundnut oil. Seven chemical parameters were measured namely PV, Totox value (TV), AV, conjugable oxidation products (COP), oxodiene value (OV), induction period (IP), IV and a sensory analytical flavour evaluation (FS). The best correlations with flavour scores were found with IP, IV and OV with correlation coefficients of 0.98, 0.97 and 0.98, respectively. PV, the primary oxidation products, showed a correlation of 0.92, which could be explained by the fact that it is continuously degraded to other oxidation products. Measurement of the secondary oxidation products, AV, unexpectedly correlated even less with the FS with a correlation coefficient of 0.74.

Volatile compounds have been found to correlate well with sensory evaluation, as previously discussed under volatile compounds. The induction period (time required before rapid

formation of peroxides and total volatiles) was compared and correlated with flavour scores (Warner and Frankel, 1985). The correlation coefficient of flavour with the induction period of PVs was 0.56 and with total volatiles 0.96. Malcolmson *et al* (1996) did a study that characterised stored regular and low-linolenic canola oils chemically and with sensory evaluation, at different levels of consumer acceptance. Correlation coefficients of the five chemical indices in relation to each other were provided, but correlation with the sensory evaluation is unfortunately absent. In a paper by Morales *et al* (1997) changes in the sensory, fatty acid composition and volatiles of virgin olive oil were studied by applying an accelerated thermoxidation process. The volatile nonanal showed a good negative correlation with the sensory quality with a correlation coefficient of -0.85 . Peroxide value did not agree with the sensory evaluation as the assessors rejected oil that was still acceptable according to the peroxide value.

2.2.3 Accelerated stability tests

Ambient shelf-life testing is very time consuming and thus not generally practical. Retailers and producers need a fast method to measure resistance to oxidation. There are several accelerated methods for testing resistance to oxidation by elevating temperatures and introducing oxygen.

* Schaal Oven Test

The test involves simply keeping a sample of oil or fatty food, normally about 50 g, in a loosely sealed glass container in an oven at 60-65°C. The progress of rancidity is monitored by sensory evaluation until the panel detects a definite rancid or off flavour. The number of days until rancidity is detected is recorded as the end point of the sample (Wan, 1995). Variations in the method use PV, AV or volatile compounds to determine the end-point. The test requires large samples and 4-8 days to complete (Wan, 1995). A drawback is that a sensory panel is not always readily available.

* Active Oxygen Method (AOM)

This has been a popular method to measure resistance to oxidative rancidity. A sample of oil (e.g. 20 g) is weighed into a glass tube. The tube is heated at 60-100°C (depending on degree of unsaturation of the oil) and air is bubbled through at a constant rate. A small sample is taken at regular intervals for PV determination (Wan, 1995). The number of hours until a PV

of 100 meq/kg (milliequivalents of peroxide per kg of oil) is obtained is reported as AOM hours for the oil (Wan, 1995). The induction period (time until PV starts to increase rapidly) can also be used for comparison of oil samples. A drawback is that periodic PV titrations are tedious and time consuming.

* Oil Stability Index (OSI)

This method is an automated replacement for the AOM. Fat or oil (2.5-5 g) is weighed into glass tubes which are held in a thermostated bath or heating block normally at 100-120°C. Purified air is bubbled through the sample at constant rate. The effluent air from the sample is then bubbled through a glass vessel containing deionised water (AOCS, 1997, Cd 12b-92). The organic volatile compounds from the heated oil are trapped in the water and their rate of formation is measured by electro-conductivity (Figure 1) (Wan, 1995). The volatile compounds formed are mainly acids of which the predominant acid formed is formic acid with significant amounts of acetic acid. To a lesser extent acids with three or more carbon atoms including propionic, butyric and caproic are also detected. (DeMan, Tie and DeMan, 1987).

