Quality parameters for the prediction of mono- and polyunsaturated oil shelf-life

by

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I declare that the dissertation herewith submitted for the PhD Food Science degree at the University of Pretoria, has not previously been submitted by me for a degree at any other university.

ABSTRACT

QUALITY PARAMETERS FOR THE PREDICTION OF MONO- AND POLYUNSATURATED OIL SHELF-LIFE

by

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The primary objective of this investigation was to establish which oil quality parameters would be best suited in the generation of rapid predictive models to predict the shelf-life of mono- and polyunsaturated oils. A secondary objective was to establish if there is a relationship between accelerated oil stability tests (Rancimat) and shelf-life at ambient temperatures. A long-term storage trial was performed on palm-olein oil, representing monounsaturated oil and on sunflower seed oil, representing polyunsaturated oil. The pro-oxidant effect of copper was assessed by addition of copper acetate to palm-olein oil at three different levels. The synthetic antioxidant, tertiary butylhydroxyquinone (TBHQ), was evaluated by addition to sunflower seed oil at three different levels. Palm-olein was stored at 50°C and sunflower seed oil at 30°C for a period of one year. Nine oil quality parameters were measured at 11 time intervals.

Palm-olein oil parameters responded in the following ways: Free fatty acids (FFA) increased gradually for all the samples but remained within acceptable limits. However, surprisingly a slower rate of increase was found in the copper-containing samples, which could be because the FFAs formed in the copper-containing samples oxidised to further oxidation products. The peroxide values (PV) of copper-containing samples were, unexpectedly, much lower than the Control, which can be explained by the fact that in a long-term oxidation study such as this, the peroxide intermediates were probably converted to secondary, more stable oxidation products within a short time span. However, the increases in anisidine value (AV) and ultra violet absorption (UV) at 268

nm for copper-containing samples were higher than the Control as would be expected. Oxidative stability index (OSI, also known as Rancimat) and total tocopherol values for samples containing copper were significantly lower than those of the Control. Delta-tocotrienol was the most stable of the four homologues. The total volatile peak areas increased for all the samples. The pentanal peak areas particularly reflected the pro-oxidant effect of copper by their higher values in comparison to the Control. Hexanal showed higher levels in the Control than the copper-containing samples. In contrast, t,t-2,4-decadienal showed no increase in the Control, whereas the copper-containing samples showed significant increases. The t-2-hexenal values were unaffected. OSI and total tocopherols proved to be valuable indices for assessing monounsaturated oil quality, whereas PV and headspace volatiles can be misleading. AV is useful and small changes in FFA were found to be significant as indicated by its selection in the models. UV absorption is effective in the presence of pro-oxidants. Sensory evaluation confirmed the differences in shelf-life of the Control and copper-containing samples.

The important parameter changes for the sunflower oil were: FFA increased beyond acceptable limits in all the samples, which indicates that hydrolysis took place during storage. There was a lower rate of increase in samples containing TBHQ which could be because TBHQ would inhibit oxidation and thereby the contribution of intermediate secondary acids formed that would be titrated as FFA, would be lower. The protective effect of TBHQ was clearly reflected in PV and AV as the Control had higher values than the TBHQ-containing samples. Higher OSI values were found for the TBHQ-containing samples in comparison to the Control, which reflects the enhanced resistance to oxidation with increased TBHQ concentrations. The decrease in total tocopherols, as well as the homologues was slight, although the TBHQ-containing samples had consistently higher values than the Control. Marginal increases in UV 232 nm and 268 nm values were observed. The total volatiles, hexanal, and pentanal values reflected the protective effect of TBHQ as the Control generally had higher values than the TBHQ-containing samples. Changes in 2-hexenal and t,t-2,4-decadienal showed no trend. Sensory evaluation made no clear differentiation between the different treatments. OSI highlighted the effect of sample treatments correlating with PV, AV and hexanal content. The importance of small changes in FFA only became apparent during modeling.

Three types of prediction models were created by multiple regression analysis: i) Ideal

model including all the variables, ii) Practical model only including easily determined variables such as FFA, PV, OSI, UV absorbance at 232 nm and 268 nm and iii) OSI model used to correlate an accelerated test with shelf-life at ambient temperatures. OSI and FFA were important predictors as they were selected repeatedly by all models. Palm-olein models emphasised secondary oxidation products (AV and UV absorbance at 268 nm), whereas sunflower seed oil models selected primary oxidation products (PV). The preferential selection of secondary oxidation products in palm-olein oil was due to the considerable increase in oxidation reactions catalysed by copper. Antioxidant content emerged as an important predictor of sunflower seed oil shelf-life. OSI did not correlate well with shelf-life for both oil types and cannot be used on its own to predict shelf-life at ambient temperatures. It needs to be complemented by other parameters. The models developed will be applicable for practical implementation in industry to predict the shelf-life of mono- and polyunsaturated oils once additional research and refining have been done. The Practical models would be the easiest to implement, giving a useful indication of shelf-life, although the Ideal models should be more accurate.

UITTREKSEL

KWALITEITS PARAMETERS VIR DIE VOORSPELLING VAN MONO- EN POLI-ONVERSADIGDE OLIE RAKLEEFTYD

deur

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Die hoofdoel van hierdie ondersoek was om te bepaal watter oliekwaliteitsparameters geskik sou wees om vinnige voorspellingsmodelle te genereer om die rakleeftyd van mono- en poli-onversadigde olies te beraam. 'n Sekondêre doelwit was om te bepaal of daar 'n verwantskap tussen die versnelde stabiliteitstoets (Rancimat) en rakleeftyd van olie by kamertemperatuur bestaan. 'n Langtermyn opbergingstoets met palm-oleïen, as mono-onversadigde olie en sonneblomolie as poli-onversadigde olie, is onderneem. Die pro-oksidanteffek van koper is bepaal deur drie vlakke van koperasetaat by palm-oleïen te voeg. Sintetiese anti-oksideermiddel, tersiêre butielhidroksiekinoon (TBHQ), se uitwerking is nagegaan deur drie vlakke by sonneblomolie te voeg. Palm-oleïen is by 50°C en sonneblomolie by 30°C vir een jaar opgeberg en nege kwaliteitsparameters oor elf intervalle ontleed.

Palm-oleïen se parameters het as volg gereageer: Vryvetsuurvlakke van alle monsters het geleidelik toegeneem, maar het binne aanvaarbare grense gebly. 'n Onverwagte waarneming was die stadiger toenametempo by die koperbevattende monsters. 'n Moontlike verklaring is dat die vryevetsure in die koperbevattende monsters verdere oksidasie ondergaan het. Peroksiedwaardes van koperbevattende monsters was heelwat laer as die Kontrole waardes. Hierdie onverwagte tendens kan toegeskryf word aan die feit dat tydens 'n langtermynstudie soos hierdie die peroksiedtussenprodukte moontlik vinnig na sekondêre stabieler, produkte omgeskakel word. Daarteenoor was die anisidienwaardes en ultravioletabsorpsie by 268 nm vir die koperbevattende monsters

hoër as vir die Kontrole en bevestig dus die voorafgaande waarneming. Oksidatiewe stabiliteitsindeks (OSI, ook bekend as Rancimat) en totale tokoferolwaardes van koperbevattende monsters was betekenisvol laer as die van die Kontrole en delta-tokotriënol was die stabielste van die vier homoloë. Die totale vlugtige komponente en pentanal piekareas het die pro-oksidanteffek van koper weerspieël. Heksanal het hoër waardes getoon in die Kontrole as in die koperbevattende monsters. Dit is in teenstelling met die t,t-2,4-dekadiënal waar die Kontrole nie meetbare vlakke getoon het nie en die koperbevattende monsters beduidende toenames getoon het. Die t-2-heksenalwaardes het geen verandering ondergaan nie. OSI en totale tokoferole se waardes was waardevolle kwaliteitsindekse vir toepassing op mono-onversadigde olies, terwyl peroksiedwaarde en dampruim vlugtige komponente misleidend kan wees. Anisidienwaardes was bruikbaar en klein veranderinge in vryvetsuurvlakke was betekenisvol soos bevestig deur hulle seleksie in die modelle. Ultravioletabsorpsie analises was nuttig wanneer daar pro-oksidant teenwoordig was. Sensoriese beoordeling het die verskil in rakleeftyd van die Kontrole en koperbevattende monsters bevestig.

Sonneblomolie het die volgende parameterveranderinge ondergaan: Vryvetsuurwaardes van al die monsters het toegeneem tot onaanvaarbare vlakke en bevestig dus hidrolietiese agteruitgang tydens opberging. Die beskermende invloed van TBHQ was opvallend en word heelwaarskynlik verklaar deur die vermindering in vorming van tussenprodukvetsure. Hierdie beskerming word ook weerspieël deur die vertraagde toename in peroksied- en anisidienwaardes teenoor die Kontrole. Hoër OSI waardes is met die TBHQ-behandeling verkry wat TBHQ se vermoë as antioksidant demonstreer. Tokoferolwaardes van olies het klein afname getoon en slegs marginale toename in ultravioletabsorpsie by 232 en 268 nm is waargeneem. Die totale vlugtige komponente, heksanal- en pentanalwaardes was weereens 'n weerspieëling van die anti-oksideermiddel se beskerming. Veranderinge in 2-heksenal en t,t-2,4-dekadiënal het geen patroon gevolg nie. Sensoriese beoordeling kon nie duidelike verskille tussen behandelings bevestig nie. OSI data en behandelings het goed ooreengestem en korrelasie met peroksied-, anisidien- en heksenalwaardes was positief. Die betekenis van die klein vryvetsuurwaarde veranderinge is eers tydens modellering besef.

Drie voorspellingsmodelle kon deur meerveranderlike regressie analises geskep word: i) Ideale model wat alle veranderlikes ingesluit het; ii) Praktiese model wat deur die maklik

bepaalbare veranderlikes, vryvetsuur-, peroksied-, OSI- en ultravioletabsorpsie-analises by 232 en 268 nm verkry is en iii) OSI model wat versnelde rakleeftydbepaling met rakleeftyd by kamertemperatuur gekorreleer het. OSI en vryvetsuurwaardes was uitstaande voorspellers want hulle is herhaaldelik deur deur alle modelle geselekteer. Palm-oleïen modelle het sekondêre oksidasieprodukte benadruk (anisidien- en ultravioletabsorpsie 268-waardes), terwyl sonneblomolie-modelle primêre oksidasieprodukte (peroksiedwaarde) geselekteer het. Die voorkeur seleksie van sekondêre oksidasieprodukte is as gevolg van die aansienlike toename in kopergekataliseerde reaksies. Anti-oksideermiddelvlakke is ook as belangrike voorspeller van rakleeftyd geïdentifiseer. OSI het nie goed met rakleeftyd van beide tipe olies gekorreleer nie en dien dus nie as goeie voorspeller op sy eie, van rakleeftyd by kamertemperatuur, nie. Dit moet deur bykomende parameters ondersteun word. Die modelle wat ontwikkel is kan prakties in die industrie toegepas word om die rakleeftyd van mono- en poli-onversadigde olies te voorspel. Die Praktiese modelle kan maklik toegepas word om 'n goeie voorspelling van rakleeftyd te gee terwyl die Ideale modelle moontlik meer akkuraat sal wees.

"Challenges make you discover things about yourself that you never really knew"

(Cecily Tyson)

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
ANNW	Artificial neural network systems
AOM	Active oxygen method
AV	Anisidine value
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
COP	Conjugable oxidation products
CV	Conjugated diene value
DFA	Discriminant function analysis
DSF	Differential scanning calorimetry
EVOO	Extra virgin olive oil
F	Test of significance between relationship between dependant variable and set of independent variables
FFA	Free fatty acids
FID	Flame ionisation detector
FS	Flavour sensory evaluation
GC	Gas chromatography
HCL	Hollow cathode lamp
HPLC	High performance liquid chromatography
IP	Induction period
IV	Iodine value
KNN	K-nearest neighbour
MHE	Multiple headspace extraction
MLR	Multiple linear regression
ND	Not detected
OSI	Oxidative Stability Index
OV	Oxodiene value
PCA	Principal component analysis
PCR	Principal component regression
PCs	Principal components
PLS	Partial least squares

PV	Peroxide value
R^2	Square of the correlation coefficient
RBD	Refined, bleached and deodorised
SIMCA	Soft independent modelling of class analogy
TBHQ	Tertiary butylhydroxyquinone
TV	Totox value
UHT	Ultra high temperature
UV	Ultra violet absorption

CHAPTER 1

INTRODUCTION

1.1 STATEMENT OF THE PROBLEM

Fats and oils are present in all our foods in varying amounts. The uses of animal and plant oils for edible purposes are numerous. The fats and oils contribute considerably to the taste, flavour and quality of the food. Should the oil be rancid or off-flavoured, the whole product would be spoiled. The quality of the fats and oils used is thus of utmost importance. The initial quality of the oil is of importance but also the stability of the oil as it indicates the resistance of the oil to possible future changes.

The stability of oils in processed foods is of importance for two reasons (Rossel, 1994). Processed food often has long distribution runs via regional warehouses and it is thus essential to have a longer shelf-life. The other reason is as a result of public awareness of nutritional issues. Foods with higher levels of polyunsaturated oils and reduced levels of food additives such as synthetic antioxidants are being created and demanded. There is also more awareness of trans fatty acids, which are formed during the hardening procedure. Stable, more solid oils, without trans fatty acids are in demand. The warm climate, along with the long transport distances and often unlimited periods of unfavourable storage conditions of South Africa, necessitates the use of stable oils in the country. There is thus a need to be able to estimate the shelf-life of oils and oil containing food to ensure realistic shelf-life dating.

The main oilseed crop produced and consumed in South Africa is sunflower. The oil is used as household oil as well as in the food industry as frying oil or as ingredient of various food products. Sunflower is high in polyunsaturated acids and thus very susceptible to oxidation (Sonntag, 1979a). It also important to take note that according to Sonntag (1979a), it is generally accepted that oil quality depends on the quality of the seed and production procedures. Antioxidants are often added to retard oxidation. The oil should be used soon after production to ensure a good stable food product. Low quality sunflower oils are on the market from either local producers or cheap imports and care should be taken to avoid the use of them.

The other oil of increasing importance in South Africa is palm oil. The oil is imported from countries such as Malaysia and Indonesia. Palm oil contains significant amounts of saturated palmitic acid and monounsaturated oleic acid and is thus very stable to oxidation (Sonntag, 1979a) although the extended transport periods after refining might affect the quality of the oil. Different fractions of the oil are used for various applications but the most commonly used is palm-olein oil. The oil is more stable than sunflower oil due to its higher level of saturation but is more expensive to use. Less or no antioxidants are needed. These are important cost saving factors for the industry.

There are various factors that influence the oxidative stability of oils. These include the level of natural antioxidants such as tocopherols present in the oil, which protects the oil against oxidation by acting as free radical scavengers (Frankel, 1996). Artificial antioxidants such as tertiary-butylated hydroquinone (TBHQ), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are often added to further protect against oxidation (Giese, 1996). The degree of unsaturation of the fatty acids in the oil plays a big role in the susceptibility to oxidation where the more unsaturated fatty acids oxidise more rapidly (Hamilton, 1994). Trace metals such as Fe and Cu also act as pro-oxidants (Berger, 1994).

Quality tests such as free fatty acids (FFA), peroxide value (PV), anisidine value (AV) and oil stability index (OSI) give an indication of the overall oil quality (Rossell, 1994) but do not predict the probable shelf-life of the oil. There is information about predicting the shelf-life or the resistance to oxidation of oils using accelerated oxidation techniques (Odumosu, Sinha, Hudson, 1979). However, there is a need to correlate and standardise the storage time at ambient temperatures against the accelerated tests. At high temperatures the mechanisms of peroxidation are different from those at low temperatures (Méndez, Sanhueza, Speisky, Valenzuela, 1996; Frankel, 1996) and the conclusions drawn from the results at elevated temperatures can be misleading as the conditions differ from that of oils stored at normal storage conditions. It has been found that the stability index of three oils (soybean oil, sunflower oil and canola oil) at different temperatures varied markedly (Frankel, 1993b). PV was used as measure of lipid oxidation. At 60°C the soybean oil was the most stable, followed by canola oil and sunflower oil. However, the OSI at 100°C indicated that the canola oil was the most stable, followed by soybean and sunflower oil. The results based on the 60°C PV tests correlated with the sensory evaluation of rancidity. This indicates that there

is a need to compare OSI data with oils stored at lower temperatures to give a true indication of oxidative stability and thus the shelf-life of oils and high fat containing products.

The contribution of other quality and characteristic parameters of the oil need to be taken into account to assess if they can contribute to the rapid prediction of the shelf-life of the oil or product.

1.2 OBJECTIVES

The primary objective of this investigation was to establish which oil quality parameters could contribute to create rapid prediction models that would be able to predict the shelf-life of two oil types, sunflower seed oil (polyunsaturated oil) and palm-olein oil (monounsaturated oil).

Secondary objectives were: To determine the correlation between accelerated tests (Rancimat) and ambient storage conditions. To determine the effects of added pro-oxidants and synthetic antioxidants on oil stability.

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 off 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):

Initiation: $RH + O_2 \xrightarrow{\text{catalyst}} R^{\bullet} + {}^{\bullet}OOH$

$RH \xrightarrow{\text{catalyst}} R^{\bullet} + {}^{\bullet}H$

Propagation: $R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$

$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$

Termination: $R^{\bullet} + R^{\bullet} \rightarrow R{-}R$

$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR$

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):

$$M^{+} + O_2 \rightarrow M^{2+} + O_2^{-} \xrightarrow{-e} {}^1O_2$$

$$O_2^{-} \xrightarrow{H^{+}} HO_2^{\bullet}$$

Both products formed are good chain initiators. It was found that singlet oxygen reacts about 1500 times faster than $^3O_2$ 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):

$$\left.\begin{array}{l} A^{\bullet} + ROO^{\bullet} \\ A^{\bullet} + A^{\bullet} \end{array}\right\} \rightarrow \text{Non-radical products}$$

$$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$$

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 (≤ 50 μg/g), α-tocopherol was a more stable and effective antioxidant than γ-tocopherol. However, at higher α-tocopherol levels (>100 μg/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):

$$M^{(n+1)+} + RH \rightarrow M^{n+} + H^{+} R^{\bullet}$$

$$ROOH + M^{n+} \rightarrow RO^{\bullet} + OH^{-} + M^{(n+1)+}$$

$$ROOH + M^{(n+1)+} \rightarrow ROO^{\bullet} + H^{+} + M^{n+}$$

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 chloropylloid 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, Brevedan 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), steatites 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, artherosclerotic 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 toxicologocal 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 of 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

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 x 10^{-3} and 100 (Snyder et al, 1986; Frankel, 1993a), nmol/ml (Frankel and Tappel, 1991) to integrator count x 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 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).

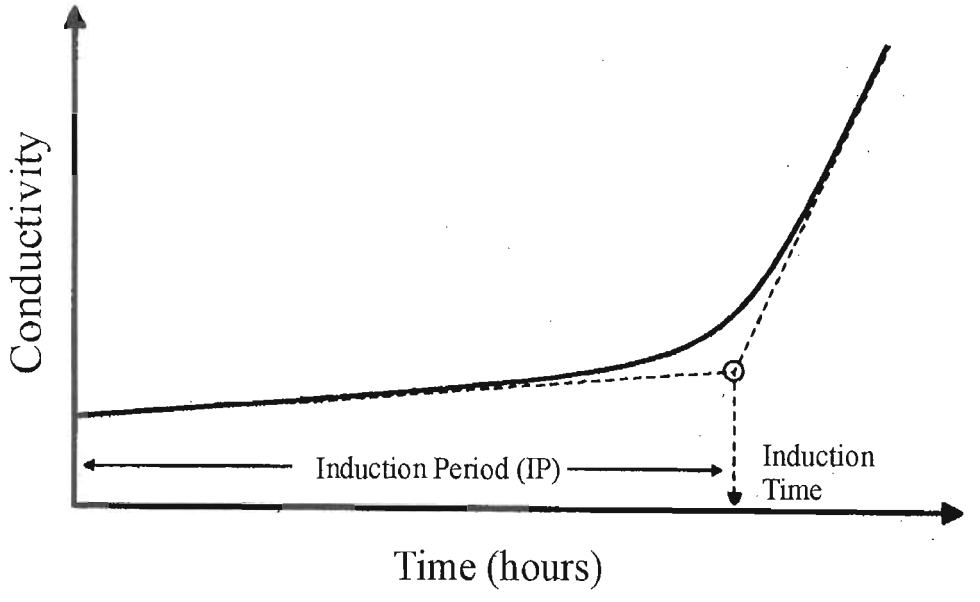


Figure 1: Course of oxidation measured by conductivity of organic volatile compounds trapped in water versus time as determined by OSI. (Wan, 1995).

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 % O_2 and 99.9 % N_2, 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, *Elaesis guineesis*, 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).

	Palm Oil Fraction		
Characteristic	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 Pagliarine, 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 Zambiazi (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):

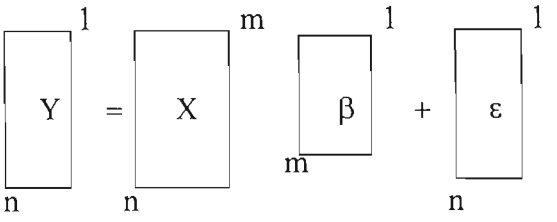
$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \varepsilon_1$$

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:

$$\mathbf{Y} = \mathbf{X}\beta + \varepsilon$$

Graphically it can be represented as:



n = the number of samples

m = the number of independent variables

Y = n x 1 vector

X = n x m matrix

β = m x 1 vector of predictor coefficients that describes the relationship and

ε = n x 1 vector of error terms which represents the difference between the dependant variable and Xβ (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.

