CHAPTER 5

DISCUSSION

The two oils will be discussed separately and a comparison and explanation of the differences found will be attempted. Under the discussion of each oil type, the variables will be dealt with individually to attempt to clarify the actions and mechanisms behind their behaviour. The interrelationships between the variables will be dealt with at the end of the individual discussions. The discussion on the different models will follow, relating their practical applicability and reliability. The relationship of the oxidative stability test (Rancimat) to shelf-life will be appraised.

5.1   PALM-OLEIN OIL

The results of the fatty acid compositions of the two oils used to prepare the blend of palm-olein oil in the shelf-life trial clearly confirmed their identity. The palm-olein oil used for the storage trial was of good quality as indicated by the results of the FFA, PV and moisture content, which correspond to guidelines by the Codex Alimentarius Commission (1999). The results of the TBHQ analyses indicate that no TBHQ had been added to the oils, which if present could have given a false perception of oil stability.
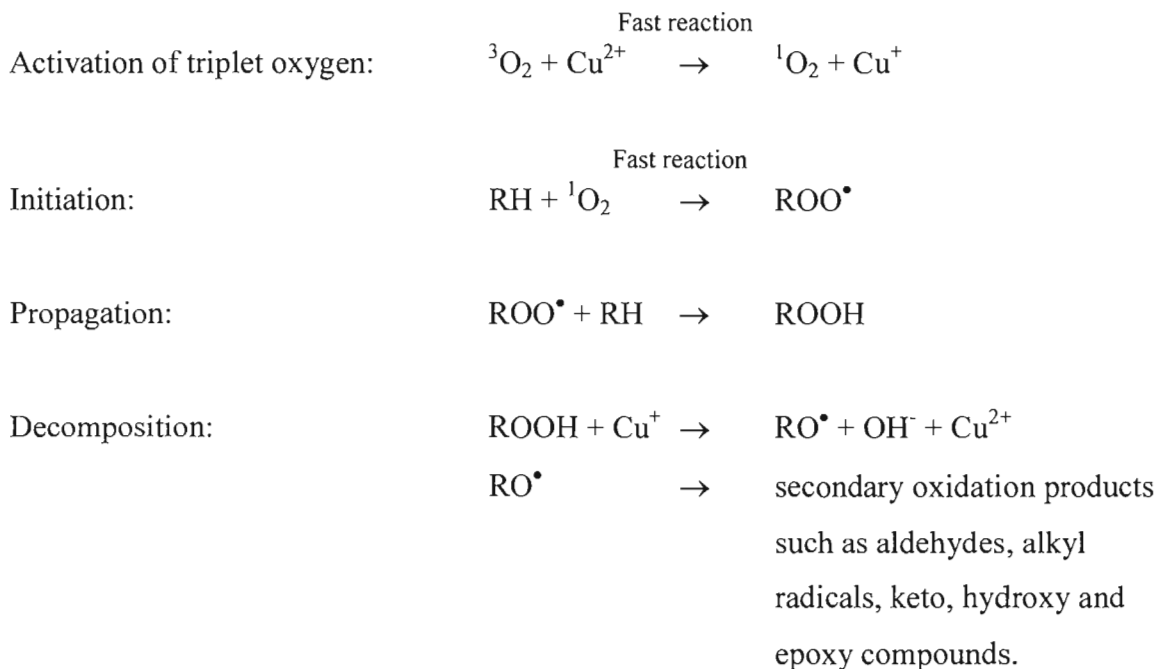
The increased FFA can be ascribed to the effect of hydrolysis when free fatty acids are liberated from the parent oil (Rossell, 1994). The slightly higher FFA of the Control than the copper-containing samples indicates that copper at those levels leads to a slightly smaller increase in FFA content than found in the Control. The fact that the Control sample showed the highest rate of increase in FFA and the highest concentration of copper-containing oil the lowest, was surprising as no effect was expected since copper is a pro-oxidant which promotes oxidation (Gordon, 1990) and not hydrolysis. If any effect in the copper containing samples was anticipated, an increase in FFA would have been more likely as copper could have acted as catalyst in some way. The general increase in FFA was very gradual and although the treatments were statistically significantly different, in practical terms one could argue that they should not be of importance as the FFA values for all the treatments are still within prescribed specifications of 0.3 % oleic acid according to the Codex Alimentarius Commision (1999). This argument has turned

out not to be valid as shown by the selection of FFA in the modelling. According to Chong and Gapor (1985) the presence of moisture can cause the hydrolysis of palm oil triglycerides, resulting in the formation of free fatty acids. They also mention that moisture present in oil hydrates trace metals, which would make the copper unavailable for further reactions and thereby decrease their catalytic activity. However, it was found in this study that variables associated with secondary oxidative products did show an enhanced response with copper thus indicating that the copper was in fact available for reaction. It indicates that the copper did not interact with the moisture in the oil in such a way to cause non-reactivity. Another explanation for the slower increase in FFA levels with copper is that the FFA formed could have oxidised to further oxidation products, as oxidation of FFAs occurs more readily than their glycerol-bound counterparts (Sonntag, 1979b; Kochhar, 1996). The copper would have accelerated the oxidation of the FFAs. Little information is available on the further breakdown of FFA hydroperoxides but according to Kochhar (1996) FFAs do degrade further. This could lead to oxidation products of FFAs that are not determined by titration, thereby causing lower FFA titration values. There is also the possibility that the FFAs formed ester linkages with the higher than normal amounts of secondary oxidation products (such as alcohols) in the copper-containing samples, or alternatively, peroxy esters could have formed with the hydroperoxides, as described by Pokorný, Rzepa and Janíček (1976).

The rate of PV formation was different for the different pro-oxidant levels. The lower PV levels in the copper-containing samples compared to the Control was contrary to what is normally expected. Abdel-Aal and Abdel-Rahman (1986) found an increase in PV level with addition of copper in polyunsaturated oils (cottonseed, sunflower and soybean) stored at 26-28°C with periodic sampling up to 119 days. Benjelloun, Talou, Delmas and Gaset (1991) and Gordon and Mursi (1994) found that metal traces in rapeseed oil increase PV when measured within hours and 20 days, respectively. Chong and Gapor (1985) added iron to palm-olein oil, stored the oil for a period of 26 days at 60°C, and found that with the addition of iron PV increased. The low levels of peroxides in the samples containing copper in the present study can be explained by the fact that this was a long-term oxidation study on monounsaturated oil where the peroxide intermediates were probably converted to secondary, more stable oxidation products within a short time span. Initiation probably occurred via the activation of triplet oxygen to singlet state oxygen. Singlet oxygen reacts 1300–1600 times faster than normal autoxidation (Frankel,
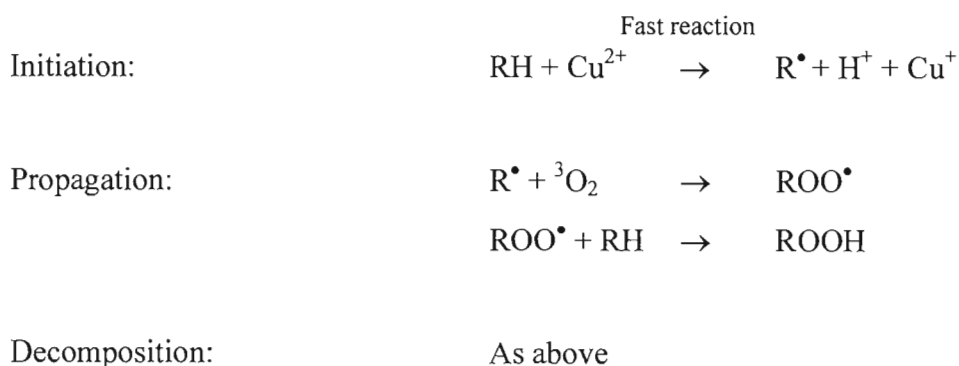
1980; Kochhar, 1996). Metals are thought to cause the activation of molecular oxygen to singlet oxygen (Labuza, 1971; Frankel, 1980; Nawar, 1985). The two proposed mechanisms are:

Mechanism 1:

Activation of triplet oxygen:

$$^3O_2 + Cu^{2+} \xrightarrow{\text{Fast reaction}} {}^1O_2 + Cu^+$$

Initiation:

$$RH + {}^1O_2 \xrightarrow{\text{Fast reaction}} ROO^\bullet$$

Propagation:

$$ROO^\bullet + RH \rightarrow ROOH$$

Decomposition:

$$ROOH + Cu^+ \rightarrow RO^\bullet + OH^- + Cu^{2+}$$

$$RO^\bullet \rightarrow \text{secondary oxidation products such as aldehydes, alkyl radicals, keto, hydroxy and epoxy compounds.}$$

The second proposed mechanism is by direct reaction of the metal with the substrate (Labuza, 1971):

Mechanism 2:

Initiation:

$$RH + Cu^{2+} \xrightarrow{\text{Fast reaction}} R^\bullet + H^+ + Cu^+$$

Propagation:

$$R^\bullet + {}^3O_2 \rightarrow ROO^\bullet$$

$$ROO^\bullet + RH \rightarrow ROOH$$

Decomposition: As above

$$^3O_2 = \text{normal triplet oxygen}$$

$$^1O_2 = \text{excited singlet state oxygen}$$

Initiation is normally the rate-controlling step as it needs large activation energies (Labuza, 1971). In the proposed mechanisms catalysed by copper and singlet oxygen, initiation reactions proceeded at an enhanced rate (1300–1600 times faster than normal autoxidation) as mentioned before. Hydroperoxides are unstable and begin to decompose as soon as they are formed (Nawar, 1985). High levels of peroxides were thus not detected, probably because they were produced within hours, or in the first few days, after addition of the copper. This is supported by the fact that the peroxides in the Control increased, as expected, and then started to decrease. The Control sample reached the suggested PV maximum guideline for a refined oil of 10 meq/kg (Codex Alimentarius Commission, 1999) by Week 6.

The higher AV levels in the copper-containing samples as compared to the Control are as expected when a pro-oxidant is added to oil. Although the differences in AV between the copper-containing samples were statistically significant, practically they would not be meaningful. The AV of the Control sample fluctuated around 10, which is deemed to be the limit for AV (White, 1995), from Week 16 up to Week 40 and reached a value of 15.2 at Week 52 which makes it difficult to assess at which stage the Control could be deemed rancid. All the samples containing copper had exceeded the suggested level by Week 6. It thus appears that the presence of copper led to the rapid formation of excess secondary oxidation products during the early stage of storage.

The significant difference in the intercepts and slopes of the Totox value of the Control versus all the copper-containing samples shows clearly the effect of the copper. The discrepancy in the PV of the Control and copper-treated samples was again reflected in the Totox value. The Totox value was therefore not a true reflection of the oxidative status of the oils in this instance and was misleading when considered on its own.

The effect of the copper can be very clearly seen in the OSI. All the samples differed significantly from each other and it was clear that different levels of copper affected the resistance to oxidation as measured by the OSI. It is evident that copper is a strong pro-oxidant from the low OSI values obtained in the samples containing copper. The resistance to oxidation was compromised from the time of addition. Increased concentrations of copper decrease the OSI concomitantly. Gordon and Mursi (1994) obtained similar results when metal ions were added to rapeseed oil.

Copper had a very pronounced effect on the total tocopherol content with the tocopherol content decreasing concomitantly with higher concentrations of copper. Tocopherols are chain reaction breaking antioxidants that donate their phenolic hydrogen atom to peroxyl (ROO$^\bullet$) or alkoxyl (RO$^\bullet$) radicals formed during the propagation step of oxidation in the presence of copper and iron, thereby quenching further reactions (Bramley et al, 2000). The tocopherols also prevent alkyl radicals (R$^\bullet$) formed during the initiation step from reacting further, by donating a hydrogen and thereby breaking the chain reaction (Frankel, 1980). Because of their antioxidant activity, the tocopherols are themselves altered to yield a number of products such as quinones, dimers, trimers and epoxides (Frankel, 1996; Bramley et al, 2000). The tocopheroxyl radical will only convert back to tocopherol if reducing power provided by water-soluble reductants such as Vitamin C or citric acid is present (Adegoke, Kumar, Krishna, Varadaraj, Sambaiah, Lokesh, 1998; Bramley et al, 2000). Presumably, the copper-containing samples generated more radicals than the Control sample and these radicals reacted with the tocopherols. This explains the rapid decrease in total tocopherol content in the samples containing copper. The small amount of total tocopherols remaining after storage shows clearly the effect of copper and the different concentrations thereof (Table 10). The slight difference in total tocopherols between higher concentrations of copper (0.17 and 0.69 mg/kg) was in practicality not meaningful. The excess radicals formed by the presence of high concentrations of copper could probably not be quenched by the low amount of tocopherols present.

There are contradictory data in literature as to which of the tocopherol homologues is the most effective as antioxidant. Most works seem to consider γ-tocopherol and γ-tocotrienol as the most effective and stable (Chow and Draper, 1974; Top, Ong, Kato, Watanabe and Kawada, 1989; Gottshein and Grosch, 1990; Pongracz, Weiser and Matzinger 1995). Peterson (1995) and Hoffman (1989) mentioned that second-order rate constants for the reaction of tocopherol homologues with free radicals and singlet oxygen decreased in the order of $\alpha > \beta > \gamma > \delta$ with the tocotrienol antioxidant activities measured in the same order. This indicates that δ-tocotrienol is the most effective antioxidant. The discrepancies in literature can be attributed to different substrates, temperatures and antioxidant concentrations of the tests (Frankel, 1996). The hydrogen donating capacity of α-tocopherol is higher than that of γ-tocopherol which means that α-tocopherol should be a more potent antioxidant than γ-tocopherol (Lampi et al, 1999).