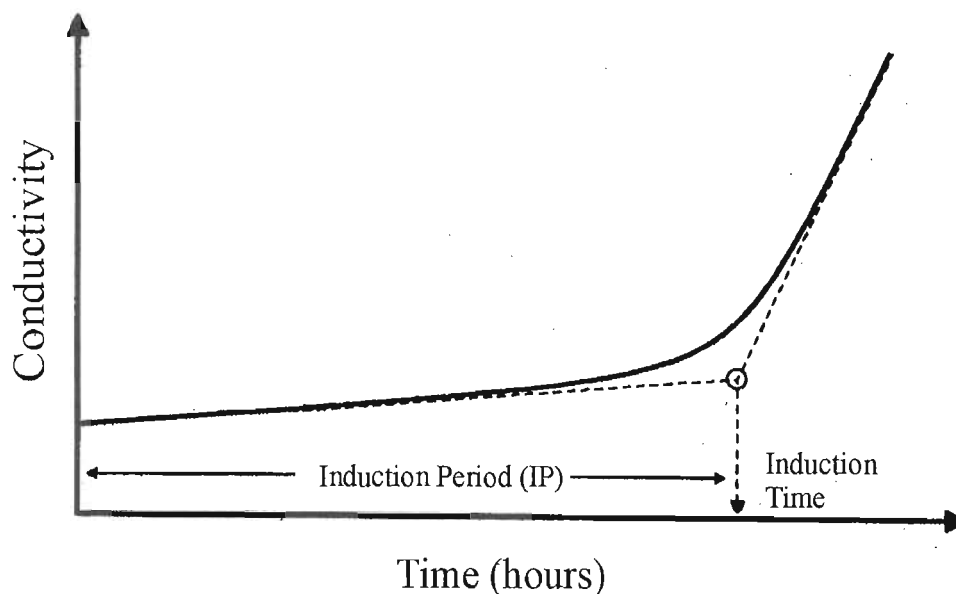


Figure 1: Course of oxidation measured by conductivity of organic volatile compounds trapped in water versus time as determined by OSI. (Wan, 1995).

Two commercially available instruments currently available are: 1) Metrohm Rancimat and 2) Omnion OSI instrument. A computer or strip chart recorder monitors the conductivity of

the water. The OSI is the time (hours) when a sharp rise in conductivity occurs (Hudson and Gordon, 1994). The period when the oil resists oxidation before the sharp rise in conductivity is the induction period or time (Figure 1) (Rossell, 1994).

Frankel (1993b) in his review of methods to evaluate oxidative stability in food lipids stated that there are limitations to high-temperature stability tests. This is because the mechanism of lipid oxidation changes at elevated temperatures. It has also been found that care has to be taken to evaluate results obtained at different temperatures as illustrated by Rossell (1992). Rossell (1992) compared four different fat and oil samples at six different temperatures ranging from 100°C to 150°C. At 100°C Brazilian cocoa butter was the most stable sample when its induction time was compared with that of hydrogenated soybean oil, Nigerian cocoa butter (A) and Nigerian cocoa butter (B). At 110°C the hydrogenated soybean had the longest induction period, followed by the Nigerian cocoa butter (B) and the Brazilian cocoa butter together with the Nigerian cocoa butter (A). Further discrepancies at the higher temperatures indicate that changes in temperature changes the relative ranking of the oils.

Frega *et al* (1999) found Rancimat induction times to be a useful tool for comparison of lipid oxidation rates when only one parameter is changed, as in their study where different levels of FFA added to oils were compared for their effect on oxidative stability. The induction times decreased with increased levels of FFAs. Warner, Frankel and Mounts (1989) used Rancimat as one of the measures to compare oxidative stability of soybean, sunflower and low erucic acid rapeseed oils after deodorising and ageing under identical conditions. Ageing conditions were conducted at 25°C, 60°C, 80°C and 100°C in the dark, as well as storage under fluorescent light at 30°C. Methods used to measure oxidative stability were volatiles, PV, AOM and Rancimat. The values obtained were correlated with sensory analyses. According to the sensory evaluation and PVs, the soybean oil was significantly better than either the sunflower or low erucic acid rapeseed oils. However, AOM and Rancimat values indicated that the low erucic acid rapeseed oil was slightly better than the soybean oil. They state that valid comparisons of oils require a variety of storage conditions and, very importantly, evaluation methods. It would be unwise to depend on a measurement such as Rancimat alone to compare oxidative stabilities.

Rossell (1992) and Frankel (1993b) state that one of the limitations of high temperature stability tests such as the Rancimat and AOM is oxidative stability testing with antioxidants present. Volatile antioxidants such as BHA and BHT are subject to losses and results can be misleading. Phenolic antioxidants in natural extracts are not stable at high temperatures and decompose at elevated temperatures (Frankel, 1993b). Reynhout (1991) investigated the effect of temperature on antioxidant-stabilised lipids. The induction times obtained from the Rancimat were over a temperature range of 80°C to 180°C against a control. Soybean oil was used fortified with the antioxidants BHT, BHA, TBHQ, rosemary extract (Herbalox® Seasoning) and tocopherol. As expected, the induction time decreased with an increase in temperature. The induction time was plotted in a logarithmic scale against temperature and a straight line was obtained for all the antioxidant systems. This logarithmic relationship held for highly volatile antioxidants at temperatures past their perceived effectiveness. This indicates that the OSI (e.g. Rancimat) data, including data of samples containing antioxidants, can be used for comparison of oxidative stability but the induction time cannot be related to a shelf-life period, as the effects of other data determining elements need to be taken into account (Reynhout, 1991).

High fat containing foods such as crisps are difficult to test directly. The fat has to be extracted first by cold extraction with a suitable solvent (Robards *et al*, 1988). Some methods of extraction such as refluxing with hexane can lead to changes in the sample (Rossell, 1992). Evaporation of the hexane led to an increase in the PV. Mashed potato chips have been analysed directly Wan (1995) where they found that because of the presence of volatile compounds, two inflection periods were seen in the Rancimat results. Their sensory results showed a correlation coefficient of 0.86 with the induction period of the second inflection period.