CHAPTER 3

MATERIALS AND METHODS

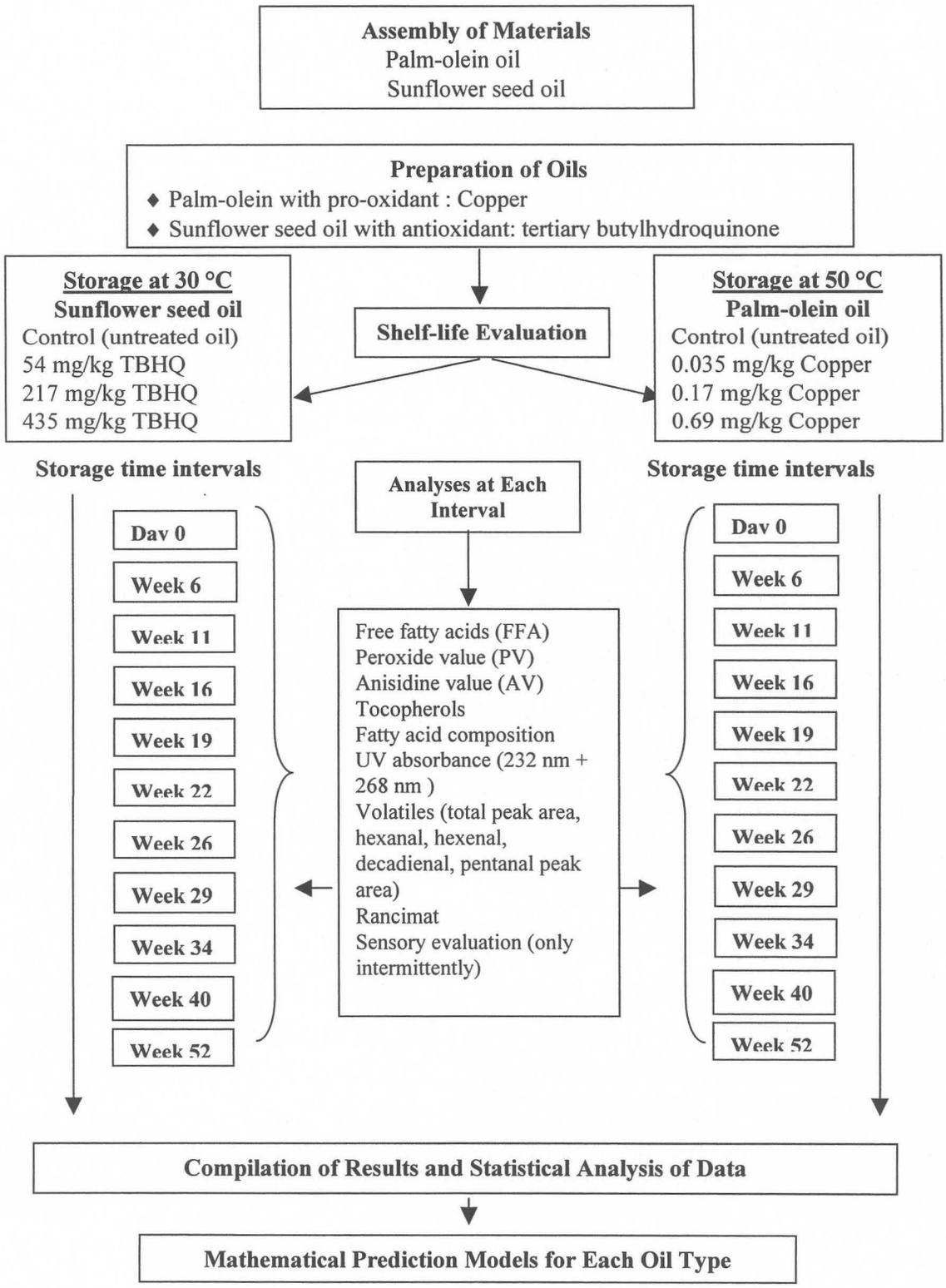


Figure 2: Summary of research methodology for development of prediction models to predict shelf-life of mono- (palm–olein oil) and polyunsaturated oil (sunflower seed oil).

3.1 MATERIALS

3.1.1 Palm-olein oil

Two different palm-olein oils were combined for the storage trial. Both oils were fully refined, bleached and deodorised (RBD) oils from Malaysia. One of the oils had been in storage in an amber glass container at – 20°C for approximately one year before the storage trial. The oil was obtained from Hudson and Knight, Durban, South Africa and the other oil (5 litre tin) had been received from Hudson and Knight, Durban, South Africa 6 weeks before the start of the storage trial. The addition of older oil was done to have oil that was comparable to oil that has been transported over a long distance and stored in bulk tankers before distribution. The oils were free of the antioxidant TBHQ. This was confirmed by testing for TBHQ as in the method from Anderson and Van Niekerk (1987) with the deviations as discussed under section 3.3. The oils were mixed in an approximate ratio of two thirds of the fresh oil and one third of the oil from storage. The combined oil was heated slightly to 50°C and stirred well to ensure proper mixing. A total of 7.0 litres were prepared. The fatty acid composition as in Official Methods and Recommended Practices of the AOCS, Method Ce 2-66 with modifications as described in section 3.3 was done on the combined oil and the profile confirmed that the oil was palm-olein oil.

3.1.1.1 Preparation of oil samples containing copper

A 0.3142 mg/ml solution of copper acetate was prepared in methanol. Copper acetate was chosen as it is soluble in methanol and is in organic form similar to fatty acids. Molecular weight of copper acetate is 199.65 g/mol and of copper (Cu^{2+}) 63.546 g/mol. The weighed copper acetate (0.1571 g) was made up to 500 ml in methanol in a volumetric flask. The concentration of copper in the solution was 0.1 mg/ml. Three concentrations of copper in oil were prepared. The first concentration contained 0.035 mg/kg copper. A solution of 0.0319 mg/L copper was prepared by adding 0.51 ml of the copper acetate solution to 1 600 ml oil. The second concentration was 0.17 mg/kg copper in oil. A solution of 1.59 mg/L copper was prepared by adding 2.55 ml of the copper acetate solution to 1 600 ml oil. The third concentration contained 0.69 mg/kg copper in oil. A solution of 6.36 mg/L copper was prepared by adding 10.18 ml copper acetate solution to 1 600 ml oil. Each of the three concentrations were mixed in a two litre glass beaker and left in the oven at 60°C for 3 h with occasional stirring to evaporate the methanol. Aliquots (100 ml) of each concentration, as

well a as control containing no copper, were measured and poured into 100 ml non-transparent (opaque) plastic containers with screw lids ready for storage. A set of four samples (0.035 mg/kg copper, 0.17 mg/kg copper, 0.69 mg/kg copper and the Control) were analysed on Day 0 (zero).

3.1.2 Sunflower seed oil

Deodorised sunflower oil was obtained from SA Oil, Randfontein, South Africa the day after production. No TBHQ was added. This was verified by testing for the presence of TBHQ as in the method of Anderson and Van Niekerk (1987) with the deviations as discussed under section 3.3. To ensure that the oil received was pure sunflower oil, a fatty acid composition determination as in Official Methods and Recommended Practices of the AOCS, Method Ce 2-66 with modifications as described in Analyses section 3.3, was done on the oil. The fatty acid composition resembled that of normal sunflower oil.

3.1.2.1 Preparation of sunflower seed oil samples containing TBHQ

A 50 mg/ml stock solution of TBHQ in ethanol was prepared by dissolving 1000 mg TBHQ in 20 ml ethanol and made up in a volumetric flask. The TBHQ solution had to be heated and shaken well until it was dissolved. Three concentrations of TBHQ in oil were prepared. The lowest concentration was 54 mg/kg TBHQ in oil. Stock solution (1.2 ml) was added to 1217 ml sunflower oil. The density of oil of 0.92 was taken into account when the TBHQ content was calculated as mg/kg oil. The second concentration of TBHQ was 217 mg/kg in oil. To prepare the solution 4.8 ml of the stock solution was added to 1217 ml sunflower oil. The third concentration of TBHQ prepared was 435 mg/kg in oil. Stock solution (480 ml) was added to 1217 ml oil. Each prepared oil sample was mixed in a two litre beaker and left in a oven at 50°C for 3 h with occasional stirring to evaporate the ethanol. Aliquots (100 ml) of each concentration, as well as a control containing no TBHQ, were measured into 100 ml size non-transparent (opaque) plastic containers with screw lids. A set of four samples (54 mg/kg TBHQ, 217 mg/kg TBHQ, 435 mg/kg TBHQ and a Control sample) was analysed on Day 0 (zero).

3.2 SHELF-LIFE METHODOLOGY

The palm-olein samples were stored in a Humidity Cabinet that was equipped with a fan where the cabinet was set at 50°C and was monitored by a thermometer. The relative humidity option was not used. A set of samples was taken out at each time interval for analyses. Samples remaining after analyses, or awaiting analyses at a later stage, were stored at – 20°C. The sunflower seed oil samples were stored in an incubator set at 30°C (the incubator was not equipped with a fan). The temperature was monitored by means of a thermometer. A set was taken out of storage at intermittent intervals for analyses. The remaining oil of the samples was stored at – 20°C until completion of analyses. The temperatures of both ovens were monitored weekly. The shelf-life test lasted for a period of 52 weeks (one year) for both oils. The sampling intervals were irregular. The intervals are set out in Figure 2.

3.3 ANALYSES

3.3.1 Chemical methods

All analyses were performed at least in duplicate except where there was insufficient equipment capacity, in which case appropriate repeat analyses were performed.

3.3.1.1 Free fatty acid value

The method determines the amount of free fatty acids present in the oil by dissolving the oil sample in a solvent and neutralising it by titration with sodium hydroxide, using phenolphthalein as indicator. The results are expressed as g/100 g oleic acid, as oleic acid is the main fatty acid present in most oils. The method used was AOCS Method Ca 5a-40, (AOCS, 1997) with the modification that 80 ml solvent (50% toluene/50% isopropanol) was used to dissolve the oil.

3.3.1.2 Peroxide value

Primary oxidation products are measured in terms of milli equivalents of peroxide per 1000 g sample. This is achieved by the addition of potassium iodide which is oxidised to iodine by the peroxides present in the oil. The iodine is measured by titration with "Titrisol"

standardised sodium thiosulphate (Merck, Darmstadt, Germany). The method used was the AOCS Method Cd 8-53, (AOCS, 1997).

3.3.1.3 Anisidine value

Secondary oxidation products were measured by determining the *p*-anisidine value. Aldehydic compounds in fats and oils react with *p*-anisidine, in the presence of acetic acid, to form yellowish reaction products. According to the method the intensity of the yellowish compounds is not related only to the amount of aldehydic compounds present, but also to their structure. A double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance four to five times. This is why 2-alkenals and dienals will contribute substantially to the value. This determines the quantity of aldehydes (principally 2-alkenals and 2,4-dienals) present in fats and oils. The AOCS Method Cd 18-90 (AOCS, 1997) was used. *p*-Anisidine was recrystallised according to the method and used for two consecutive days only, after which fresh *p*-anisidine was prepared.

The Totox value was calculated from the peroxide value and the *p*-anisidine value with the formula 2PV +AV.

3.3.1.4 Oxidative stability index (Rancimat)

The time from the start of the accelerated stability test to the point at which rapid oxidation occurs is called the induction period and gives an indication of the relative oxidative stability of the oil or fat. This is measured by heating the sample in a thermostated heater, while bubbling purified air through the sample at a constant rate. The effluent air is passed through deionised water and the conductivity of the water is measured for polar oxidation products (mainly formic acid) by an electrode. The method used was AOCS Method Cd 12b-92 (AOCS, 1997). A Rancimat 679 Metrohm Ltd, Switzerland was used. Sample (2.50 g) was weighed and air was bubbled through at a rate of 20 l/h. The temperature used to conduct the test for the sunflower seed oil samples was 110°C and for the palm-olein oil samples 120°C. This was because monounsaturated oils such as palm-olein are much more stable than polyunsaturated oils such as sunflower seed oil and are therefore normally subjected to higher temperature to ensure an induction period less than 48 h, which is the normal maximum run time for the Rancimat instrument.

3.3.1.5 Tocopherols

Tocopherols were measured by normal phase HPLC using a fluorescence detector with excitation at 295 nm and emission at 330 nm as described by Van Niekerk (1973) and Van Niekerk (1975) and amended as in AOCS Method Ce 8-89 (AOCS, 1997). The oil was made up to volume in the mobile phase, 1 % isopropanol in hexane. The mobile phase was dried over sodium sulphate, as the water content of the mobile phase is of critical importance and drying with sodium sulphate regulates it. Separation was obtained by using a 25 cm Lichrosorb Diol 5µm column (Merck) and applying a flow rate of 0.8 ml/min. The detection limit for tocopherols is 0.1 mg/100 g.

3.3.1.6 Volatile compounds

Volatile compound analyses were determined using an in-house method of static headspace Gas chromatography (GC)-Flame ionisation (FID) analysis. A Genesys equilibrium headspace sampler and a Varian 3800 GC with a Restek Rtx-5, 30 m, 0.25µm film thickness, 0.32 mm ID (poly-5 % diphenyl / 95 % dimethylsiloxane) (Chromspec, PA, USA) column was used. Two g sample (accurate to four decimal places) was weighed in a 20 ml headspace vial and sealed with a crimp cap. The samples were equilibrated at 80°C for 240 min. after which 120 µl of the headspace was injected splitless to the column. The line temperature was 150°C and the loop temperature 120°C. The temperature program of the oven was 40°C, held for one min, and increased to 240°C at a rate of 10° C/min. The detector was set at 240°C. Three volatiles: hexanal, trans-2-hexenal and trans,trans-2,4-decadienal, were determined and standards were prepared with all three in one sample. The following approximate concentrations were used for the calibration: 0.5, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg/kg. These were made up accurately to three decimal places in fresh oil. Fresh sunflower oil was obtained from Unifoods, Boksburg, South Africa and was collected directly after the deodorisation step. A blank run of the oil was performed to ensure that the three volatile peaks that would be determined were absent or minimally present in the oil. Two palm-olein oils were tested and the cleaner oil was selected as blank and was used to make up the standards.

3.3.1.7 Fatty acid composition

The determination of the fatty acid composition procedure was based on the Official Methods and Recommended Practices of the AOCS Method Ce 2-66 (AOCS, 1997) by preparing

methyl esters which are separated and determined by capillary GC with FID. BF_3-methanol reagent was used for the derivitisation after transesterification with 0.5 M NaOH in methanol. Three drops of oil sample were derivatised and taken up in 2 ml. heptane. One µl of the prepared sample was injected onto an Omegawax™ 320 (Supelco, Johannesburg, South Africa), fused silica capillary 30m column, 0.32 mm ID and 0.25 µm film thickness as supplied by Supelco. The oven temperature program was 180°C for 15 min, after which it increased to 210°C at 5 °C/min and held at 210°C for 18 min. The injector was set at 230°C and the detector at 240°C. The fatty acids were expressed as g/100 g fatty acids.

3.3.1.8 Tertiary butylhydroquinone (TBHQ)

The oils were tested for the presence of the synthetic antioxidant TBHQ before using the oils in the shelf-life tests. The method used was that of Anderson and Van Niekerk (1987) with modifications to the mobile phase. TBHQ was determined in the oil directly using normal-phase HPLC with fluorescence detection. One g (weighed accurately to four decimals) of oil was dissolved in the mobile phase consisting of 6 % isopropanol in hexane and made up to 10 ml. The mobile phase was dried over sodium sulphate, to adjust the moisture below a critical level. The excitation of the fluorescence detector was set at 309 nm and the emission at 340 nm and separation was done by a 25 cm Lichrosorb Diol 5µm column supplied by Merck.

3.3.1.9 Conjugated diene and triene values

The shift in the position of the double bond of polyunsaturated oils, that results as one of the first steps of oxidation (primary oxidation), is measured by the conjugated diene value (CV) (White, 1995). The determination of the CV was done according to ISO/DIS 3656 method (ISO/DIS, 1989). The oil sample was dissolved in 25 ml iso-octane and the absorbance was measured at 232 (diene) and 268 (triene) nm. The CV will also be referred to as UV at 232 and the conjugated trienes as UV at 268 nm.

3.3.1.10 Moisture

The initial moisture contents of the two oils were determined by a vacuum oven method where the moisture loss was measured gravimetrically. The sample was weighed (2 g) accurately to 4 decimal places into a dried, clean, weighed aluminium dish. The dish, covered partially with the lid, was placed in a vacuum oven at 70 °C (± 5 °C) under pressure of *c.* 280

mm Hg. Whilst drying, a slow stream of dry air (approx. 2 bubbles/s) was introduced into the oven. The samples were dried overnight (16-18 h) or longer until constant weight. Once the drying period was completed the dish was cooled in a dessicator and weighed.

The moisture content was calculated as follows:

$$\text{g/100 g moisture} = \frac{(\text{dish + lid + wet sample}) - (\text{dish + lid + dry sample}) \times 100}{\text{mass of wet sample}}$$

3.3.1.11 Iron and copper

The iron and copper contents were determined by atomic absorption spectroscopy (AAS) after dry ashing to convert organic matter into inorganic compounds. Ashing was done by weighing 5-10 g oil sample into clean, preweighed silica dishes. Samples were placed in a cold muffle furnace that was heated very slowly 10 °C/h to 520 °C. On reaching temperature the samples were left overnight. The resulting ash was moistened with 2 ml conc. HNO_3, dried, dissolved by the addition of a ml conc. HCl and made up to volume with deionised water. The mineral determinations were carried out according to the instructions given by the manufacturer of the Perkin-Elmer Model 5000 AAS instrument, Buckinghamshire, England. The operating procedures for each element were as follows:

Element	**Flame type**	**Wavelength**	**Slit setting**	**Light source**
Copper (Cu)	Air acetylene	324.8 nm	4 (0.7 nm)	HCL*
Iron (Fe)	Air acetylene	248.3 nm	3 (0.2 nm)	HCL

* HCL = Hollow Cathode Lamp

$$\text{Calculation: mg/100 g} = \mu\text{g/ml} \times \frac{\text{final vol}}{\text{mass}} \times \text{dilution} \times \frac{100}{1000}$$

where µg/ml = obtained from standard curve (Y = A + Bx).

3.3.2 Sensory evaluation

The method used was by odour evaluation of the oils. The four samples of one of the oil types, from a specific storage time interval, were evaluated at a time. For example, the four sunflower oil samples, Control, 54 mg/kg TBHQ, 217 mg/kg TBHQ and 435 mg/kg TBHQ, taken out at Week 22, were evaluated at the same time. The panel consisted of people

working at CSIR Bio/Chemtek, Pretoria at the time of the evaluation. Half of the panellists selected had previous experience in oil odour evaluation and the rest of the panellists had to be informed of the characteristics of rancid oil and were presented with rancid oil for evaluation in order to identify typical rancidity odour in oil. A panel of 10-12 people was used. Sample (15 ml) was measured into a 50 ml glass beaker and covered with aluminium foil. Fresh oil was used as a standard to remind the panellist of the characteristics of good quality oil. The standard sample, also measured into a 50 ml beaker, was presented to the panellist at the same time as the test samples. The samples were heated to approximately 50°C before being presented to the panellist. The score sheet listed a choice of four categories to guide the panellist in the evaluation of the samples (as compared to the standard sample). The four categories were as follows:

1) Odour bland, weak characteristic odours
2) Weak off-odour or loss of characteristic odour
3) Moderate off-odour, slightly rancid
4) Strong off-odour, rancid, painty

In the case of the odour evaluation of palm-olein oil an additional standard sample was presented to the panellists. The sample contained a small amount of added copper acetate. This was done because some of the samples presented to the panellists contained copper acetate and it was necessary that they could differentiate between the copper acetate odour, if they could detect it, and normal off-odours due to rancidity. This was not done with the sunflower oils, which had TBHQ added, as TBHQ does not have a strong characteristic odour.

3.4 MODELLING

Mathematical modelling was done by multiple regression analysis on STATISTICA® Kernel release 5.1M 1998 Edition, StatSoft Inc., Tulsa, USA. Multiple regression was performed where the dependant variable was the shelf-life, and the independent variables were the values obtained for the various analyses such as PV, FFA, OSI, etc at the determined shelf-life. The programme selected relevant variables with forward stepwise regression where the independent variables were individually added or deleted from the model at each step until the "best" regression model was obtained. The *F to enter* value, which determines how significant the contribution of a variable in the regression equation has to be in order to be added to the equation, was set at 3.0 where the minimum *F to enter* value is 0.0001. The *F to remove* value, which determines how "insignificant" the contribution of a variable in the regression equation has to be in order to be removed from the equation, was set at 1.5, where the minimum *F to remove* value is 0.0. The *tolerance* of a variable, which is defined as one minus the squared multiple correlation of this variable with all other independent variables in the regression equation, was set at 0.0001.