However, Chow and Draper (1974) and Pongracz et al (1995) found the order of antioxidant activity of the tocopherol isomers was $\gamma > \delta > \beta > \alpha$. Frankel (1996) also confirms this in an article where various sources report γ- and δ- to be more effective than the β- and α-tocopherols. The fast reaction of α-tocopherol is confirmed by the results of the present study, as it decreased rapidly within the first 6 weeks of storage in the copper-containing samples with none remaining in the two higher concentrations of copper after Week 22. All the α-tocopherol probably reacted with the excess radicals formed by the copper and had been broken down to further oxidation products. It is also possible that a radical formed by the α-tocopherol promoted and enhanced oxidation, as the presence of metals induces the pro-oxidant effect of α-tocopherol (Huang et al, 1995). The small amount of α-tocopherol remaining after 6 weeks in the lowest concentration of copper indicates that rapid reaction occurred within the first 6 week period as the α-tocopherol remained fairly constant after that. This could mean that all the radicals formed at this level of copper had been quenched by the α-tocopherol within the first 6 weeks, after which no further significant amounts of radicals were present to react with. Another possible explanation for the rapid decrease in α-tocopherol is that rapid oxidation could have occurred because of singlet oxygen and α-tocopherol is a singlet oxygen quencher (Frankel, 1980). The α-tocopherol would have reacted with the singlet oxygen, possibly been broken down to further oxidation products and would not have been detected by tocopherol analysis. The decrease in α-tocopherol in the Control is as expected, when normal slow autoxidation, without the presence of pro-oxidants, takes place (Bramley et al, 2000). The α-tocopherol probably reacted with radicals as and when they formed as indicated by the slow decrease of α-tocopherol to approximately half at the end of storage.

Cetin (1989) observed that most studies established the order of antioxidant activity of the tocopherol and tocotrienol homologues to be as follows: α-T $< \alpha$-T3 $< \beta$-T $< \beta$-T3 $< \gamma$-T $< \gamma$-T3 $< \delta$-T $< \delta$-T3 where T = tocopherol and T3 = tocotrienol. It suggests that the tocotrienols are more effective than their tocopherol counterparts. Contrary to this Top et al (1989) suggested that the tocopherols have slightly better or equal antioxidant activity than the corresponding tocotrienols. According to Pongracz et al (1995) the unsaturated side chain of tocotrienols causes only slight differences in their antioxidant effect. They noted that the tocotrienols are slightly more effective in low concentrations and in

contrast in high concentrations less efficient than their counterparts. These contradictory results suggest that the antioxidant activity and reactivity of the tocotrienol homologues are similar to their tocopherol counterparts. This is confirmed by the similar results obtained in this study for α-tocopherol and its counterpart α-tocotrienol.

Gamma-tocotrienol would be expected to be more effective than α-tocopherol and α-tocotrienol (Mäkinen, Eldin, Lampi and Hopia, 2000) at the levels found in this study. However, the γ-tocotrienol decreased at a slower rate than the α-tocopherol and α-tocotrienol. The better stability of γ-tocotrienol when compared to α-tocopherol and α-tocotrienol is clear in that the γ-tocotrienol was only depleted by Week 52 for the two highest concentrations of copper. The γ-tocotrienol had approximately twice the stability of α-tocopherol and α-tocotrienol in the Control and 0.035 mg/100g copper samples. Huang et al (1995) suggested that the depletion period of tocopherols is related to the period that they remain effective as antioxidants. This means that the γ-tocotrienol in this study was generally not as effective as α-tocopherol and α-tocotrienol.

The small and slow decrease in δ-tocotrienol in the Control and 0.035 mg/100g copper samples indicate that δ-tocotrienol was the most stable homologue. The effect of copper on the samples containing higher concentrations of copper was not severe, as the δ-tocotrienol of the two high copper-containing samples had only decreased by approximately half at the end of storage. If, as according to Huang et al (1995), the depletion period of tocopherols is related to the period during which they remain effective as antioxidants, the δ-tocotrienol was still effective as antioxidant at the end of the storage period even with the high concentrations of copper present. This means that δ-tocotrienol is surprisingly stable against the pro-oxidant effect of copper. This can be ascribed to various reasons, either the products formed from the δ-tocotrienol-peroxy radicals acted themselves as antioxidants, or α-tocopherol and α-tocotrienol could have reacted with the δ-tocotrienol-peroxy radicals to form radicals themselves and thereby spared the δ-tocotrienol (Huang et al, 1995). It seems contradictory that δ-tocotrienol, which is thought to be very effective as antioxidant (Cetin, 1989), remained at such high levels when a strong pro-oxidant was present. Yoshida et al (1993) evaluated the antioxidant effects of tocopherol homologues at different concentrations on the oxidative

stability of purified substrate oils when heated in a microwave oven. They found that δ-tocopherol was significantly less effective than the other tocopherols. The highest antioxidant activity was seen in α-, followed by β- or γ- and δ-tocopherols. Delta-tocopherol had the highest relative stability, followed by β- and α-tocopherols in decreasing order. These findings are in accordance with the results of this study and it can be concluded that δ-tocotrienol is not an effective antioxidant in the circumstances of this study, whereas α-tocopherol and α-tocotrienol were the most reactive. This was not only in the instances where copper was present but also for the Control sample.

Conjugated dienes is a measure of the shift in the double bond in the initial stages of oxidation of linoleate (18:2) or higher polyunsaturated fatty acids (White, 1995). High CV values are thus not expected for monounsaturated oils. This is supported by Yoon, Kim, Shin and Kim (1985) who found that this method was not a suitable assay for oxidation of palm-olein oil when they heated the oil at 180°C for 50 h. The fact that the CV increased only slightly during the storage period of palm-olein oil in this study was thus expected. The slightly higher values of the Control oil can be explained as a result of rapid breakdown of the hydroperoxides formed in the copper-containing samples to secondary oxidation products, caused by the catalytic effect of copper. The initial higher CVs of the copper-containing samples support this, as it indicates that conjugated compounds were formed during initial oxidation as measured at Day 0. The CVs were subsequently been broken down to secondary oxidation products, as the lower values indicate in the following weeks. The small differences found between the copper-containing samples would not be of practical importance.

Absorbance at 268 nm measures conjugated trienes, as well as secondary oxidation products such as ethylenic diketones and conjugated ketodienes (White, 1995). It can generally be correlated with *p*-anisidine (Noor and Augustin, 1984). The slight increase in conjugated triene value of the Control during storage is as expected in oil with low levels of C18:3 (0.17 %). Yoshida, Hirooka and Kajimoto (1990) correlated an increase in conjugated diene and triene values with increased C18:3 content. The higher increase in conjugated triene values of the copper-containing samples cannot be attributed to C18:3 content and is most likely due to secondary oxidation products such as ethylenic diketones, conjugated ketodienes and dienals (Noor and Augustin, 1984).