* Oxygen consumption methods

In oxygen consumption methods the sample is sealed under air or oxygen and stored at a constant elevated temperature (Tian and Dasgupta, 1999). Oxygen concentration in the headspace is monitored by taking regular samples through multiple sealed septa affixed to the container and analysing them for oxygen concentration or the oxygen pressure is monitored.

In the oxygen bomb method the sample is placed in a glass container, which is inserted in a stainless steel bomb. The bomb is sealed and pressurised with oxygen. The whole bomb is immersed in a bath of boiling water. The oxygen pressure is measured over a period of time, recorded and plotted. The time, in minutes, from when the oxygen pressure reaches a plateau at the bath temperature until a sudden drop in oxygen pressure occurs is the measurement of the oxidative stability of the sample (O'Brien, 1998). Fats and oils do not have a sharp drop in oxygen pressure so that an arbitrary endpoint based on comparative pressure drops is generally used. This method is 2-10 times faster than the AOM or OSI methods, although only one sample at a time can be analysed (Wan, 1995; O'Brien, 1998). Fatty foods and oils containing volatile antioxidants can be analysed directly and results are very reproducible.

The Leatherhead Food Research Association in the United Kingdom (UK) developed the FIRA-Astell apparatus. Pressure balance flasks for each test flask are coupled to a balancing diaphragm to eliminate the effect of atmospheric pressures. The balancing diaphragm is connected to an automatic recorder that records the pressure drop in the test flasks. The controlled temperature range for the apparatus is from 50-150°C with constant stirring by magnetic followers (Rossell, 1994).

Various variations on the principle of oxygen absorption are available. Tian, Dasgupta and Shermer (2000) developed a gas-phase flow injection analysis method for the direct determination of the oxidative stability of solid high fat containing samples. It is an automated stopped-flow gas-phase system with an oxygen sensor and a programmable temperature reactor that measures oxygen consumption of samples at various programmed temperatures. The reactor containing the sample is flushed with a carrier gas of 0.1 % O₂ and 99.9 % N₂, heated to intended temperature, and with the use of a valve system the reactor vessel is connected on-line at set time intervals with the carrier gas and the amount of oxygen uptake in the reactor vessel is measured by an oxygen sensor.

Advantages of oxygen absorption methods are that they correlate well with ambient storage temperature shelf-life, have good reproducibility and are faster than OSI or AOM (Wan, 1995, O'Brien, 1998). However, despite the advantages of oxygen absorption methods they are not as popular and are seldom used in quality control or comparison of samples. According to Tian *et al* (2000) the close correspondence to Arrhenius behaviour makes it

possible to predict the relative stability of samples at temperatures different from the experimental conditions used. However, no mention has been found in literature on the use of oxygen absorption methods to predict the shelf-life of oils. Comparison of costs involved in acquiring the instruments has also not been found but the analysis costs should be similar.

* Other

There is a variety of other accelerated oxidative stability testing methods that are not used as routine and will be mentioned briefly.

Thermal analysis can be used to follow the oxidative and thermal (e.g. frying) degradation changes under isothermal conditions (Buzás and Kurucz, 1979). Oxidative changes are followed quantitatively by weight gain of the sample and the rate thereof. Samples are dispersed as a thin film on a ceramic block under airflow (20 l/h). The block is heated up to 400°C. Accelerated oxidation of edible oils by thin-film oxidation with UV irradiation at different temperatures (80 and 100°C) has been measured by PV, headspace volatile peak areas and UV absorbance at 232 nm (Gordon *et al.*, 1994). An oil sample (2.5 g) in a crucible was irradiated from a distance of 3 cm with a six-Watt short wave UV lamp (200-280 nm). Tan, Che Man, Selamat and Yusoff (2002) used differential scanning calorimetry (DSC) to compare oxidative stability of oils. The technique involves oxidation of oil samples in an oxygen-flow DSC cell with the cell temperature set at four isothermal temperatures (110, 120, 130 and 140°C). Initiation of the oxidation reaction is observed by a dramatic increase of evolved heat. Extrapolation of the downward portion of the DSC oxidation curve is taken as the oxidative induction time.

2.3 STABILITY OF SPECIFIC OILS

Stability of oils is a broad term and generally means the resistance of oil to chemical change or to physical disintegration (Smouse, 1995). Evaluation of stability includes characteristics such as oxidative, flavour, colour, hydrolytic, crystal, light, enzymatic, foam or emulsion stability. The main characteristics generally looked at when considering oil stability are oxidative and flavour stability. The two factors can be independent of each other as an oil can show good oxidative stability but less flavour stability (Smouse, 1995). Cottonseed oil and soybean oil are good examples of this. Soybean oil shows better oxidative stability than