The storage time at which the oils were considered rancid was chosen by a combination of PV and AV. A PV higher than 20-25 meq/kg (Yousuf Ali Khan et al, 1979; Tian et al, 1999) and an AV higher than 10 mmol/kg (White, 1995) were used as criteria to determine at which storage time the oils were rancid. The different cases used in the modelling were generated by taking the time at which the oil was deemed rancid, e.g. at Week 22 the Control of palm-olein was deemed rancid, and attributing the agreeing shelf-life of 0 weeks to the case. The oil was rancid at Week 22 and thus had no shelf-life. The remainder of the cases were calculated by working backwards to the start of the storage trial so that as in this example, the next case for the Control was at Week 19 with a shelf-life of 3 weeks followed by Week 16 with a shelf-life of 6 weeks and so on. In the same manner all the cases for each treatment is calculated from the time it was deemed rancid with its agreeing shelf-life up till Day 0. The Totox value was excluded from the modelling, as it is a combination of the AV and the PV. Models that based onset of rancidity on the sensory evaluation were also done. The following models had been designed to predict the shelf-life of the oils:

Palm-olein oil:

Model 1 Normal function values of all the variables

Model 2 Squared values of all the variables

Model 3 Weighted values of normal function values of all the variables (done by the division of all the values by the standard deviation of each analysis)

Model 4 Weighted values of squared values of all the variables

Model 5 Ideal model including all the variables, normal and squared values

Model 6 Practical model using only well-known, easy to use methods, namely FFA, PV, OSI, conjugated diene and triene value

Model 7 OSI and its squared value

Model 8 Ideal model, based on sensory evaluation with all variables, normal and squared values

Model 9 Practical model based on sensory evaluation

Model 10 OSI and its squared value based on sensory evaluation

Sunflower seed oil:

The same models were done for the sunflower seed oil. The Ideal Model 5 was additionally performed with the exclusion of the TBHQ values.

The models selected, based on their correlation coefficients and p-values defined in the Results 4.1.3.1 section, were validated by the jackknife approach (Hair et al, 1998). The method is based on the "leave-one-out" principle, where the modelling was performed with the exception of one case. All the cases used in the models were excluded one by one and the models were then tested with the excluded case.

CHAPTER 4

RESULTS

4.1 PALM-OLEIN OIL

4.1.1 Composition of oils

The fatty acid composition of the two palm-olein oils that were used to prepare the blend of palm-olein oil used for the shelf-life test is given in Table 7. The fatty acid composition of typical palm-olein oil (Codex Alimentarius Commission, 1997) has been included.

Table 7: Fatty acid composition (g/100 g fatty acids) of the two palm-olein oils used and that of typical palm-olein oil (Codex Alimentarius Commission, 1997).

Fatty acids	Palm-olein oil fresh	Palm-olein oil from storage	Typical palm-olein oil
C12 : 0	0.26	0.23	0.1-0.5
C14 : 0	0.90	0.97	0.9-1.4
C15 : 0	0.05	0.04	ND*
C16 : 0	36.95	37.15	38.2-42.9
C16 : 1	0.17	0.21	0.1-0.3
C18 : 0	4.29	4.45	3.7-4.8
C18 : 1	45.15	41.38	39.8-43.9
C18 : 2	10.75	13.86	10.4-13.4
C18 : 3	0.17	0.17	0.17-0.6
C20 : 0	0.42	0.37	0.2-0.6
C20 : 1	0.18	0.17	ND*
C22 : 0	0.09	0.14	ND*
C24 : 0	0.11	0.10	ND*

- ND – Non detectable, defined as ≤ 0.05 g/100 g fatty acids

The detection limit (< 0.1 mg/kg) of the iron and copper content, which were determined to assess the initial quality content of the oils, was too high to be of practical value. The moisture and TBHQ contents of the oil are given in Table 8.

Table 8: Moisture and TBHQ content of composite palm-olein oil.

	Moisture g/100 g	TBHQ (mg/kg)
Palm-olein oil	0.13	Not detected*

* Non detectable defined as ≤ 2.0 mg/kg TBHQ

4.1.2 Shelf-life tests

The four sample treatments will in future be identified as Control (no addition of copper), 0.035 mg/kg, 0.17 mg/kg and 0.69 mg/kg copper-containing samples.

The data showed that the sample treatment with the sample containing 0.17 mg/kg copper, at Week 29, was suspect. The data was subjected to Dixon's test for outliers on the residuals of the regressions lines of the variables (Snedecor and Cochran, 1980). The data for Week 29 qualified as an outlier, at 99 % confidence level and was then omitted. All the curves of that sample were interpolated between Weeks 26 and 34.

Values shown in the figures are means of at least duplicates (see section 3.3.1). Error bars and standard deviation in figures and tables are not shown in order for legible interpretation of results. Statistical analysis of data to compare treatments of each parameter is given in section 4.3.

4.1.2.1 Free fatty acids

The effect of storage at 50°C on the four sample treatments over a period of 52 weeks is given in Figure 3.

The FFA content of the samples increased over the storage period. The FFA increased gradually up to Week 40, after which a sharp increase occurred in all the samples. The FFA of the Control increased the most, followed by a slightly lower rate of increases in FFA in the copper-containing samples. The sample with the highest concentration of copper (0.69 mg/kg) had the lowest FFA content after the storage period.

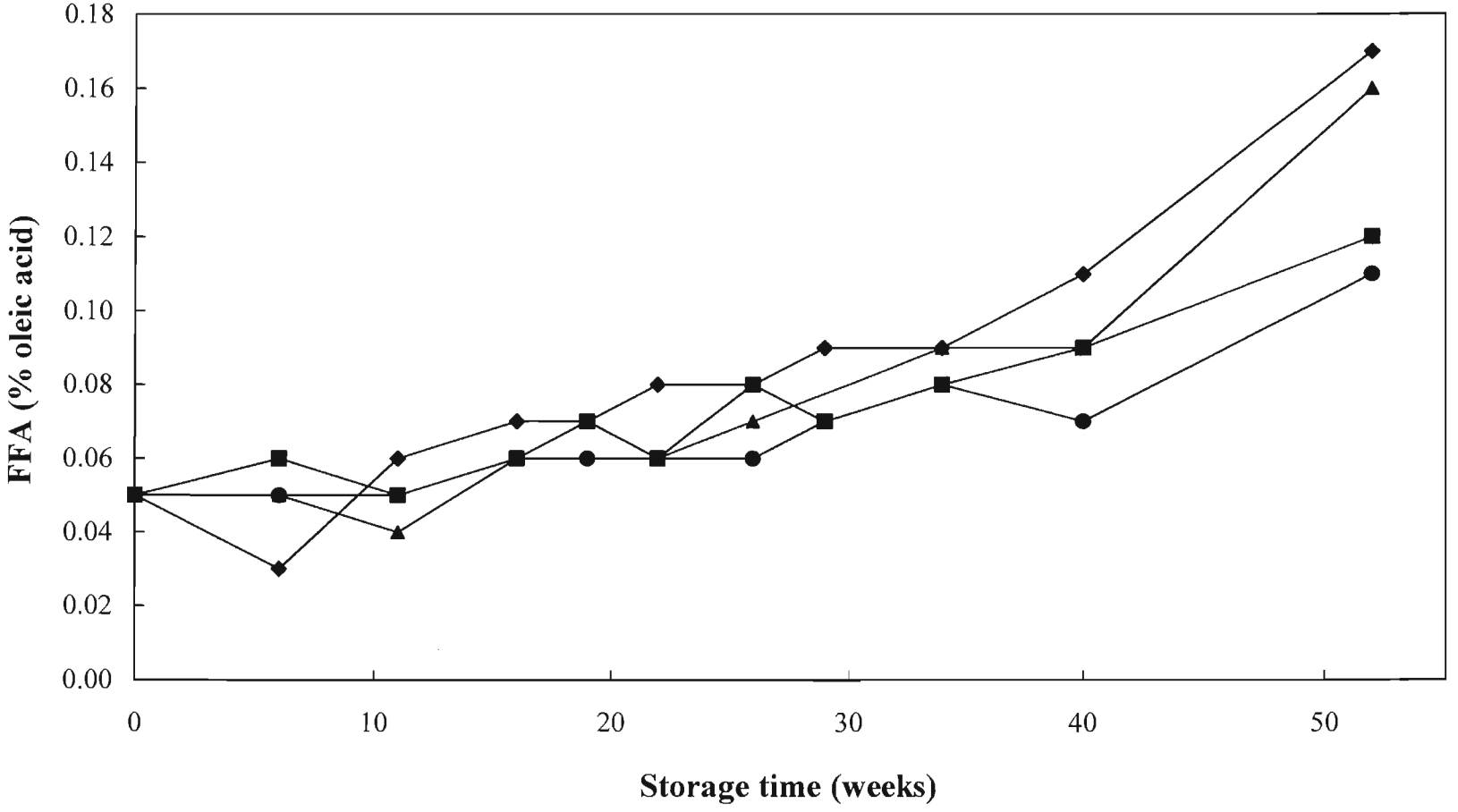


Figure 3: The effect of storage on the FFA content (% oleic acid) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.2 Peroxide value

The effect of storage at 50°C on the PV of the four sample treatments is given in Figure 4.

The PV of the Control increased steadily and reached a plateau at Weeks 34-40, after which it appeared to decrease. The highest PV obtained for the Control was 28.8 meq/kg at Week 40. The samples containing copper had low peroxide values (< 5.0 meq/kg) up to Week 26, after which the values for the samples containing 0.035 mg/kg and 0.17 mg/kg copper gradually increased, whereas the values for the sample containing 0.69 mg/kg copper remained stable. The PV of the sample containing 0.17 mg/kg copper increased sharply after Week 40 to 14.0 meq/kg, whereas the sample containing 0.035 mg/kg copper increased steadily after week 26 to reach a value of 10.7 at Week 52. The sample with the highest concentration of copper (0.69 mg/kg) had the lowest PV after the 52 week storage period of 1.6 meq/kg.

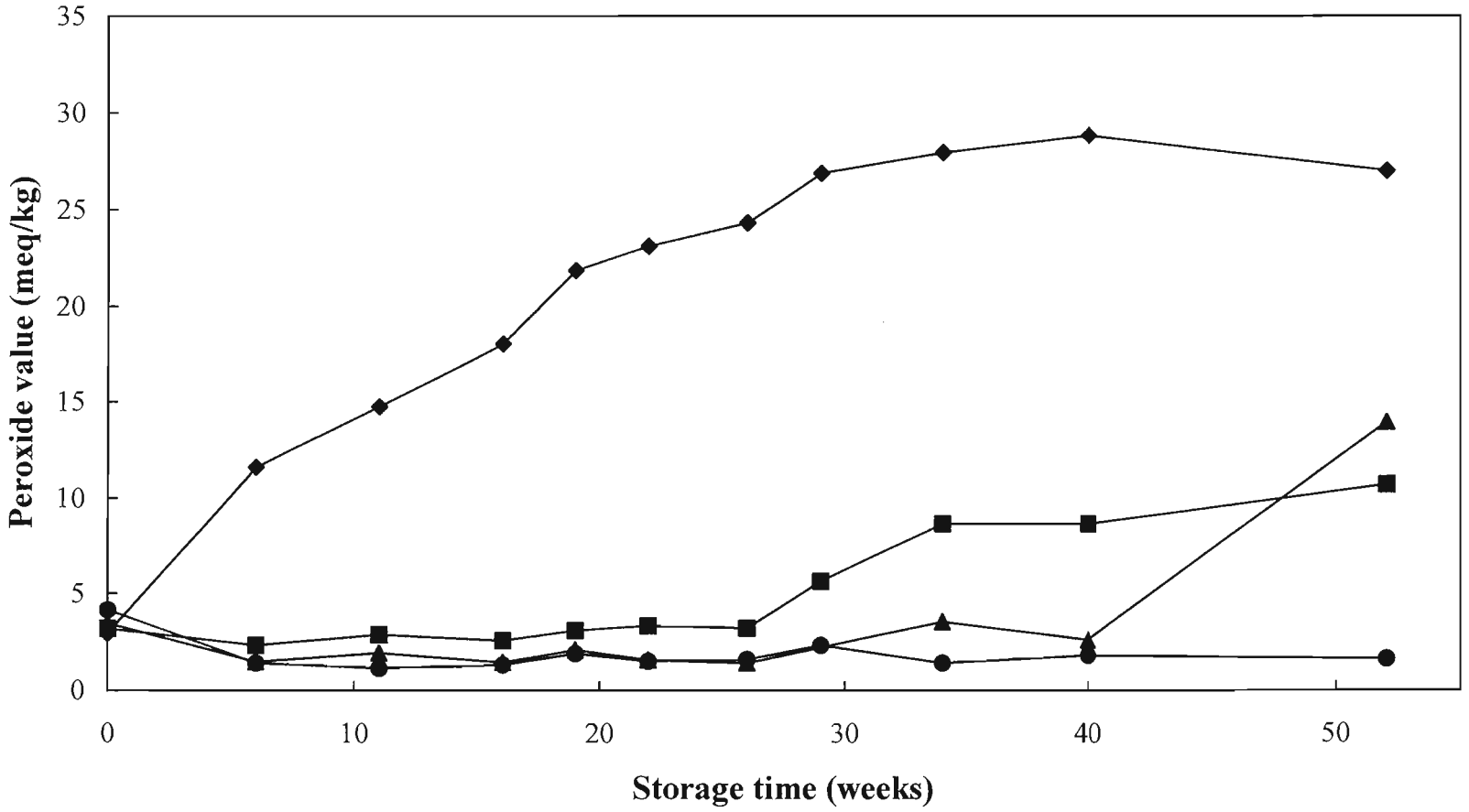


Figure 4: The effect of storage on the peroxide value (meq/kg) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.3 Anisidine value

The effect of storage at 50°C on the AV of the four sample treatments is given in Figure 5.

In general, the AV of all the samples increased. The AV of the Control increased steadily up to Week 40, after which there was a sharper increase to a value of 15.2. The AV of the samples containing copper increased at a much faster rate. The AV increased sharply after Week 19 for the samples with 0.035 and 0.17 mg/kg copper, whereas the sample with 0.69 mg/kg copper increased steadily. At Week 52 the AV of the samples containing copper was approximately double than that of the Control.

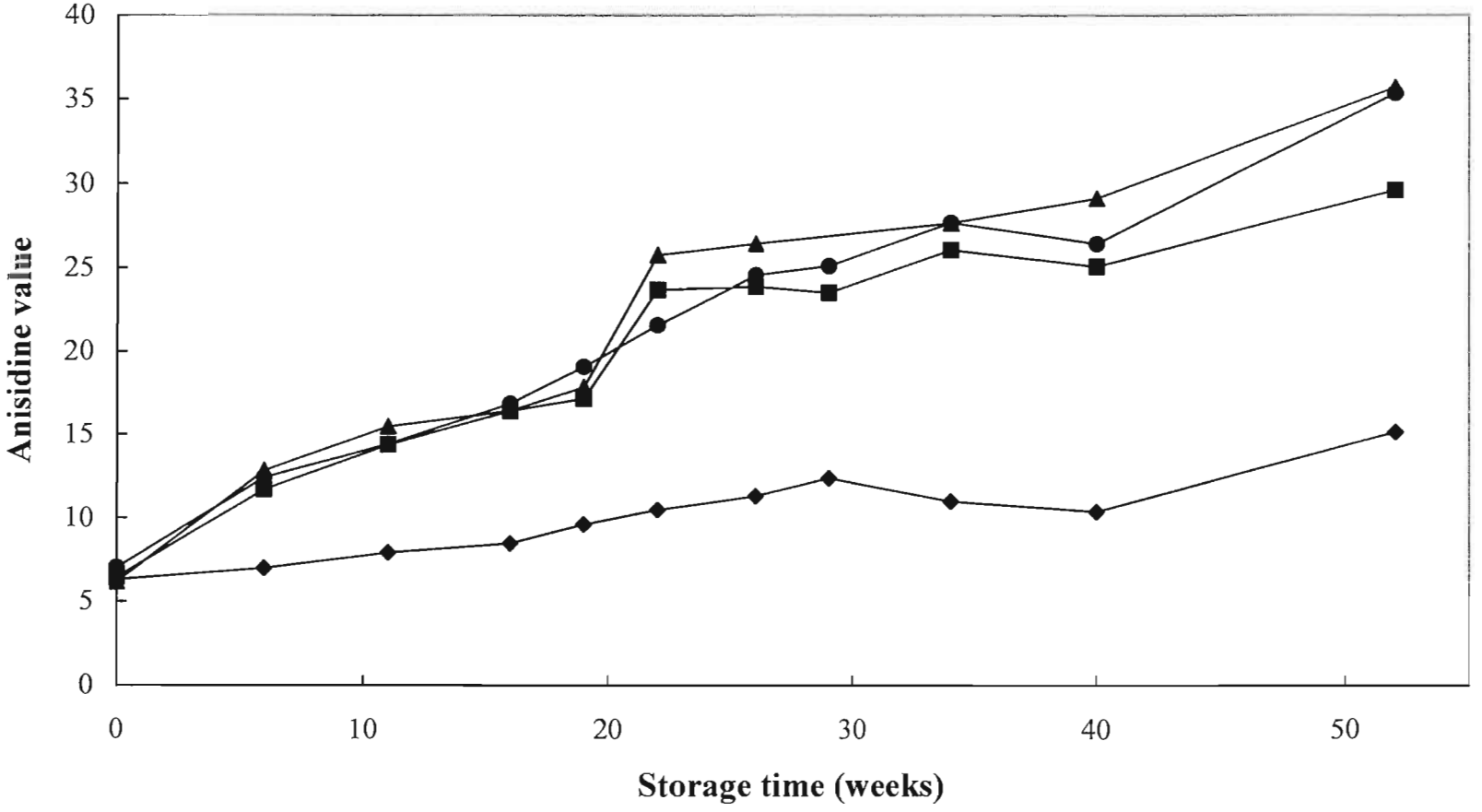


Figure 5: The effect of storage on the anisidine value of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.4 Totox value

The effect of storage at 50°C on the Totox value of the four sample treatments is given in Figure 6.

The calculated Totox values (2PV +AV) of all the samples increased gradually during the 52 week storage period. The Totox value of the Control increased at a much faster rate than the copper-containing samples and had consistently higher values than the copper-containing samples. The different concentrations of copper did not appear to affect the rates of increase in the samples up to Week 26, after which the highest concentration of copper (0.69 mg/kg) had the lowest Totox values and the lowest concentration of copper (0.035 mg/kg) the highest values of the copper-containing samples. However, at Week 52 the 0.17 mg/kg sample had the highest Totox value.

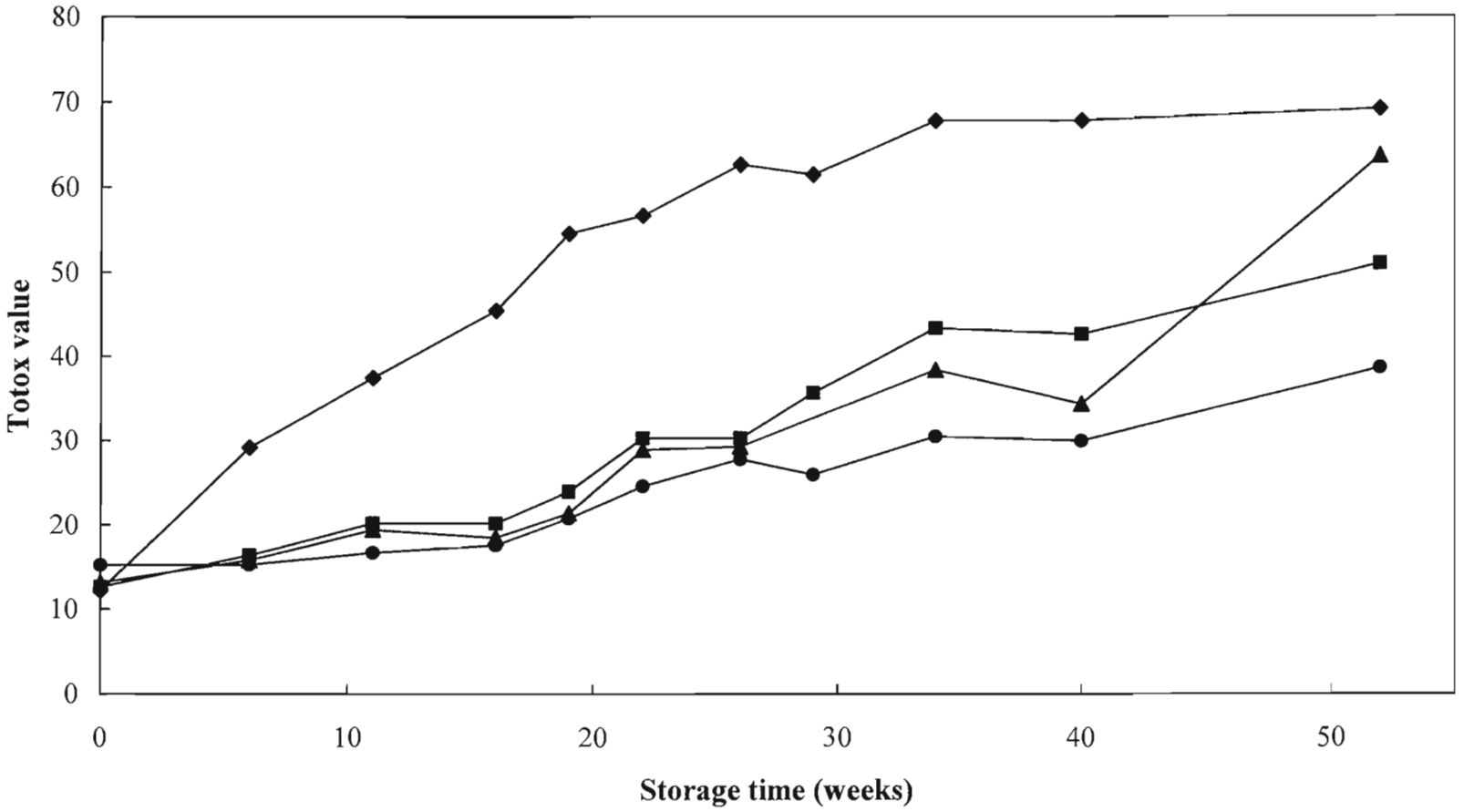


Figure 6: The effect of storage on the Totox value of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.5 Oxidative Stability Index

The effect of storage at 50°C on the oxidative stability conducted at 120°C of the four sample treatments is given in Figure 7.