The iodine value is not a very sensitive measure of oxidation, as can be seen by the fact that oxidation of the unsaturated fatty acids was not to such an extent that it could be detected by the iodine value, whereas most of the other variables did show definite changes during storage. It is also generally accepted that a noticeable change in IV will only be noticeable once gross deterioration of an oil has occurred (Noor and Augustin, 1984).

The increase in total volatile peak area was to be expected, as volatiles are formed from the decomposition of hydroperoxides of unsaturated fatty acids (Przybylski and Eskin, 1995). The indistinct differences in response between the sample treatments was probably because of different volatile compound ratios and possible breakdown of volatiles formed in the copper-containing samples. Typical palm-olein contains 40-44 % 18:1 (oleic acid) and 10.4-13.4 % 18:2 (linoleic acid) and only a small amount of 18:3 (linolenic acid) 0.17-0.6 % (Codex Alimentarius Commission, 1997). Two- or 3-Hexenal are formed mainly from linolenic acid (Przybylski and Eskin, 1995; Kochhar, 1996) and this explains why little change in trans-2-hexenal concentration was observed over the storage period. According to Kochhar (1996) the formation of hexanal is from the decomposition of linoleate 13-OOH hydroperoxide and the formation of t,t-2,4-decadienal is because of decomposition of 9-OOH hydroperoxide. Secondary decomposition of t,t-2,4-decadienal leads to hexanal formation (Przybylski and Eskin, 1995). The high initial hexanal values for the sample containing 0.69 mg/kg copper could have been due to immediate decomposition of t,t-2,4-decadienal to hexanal, followed in time by further decomposition of hexanal to hexanoic acid and peroxides (Przybylski and Eskin, 1995). The increase in t,t-2,4-decadienal in the samples containing copper was most likely due to the decomposition of 9-OOH hydroperoxides (Kochhar, 1996) that were formed in the presence of copper. Some of the t,t-2,4-decadienal would have decomposed further to hexanal. The Control probably oxidised preferentially via the formation of 13-OOH hydroperoxides to hexanal. Andersson and Lingnert (1998) found that the hexanal as well as 2-hexenal content increased with addition of copper during storage of rapeseed oil at 40°C for a period of 35 days. This is expected in oils that contain high levels of linoleic and linolenic acids. It is clear that monounsaturated oils behave differently. According to Przybylski and Eskin (1995) an increase in pentanal is because of decomposition of linoleate 13-OOH hydroperoxide, which explains the increase in pentanal peak areas of all the samples. The higher pentanal formation in the copper-containing samples than the

Control was possibly because of initiation by singlet oxygen since metals are thought to cause the activation of molecular oxygen to singlet oxygen as mentioned earlier.

The sensory evaluation results for Option 1 (Fresh 1, Rancid 2+3+4) were more discriminatory than Option 2 (Fresh 1+2, Rancid 3+4) (Table 10). Increasing the number of panelists would have probably enabled more convincing results. The samples had been stored at –20°C at the end of their individual storage times, for periods up to 7 months. Slight freezer odours could possibly have been imparted to the samples, thereby obscuring differences between sample treatments and the differences between samples at each storage time. This could explain the disagreement between the rancidity point as judged by chemical analyses (PV = 20-25 meq/kg according to Yousuf Ali Khan et al (1979) and AV of 10, according to White (1995) and the sensory evaluation. According to the chemical parameters the Control was rancid at Week 22 and all the copper-containing samples at Week 6, contrary to the sensory evaluation where the Control was deemed rancid at Week 52, the 0.035 mg/kg copper sample at Week 40 and the 0.17 and 0.69 mg/kg copper samples at Week 26.

There were clear interrelationships between the different variables, as the reactions that occurred were dependant on each other. Garrido et al, (1994) found that a positive correlation exists between oil acidity and PV, as both parameters are related to the occurrence of rancidity. The type of rancidity is different though, as FFA is associated with hydrolytic rancidity and PV with oxidative rancidity. In this study both the FFAs and PVs were found to be less in the copper-containing samples than the Control, which was an unexpected result for both. The PV, hexanal and conjugated diene values followed similar trends with low values for the samples containing copper and higher values for the Control sample. It is of interest to note that the PV-hexanal relationship is confirmed by the 0.17 mg/kg copper sample that displayed increased values at Week 52 for both variables, as well as a decreased OSI value. The increase in PV and hexanal towards the end of the storage trial in the copper-containing samples (apart from PV of the 0.69 mg/kg sample) can be explained by oxidation of oleic acid and its further degradation to hexanal via its oxidation products. PV and conjugated diene value are measures of primary oxidation products (White, 1995; Hahm and Min, 1995) and according to these results it appears that hexanal formation was also caused by primary oxidation of linoleic acid, which is more susceptible to oxidation than oleic acid and would thus be oxidised

preferentially before oleic acid (Kochhar, 1996). AV, t,t-2,4-decadienal, pentanal and conjugated triene value had inverse trends compared to those for PV, hexanal and conjugated dienes. The AV and t,t-2,4-decadienal relationship is expected as the AV procedure measure aldehydes and primarily 2-alkenals such as t,t-2,4-decadienal (Chong and Gapor, 1985). AV and conjugated trienes measure the secondary oxidation products (White, 1995) and thus it appears that pentanal formation was due to secondary oxidation of the primary oxidation products formed. It is therefore evident that oxidation in the copper-containing samples progressed rapidly from primary oxidation products to the secondary oxidation products. The relationship between the OSI value and the tocopherols is evident in that the OSI value decreased with a decrease in tocopherols. According to Frankel (1985) t,t-2,4-decadienal is one of the more significant flavour volatiles with low threshold values (0.04-0.3 mg/kg), followed in order by n-hexanal, n-pentanal and 2-hexenal. The sensory evaluation test conducted on the palm-olein was not as sensitive and panelists only detected rancidity once levels of approximately 50 mg/kg t,t-2,4-decadienal had been reached for the copper-containing samples. However, the Control did not appear to have formed t,t-2,4-decadienal, although it might have been present at levels below the detection limit.

It had to be decided which were the best values to use in the modelling, either normal values, squared values or weighted normal or weighted squared values (Models 1-4) to create the Ideal, Practical and OSI Models (Tables 11-14). The deciding factors used to select the values to be used were based on the R^2, F, Std error of estimate values and the general p-levels obtained for the regression coefficients. The R^2, F, Std error of estimate values and the range of p-levels obtained for the regression coefficients for the normal versus weighted normal variable values were the same. The values of the deciding factors of the squared versus weighted squared variable values were also all the same. The normal and squared normal values were used in further modelling as the weighted values did not appear to markedly improve the modelling. The combination of normal and squared values did give an improvement in the modelling correlation coefficient as some variables did not increase or decrease linearly, but with a curved regression line during the storage period.