cottonseed oil as measured by accelerated oxidative tests such as OSI or AOM. However, cottonseed oil shows better flavour stability than soybean oil as measured by Schaal Oven test at 63°C (Smouse, 1995). There are various inherent factors of an oil type that influence oil stability, as well as extrinsic factors. These factors are best explained by using two specific oils as examples to show how their inherent and intrinsic factors affect the oil's stability. The two oil types that will be discussed in the following section are monounsaturated oil and polyunsaturated oil. The first type, a monounsaturated stable oil, as represented by palm-olein oil, an important oil in South Africa as it is increasingly imported for local use due to its stability. It is used especially as a frying oil for chips and other frying purposes, manufacture of shortenings, ice cream, coffee creamers and cocoa butter extenders (Pike, 1980). The second type, a polyunsaturated non-stable oil, as represented by sunflower oil, will be discussed. Sunflower is the second largest world source of plant oil with the majority of production in Russia (O'Brien, 1998). Sunflower is also South Africa's main source of locally produced plant oil and is used widely throughout South Africa for many purposes such as frying, household oil, margarines and in high fat products such as salad dressings and mayonnaise.

2.3.1 Palm-olein oil and similar oils

Palm oil is derived from the fruit of the oil palm tree, *Elaeis guineensis*, which looks similar to date fruit and is carried on large fruit bunches with 400-2000 individual fruit (Rajanaidu, 1994; O'Brien, 1998). Each fruit consists of an outer fleshy part (mesocarp) and an inner shell containing the palm kernel. The fresh fruit bunches are harvested with care so as not to cause excessive bruising. They are then immediately processed to minimise free fatty acid formation through enzyme activation (Pike, 1980; O'Brien, 1998). Two products are produced from the oil palm fruit namely palm oil from the mesocarp and palm kernel oil from the kernels (Chong, 1994). The two oils have different chemical and physical properties. Palm oil has high levels of palmitic acid (C16:0) and is solid at ambient temperatures in temperate climates and fluid with a small fraction present in crystalline form in tropical countries (Pike, 1980). Palm kernel oil is amongst the most stable fats and oils, due to its high levels of lauric acid (C12:0) and low level of unsaturated fatty acids (O'Brien, 1998). O'Brien (1998) further states that it is a solid fat at room temperature but melts sharply and completely below body temperature and can develop off-flavours characterised as astringent and coarse.

A large portion of palm oil is fractionated, to cater for a wide range of markets, into products such as palm stearin (“harder”) and palm-olein (“more liquid”) (Mohd Suria Affandi, 1994). The palm oil is fractionated by separating a higher melting, crystalline and a lower melting liquid fraction. The crystalline form is the solid stearin fraction, while the liquid oil is the olein fraction (Pike, 1980; Mohd Suria Affandi, 1994). Minor components such as free fatty acids, diglycerides, carotenes, sterols, tocopherols, peroxides and oxidised products remain with the palm-olein fraction, while the phospholipids and metals migrate to the stearin fraction (O’Brien, 1998). Physical characteristics of palm, palm-stearin and palm-olein oils are compared in Table 3.

Table 3: Fractionated palm oil characteristics (adapted from O’Brien, 1998).

Characteristic	Palm Oil Fraction		
	Whole	Olein	Stearin
Softening point (°C)	3.0-38.0	19.0-24.0	44.0-56.0
Density at 25 °C	0.892-0.893	0.909-0.903	
Density at 25 °C			0.882-0.891
Saponification value	190-202	194-202	193-206
Cloud point (°C)		6.0-12.0	
Unsaponifiable matter (%)			0.1-1.0
Iodine value	51.0-55.0	51.0-61.0	22.0-49.9
Fatty acid composition (%)			
Myristic (C14:0)	1-1.5	1-1.5	1-2
Palmitic (C16:0)	42-47	38-42	47-74
Stearic (C18:0)	4-5	4-5	4-6
Oleic (C18:1)	37-41	40-44	16-37
Linoleic (C18:2)	9-11	10-13	3-10

Palm-olein oil with its high proportion of saturated (approximately 44 % C16:0 and C18:0) and monounsaturated (approximately 42 % C18:1) fatty acids and low polyunsaturated (approximately 12 % C18:2) fatty acids is more stable against oxidation than polyunsaturated oils such as soybean and maize oil (Leong, 1994). Other oils that contain higher levels of monounsaturated fatty acids than polyunsaturated fatty acids include the following; olive oil

with an oleic acid content of approximately 80.3 %, high-oleic sunflower oil with an oleic acid content of approximately 81.3 %, high-oleic safflower oil with an oleic acid content of approximately 81.5 % and canola oil with an approximate oleic acid content of 60.9 % (O'Brien, 1998). Palm-olein oil is different in that the saturated and monounsaturated fatty acids content are more or less the same, whereas the other monounsaturated oils mentioned have saturated fatty acid content ranging from 5.9-11.7 %. Oil stability is often compared by determining the OSI using the Rancimat instrument. Table 4 lists the induction periods determined by Rancimat that were conducted at 100°C (Personal communication, Malaysian Palm Oil Board, 2000). The induction period of palm-olein oil (36-58 hrs) is three times as long as for sunflower seed oil (12-16 hrs) illustrating clearly the higher resistance to oxidation of palm-olein oil.