The copper-samples showed marked differences from the Control at Day 0 where progressively higher concentration of copper have concurrent lower OSI values. There was a steady decline in OSI (in hours) of the Control and the sample with 0.035 mg/kg copper, that was very similar apart from the initial values. The values for the Control decreased by half the number of hours (11 hours) from Day 0 to Week 52, whereas the values for the sample with 0.035 mg/kg copper decreased by 2.5 hours from 0 to Week 52. The oxidative stability of the sample with 0.17 mg/kg copper remained fairly stable up to Week 40, after which it decreased significantly to half the number of hours. The oxidative stability of the sample with 0.69 mg/kg copper remained stable from the start of the shelf-life test up until Week 52 with an oxidative stability index of only 3–4 hours.

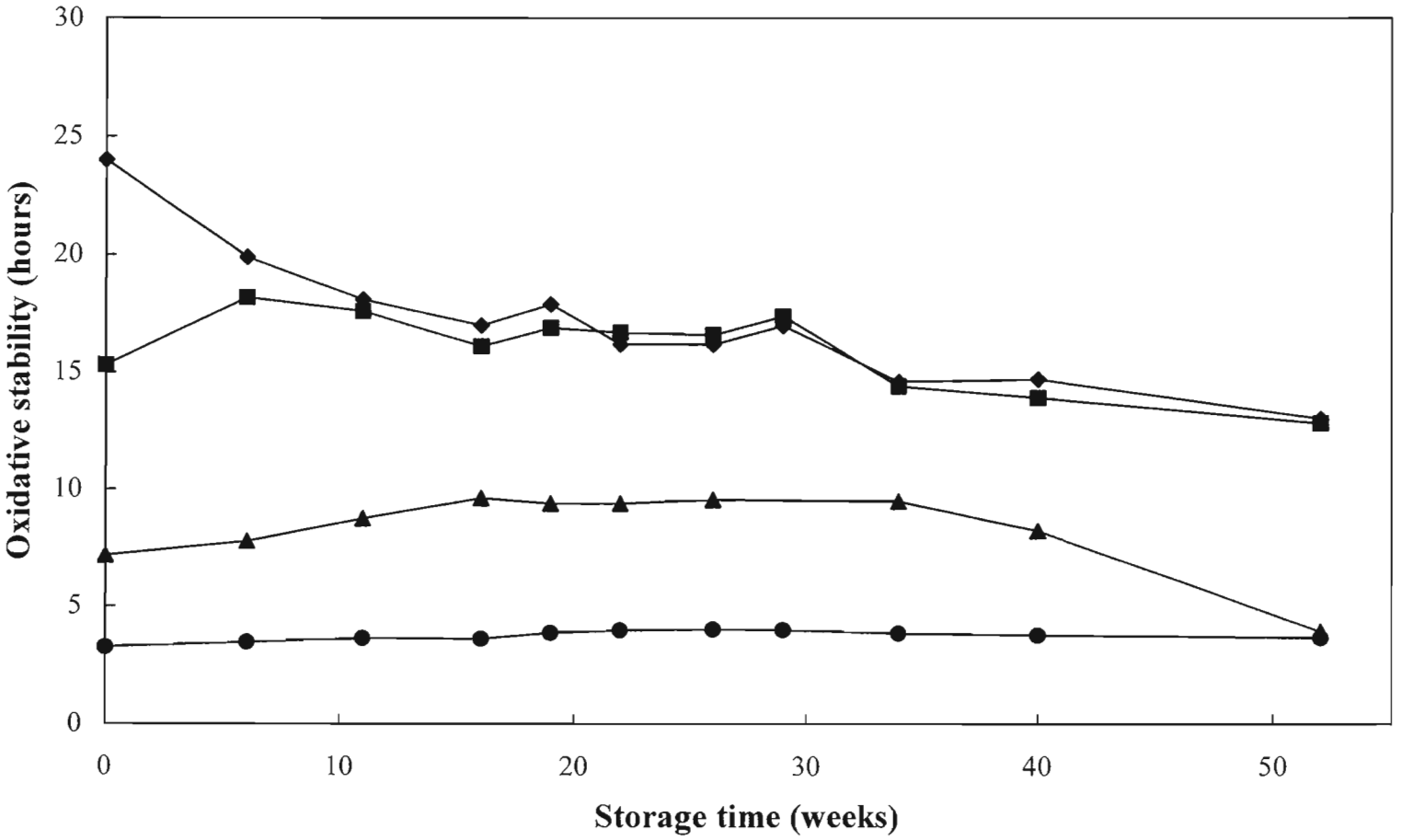


Figure 7: The effect of storage at 50°C for 52 weeks on the oxidative stability (hours) conducted at 120° of palm-olein with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.6 Tocopherols

The detection limit for tocopherols is 0.1 mg/100 g and therefore tocopherols such as β- and γ-tocopherol that might have been present at levels less than the detection limit were not reported.

Total tocopherols

The effect of storage at 50°C on the total tocopherol content of the four sample treatments is given in Figure 8. The total tocopherols of the Control remained the highest of all the samples over storage, but declined from 53.7 mg/100 g to 32.2 mg/100 g by Week 52. The total tocopherol contents of the 0.17 and 0.69 mg/kg copper samples showed a slight decrease at Day 0 when the copper was added. All the copper-containing samples showed a sharp decline within the first 6 weeks as measured at Week 6. After Week 6 the treatments containing 0.17 and 0.69 mg/kg copper maintained a gradual decrease and the sample with the lowest concentration of copper (0.035 mg/kg) remained constant up to Week 52. The decrease in total tocopherols was concomitant with concentration of copper added. The change in total tocopherols and the individual tocopherols content (mg/100 g) from the initial values to the values at Week 52 is given in Table 9.

Table 9: Total and individual tocopherols (mg/100 g) content of palm-olein at Day 0 and after 52 week storage period at 50°C.

Tocopherol content (mg/100 g)					
Tocopherols	**Day 0**	**Week 52**			
		Control	0.035 mg/kg copper	0.17 mg/kg copper	0.69 mg/kg copper
Total tocopherols	53.7	33.2	15.7	2.5	3.4
Alpha-tocopherol	14.8	7.3	2.6	0	0
Alpha-tocotrienol	14.2	6.3	2.2	0	0
Gamma-tocotrienol	19.2	14.2	6.9	0	0
Delta-tocotrienol	5.5	5.4	4.1	2.5	3.4

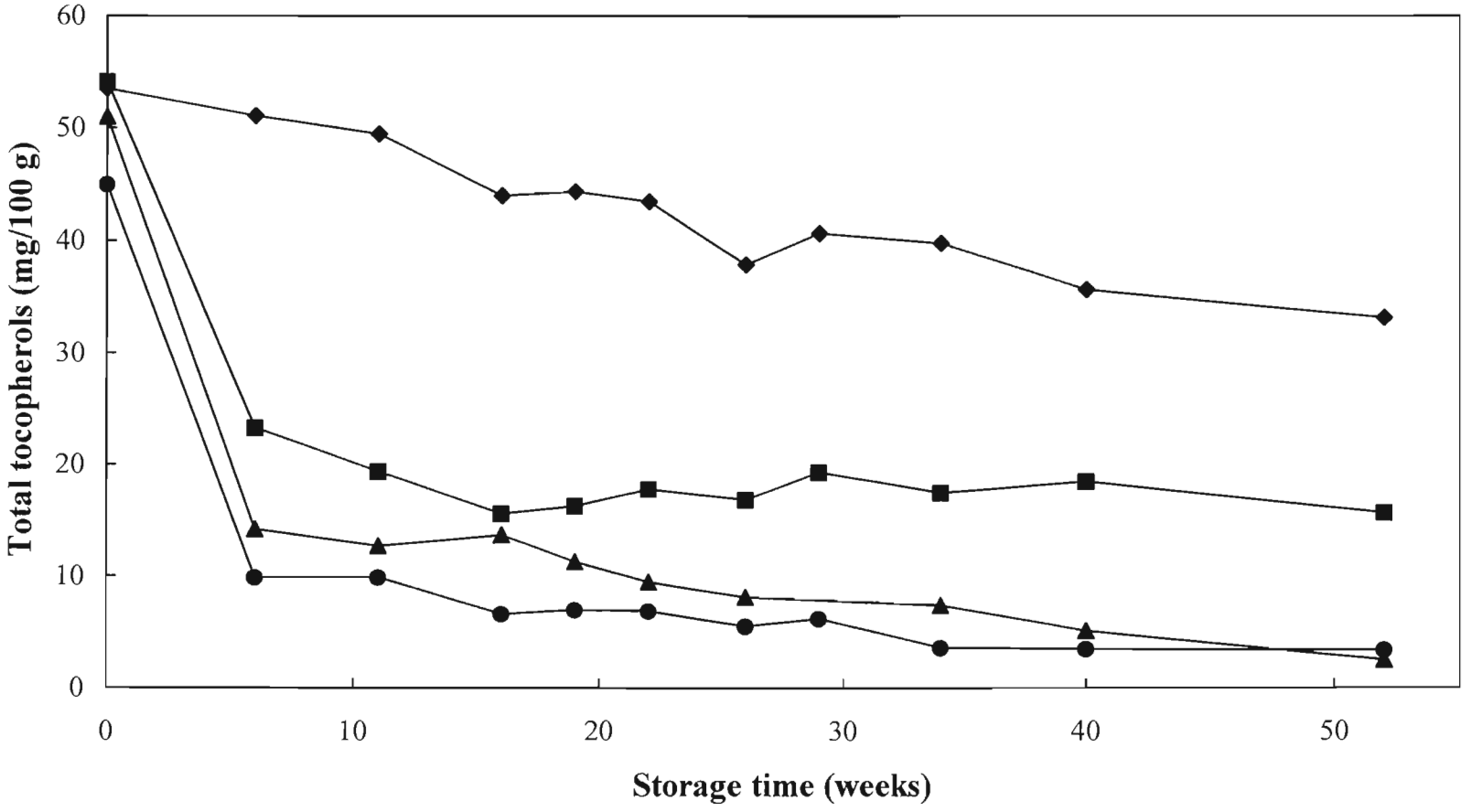


Figure 8: The effect of storage on the total tocopherol content (mg/100 g) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

Alpha-tocopherol

The effect of storage at 50°C on the alpha-tocopherol content of the four sample treatments is given in Figure 9.

The Control sample decreased gradually from Day 0 to Week 52. The alpha-tocopherol contents of the 0.17 and 0.69 mg/kg copper samples revealed slight decreases at Day 0 with further sharp decreases until Week 6 and no alpha-tocopherol remained in the 0.17 and 0.69 mg/kg copper samples after Week 22. The alpha-tocopherol of the sample with the lowest concentration of copper (0.035 mg/kg) remained reasonably constant after Week 6 with the values fluctuating between 0.8-2.9 mg/100 g.

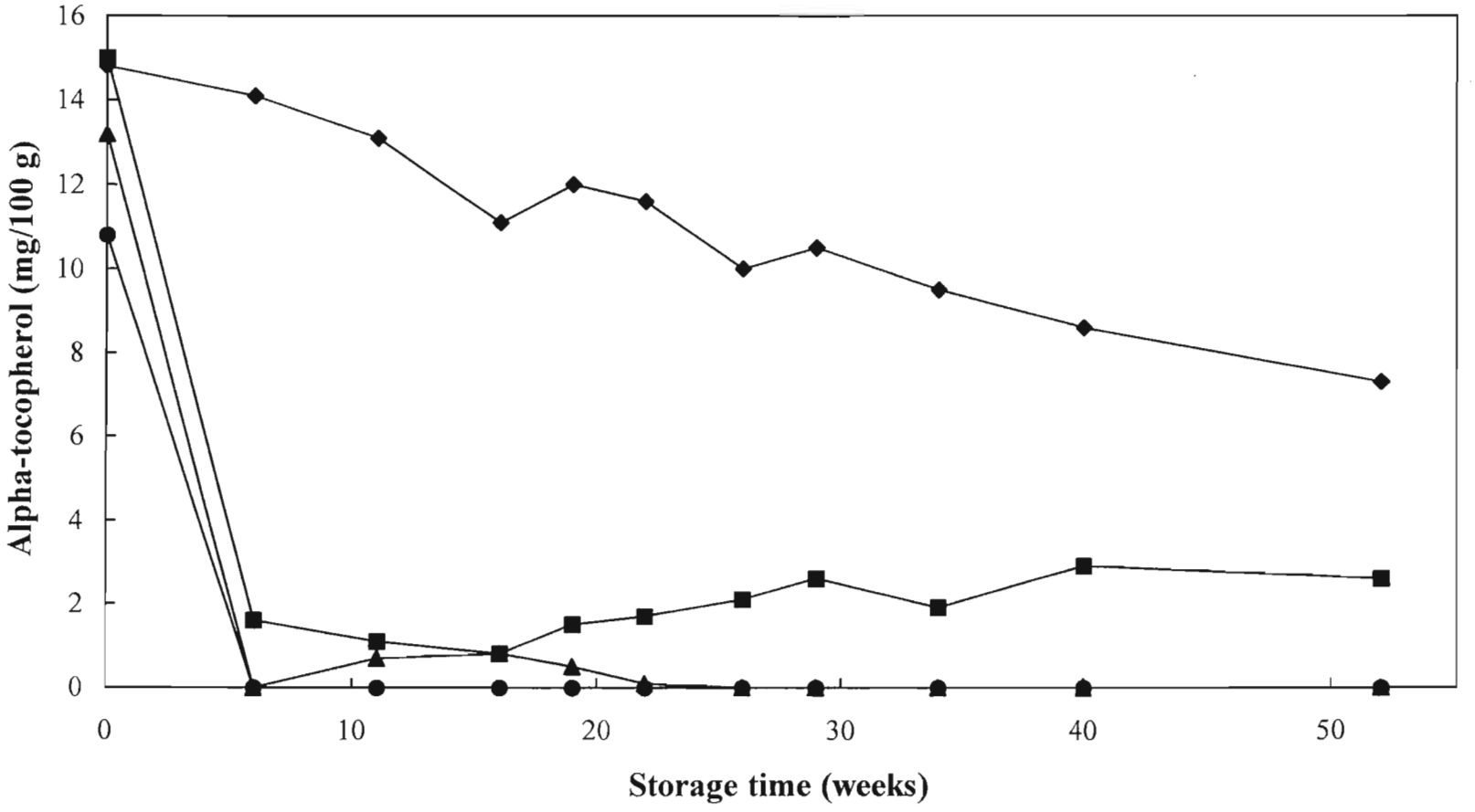


Figure 9: The effect of storage on the alpha-tocopherol content (mg/100 g) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

Alpha-tocotrienol

The effect of storage at 50°C on the alpha-tocotrienol content of the four sample treatments is given in Figure 10.

The alpha-tocotrienol decreased in a similar manner to the alpha-tocopherol, as can be seen from Figure 10. The Control sample decreased gradually from Day 0 to Week 52. The alpha-tocotrienol content of the 0.69 mg/kg copper sample had decreased somewhat at Day 0 after addition of copper. However, the alpha-tocotrienol content of the 0.17 mg/kg copper sample did not show a slight decrease at Day 0 as it had with the alpha-tocopherol. The alpha-tocotrienol in the samples containing copper decreased drastically within the first 6 weeks and no alpha-tocotrienol remained in the 0.17 and 0.69 mg/kg copper samples after Week 22. The alpha-tocotrienol of the sample with the lowest concentration of copper (0.035 mg/kg) remained reasonably constant after Week 6 with the values fluctuating between 0.7-2.4 mg/100 g in similar fashion to alpha-tocopherol.

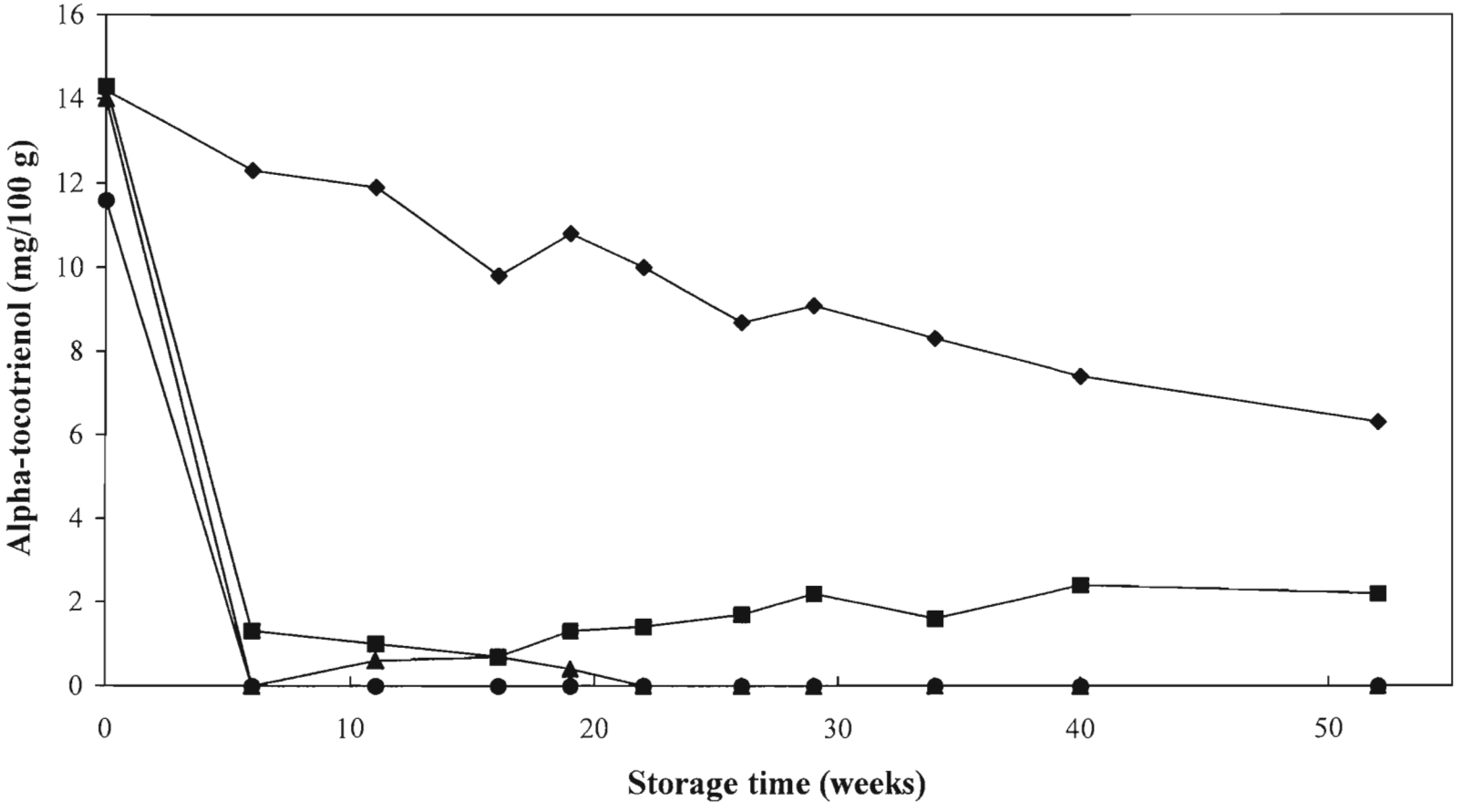


Figure 10: The effect of storage on the alpha-tocotrienol content (mg/100 g) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

Gamma-tocotrienol

The effect of storage at 50°C on the gamma-tocotrienol content of the four sample treatments is given in Figure 11.

The gamma-tocotrienol decreased in a different manner when compared to alpha-tocopherol and alpha-tocotrienol. The decrease was gradual from Day 0 to Week 52 for all the samples, although the gamma-tocotrienol of the Control sample decreased at a slower rate than the samples containing copper. The decrease in gamma-tocotrienol was concomitant with concentration of copper added. At Week 52 the two samples with the highest concentrations of copper (0.17 and 0.69 mg/kg) did not have any gamma-tocotrienol remaining, whereas the samples with 0.035 mg/kg copper and the Control still had 7.0 and 14.0 mg/kg gamma-tocotrienol remaining, respectively.