The Practical model (Model 6), surprisingly seems to be a slightly better model than the Ideal model (Model 5). This can be seen from the correlation coefficients and the spread

around the regression line of the observed versus predicted values (Figs. 27 and 28). The jackknifing testing of the two models indicated that the two models turned out to be very similar with the Practical model having slightly fewer predicted values that exceeded the more than 4 week prediction fault (Table 21 and Fig. 33). The Practical model with a R^2 of 0.9546 would be the preferred model to use in this instance. The OSI values could not be used on their own to predict the shelf-life of palm-olein oil, as can be seen in the OSI model (Model 7). This is clear from the poorer R^2 (0.6485), higher standard error of estimate (4.56) and dispersion of observed versus predicted values around the regression line (Table 17 and Fig. 29). The jackknifing results showed significant dispersion of predicted values against observed values (Table 21 and Fig. 33) and compared poorly with the Ideal and Practical models.

The Ideal model based on sensory evaluation (Model 8) had the best R^2 (0.9891) and high F-values (350.4) (Table 18) when compared to the other models. The jackknifing results indicated that the Ideal model (based on sensory evaluation) fared marginally better compared to the Practical model (based on sensory evaluation) (Model 9) as the lower standard error of estimate values, 3.60 versus 4.09, respectively (Table 22) and distribution around the mean (Fig. 34) indicated. However, the Ideal model did show a high percentage cases where the shelf-life was overpredicted (< -4 weeks). The OSI model (based on sensory evaluation) (Model 10) once again was not a good prediction model when the OSI values were used on their own with a R^2 of 0.5495 (Table 20) and poor distribution around the regression line of predicted versus observed values (Fig. 32). The jackknifing results compared poorly to the other two models (Table 22 and Fig. 34).

The drawback of the models based on the PV and AV values is that the number of cases is very limited (only 12) which causes the models to be sensitive to small changes. The models based on sensory evaluation probably did better because of the higher number of cases (35) used in the modelling.

The importance of OSI is clear as all the models consistently selected OSI, either the straight value, squared value or both, as a predictor but, as discussed, it cannot be used on its own in a prediction model, therefore confirming the claims of Warner et al (1989) and Reynhout (1991). Warner et al (1989) used PV, volatiles, Rancimat and AOM to measure oxidative stability, which were correlated with sensory analysis and found that a variety

of evaluation methods is necessary to enable prediction of oil stability. Reynhout (1991) stated that correlating the induction period to a shelf-life period for a food system would not take into account the effect of other data determining elements. The FFA (straight or squared value) was also, surprisingly, included in the all the models. It thus appears that small changes in FFA content are highly important. The selected variables for the Ideal model (based on AV and PV) (Model 5) were AV, OSI, OSI^2 and FFA, whereas the selected variables for the Practical model (based on AV and PV) (Model 6) were conjugated triene value, OSI, OSI^2 and FFA^2. The Practical model based on sensory evaluation (Model 9) correspondingly selected the conjugated triene value, OSI^2 and FFA^2 as predictors. These results were very similar as both AV and conjugated triene value determine secondary oxidation products (White, 1995). It seems that a variable that measures secondary oxidation products, a variable that measures hydrolysis and a variable that determines resistance to oxidation or oxidative stability were included in prediction modelling. No primary oxidation products were included in the models, presumably because of the rapid degradation effect of copper on the primary oxidation products. The only tocopherol homologue that was selected was alpha-tocopherol and it was found in models (Models 2 + 4) that used the squared values (normal and weighted) as well as in the Ideal model based on sensory evaluation (Model 8). This could be because alpha-tocopherol is the most reactive to oxidation of the homologues determined. The short chain volatiles were also found not to be important predictors. The two volatiles determined that were selected were total volatiles and pentanal. Both were selected in the Ideal model based on sensory evaluation (Model 8) along with OSI, FFA, AV, alpha-tocopherol^2 and copper^2. Notably is that this is the only model that found a correlation between copper content and shelf-life. The inclusion of the two volatiles can be explained by the fact that this model was based on sensory evaluation and it is thus not surprising that the total aromatic volatiles would correlate with sensory evaluation. In addition, pentanal has a low odour threshold of 0.24 mg/kg (Kochhar, 1996), which indicates that it could be detected by a sensory panel at low levels. The shelf-life prediction model of monounsaturated olive oil developed by Pagliarini et al, (2000) selected hydroxytyrosol and tyrosol, carotenoid absorbance at 475 nm and 448 nm (which were only applicable for olive oil) and alpha-tocopherol content, OSI and UV at 232 nm. The selection of OSI and alpha-tocopherol is in agreement with this study, whereas the UV at 232 as a measure of primary oxidation products was not included in our study probably due to the effect of copper addition.

The present study suggests that copper addition modified the normal rate and/or route of oxidation. The types and levels of oxidation products found in the different treatments indicated an altered route of oxidation. It was shown that sensory evaluation was very valuable to detect the point of rancidity when traditional chemical parameters tended to be misleading. However, it is known that sensory evaluation is time consuming and costly and relevant chemical parameters should ideally be applied as practical alternative.

5.2   SUNFLOWER SEED OIL

The results of the fatty acid analysis of the sunflower seed oil used in the shelf-life trial clearly confirmed its identity (Results, Table 23). Further, the oil used for the trial was of good quality as indicated by its FFA, PV and moisture contents, which conform to the guidelines of the Codex Alimentarius Commission (1999). The results of the TBHQ analyses indicate that no TBHQ had been previously added to the oil.

The increase in FFA content in all the samples, namely the Control, 54, 217 and 435 mg/kg TBHQ containing samples, indicates that hydrolysis took place during storage. The lower rate of increase in samples containing TBHQ concurrent with increased TBHQ concentrations suggest that TBHQ has some protective effect against hydrolysis of oil. During oxidation acids such as formic and acetic acid are normally formed from intermediate oxidation products (Kochhar, 1996). These acids would be titrated as FFA, thereby increasing the apparent FFA value, although it would not necessarily all be contributed because of FFA formed by hydrolysis. The lower FFAs in the TBHQ-containing samples could be explained by the fact that TBHQ would inhibit oxidation and thereby the contribution of intermediate secondary acids formed, leading to lower FFA values. Little information is available in the literature on the mechanism of protection of antioxidants against hydrolytic rancidity, as most studies focus on the protective effect of antioxidants against oxidative rancidity. However, Robards et al, (1988) did mention in a review that there is no additive which will effectively prevent FFA formation, as antioxidants capable of delaying the onset of oxidative rancidity will not prevent chemical hydrolysis. As discussed, TBHQ inhibited oxidation and thereby formation of the secondary oxidation products such as short chain acids that would normally be tritrated as FFAs.