Table 4: Rancimat values of oils at 100°C (personal communication, Malaysian Palm Oil Board, 2000).

Oil type	Rancimat values at 100°C
Coconut oil	38 - 80
Palm-olein	36 - 58
Palm oil	30 - 44
Groundnut oil	18 – 23
Cottonseed oil	17 – 21
Maize oil	16 – 20
Olive oil	14 – 16
Sunflower oil	12 – 16
Soybean oil	8 - 13

Minor components present in palm oil affect the stability and quality of the oil (Sundram, 1999). The minor components include the carotenoids, tocopherols, tocotrienols, sterols, phosphatides, triterpenic and aliphatic alcohols. They account for less than 1 % of the oil's constituents (Sundram, 1999). The tocopherols and tocotrienols are the most important of these components as the tocopherol and tocotrienol content affects the oil stability as discussed earlier in section 2.1.2. The tocopherol and tocotrienol content is reduced during the refining, bleaching and deodorising process of crude palm oil by 28 - 33 % (Sundram, 1999). Palm oil is one of the few oils that contains high levels of tocotrienols (Lin, 1999). According to Lin (1999) the total tocopherol (including tocotrienols) content in refined palm-

olein oil is 468-673 mg/kg. The composition of tocopherols and tocotrienols in oil is also of importance as discussed earlier in section 2.1.2. The composition of tocopherols and tocotrienols in crude palm oil as percentage of the total tocols is given in Table 5. According to Rossell, King and Downes (1985) there are no differences in tocopherol, tocotrienol and sterol composition between palm oil and its fractions.

Table 5: Composition of tocopherols and tocotrienols in crude palm oil (% of total) (Lin, 1999).

Tocol	Percentage of total
α -tocopherol	21.5
β -tocopherol	3.7
γ -tocopherol	3.2
δ -tocopherol	1.6
α -tocotrienol	7.3
β -tocotrienol	7.3
γ -tocotrienol	43.7
δ -tocotrienol	11.7

The depletion of the tocopherols is in the order $\alpha > \beta \cong \gamma > \delta$ with δ -tocopherol as the most potent antioxidant of the homologues (Hoffman, 1989). Little information is available on the potency and stability of the tocotrienols. This would be of interest in palm-olein oil as a high percentage of its total tocol content consists of γ -tocotrienol (43.7 %) and other tocotrienols (α -, β -, δ -tocotrienols accounting for 26.3 %) according to Table 5.

The other minor components present in most oils, such as sterols, do not have a significant influence on stability but are of nutritive value (Lin, 1999). Carotenoids are another minor component present in crude palm oil but due to its strong yellow colour it is thermally degraded and removed during the deodorising stage of refining (Sundram, 1999).

As can be seen, palm-olein oil is unique from other monounsaturated oils such as olive oil, high-oleic sunflower oil and canola oil due to its high degree of saturated fatty acids and its tocol composition.

2.3.2 Sunflower oil and similar oils

Sunflower oil is the second largest world source of plant oil according to O'Brien (1998) whereas Basiron and Balasundram (1999) state that sunflower is the fourth largest plant oil produced worldwide. According to Basiron and Balasundram (1999), soybean oil was the major oil produced in 1998 at 29.4 % of total plant oils produced, followed by palm oil at 20.4 %, rapeseed oil at 15.0 % and sunflower oil at 10.5 %. Spurling (2000) states that South Africa is the world's tenth largest producer of sunflower seed oil. Sunflower oil is obtained from the seed of the plant *Helianthus annuus*. The flower heads with the seeds are harvested mechanically once the moisture content of the seeds has dropped to approximately 9-10 % (Padley, 1994). The seed has a hard woody pericarp, which is removed with a decorticator, although some smaller producers press the seeds without decortication. The oil is obtained by hydraulic or screw pressing, which is generally followed by solvent extraction (Sonntag, 1979a). Few plant oils reflect the effect of climate, temperature, genetic factors and location of seed in the flower head so significantly in the composition of the oil as sunflower seed oil (Sonntag, 1979a). Oil content in the seeds has been improved from 20-32 % in the old strains to 40-50 % in the new strains (Sonntag, 1979a, Padley, 1994). The oil is known for its high linoleic acid content, although there is increasing interest in the cultivated high-oleic acid varieties (Padley, 1994). Average characteristics of sunflower oil are given in Table 6.

Table 6: Characteristics of sunflower oil (adapted from O'Brien, 1998).