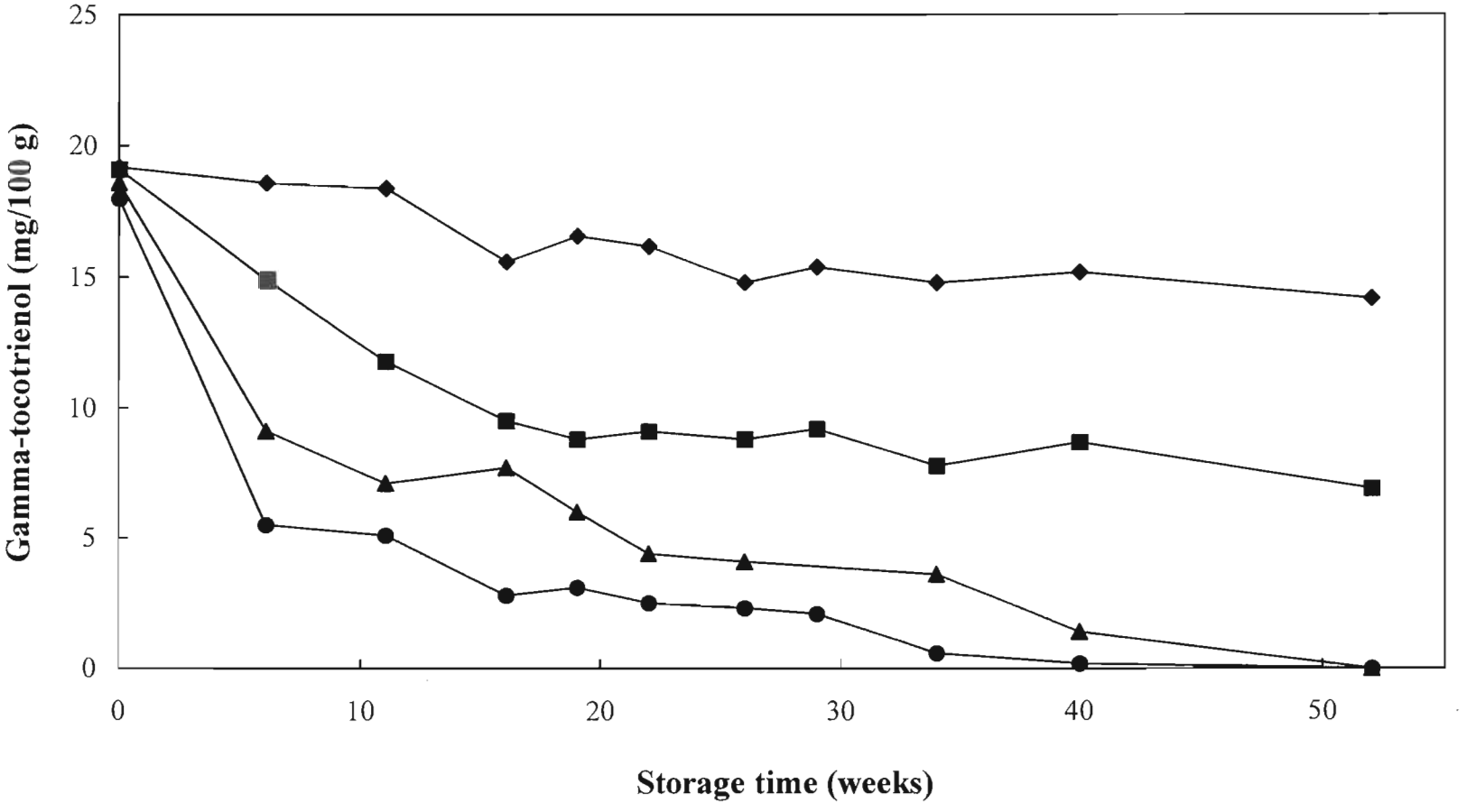


Figure 11: The effect of storage on the gamma-tocotrienol content (mg/100 g) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

Delta-tocotrienol

The effect of storage at 50°C on the delta-tocotrienol content of the four sample treatments is given in Figure 12.

The delta-tocotrienol showed the least decrease during the storage period of all the tocopherols. The delta-tocotrienol decreased very slowly over the storage period in the Control as well as the copper-containing samples. The delta-tocotrienol of the Control sample did not decrease substantially during the storage period, whereas in the samples containing copper the delta-tocopherol did decrease somewhat. The delta-tocotrienol content of the 0.035 mg/kg copper sample decreased by approximately a quarter and the 0.17 mg/kg by half and the 0.69 mg/kg by less than half (Table 9).

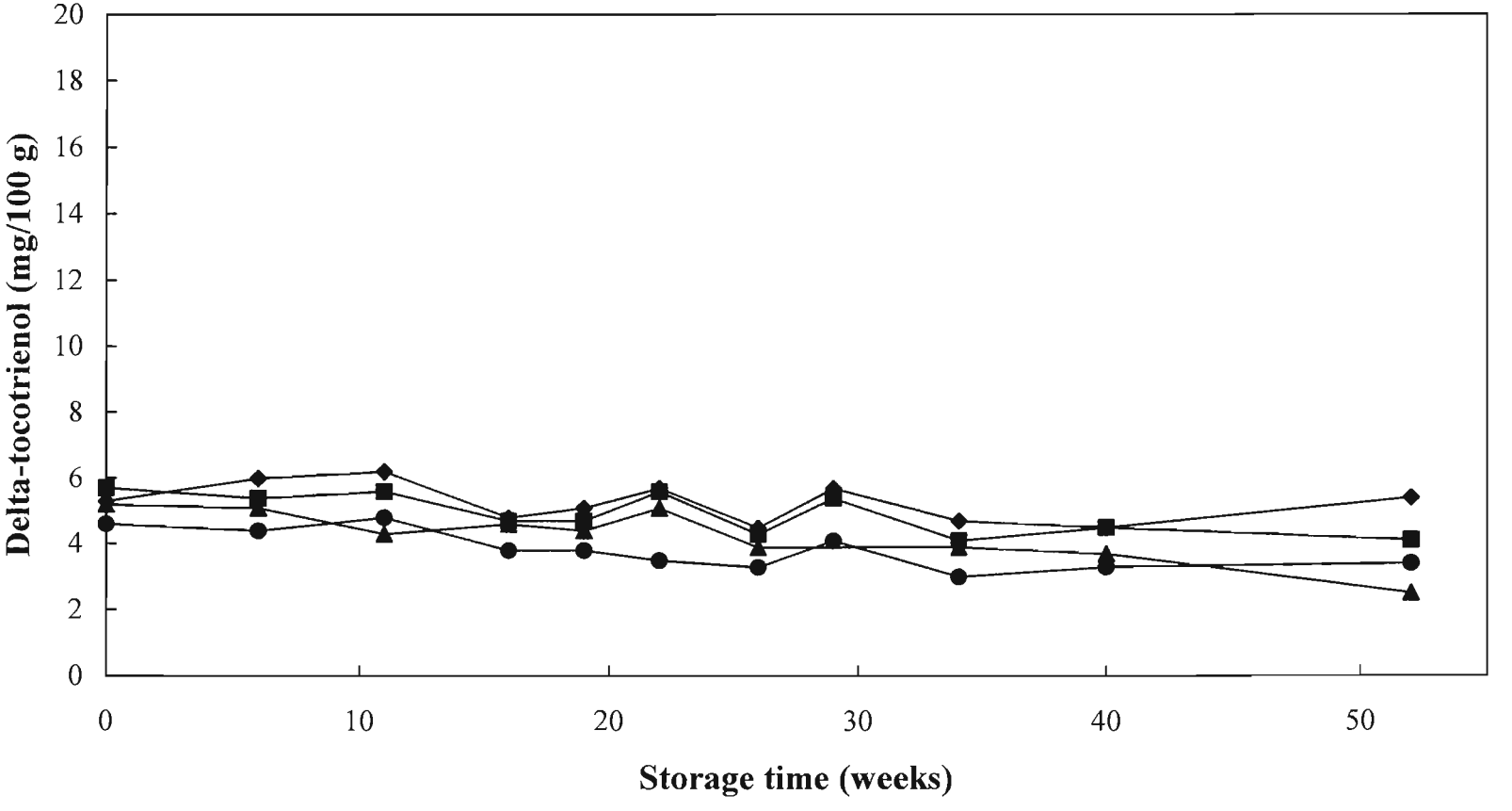


Figure 12: The effect of storage on the delta-tocotrienol content (mg/100 g) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.7 Conjugated diene and triene values

Conjugated diene (UV 232 nm)

The effect of storage at 50°C on the conjugated diene value of the four sample treatments is given in Figure 13.

There was a slight increase in the conjugated diene value during the storage period for all the samples. The Control generally had slightly higher values than the samples containing copper, although two copper-containing samples had slightly higher values at Day 0 than the Control. The values of all copper-containing samples decreased to levels below that of the Control at Week 6 and remained below the Control for the rest of the storage period, apart from the 0.17 mg/kg copper sample that reached the same value at Week 52 than the Control.

Conjugated triene (UV 268 nm)

The effect of storage at 50°C on the conjugated triene value of the four sample treatments is given in Figure 14.

The conjugated triene value of the Control increased only slightly over the storage period from 0.74 at Day 0 to 1.33 at Week 52 However, the values of the copper-containing samples increased up to 2.65, 2.73 and 2.84 for the 0.035, 0.17 and 0.69 mg/kg copper samples, respectively from initial values of 0.81, 0.87 and 0.96. The differences between the three concentrations of copper were minimal.

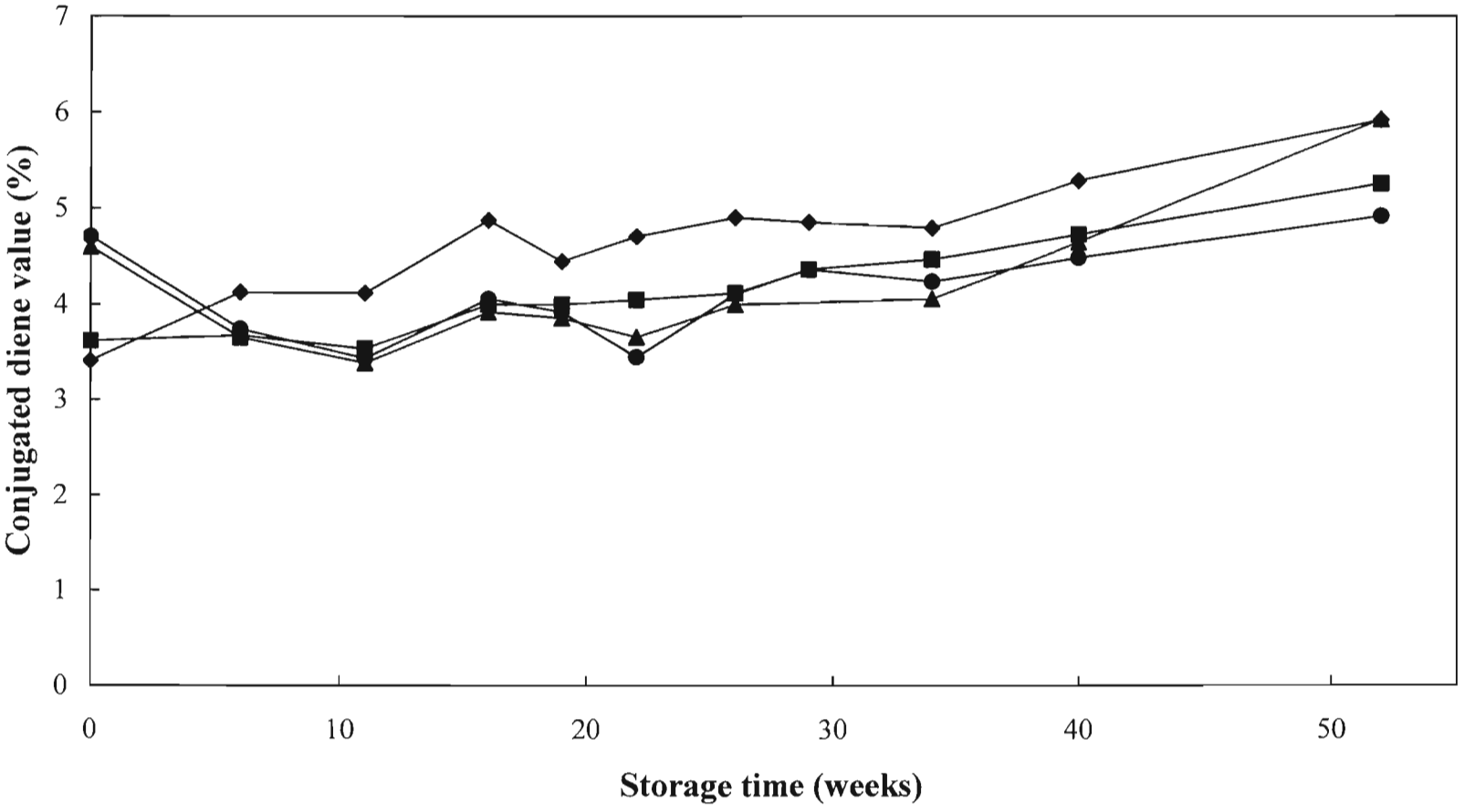


Figure 13: The effect of storage on the conjugated diene value (%) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

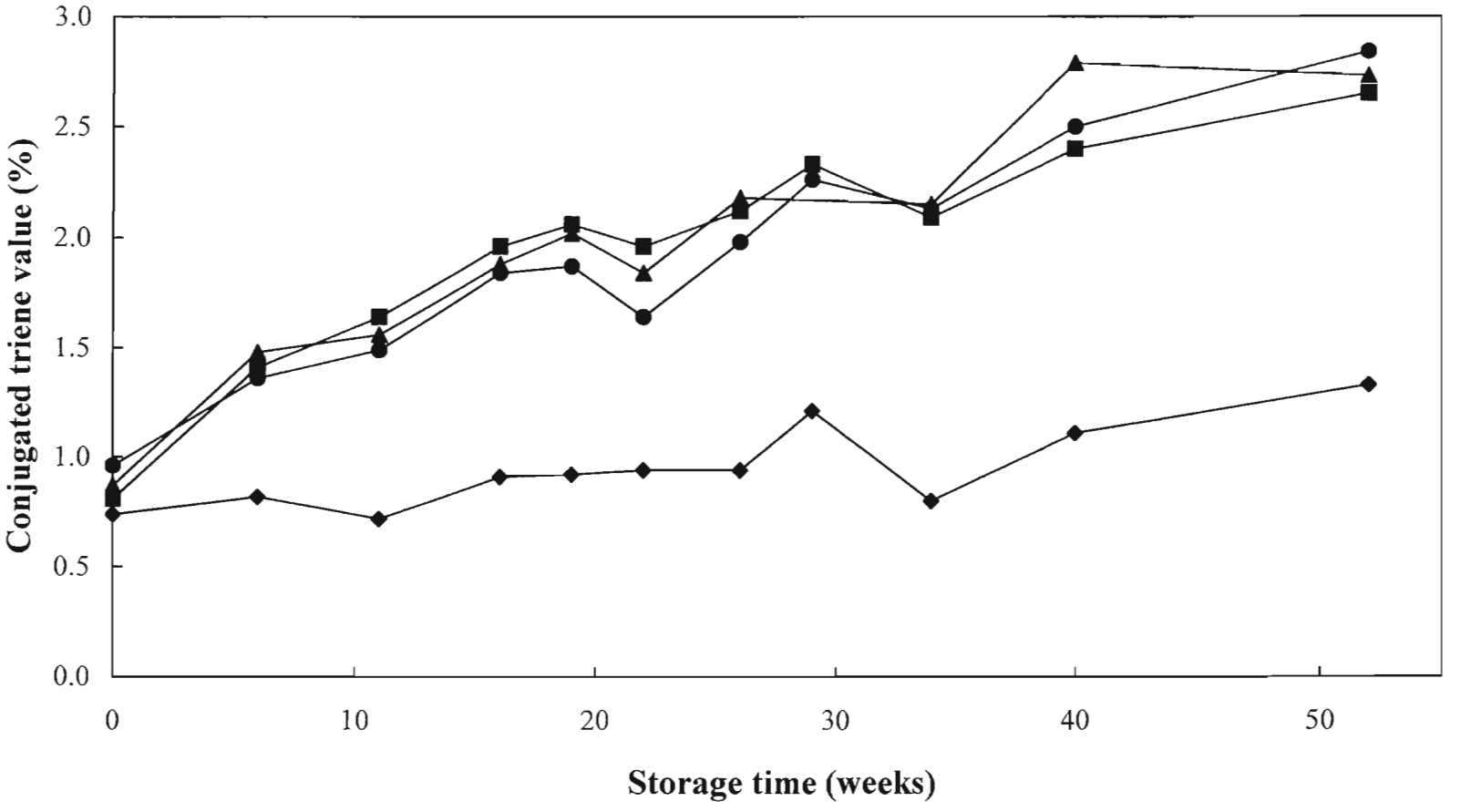


Figure 14: The effect of storage on the conjugated triene value (%) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.8 Iodine value

The effect of storage at 50°C on the IV of the four sample treatments is given in Figure 15.

Very little change occurred in the iodine value during storage at 50°C. The initial IV was 58.8 and at Week 52 the values obtained were 58.4, 58,2, 57.3 and 58.1 for the Control, 0.035, 0.17 and 0.69 mg/kg copper samples, respectively. The IVs of the copper-containing samples did not appear to differ markedly from the IV of the Control.

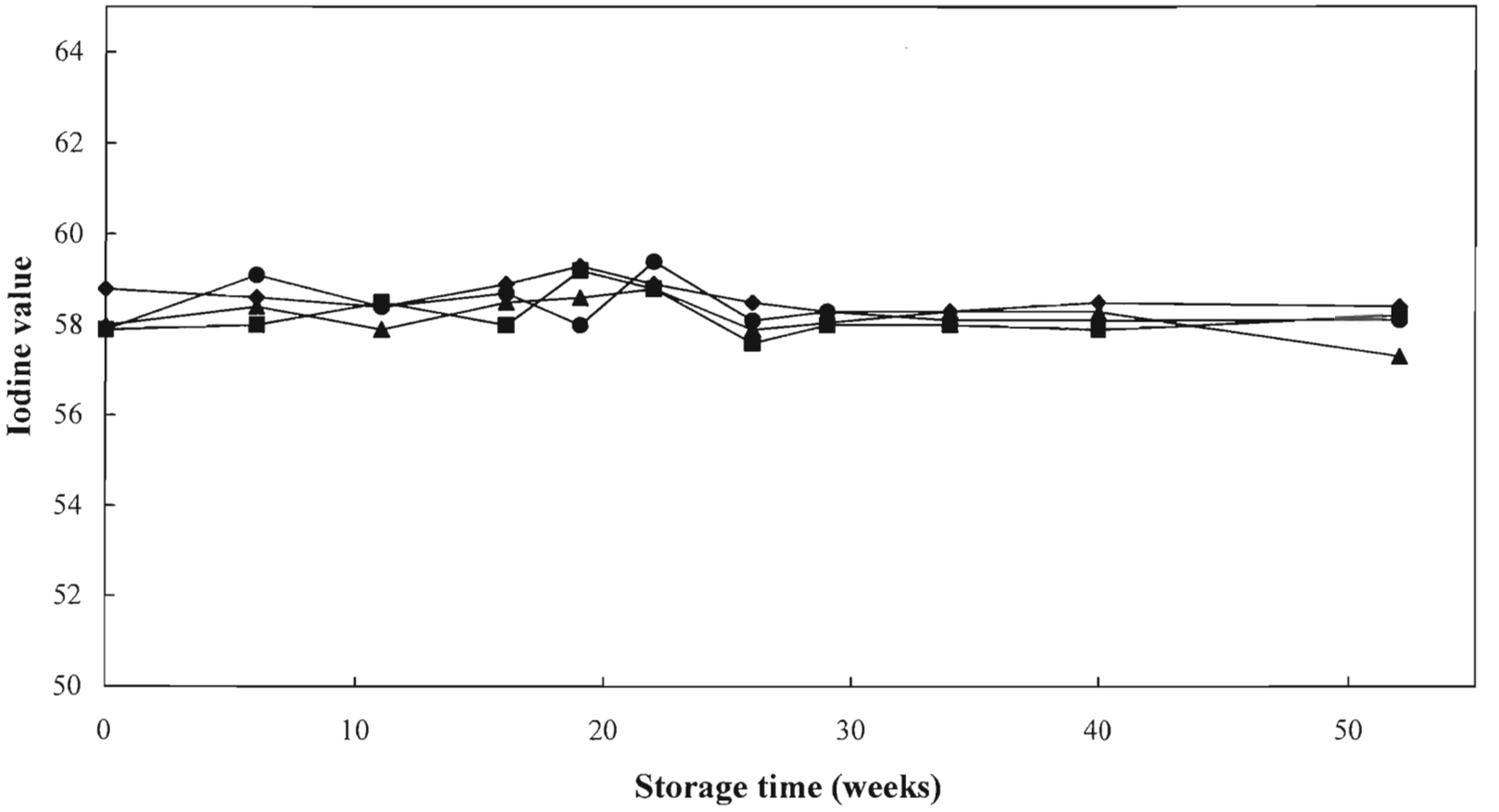


Figure 15: The effect of storage on the iodine value of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.9 Headspace volatile components

Total volatile peak area

The effect of storage at 50°C on the total volatile peak area of the samples is shown in Figure 16. The total volatile peak area for all the samples increased gradually during the storage period. The sample with the most copper generally had the highest total volatile peak area. The order of the rest of the samples' peak areas is not clear as the values fluctuated between the samples, although it does appear as if the 0.035 mg/kg copper sample generally had the lowest peak area.

trans-2-hexenal

The effect of storage at 50°C on the t-2-hexenal content of the four sample treatments is given in Figure 17. Low values of trans-2-hexenal were obtained for all the samples stored at 50°C. The values showed no pattern within the narrow fluctuation range of 0-2.3 mg/kg.