The protective effect of TBHQ on oxidative rancidity was clear from the slower rate of increase in PV during storage of the TBHQ-containing samples. The slower rate of increase was concurrent with higher concentrations of TBHQ. In fact, oxidation of the Control at Week 40 had reached a stage where secondary oxidation products were formed at a slightly increased or constant rate, in contrast to the decrease in primary oxidation products. Crapiste et al, (1999) also reported sharp AV increases following the decomposition of peroxides. This could account for an increased rate of secondary oxidation products formed from primary oxidation intermediates, leading to decreased PV with constant or increased levels of AV. In contrast, 18 weeks after the Control had reached its plateau, the TBHQ-containing samples had not reached that stage and their PVs were still increasing slightly with signs of leveling off.

Similar trends were observed for AV. The protective effect of TBHQ was concurrent with higher concentrations thereof. As AV measures secondary oxidation products it would be expected that AV would increase with a decrease in PV. However, in the Control this was not the case and it could be that the secondary oxidation intermediates formed from peroxides were not 2-alkenals. The AV method mainly measures 2-alkenals as the molar absorbance increases by a factor of four to five if the aldehyde contains a conjugated double bond to the aldehyde (White, 1995; O'Brien, 1998). Other secondary oxidation products formed such as saturated aldehydes and alkenes would thus not be detected at the same level by the AV method.

The higher OSI values of the TBHQ-containing samples in comparison to the Control reflect the enhanced resistance to oxidation with increased TBHQ concentrations. However, interpretation can be difficult as several papers indicate that OSI is misleading when volatile antioxidants are present (Rossell, 1992; Frankel, 1993b; Hill, 1994). This is because they exert an influence only in the early stages of the analysis, up to the time that they have been volatilised and swept over from the oil into the conductivity cell (Rossell, 1992). According to Frankel (1993b) the mechanism of lipid oxidation at elevated temperatures is significantly different from ambient temperatures and OSI results would thus be unreliable and unrepresentative of actual oxidation at ambient temperatures. Another concern regarding OSI is that the induction period measurement is based on volatile secondary oxidation products that are formed after the onset of oxidation and can thus slightly overestimate the resistance of oil to rancidity (Rossell, 1994). OSI is thus

useful as a comparative measurement between the same oil types but is difficult to correlate to actual shelf-life on its own.

The consistently slightly higher tocopherol contents of the TBHQ-containing samples than in the Control show that TBHQ either had a protective effect on tocopherol oxidation or that peroxyl radicals reacted preferentially with TBHQ, thereby sparing the tocopherols. Tocopherols and TBHQ both have the same antioxidant mode of action, whereby they act as free-radical terminators by donating hydrogen from their phenolic hydroxyl groups (Giese, 1996). They could thus have competed as antioxidants for the peroxyl radicals and it seems that TBHQ was the more reactive, as seen by the lower tocopherol content in the Control and preserved tocopherol contents in the TBHQ-containing samples. The fact that the total tocopherols in all the samples only decreased marginally during storage indicates that the tocopherols had either not reacted significantly with the free radicals formed, or reacted and the tocopheroxyl radicals formed subsequently reconverted back to their original structure, as described by Bramley et al (2000). Alpha-tocopherol was the predominant tocopherol homologue present and thus the trend and interpretation of its changes is the same as for the total tocopherols. No conclusions could be drawn from the β-tocopherol as it was present at low levels, which lead to poor analytical repeatability. The same applies for the changes in γ-tocopherol, preventing correlation with TBHQ levels. Chow and Draper (1974) found that α-tocopherol and α-tocotrienol were destroyed faster than γ-tocopherol and γ-tocotrienol when oil was heated at 70°C with aeration. The present study suggests that γ-tocopherol was less affected by the oxidative conditions than α-tocopherol.

The lower conjugated diene values in the TBHQ-containing samples when compared to the Control again illustrates the protective effect of TBHQ against primary oxidation. The increase in conjugated diene values was expected since polyunsaturated fatty acids such as linoleic acid, which is high in sunflower seed oil (48.3-74.0 %; Codex Alimentarius Commission, 1997), form linoleic hydroperoxides that contain a conjugated diene group which absorbs at 234 nm (Nawar, 1985; White, 1995). The fact that the conjugated triene values were fairly constant during storage, in contrast to the increase in AV, was surprising as both measure secondary oxidation (White, 1995). This could be due to the different secondary oxidation products measured by these two tests, namely primarily 2-

alkenals in the case of AV, whereas the conjugated triene test measures ethylenic diketones, dienals, trienes and conjugated ketodienes (Noor and Augustin, 1984). According to Robards et al (1988) conjugated ketodienes absorb in the 260 nm region. Wanasundara, Shahidi and Jablonski (1995) mention that it is particularly the diketones that absorb at 268 nm. This corroborates that different secondary oxidation products are determined by the two methods.

The insignificant change in IV shows that, as with the palm-olein, it is not a sensitive assay to determine oil oxidation, as gross deterioration of oil has to occur before it will be reflected in the IV (Noor and Augustin, 1984).

The total volatile peak areas show that TBHQ had a protective effect on the sunflowerseed oil during storage with the slower volatile formation in the TBHQ-containing samples. The general increase in volatiles was expected as volatiles are formed by decomposition of unsaturated fatty acid hydroperoxides (Przybylski and Eskin, 1995). The decrease in total volatiles in the Control at Week 52 is probably due to decomposition of the volatiles to further breakdown products such as very short chain volatiles (< C4), not determined by the volatile analysis method. The predominant volatile produced from the 13-OOH hydroperoxide of linoleate (C18:2) is hexanal (Przybylski and Eskin, 1995; Kochhar, 1996), which is in agreement with the results found and is consistent with the high levels of linoleic acid in sunflower seed oil (Codex Alimentarius Commission, 1997). The protective effect against oxidation of increased concentrations of TBHQ is clear. The decrease or plateau reached in hexanal content can be explained by its known further decomposition to other compounds such as hexanoic acid and peroxides (Przybylski and Eskin, 1995). The low values obtained for t-2-hexenal in all the samples seems to be because linolenic acid (C18:3), of which the content in sunflower seed oil is very low (0.08%, Codex Alimentarius Commission, 1997), is the main precursor for t-2-hexenal formation (Przybylski and Eskin, 1995; Kochhar, 1996). Trans,trans-2,4-decadienal is produced from 9-OOH linoleate (Przybylski and Eskin, 1995; Kochhar, 1996). The preferential mechanism of peroxide formation was possibly at the C13 carbon that produces hexanal (Przybylski and Eskin, 1995; Kochhar, 1996) and not at the C9 carbon, thus leading to lower levels of t,t-2,4-decadienal in comparison to hexanal. The low levels of t,t-2,4-decadienal found in all the samples could also be explained by low sensitivity of the volatile method, as t,t-2,4-decadienal consists of a

longer carbon chain than the other volatiles determined and would consequently not be as volatile or easily removed from the oil as the shorter chain volatiles determined. Differences in solubility and partial pressures can affect the transfer of volatiles (Przybylski and Eskin, 1995). This means that volatiles in the headspace may not always accurately reflect the composition of the same components in the lipid sample (Przybylski and Eskin, 1995). In addition, secondary decomposition of t,t-2,4-decadienal to hexanal (hexanal being the preferential decomposition volatile compound formed) (Przybylski and Eskin, 1995) would lead to increased levels of hexanal with lower levels of t,t-2,4-decadienal. The increase in pentanal formation was probably due to decomposition of 13-OOH linoleate, confirming the preferential mechanism suggested above by Przybylski and Eskin (1995). The decrease at the end of storage in the Control and 54 mg/kg TBHQ samples could possibly be due to further decomposition of the pentanal to shorter chain components.