Characteristic		
Iodine value		125-136
Cloud point (°C)		-9.5
Melting point (°C)		-18.0 to -16.0
Specific gravity at 25°C		0.915-1.474
Refractive index at 25°C		1.472-1.474
Wax (%)		0.2-3.0
Saponification number		188-194
Unsaponifiable matter (%)		1.5 max
Fatty acid composition (%)		
Myristic	(C14:0)	0.1
Palmitic	(C16:0)	7.0
Palmitoleic	(C16:1)	0.1
Margaric	(C17:0)	0.1
Stearic	(C18:0)	4.5
Oleic	(C18:1)	18.7
Linoleic	(C18:2)	67.5
Linolenic	(C18:3)	0.8
Arachidic	(C20:0)	0.4
Gadoleic	(C20:1)	0.1
Behenic	(C22:0)	0.7

Oils similar in fatty acid composition (that is high in linoleic acid) are safflower oil with 67.8 – 83.2 %, maize oil with 34.0-65.6 % and soybean oil with 49.8-59.0 % linoleic acid, respectively (Codex Alimentarius Commission, 1999).

Oils with high levels of polyunsaturated fatty acids such as sunflower, soybean, safflower and maize are not as stable as oils with higher levels of monounsaturated fatty acids as illustrated earlier in Table 4. When comparing the hours of resistance to oxidation at high temperature as in Table 4, sunflower and soybean oil have the least resistance to oxidation at 100 °C.

Minor components present in sunflower oil are waxes, hydrocarbons, sterols at relatively high levels and natural antioxidants at relatively low levels (Sonntag, 1979a). The natural antioxidants are of interest as they have some effect on oil stability, as discussed earlier in section 2.1.2. The level of natural antioxidant present distinguishes between good quality sunflower oil and sunflower oil that has been pressed from aged, bad quality seed or that underwent harsh processing conditions. Alpha-tocopherol is the principal natural antioxidant present in sunflower oil at levels of 403-935 mg/kg (96 %), followed by β -tocopherol at not detected (ND)-45 mg/kg (2.5 %) and γ -tocopherol at ND-34 mg/kg (1.5 %) where the values in brackets are % of total tocopherols (Sonntag, 1979a). The total tocopherols range from 440 to 1520 mg/kg (Codex Alimentarius Commission, 1999).

2.4 SHELF-LIFE PREDICTION AND MODELLING METHODS

According to Giese (2000), the shelf-life of food is the period of time that a product is acceptable and meets consumer expectations regarding its quality. During storage of food products, various physico-chemical reactions may occur, resulting in changes in sensory and nutritional qualities (Rustom, López-Leiva and Nair, 1996). There is therefore a need to determine how long a product will maintain its commercial value on the shelf. Predictive modelling can do this by estimating shelf-life. Modelling is when a specified set of dependency relationships is tested empirically and a comprehensive representation, e.g. by a set of structural equations, of the relationships is formalised (Hair, Anderson, Tatham and Black, 1998). The equation of the relationship between the dependant variable (e.g. shelf-life) and the set of independent variables is then used to predict the changes in the dependant variable (e.g. shelf-life) in response to changes in the independent variables (Hair *et al.*, 1998). Predictive modelling is a tool that can be applied to a variety of food types.

2.4.1 Value of predictive tests

Predictive modelling is used to estimate the shelf-life of foods. It is often used in the prediction of microbiological deterioration, where it relies on mathematical equations that predict the rate of growth or decline of micro-organisms under a given set of conditions (Garcia-Gimeno and Zurera-Cosano, 1997). Garcia-Gimeno *et al.* (1997) investigated the use of predictive modelling to estimate the shelf-life of ready-to-eat plant salads. The shelf-life of vegetable salads is normally determined by loss in sensory qualities and a more objective

method to establish shelf-life was desired. This was done by taking the growth rate of the spoilage bacteria and the storage temperature into account in order to prepare a predictive model. Sensory evaluation and the maximum bacterial counts were used as acceptability criteria. An equation used by Ratkowsky as described in the study was used to prepare the predictive model. Rustom *et al.*, (1996) developed shelf-life prediction models for UHT-sterilized peanut beverages based on data of sensory properties and kinetics of physicochemical changes such as pH, viscosity, homogenisation index, colour lightness and sedimentation index. Correlation coefficients between sensory attributes and physicochemical properties were calculated and regression analysis was used for the prediction modelling. A similar study on ultra-high temperature soy beverage was done by Narayanan, Kumar and Patil (1993), whereby various physico-chemical and sensory changes were monitored during storage and a shelf-life prediction model was developed based on the data. Marsili (2000) used multivariate analysis to decipher meaningful trends in the solid-phase microextraction mass spectra data of volatiles extracted from two types of processed milk. Prediction models based on partial least squares (PLS) regression of the two milk types were able to predict the shelf life of the two products within ± 1 day.