Hexanal

The effect of storage at 50°C on the hexanal content of the four sample treatments is given in Figure 18. Hexanal content increased over time for all the samples. The hexanal content of the Control sample increased most, whereas the hexanal of the samples containing copper increased at a slower rate over the storage period. There was no difference in the rate of increase between the copper-containing samples. However, there were higher initial concentrations of hexanal in the copper-containing samples and a higher final concentration of hexanal in the 0.17 mg/kg copper sample.

trans,trans-2,4-decadienal

The effect of storage at 50°C on the t,t-2,4-decadienal content of the four sample treatments is given in Figure 19. The Control sample did not form t,t-2,4-decadienal over the storage period. The samples containing copper all showed an increase in t,t-2,4-decadienal over time. There was hardly any t,t-2,4-decadienal formed up to Week 6 but this was followed by a rapid increase in t,t-2,4-decadienal up to Week 11, after which it increased gradually up to Week 52. After Week 16 the 0.17 mg/kg copper sample generally had the lowest values of the three copper-containing samples.

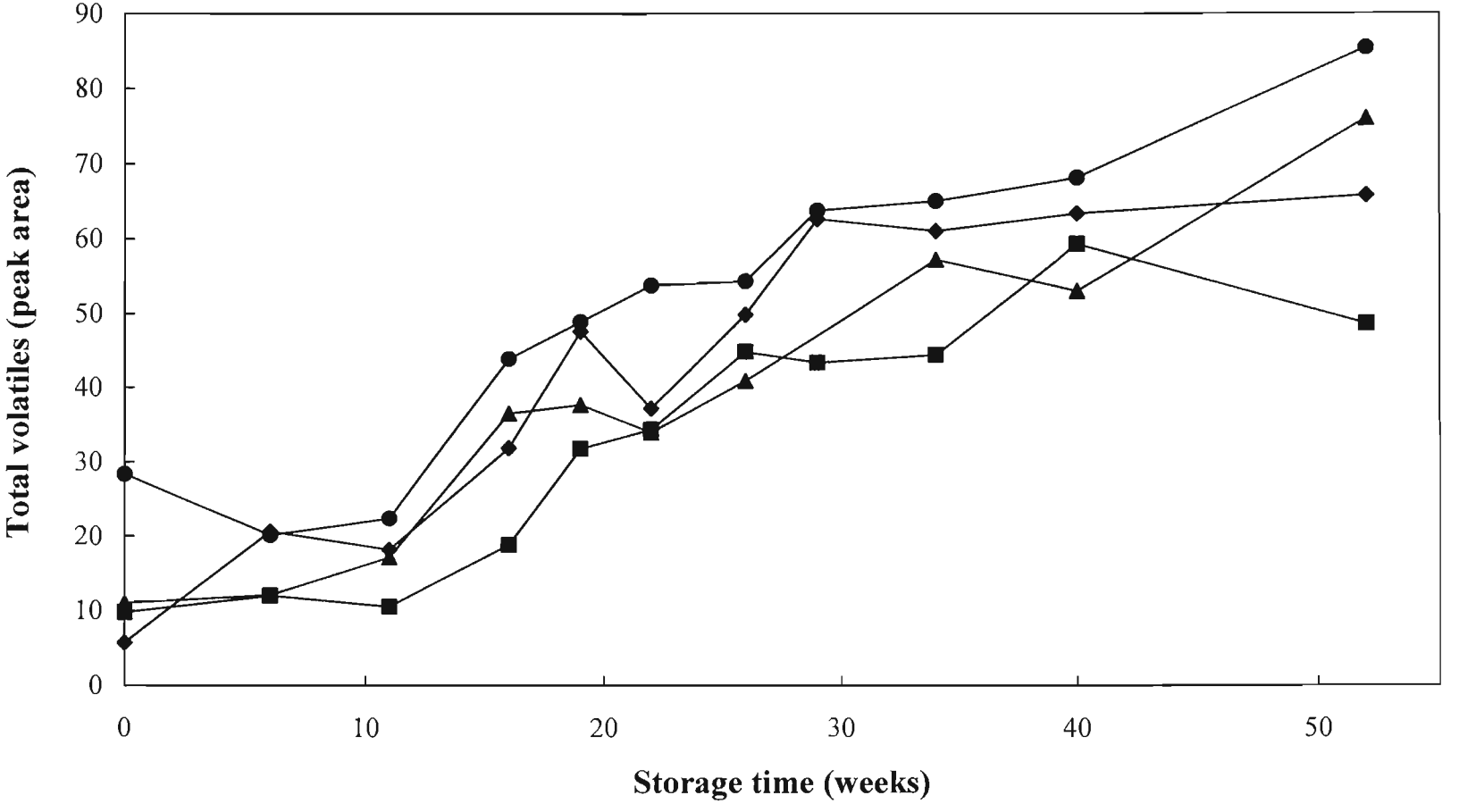


Figure 16: The effect of storage on the total volatile peak area of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

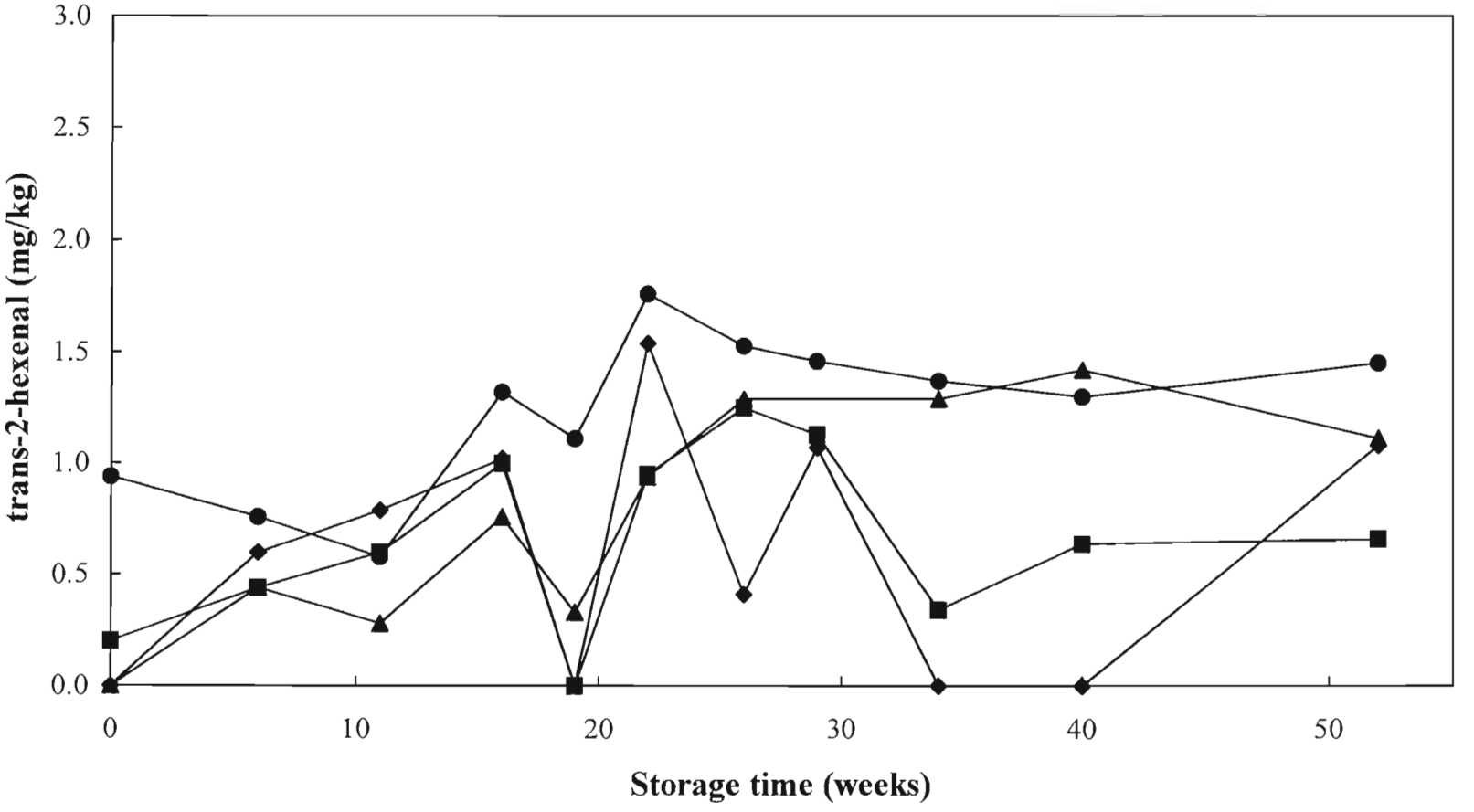


Figure 17: The effect of storage on the trans-2-hexenal content (mg/kg) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

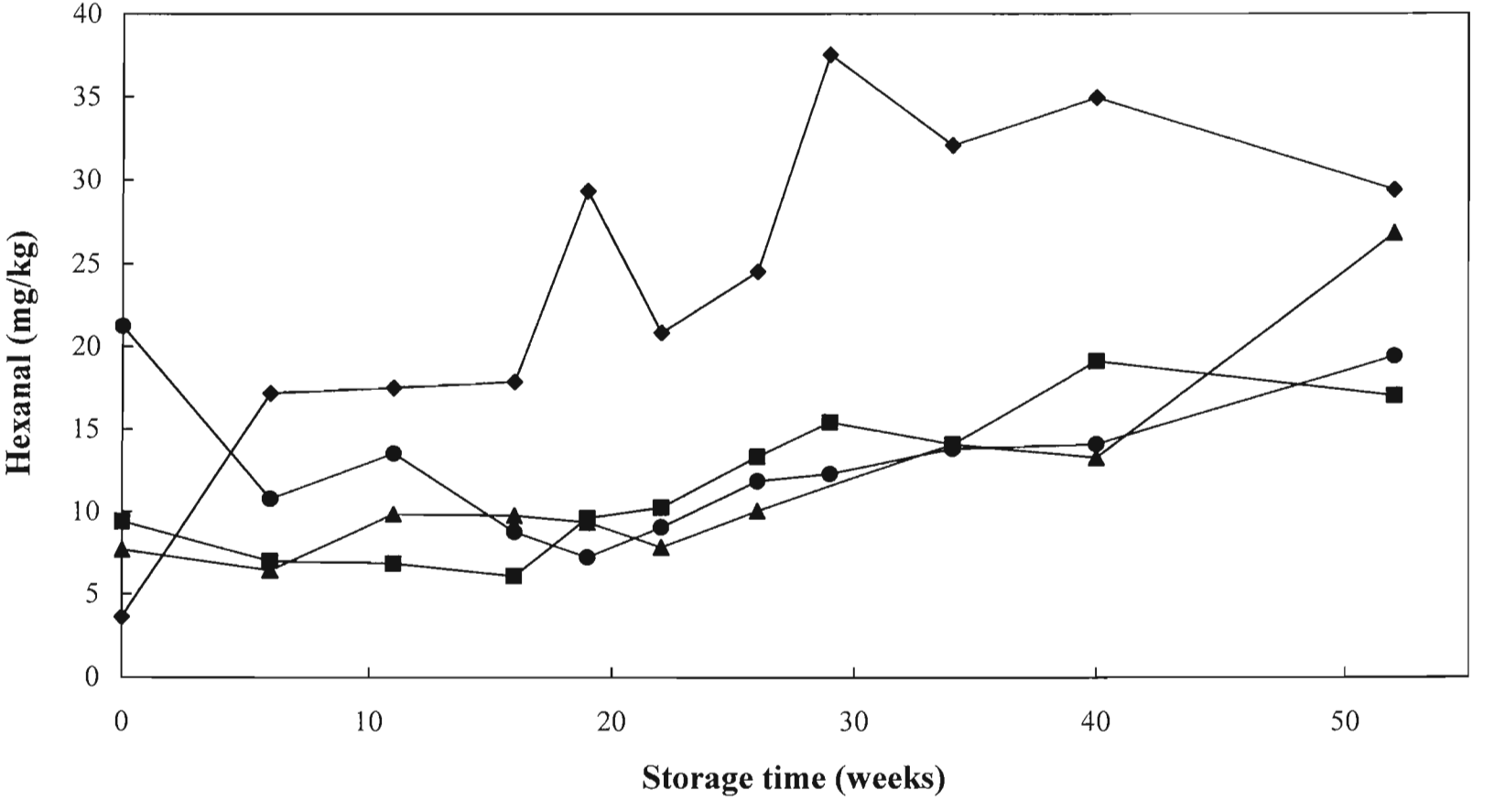


Figure 18: The effect of storage on the hexanal content (mg/kg) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

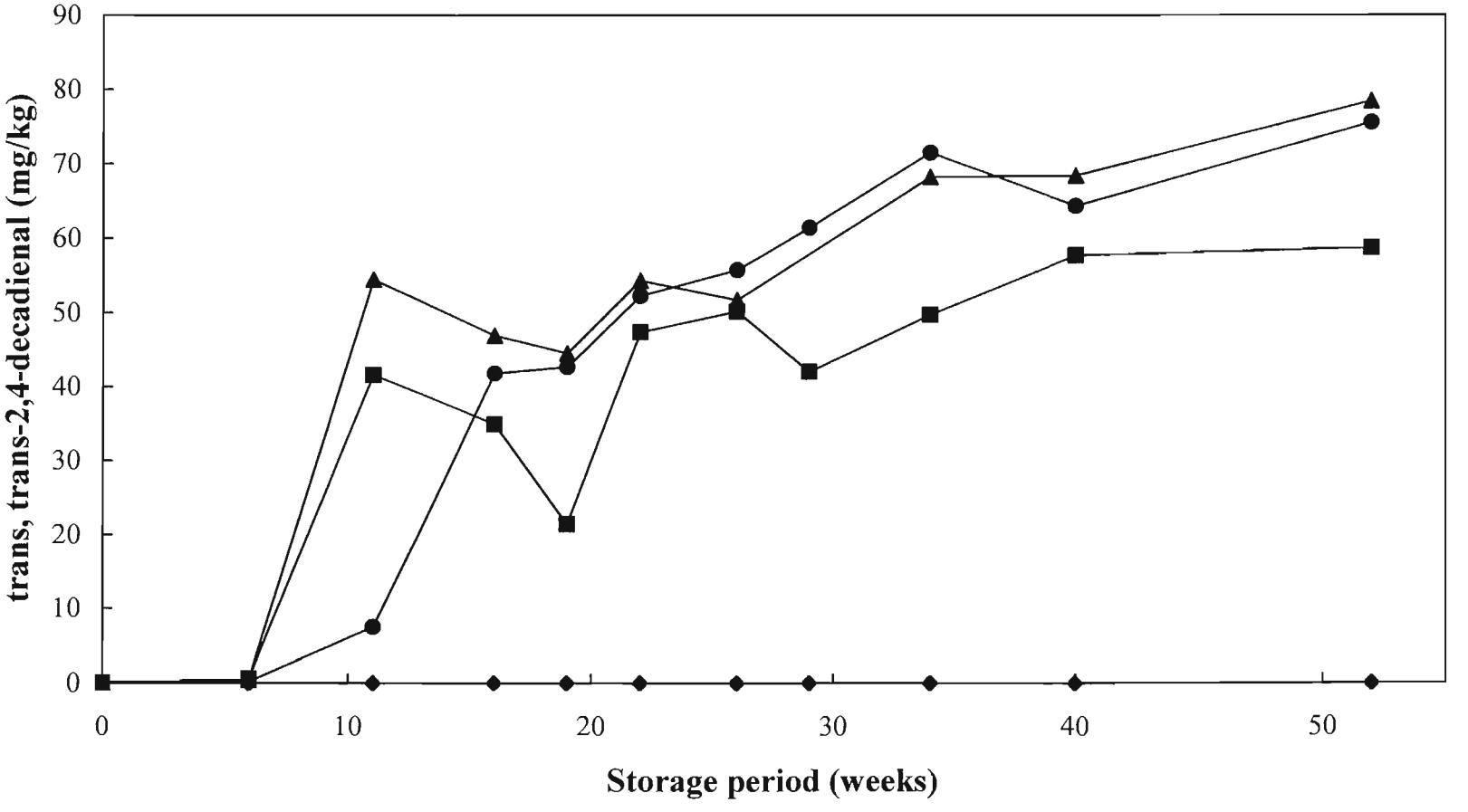


Figure 19: The effect of storage on the trans, trans-2,4-decadienal content (mg/kg) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

Pentanal peak area

The effect of storage at 50°C on the pentanal content of the four sample treatments is given in Figure 20. The Control had the lowest values and the values increased very gradually from Day 0 to Week 52. The 0.69 mg/kg copper-containing sample appeared to have the highest values that increased at a faster rate than the values of the Control from Day 0 to Week 52. The 0.035 mg/kg copper-containing sample had values higher than the Control and increased steadily from Day 0 to Week 40 after which the values decreased at Week 52. The 0.17 mg/kg copper-containing sample had values that increased between those of the 0.69 mg/kg and 0.035 mg/kg copper-containing samples from Day 0 up to Week 34, after which the values decreased.

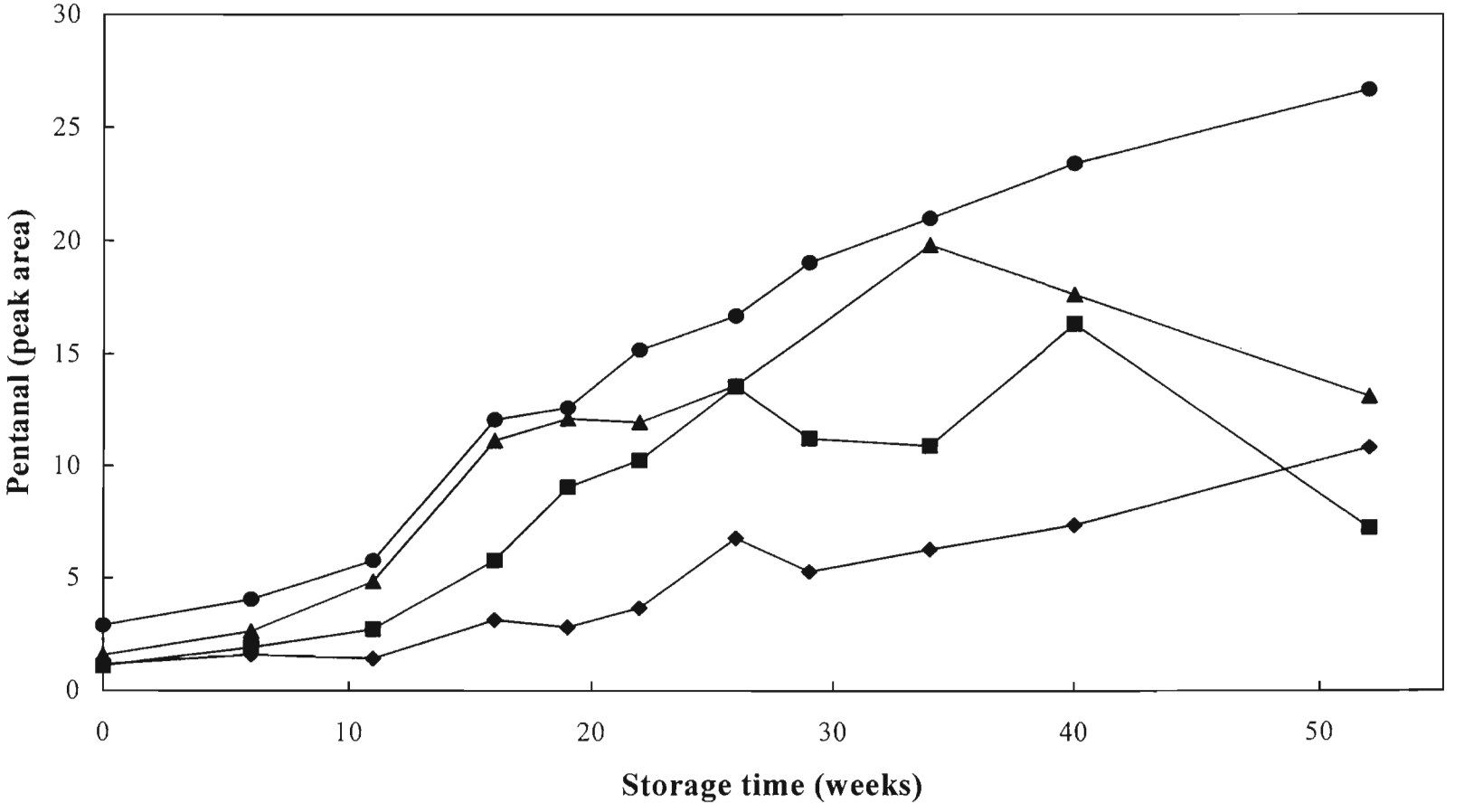


Figure 20: The effect of storage on the pentanal (peak area) of palm-olein stored at 50°C for a period of 52 weeks with different concentrations of copper added to the oil. Control sample (◆), 0.035 mg/kg copper (■), 0.17 mg/kg copper (▲) and with 0.69 mg/kg copper (●).