As with the palm-olein oil the sensory evaluation results of Option 1 (Fresh 1, Rancid 2+3+4) were more discriminatory than Option 2 (Fresh 1+2, Rancid 3+4). The same reasoning applies for the sunflower seed oil sensory evaluation as for the palm-olein oil in terms of the necessity to increase the number of panelists and avoid freezer odours that could have been imparted on samples stored at –20°C until time of sensory evaluation. Sensory evaluation deemed the oils rancid at an earlier stage in the shelf-life study compared to when rancidity was based on chemical parameters. According to the chemical parameters (PV = 20-25 meq/kg according to Yousuf Ali Khan et al, (1979) and AV = 10 according to White (1995) the 435 and 217 mg/kg TBHQ samples were acceptable for 23 and 5 weeks longer, respectively, than in the sensory evaluation. However, the Control and 54 mg/kg TBHQ samples gave only three weeks difference between onset of rancidity based on sensory and chemical parameters.

Clearly the different parameters are linked to some extent, as the reactions that occurred were dependent on each other. The positive correlation between acidity and PV is because both parameters are related to the occurrence of rancidity, although as discussed different aspects of rancidity, namely hydrolytic rancidity and oxidative rancidity are involved (Garrido et al, 1994). The primary oxidation parameters, namely PV and conjugated diene values, both increased although PV increased faster than the conjugated diene value. The determination of primary oxidation in sunflower seed oil by PV is

therefore a more sensitive parameter than conjugated diene value. The relationship between the secondary oxidation parameters, namely AV and conjugated triene value was not significant as AV increased, whereas conjugated triene value hardly changed during storage. This could be due to different secondary oxidation products measured by the two methods (Noor and Augustin, 1984) as mentioned earlier. AV would be a more reliable method to determine the occurrence of secondary oxidation in sunflower seed oil. As Totox is a combination of primary and secondary oxidation products (PV and AV) it should give a good indication of oil rancidity in sunflower seed oil. PV and AV were also related to each other as both increased during storage, although PV seemed to decrease towards the end of the storage period, whereas AV continued to increase as the rate of decomposition of primary oxidation products to secondary oxidation products increased. The resistance to oxidation, as determined by OSI, illustrates the protective effect of TBHQ well but did not seem to correlate with tocopherol content, which is also related to resistance to oxidation. Hexanal content seemed to correspond well with AV, as both increased initially and had reached a plateau or started to decrease towards the end of storage. Similar trends for total volatile and pentanal peak areas were observed. This would be expected as volatiles are secondary oxidation products like AV (Kochhar, 1996; White, 1995). The prescribed maximum level of TBHQ according to Codex Alimentarius Commission (1999) is 120 mg/kg. Levels used in this study indicated that higher levels of TBHQ gave progressively better protection against oil oxidation. However, relatively low levels of 54 mg/kg showed marked protection against oil oxidation compared to the Control, which would indicate that levels of the prescribed 120 mg/kg TBHQ should give adequate protection against oxidation.

The sensory evaluation data agrees to some extent with the volatile analyses, as at Week 29 when the oils were generally deemed rancid by sensory evaluation, the total volatile peak area started to increase gradually. Trans,trans-2,4-decadienal, which in soybean oil, is thought to be one of the volatiles with a low flavour threshold (Frankel, 1985), was detected at Week 34 but could have been present at Week 29 at very low levels without being detected. Pentanal peak area also started to increase gradually at Week 29, whereas hexanal only showed a clear increase at Week 40. No clear distinction was made between the Control and TBHQ-containing samples in the sensory evaluation, whereas chemical parameters did distinguish between the sample treatments, although as mentioned according to the chemical parameters the oils reached rancidity levels a few weeks after

the sensory evaluation deemed the oils to be rancid. In this study, sensory evaluation was more sensitive than the chemical parameters and the sensory evaluation seemed to be supported by the volatile analyses, which is logical, as volatiles are the compounds that impart rancid flavour to oil.

It was decided, based on the R^2, F, Std error of estimate values and the general p-levels obtained for the regression coefficients of Models 1-4 (Tables 11-14), to use normal and their squared values in the Ideal, Practical and OSI models. The R^2, F, Std error of estimate values and the range of p-levels obtained for the regression coefficients for the normal and squared values versus weighted normal and squared variable values were the same. The combination of normal and squared values did give an improvement in the modelling correlation coefficient, as some parameters increased or decreased in a non-linear, as opposed to linear, manner.

Of the models where onset of rancidity was decided on by using chemical values, the Ideal model (Model 5) including TBHQ was found to be the best in terms of its R^2 and spread around the regression line of the observed versus predicted values. When TBHQ was excluded from the Ideal model (Model 6) its R^2 decreased to a similar value than the Practical model (Model 7). This decrease in R^2 shows that antioxidant levels are an important indicator of polyunsaturated oil stability and shelf-life. However, TBHQ content can be difficult to measure accurately in oils and fats after bulk dosing, as TBHQ tends to be unevenly distributed (own observation). The Practical model (Model 7) seems to be promising with a R^2 of 0.9230 (Table 33) and a standard error of estimate of approximately 4 weeks. The jackknifing results (Table 39, Fig. 66) indicate that the Ideal model including TBHQ values (Model 5) would be the best model, as seen by low standard error of estimate value and the high percentage cases in the 0 to $\pm$ 2 weeks prediction error range. The Practical model (Model 7) performed less well than the Ideal model excluding TBHQ (Model 6), as can be seen from the spread around the plot that shows the prediction error in percentage cases in each week-category (Fig. 66). The Ideal model excluding TBHQ (Model 6) had fewer cases in the $\pm$ 2 to $\pm$ 4 and more than 4 - week categories than the Practical model (Fig. 66). The OSI model (Model 8) showed once again, as seen previously in palm-olein oil, by its low R^2 (0.5483) and spread around regression line of observed versus predicted values (Fig. 61) that OSI could not be used on its own to predict the shelf-life of oil. The standard error of estimate of the jackknifing

results of 9.8 weeks is too high to give a useful indication of shelf-life. The modelling results based on sensory evaluation appeared to give more reliable results, as can be seen by the smaller percentage cases outside the 0 to ± 2 week prediction error range of the jackknifing results (Table 40, Fig. 67).