Shelf-life prediction models for fats and oils are not based on bacterial deterioration but on intrinsic and extrinsic factors as described earlier under section 2.1.2. A predictive model of Tuscan extra virgin olive oil (EVOO) stability has been developed by Pagliarini, Zanoni and Giovanelli (2000) to monitor product changes during commercial activities. End consumers could apply the model for practical use. Five lots of oil were taken from one batch of EVOO and were subjected to different conditions and treatments to simulate different commercial activities. The different lots were sampled approximately every two months and analysed for the following: FFA, PV, polyphenol content, tyrosol and hydroxytyrosol, alpha-tocopherol, spectroscopic indices in UV and visible regions, induction time as measured by Rancimat and sensory evaluation. Principal component analysis (PCA) and PLS analysis were used to process the data. PCA was aimed at finding the simplest model to describe the data set and PLS was aimed at detecting cause-effect relationships. The following parameters were found to be significant for use in the prediction model: hydroxytyrosol and tyrosol contents, carotenoid absorbance at 475 and 448 nm, alpha-tocopherol content, Rancimat induction period and UV absorbance at 232 nm.

Przybylski and Zambiasi (2000) used Artificial Neural Network Systems (ANNW) to predict the stability of plant oils. Thirty-three plant oils were used in the study. The oils were tested for their fatty acid composition and the following endogenous components: neutral lipids, phospholipids, glycolipids, tocopherols and tocotrienols, sterols, chlorophyll, carotenoids, metals, phenolic acids, triglycerides and FFAs. The oxidative stability of the oils was evaluated by measuring the oxygen consumption during accelerated storage. They found that oil stability could be successfully predicted from a few oil components. The fatty acid composition and the total tocopherol and tocotrienol content were found to be good predictors of oil stability.

Shiers and Adechy (1998) undertook storage trial studies with a number of oils in which the rancidity of the oils was monitored by conventional chemical means (PV and AV) as well as electronic nose technology. Three different electronic nose instruments were compared. The electronic nose instruments were evaluated for their ability to assess the oxidative state of edible oil and from the accumulated data and further investigation, these instruments could possibly be used to predict shelf-life. Data were processed using PCA or discriminant function analysis (DFA).

Shelf-life prediction has a very important place in the food industry as can be seen by the various studies done on different food types. There is a definite need for a simple, rapid test that would provide an assessment of oil quality resulting in a reliable estimate of edible oil shelf-life.

2.4.2 Chemometric techniques

Massart, Vandeginste, Deming, Michotte and Kaufman (1988), and Bailey and Rohrback (1994) define chemometrics as the chemical discipline that uses mathematical, statistical, and other methods employing formal logic:

- (a) to design or select optimal measurement procedures and experiments, and
- (b) to provide maximum relevant chemical information by interpretation of patterns in multivariate data.

Shelf-life prediction could be achieved by using different chemometric techniques.

Multivariate analysis generally refers to all statistical methods that simultaneously analyse multiple measurements on each individual or object under discussion (Hair *et al*, 1998). Thus, any simultaneous analysis of more than two variables can generally be considered as multivariate analysis. The discussion of different multivariate analysis methods follows:

2.4.2.1 Multiple regression analysis

Multiple regression analysis is the simplest of all the multivariate analyses techniques. In multiple regression there is one dependant variable and many independent variables. The objective of regression analysis is to predict the single dependant variable from a set of independent variables (Flury and Riedwyl, 1988; Hair *et al*, 1998). Multiple linear regression (MLR) can be represented mathematically as shown (Geladi and Kowalski, 1986; Hair *et al*, 1998; Kleinbaum, Kupper and Muller, 1998):

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k + \varepsilon_1$$

y = observation is the response (dependant variable),

x_k = the independent variables

k = the number of independent variables

β_0 = the constant term that describes the intercept

β_k = the regression coefficients for each independent variable and

ε = the residual or error between the observed response and the true value.

The equation describes multilinear dependencies for only one set of variables. Where there are n samples, the y vector can be written as a column vector Y , β remains the same and the vectors x form the rows of a matrix X (Geladi and Kowalski, 1986) as follows:

$$Y = X\beta + \varepsilon$$

Graphically it can be represented as:

$$\begin{array}{c} \boxed{Y} \\ n \end{array} \begin{array}{c} 1 \\ \\ \\ \end{array} = \begin{array}{c} \boxed{X} \\ n \end{array} \begin{array}{c} m \\ \\ \\ \end{array} \begin{array}{c} \boxed{\beta} \\ m \end{array} \begin{array}{c} 1 \\ \\ \\ \end{array} + \begin{array}{c} \boxed{\varepsilon} \\ n \end{array} \begin{array}{c} 1 \\ \\ \\ \end{array}$$

n = the number of samples

m = the number of independent variables

Y = $n \times 1$ vector

X = $n \times m$ matrix

β = $m \times 1$ vector of predictor coefficients that describes the relationship and

ε = $n \times 1$ vector of error terms which represents the difference between the dependant variable and $X\beta$ (Van Niekerk, 1990).

An important issue in multivariate analysis is the measurement error. As Hair *et al* (1998) state, all variables used in multivariate techniques must be assumed to have some degree of measurement error. The reliability of the measurement can be incorporated into the relationship between the dependant and independent variables.