4.1.2.10 Sensory evaluation

The four categories used to evaluate the oils were:

1) Odour bland, weak characteristic odours
2) Weak off-odours or loss of characteristic odours
3) Moderate off-odours, slightly rancid
4) Strong off-odours, rancid, painty

Graphs were drawn with two combinations (options) of the categories: Oil identified as **Fresh** was grouped by either category **1)** only (Option 1) or the combination of categories **1)** and **2)** (Option 2). Oil identified as **Rancid** as a combination of categories **2)**, **3)** and **4)** or **3)** and **4)**.

The reasoning behind this was that for Option 1, anything that was not bland or characteristic of the oil, would be deemed off-odoured or rancid. The reasoning for Option 2 was that some panelists would seldom or never categorise an oil as fresh and would thus select category **2)** even though the oil was deemed fresh.

Thus:	**Option 1:**	Fresh	1)
		Rancid	2), 3) and 4)
	Option 2:	Fresh	1) and 2)
		Rancid	3) and 4)

According to O'Mahoney (1986) the statistical guideline would be that if 8 or 9 out of 12 panelists regard the oil to be rancid it would be significant at the 20 % and 10 % level of significance, respectively. This applies for a one-tailed paired-comparison difference test of rancid and fresh oil. For the 5 % level of significance 10 out of 12 panelists must deem the oil to be rancid.

The results of the sensory evaluation conducted by 12 panelists are given in Figures 21 and 22 where Figure 21 shows results of Option 1 and Figure 22 the results of Option 2.

Control

At the storage time where the number of panelists that judged the oil to be rancid surpassed the number of panelists that judged the oil to be fresh, thus where the Fresh and Rancid lines cross and part permanently, the oil can be deemed rancid. Thus, according to Option 1, the majority of the panelists (8) judged the Control rancid by Week 40, although the oil was deemed rancid more convincingly at Week 52 by a majority of panelists (11). According to Option 2, the Control oil was rancid by Week 52 according to a majority of panelists (9). The lines of the Control and the 0.17 mg/kg copper sample (Fresh and Rancid) of Option 2 overlapped from Week 34, which resulted in the Control lines to be hidden underneath the 0.17 mg/kg copper sample lines.

Sample with 0.035 mg/kg copper

According to Option 1, the majority of the panelists (10) judged the oil to be rancid by Week 40. However, according to Option 2 the oil had not reached rancidity at Week 52.

Sample containing 0.17 mg/kg copper

According to Option 1, the oil was rancid by Week 26 with a majority of panelists (10). However, according to Option 2 the majority of panelists (9) judged the oil to be rancid at Week 52. The data point at Week 29 has been excluded from all the previous data as it was clearly shown to be an outlier, but for the sensory evaluation it has been included to demonstrate that most of the panelists could pick up that the oil at Week 29 was rancid.

Sample containing 0.69 mg/kg copper

According to Option 1, the oil was clearly rancid by Week 26 according to a majority of panelists (10). However, according to Option 2 the oil only appeared to be convincingly rancid by Week 52 with a majority of panelists (9).

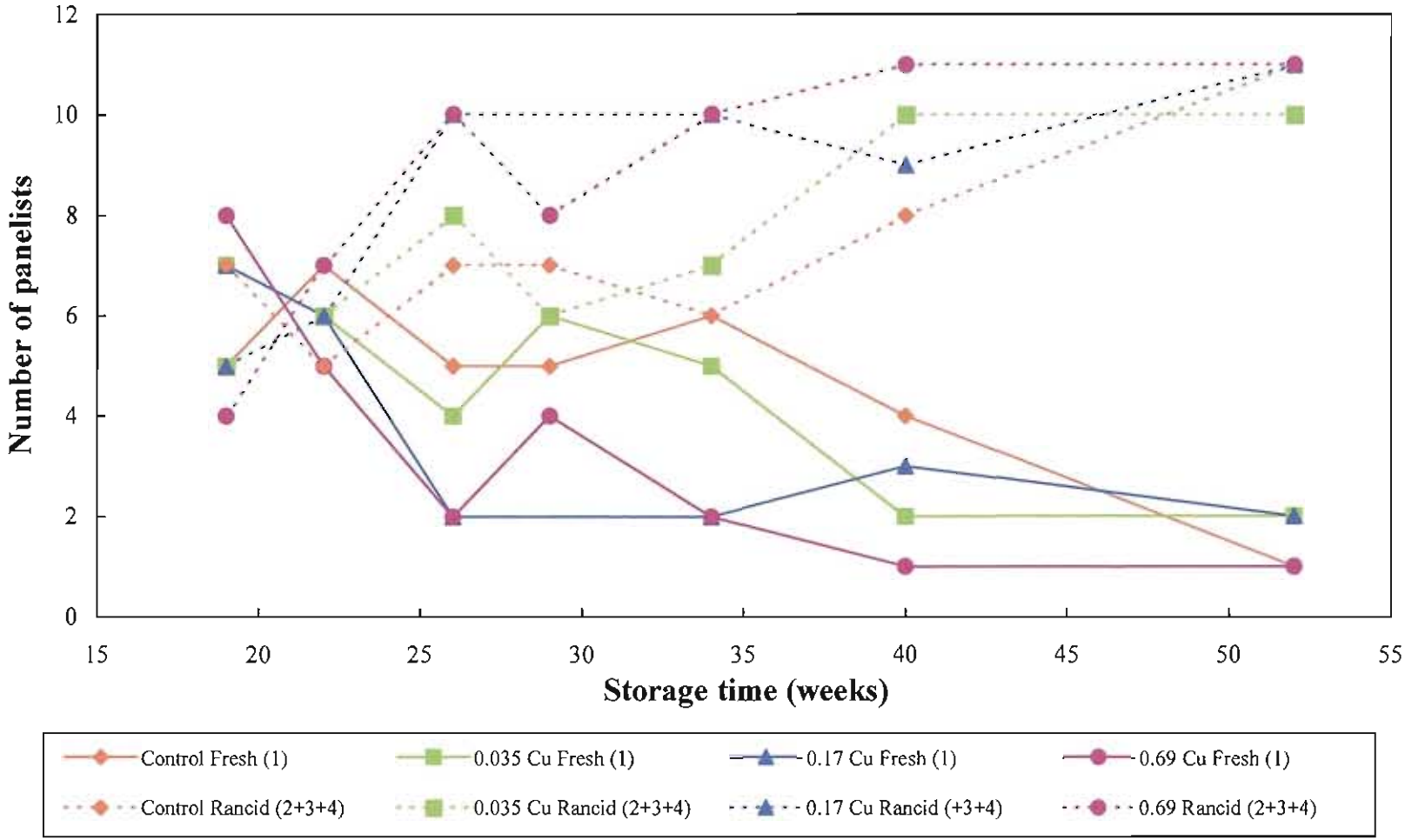


Figure 21: The effect of storage on the sensory quality of palm-olein oil stored at 50°C for a period of 52 weeks. (Option 1).

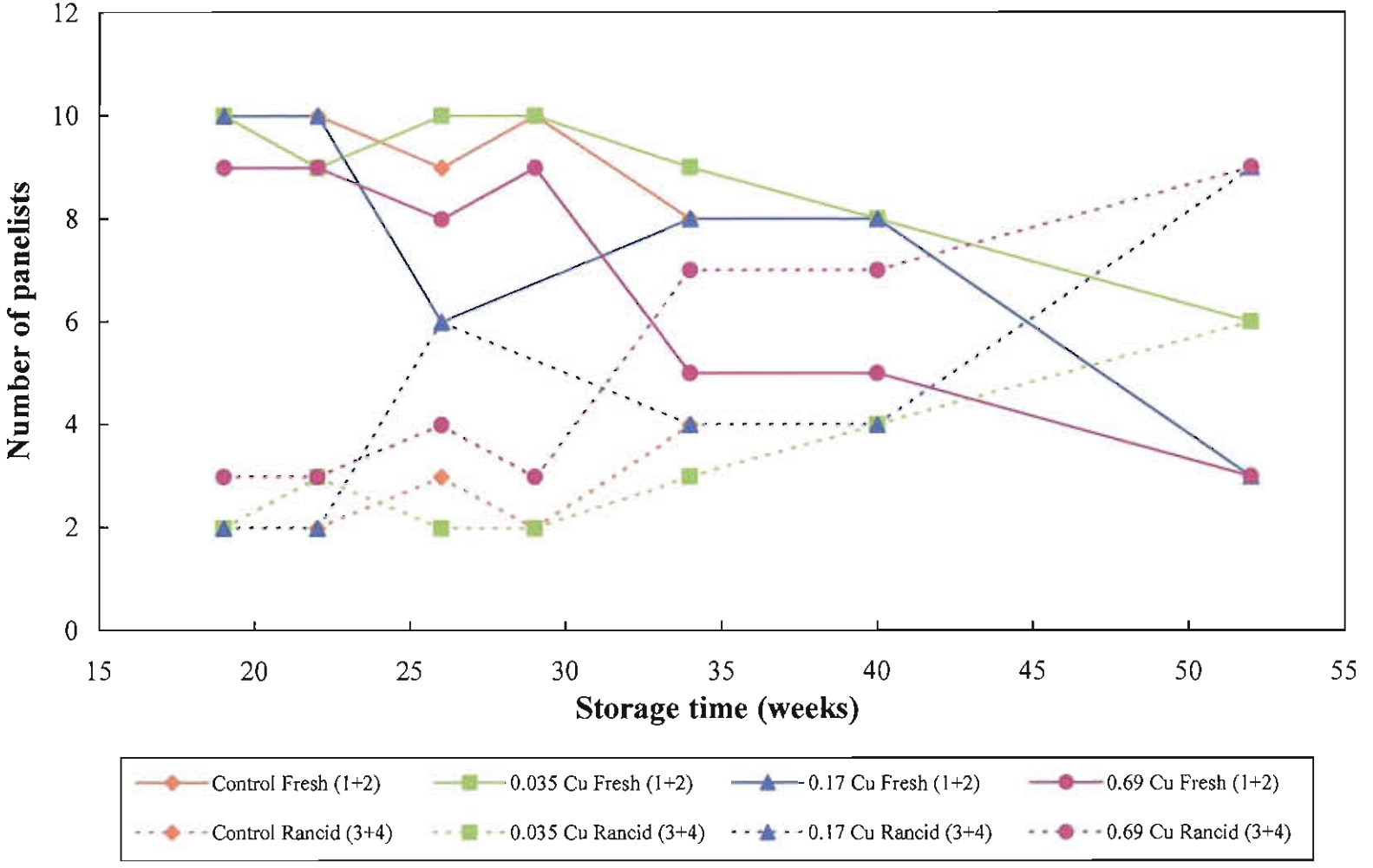


Figure 22: The effect of storage on the sensory evaluation of palm-olein oil stored at 50°C for a period of 52 weeks. (Option 2).

A summary of the results is presented in Table 10.

Table 10: Estimation of rancidity onset time by means of sensory evaluation and chemical parameters.

Samples	Storage time (weeks) at which oil is deemed to be rancid		
	Sensory evaluation*		Chemical parameters♦
	Option 1 Fresh (1), Rancid (2+3+4)	Option 2 Fresh (1+2), Rancid (3+4)	
Control	52	52	22
0.035 mg/kg copper	40	>52	6
0.17 mg/kg copper	26	52	6
0.69 mg/kg copper	26	52	6

* A majority of 9 out of 12 sensory panelists deemed the oil rancid

♦ Chemical parameters defined in Section 3.4 Modelling

4.1.3 Modelling

4.1.3.1 Models

The models are based on the following equation:

$$\text{Shelf-life (weeks)} = B\,(\text{Intercept}) + B_1\text{Variable}_1 + B_2\text{Variable}_2 + \cdots\cdots B_i\text{Variable}_j$$

where B_i regression coefficients

Variable_j independent variables such as FFA, PV etc., selected for model by multiple regression.

Model 1

All the data were used to create Model 1. The results of the variables selected by multiple regression to be used in the model are shown in Table 11. The variables were FFA, PV, AV, UV 232 nm, UV 268, OSI, total volatile components, hexanal, t-2-hexenal, t,t-2,4-decadienal, pentanal, total tocopherols, α-tocopherol, α-tocotrienol, γ-tocotrienol, δ-tocotrienol, IV, polyunsaturated fatty acids and copper content.

Table 11: Regression summary for dependant variable: shelf-life of Model 1.

N = 12 cases	**B** regression coefficients	Standard error of B coefficients	p-level
Intercept	23.0	5.85	0.004
AV	-1.31	0.483	0.026
OSI	0.507	0.170	0.017
FFA	-217.4	89.3	0.041

Where R^2 = 0.8115

F (3, 8) = 18.1

Standard error of estimate = 3.54

Explanation of the symbols used (Flury and Riedwyl, 1988; Miller and Miller, 1988; Farrant, 1997):

R^2: square of the correlation coefficient measures the degree of association between the dependant and the independent variables

F: a test of the significance of the relationship between the dependant variable and the set of independent variables. In this case the numerator was calculated to have 3 (4 regression coefficients – 1) degrees of freedom and the denominator had 8 (12 cases – 4 regression coefficients) degrees of freedom.

p-level: resulting probability value from t- and F-test indicates the significance of the values obtained

Std error of estimate: measurement of the dispersion of the observed values about the regression line

The graph of the predicted versus the observed values is shown in Figure 23.

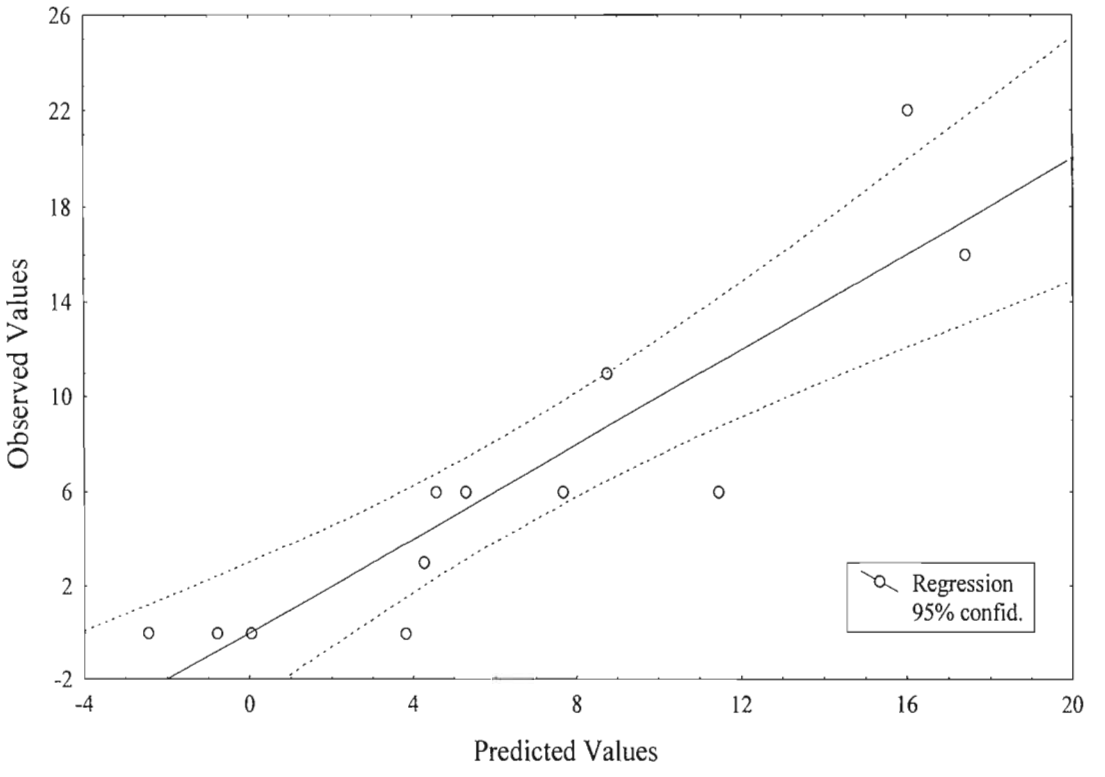


Figure 23: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 1. The dependant variable is the shelf-life.

Model 2

The squares of the values obtained were used to create Model 2. The multiple regression results are shown in Table 12.

Table 12: Regression summary for dependant variable: shelf-life of Model 2.

N = 12 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	28.7	10.9	0.034
Alpha-tocopherol^2	-0.05	0.038	0.243
FFA^2	-2975	630	0.002
OSI^2	0.02	0.005	0.003
Conjugated triene^2	-11.5	5.00	0.055

Where R^2 = 0.9146

F (4, 7) = 18.7

Standard error of estimate = 2.549

The graph of the predicted versus the observed values is shown in Figure 24.

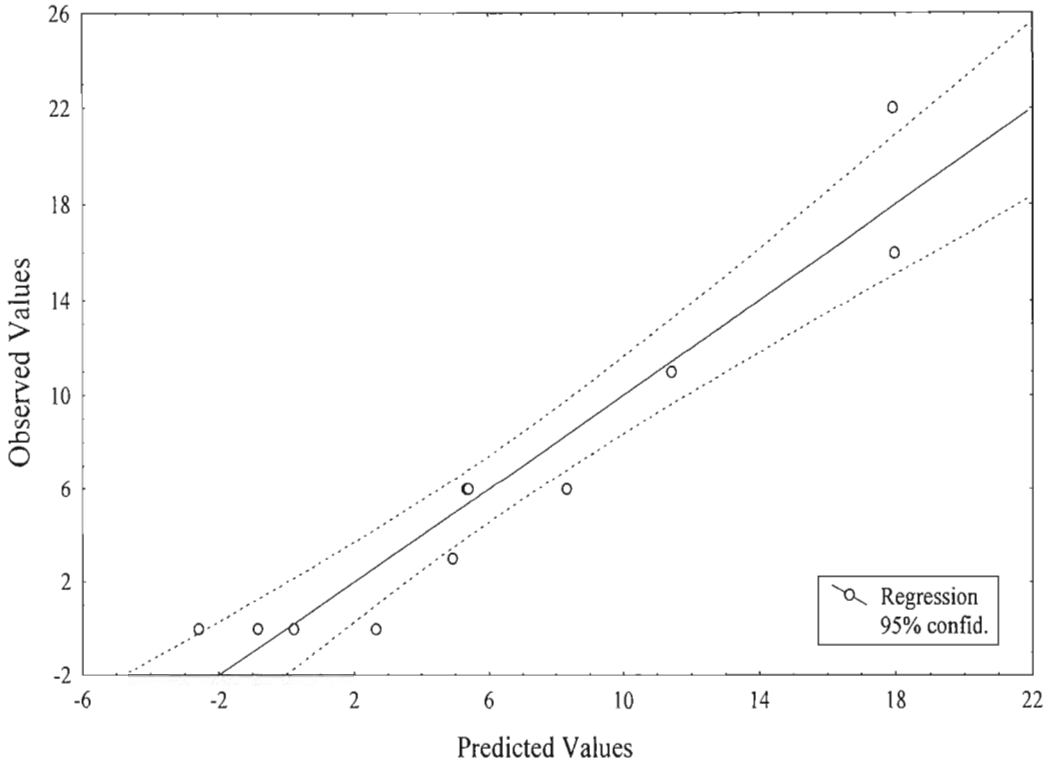


Figure 24: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 2. The dependant variable is the shelf-life.

Model 3

The weighted values (see Materials and Methods, section 3.4) obtained for each variable was used to create Model 3. The multiple regression results are shown in Table 13.

Table 13: Regression summary for dependant variable: shelf-life of Model 3.

N = 12 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	23.0	5.84	0.004
Weighted AV	-2.29	0.844	0.026
Weighted OSI	0.197	0.066	0.017
Weighted FFA	-3.33	1.37	0.041

Where R^2 = 0.8115

F (3, 8) = 11.5

Standard error of estimate = 3.54

The graph of the predicted versus the observed values is shown in Figure 25.

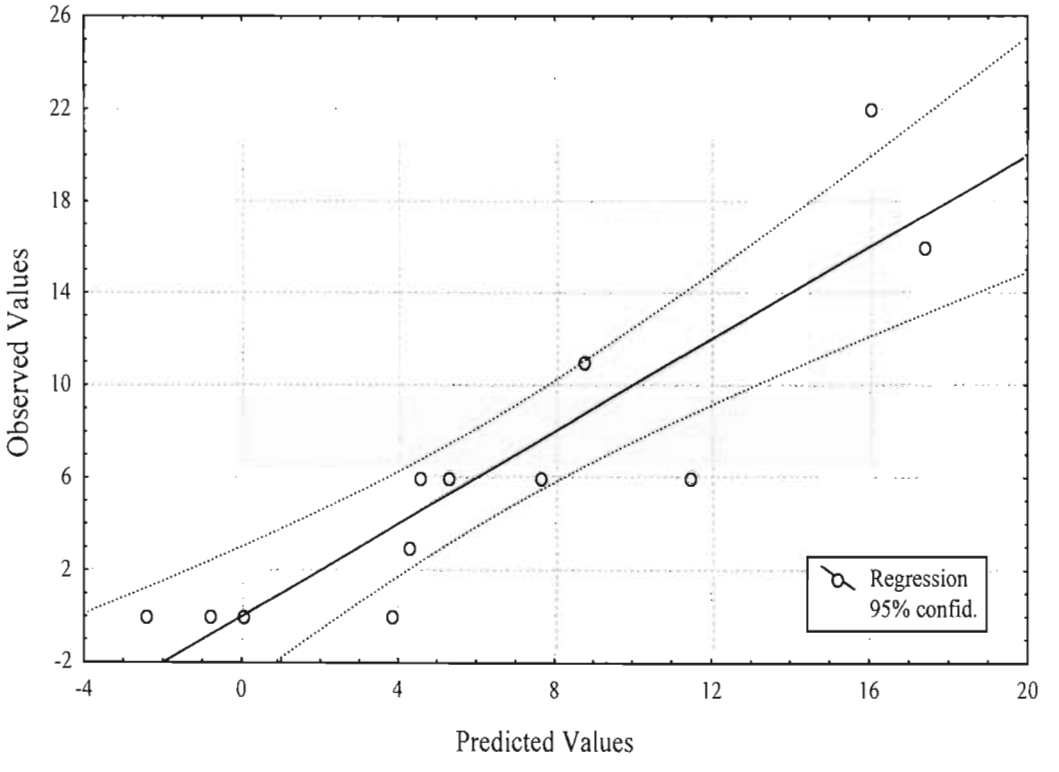


Figure 25: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 3. The dependant variable is the shelf-life.