The parameters that were present in all the models, where they were included in the selected modelling parameters, were FFA and TBHQ content. The importance of FFA as predictor is surprising, as discussed previously, since it is regarded as an indication of hydrolytic rancidity and not oxidative rancidity. The latter normally leads to the volatile components that cause rancid flavours in oil (Przybylski and Eskin, 1995). OSI and/or its square value were clearly also a very important predictor, as it had been included in 10 out of 12 models. However, once again as with palm-olein oil, it has been shown that it cannot be used on its own as a reliable predictor of the shelf-life of oil. Secondary oxidation products such as AV and conjugated triene value failed as predictors, since AV had not been included in any model and conjugated triene value was included in only 2 out of 10 models, namely Model 1 and Model 3, which were the normal and weighted normal values, respectively. However, primary oxidation products as measured by PV were included in 6 out of 10 models and thus are a better predictor of shelf-life than secondary oxidation products. This is probably because primary oxidation products give an indication of oil quality and its potential for oxidation before the oil has reached rancidity. The volatile components were not included in many models; pentanal peak area in 4 out of 10, total volatile peak area in 3 out of 10 and hexanal content also in 3 out of 10 models. The volatile components coincide with the secondary oxidation products measured by AV, as previously discussed and this could possibly explain their exclusion, since AV was also not selected as predictor of oil quality. Surprisingly, the models based on sensory evaluation seldom included the volatile components. Only the Ideal sensory model including TBHQ (Model 9) selected a volatile component, namely pentanal (Table 35). Another unexpected result was the selection of α-tocopherol and total tocopherols in the Ideal Model 9 (Table 35) and α-tocopherol in the Ideal Model 10 (Table 36). Both models were based on sensory evaluation. Thus it appears that the small changes in the tocopherols that occurred during storage must be significant in relation to polyunsaturated oil shelf-life assessment by sensory evaluation. Przybylski and Zambiazi (2000) used mainly oil composition factors instead of oil quality factors to predict the shelf-life of a variety of oils types and found that fatty acid composition, amount of tocopherols and

tocotrienols, chlorophylls and metals were good predictors of oil stability. This can unfortunately not be related to this study as the focus was mainly on oil quality parameters.

Primary oxidation products, as measured by PV, seem to be one of the important predictors of shelf-life. However, the Practical model based on chemical parameters (Model 7) did not include PV as a predictor, whereas the Practical model based on sensory evaluation (Model 11) did. The Practical model based on sensory evaluation appeared to be more accurate in predicting shelf-life (Table 40, Fig. 67) than the Practical model based on chemical parameters (Table 39, Fig. 66) and would be the preferred model if sensory evaluation could be done accurately. This is perhaps because sensory evaluation is said to integrate all factors, as measured by instrumental and chemical methods, into a total perception of flavour intensity and overall quality (Warner, 1995). With a sufficient number of well-trained and experienced panelists, sensory evaluation of oil can be done accurately, and thus might be found to correlate better with the chemical values used to assess oil rancidity. This might not always be practical though, as sensory evaluation is a costly, labour intensive and time consuming undertaking. According to Malcolmson (1995) the basic requirements for a sensory evaluation facility include preparation area, booth area, and training area, which would often not be available.

Table 47: Summary of predictive models obtained for the palm-olein oil (monounsaturated type) and sunflower seed oil (polyunsaturated type)

	Palm-olein oil			Sunflower seed oil		
	Parameters selected	R^2 value	Standard error of estimate	Parameters selected	R^2 value	Standard error of estimate
Models based on chemical parameters:						
Ideal model (all)	AV, OSI^2, OSI, FFA	0.9316	2.28	PV, $TBHQ^2$, FFA^2, OSI^2, $hexanal^2$, total $volatiles^2$	0.9875	1.69
Ideal model (excl. TBHQ)	n/a	n/a	n/a	OSI^2, FFA^2, pentanal	0.9233	3.98
Practical model	UV 268 nm, FFA^2, OSI^2, OSI	0.9546	1.86	OSI^2, FFA^2, FFA	0.9230	3.98
OSI model	OSI^2, OSI	0.6485	4.56	OSI^2, OSI	0.5483	9.50
Models based on sensory evaluation:						
Ideal model (all)	Pentanal, FFA, AV, OSI, alpha $tocopherol^2$, $copper^2$, total $volatiles^2$	0.9891	1.62	FFA, TBHQ, PV, OSI^2, $TBHQ^2$, pentanal, α-$tocopherol^2$, FFA^2, total tocopherols	0.9931	0.92
Ideal model (excl. TBHQ)	n/a	n/a	n/a	FFA, OSI, OSI^2, PV, FFA^2, α-$tocopherol^2$	0.9839	1.32
Practical model	UV 268 nm, OSI^2, FFA	0.9306	3.83	FFA, OSI, OSI^2, PV	0.9691	1.76
OSI model	OSI^2, OSI	0.5495	9.60	OSI^2, OSI	0.1437	8.91

5.3 MONOUNSATURATED OIL *VERSUS* POLYUNSATURATED OIL

Direct comparison between the monounsaturated oil, represented by palm-olein oil, and polyunsaturated oil, represented by sunflower seed oil, is complicated as the oils were stored at different temperatures, 50°C and 30°C, respectively and one was spiked with a pro-oxidant and the other with an antioxidant. If the fatty acid composition of the two oils were used as indicator of stability it would be expected that the polyunsaturated sunflower seed oil should be more susceptible to oxidation than the monounsaturated palm-olein oil (Sanders, 1994). The fact that the Control of palm-olein oil was rancid at Weeks 22 and 52 based on chemical values and sensory evaluation (Table 10), respectively, whereas the Control of sunflower seed oil was rancid at Week 26 for both chemical and sensory rancidity determinations (Table 26), is not a good reflection of their respective stabilities. It has to be kept in mind that storage at 50°C would have accelerated oxidation of palm-olein oil considerably when compared to storage at 30°C. The storage temperatures selected for both oil types were harsh but they were specifically selected to evaluate the two oil types under such harsh but real conditions. If the monounsaturated oil had been stored at 30°C rancidity would have occurred at a much later stage and this shows that palm-olein oil was indeed the more stable oil. However, what did become clear was that FFA and OSI are very valuable predictors of shelf-life for both oil types (Table 47). The fact that the palm-olein oil model selected secondary oxidation products in preference to primary oxidation products in its models, whereas sunflower seed oil selected primary oxidation products in preference to secondary oxidation products (Table 47), is probably due to the acceleration effect of copper on oxidation leading to rapid decomposition of primary oxidation products. The presence of copper in the palm-olein oil imparts important information regarding the mechanism of the adverse effect copper has on oxidation, even on stable monounsaturated oils, since copper is an ever present potential hazard that can be picked up during transport and storage (Willems and Padley, 1985). The suggested maximum limit for the company Unilever's acquisition purposes of 0.05 mg/kg copper in a refined palm-olein could be too lenient and probably needs revision.