The difference between multiple regression and other latent multivariate techniques, such as factor analysis and cluster analysis, is that multiple regression generally has one dependant variable and many independent variables, whereas latent variable techniques have many dependant variables. Thus latent variable techniques emphasise the analysis of interdependence among data sets (Green, 1978).

2.4.2.2 Principal component analysis

PCA is a technique to identify and combine variables that are correlated into linear principal components, which leads to a reduced number of significant combinations that are independent of the other principal components (PCs) (Flury and Riedwyl, 1988; Van Niekerk, 1990; Aries, Lidiard and Spragg, 1991). This reduces large data sets into a new set of variables (PCs). According to Aries *et al* (1991) the potential applications of PCA are:

- a) as a display and classification method by comparing plots of data, which shows variables that are grouped together and when applied to different samples can classify samples into groups
- b) to interpret chemical information where the underlying chemistry is highlighted which would otherwise not been noticed
- c) quantitative analysis of individual components.

PCA is particularly useful in cases where the variables are highly correlated, but makes little sense for weakly related variables (Flury and Riedwyl, 1988). PCA is often an intermediate step towards further objectives and techniques of multiple regression can be applied to it such as principal component regression (PCR) (Aries et al., 1991; Johnson and Wichern, 1992).

2.4.2.3 Factor analysis

Factor analysis is thought to be an extension of PCA and differs from multiple regression by being an interdependent technique, in which all variants are simultaneously considered to describe the possible covariant relationship among many variables in terms of underlying random quantities called factors (Johnson and Wichern, 1992; Hair et al., 1998). The correlations or interrelationships between variables are defined by a set of common underlying dimensions called factors (Hair et al., 1998). The factors are formed to accentuate their explanation of the entire variable set and not to predict a dependant variable (Hair et al., 1998). Factor analysis originated as a way to define and measure intelligence where characteristics such as “verbal ability”, “memory”, “numerical ability” and “intelligence” could not be measured directly (Krzanowski, 1988; Hair et al., 1998). The scores for a battery of various tests would be grouped into factors such as “numerical ability” to best explain the different characteristics. Factor loadings describe the correlations between the original variables and the factors that emerged from factor analysis (Hair et al., 1998; Kleinbaum et al., 1998). The “eigenvalue” represents the amount of variance accounted for by a factor and is the sum of squared loadings for a factor (Hair et al., 1998).

2.4.2.4 Other multivariate techniques

In PLS regression a block of independent variables (X) are related to a block of dependant variables (Y) through a process where the variance structure in the Y block influence the calculation of the linear combination components in the X block and *vice versa* (Vogt, 1987).

It can thus be considered as consisting of two outer relations (X and Y blocks individually) and an inner relation (linking both blocks) (Geladi and Kowalski, 1986).

SIMCA (soft independent modelling of class analogy) is a classification method that is based on latent factors (Martens, Wold and Martens, 1983). It involves separate PCA modelling of each class and the obtained class models are then used for classification by “curve-fitting” of the data of each test object.

There are many more multivariate techniques such as k -nearest neighbour (KNN), hierarchical clustering, ANNW and an important decision is the choice of which multivariate technique to use (Martens *et al.*, 1983). According to Martens *et al.* (1983) this depends on the aim of the analysis, whether it is to predict an unknown response from measured predictors, for data reduction purposes, as classification or as pattern recognition. In this study a dependant variable, namely shelf-life, needs to be predicted from a set of independent variables such as the FFA, PV, AV, OSI, tocopherols, short chain volatiles, IV, UV absorbance at 232 and 268 and sensory evaluation. It appears that the most suitable multivariate technique with a single dependant variable is multiple linear regression.

2.5 SUMMARY

It is clear that the mechanism of oxidation and factors influencing oxidative stability of edible oils have been studied extensively and are reasonably well understood. It is important to differentiate between the two types of rancidity, namely hydrolytic and oxidative rancidity, as the effect on oil is different. Pro-oxidants such as metals, light and temperature promote oxidation and the inherent characteristics of oil namely fatty acid composition and natural antioxidants present affect the stability of oil significantly. However, the influence of a pro-oxidant such as copper on oil during a long-term storage study has not been investigated properly, as most studies on pro-oxidants were conducted with short term storage studies. Similarly, the effect of artificial antioxidants that have been added to oil needs to be investigated during a long-term storage study.

Traditional methods to determine oil quality such as FFA, PV, AV, UV absorbance and accelerated stability tests such as Rancimat are still popular, although direct determination of

short chain volatile components is increasing in importance especially as it correlates with sensory evaluation. There is a clear need to determine how accelerated tests such as Rancimat, correlate with actual shelf-life of oil.

Very few studies have been conducted on predicting the shelf-life of oils. The multivariate methods used were either PCA, PLS and ANNW, which are based on classification and pattern recognition of the parameters determined. Further shelf-life prediction studies are needed for economic reasons and to enlighten our knowledge on this important field of study. Multivariate analysis by multiple regression analysis is an obvious technique to implement in prediction of the single dependant variable, shelf-life, from a set of data and needs to be put to the test.