Model 4

The weighted values obtained for the square value of each variable was used to create Model 4. The multiple regression results are shown in Table 14.

Table 14: Regression summary for dependant variable: shelf-life of Model 4

N = 12 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	28.7	10.9	0.034
Weighted alpha-tocopherol^2	-0.005	0.004	0.243
Weighted FFA^2	-45.5	9.64	0.002
Weighted OSI^2	0.009	0.002	0.003
Weighted conjugated triene^2	-0.682	0.296	0.055

Where	R^2	=	0.9146
	F (4, 7)	=	18.7
	Standard error of estimate	=	2.55

The graph of the predicted versus the observed values is shown in Figure 26.

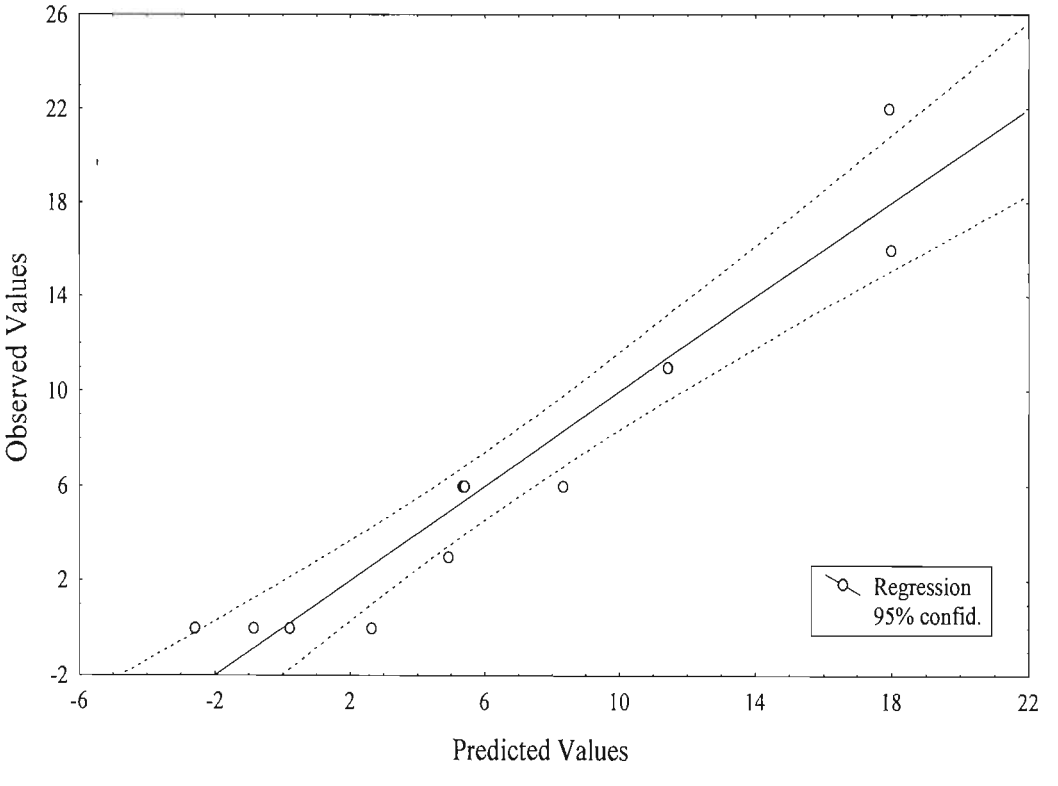


Figure 26: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 4. The dependant variable is the shelf-life.

Model 5

A complex model (Ideal model) where the measured values and the square of those values of all of the variables were used to create Model 5. The results of the variables selected by the multiple regression program are shown in Table 15.

Table 15 Regression summary for dependant variable: shelf-life of Model 5

N = 12 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	26.0	3.86	0.000
AV	-1.23	0.312	0.006
OSI^2	0.072	0.020	0.010
OSI	-1.31	0.530	0.042
FFA	-136	61.9	0.063

Where	R^2	=	0.9316
	F (4, 7)	=	23.8
	Standard error of estimate	=	2.28

The graph of the predicted versus the observed values is shown in Figure 27.

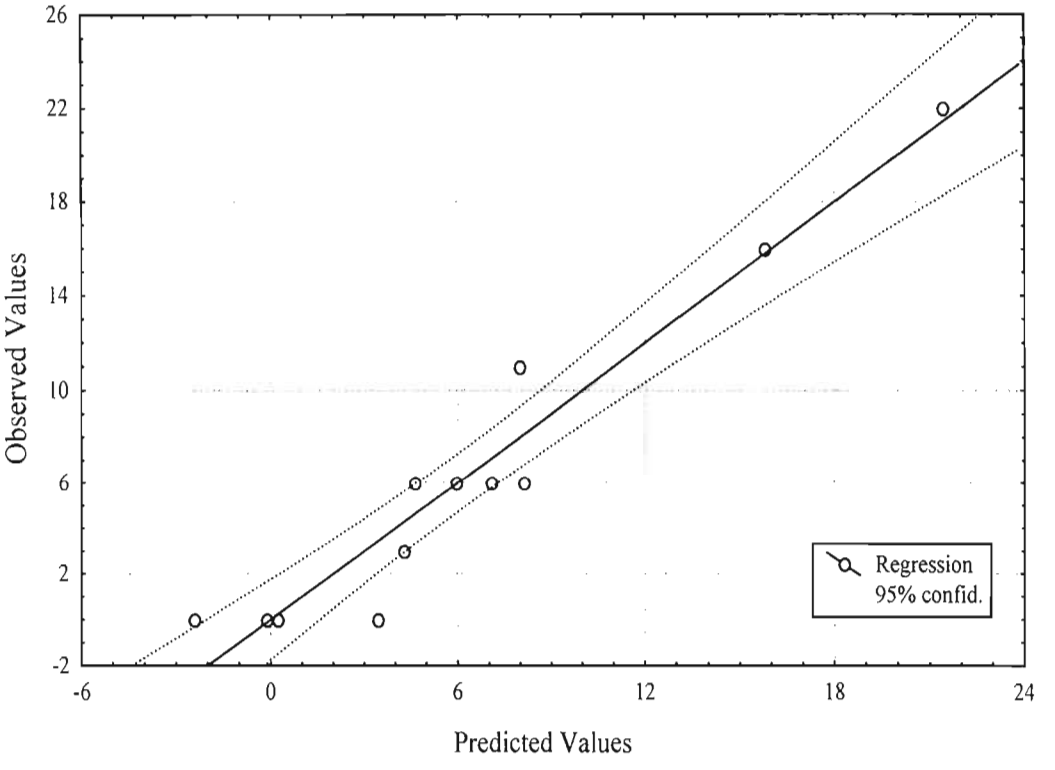


Figure 27: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 5. The dependant variable is the shelf-life.

Model 6

A simple model (Practical model) with variables that are simple methods of analyses was used to create Model 6. The measured values and the square of those values were used to create the model. The measured values for FFA, PV, OSI and conjugated diene/triene value, as well as the square of these values were utilised to create Model 6. The multiple regression results are shown in Table 16.

Table 16: Regression summary for dependant variable: shelf-life of Model 6.

N = 12 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	26.7	3.54	0.000
Conjugated triene value	-12.6	2.41	0.001
FFA^2	-1840	430	0.004
OSI^2	0.070	0.017	0.005
OSI	-1.30	0.440	0.021

Where R^2 = 0.9546
F (4, 7) = 36.8
Standard error of estimate = 1.86

The graph of the predicted versus the observed values is shown in Figure 28.

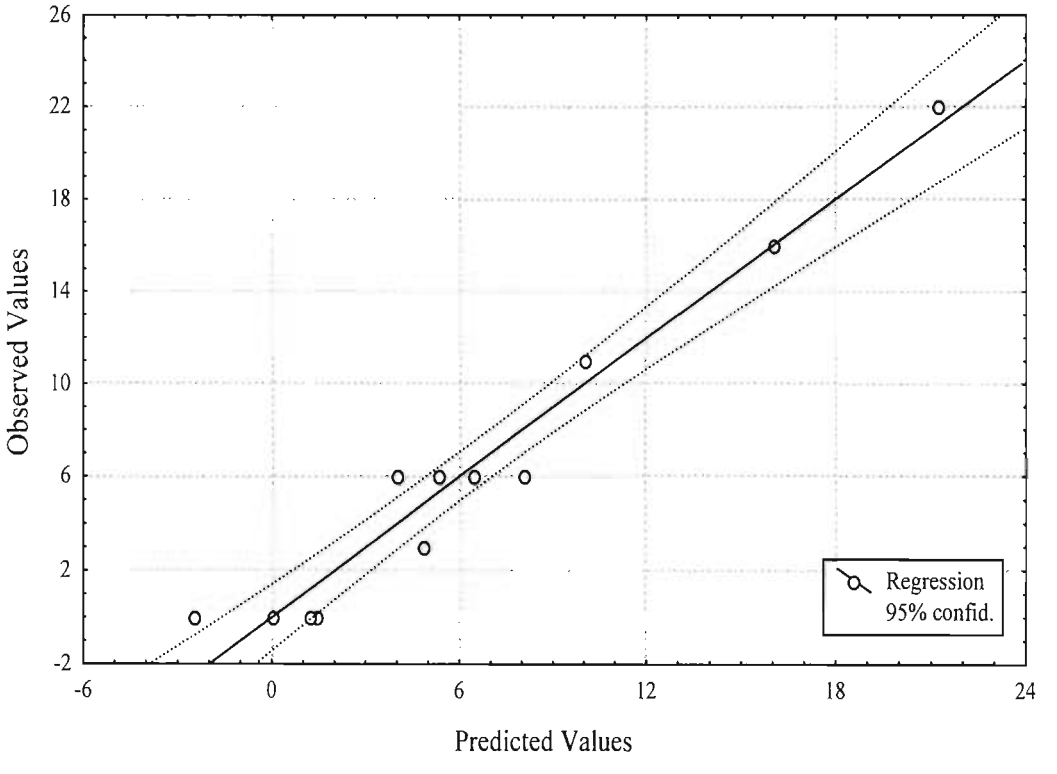


Figure 28: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 6. The dependant variable is the shelf-life.

Model 7

The OSI value and its square were used to create Model 7. The multiple regression results are shown in Table 17.

Table 17: Regression summary for dependant variable: shelf-life of Model 7.

N = 12 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	10.6	5.18	0.071
OSI^2	0.11	0.037	0.016
OSI	-2.18	0.951	0.048

Where R^2 = 0.6985
F (2, 9) = 8.30
Standard error of estimate = 4.56

The graph of the predicted versus the observed values is shown in Figure 29.

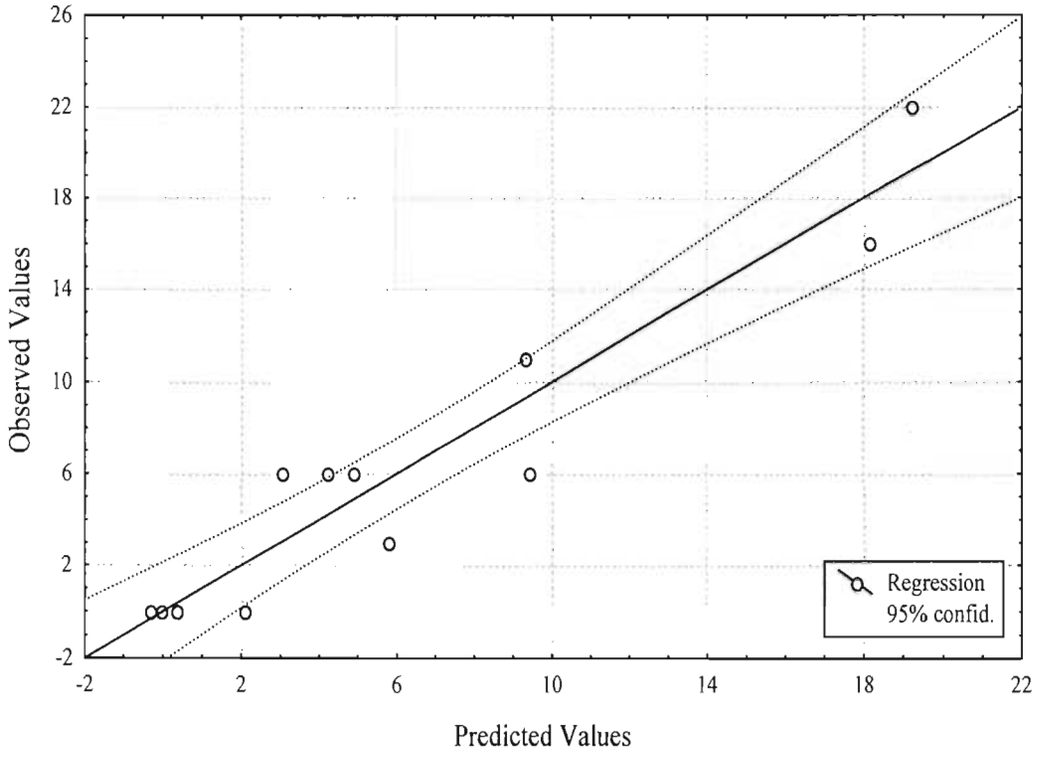


Figure 29: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 7. The dependant variable is the shelf-life.

Model 8

A complex model (Ideal model) as in Model 5, but based on the sensory evaluation, was used to create Model 8. The multiple regression results are shown in Table 18.

Table 18: Regression summary for dependant variable: shelf-life of Model 8.

N = 35 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	20.5	2.33	0.000
Pentanal peak area	-0.446	0.188	0.025
FFA	-95.4	21.8	0.000
AV	-0.728	0.131	0.000
Alpha-tocopherol^2	0.030	0.006	0.000
Copper^2	24.7	3.19	0.000
Total volatile peak area^2	-0.002	0.001	0.001
OSI	1.51	0.093	0.000

Where R^2 = 0.9891

F (7, 27) = 350.4

Standard error of estimate = 1.62

The graph of the predicted versus the observed values is shown in Figure 30.

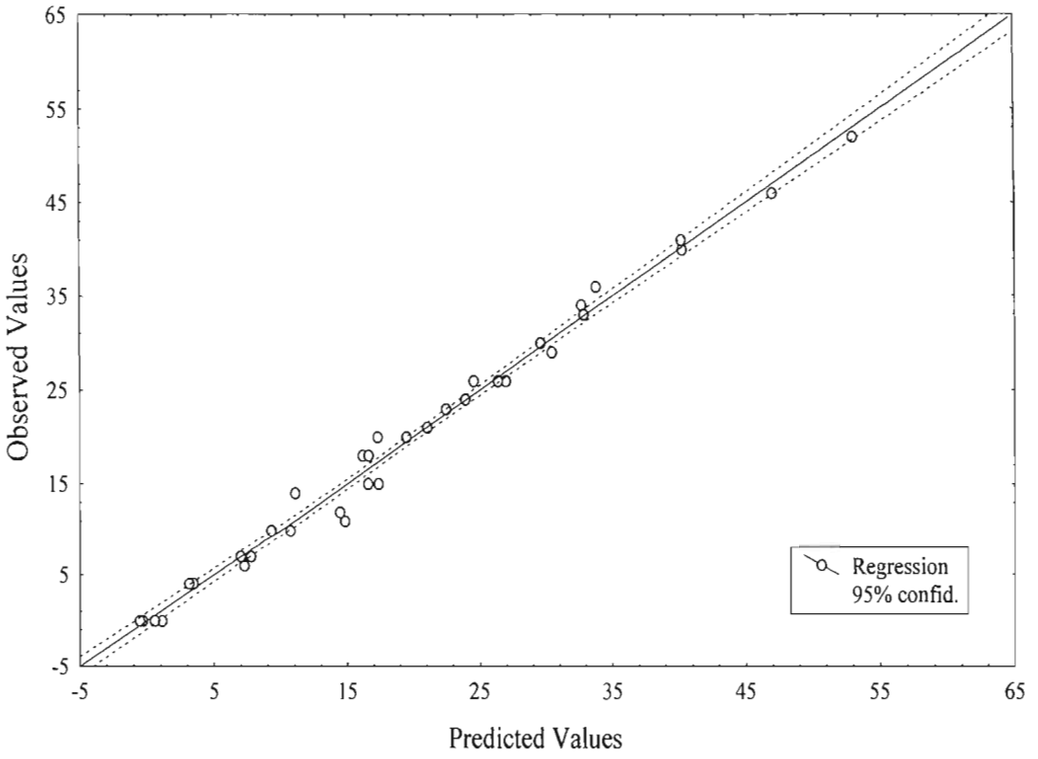


Figure 30: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 8. The dependant variable is the shelf-life.

Model 9

A simple model (Practical model) as in Model 6, but based on the sensory evaluation, was used to create Model 9. The multiple regression results are shown in Table 19.

Table 19: Regression summary for dependant variable: shelf-life of Model 9.

N = 35 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	46.2	2.93	0.000
Conjugated triene value	-14.4	1.34	0.000
OSI^2	0.053	0.005	0.000
FFA	-220	27.7	0.000

Where R^2 = 0.9306

F (3, 31) = 138.6

Standard error of estimate = 3.83

The graph of the predicted versus the observed values is shown in Figure 31.

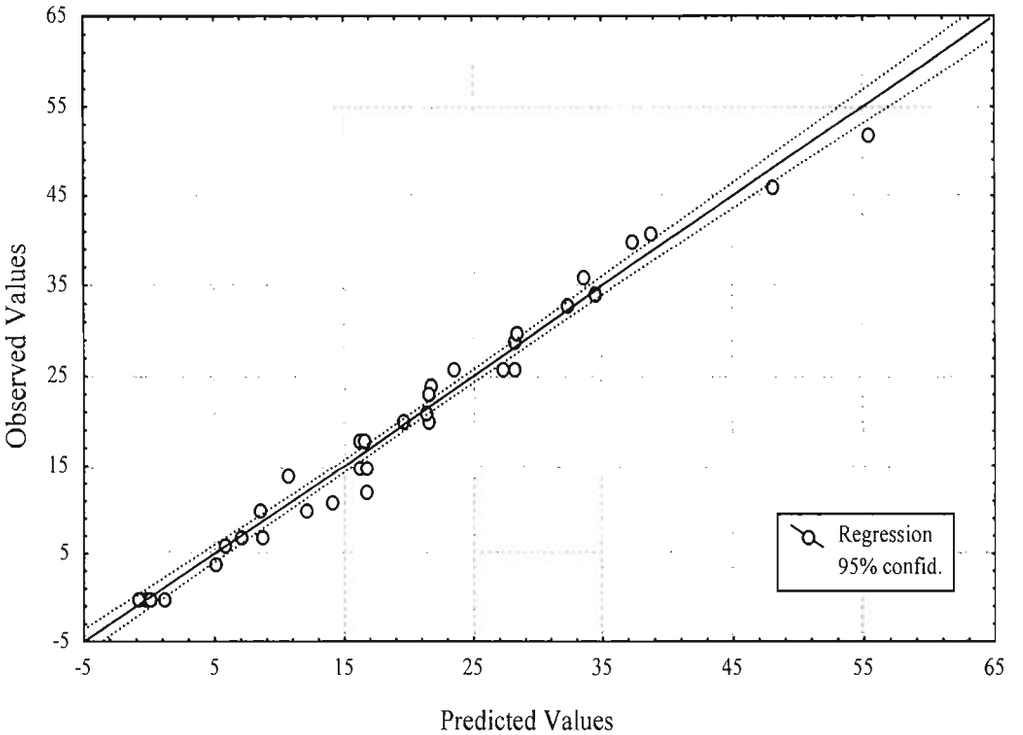


Figure 31: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 9. The dependant variable is the shelf-life.

Model 10

A model using only OSI and OSI^2 as in Model 7, but based on the sensory evaluation, was used to create Model 10. The multiple regression results are shown in Table 20.

Table 20: Regression summary for dependant variable: shelf-life of Model 10.

N = 35 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	23.1	6.80	0.002
OSI	-3.45	1.30	0.012
OSI^2	0.209	0.054	0.001

Where R^2 = 0.5495

F (2, 32) = 19.5

Standard error of estimate = 9.60

The graph of the predicted versus the observed values is shown in Figure 32.

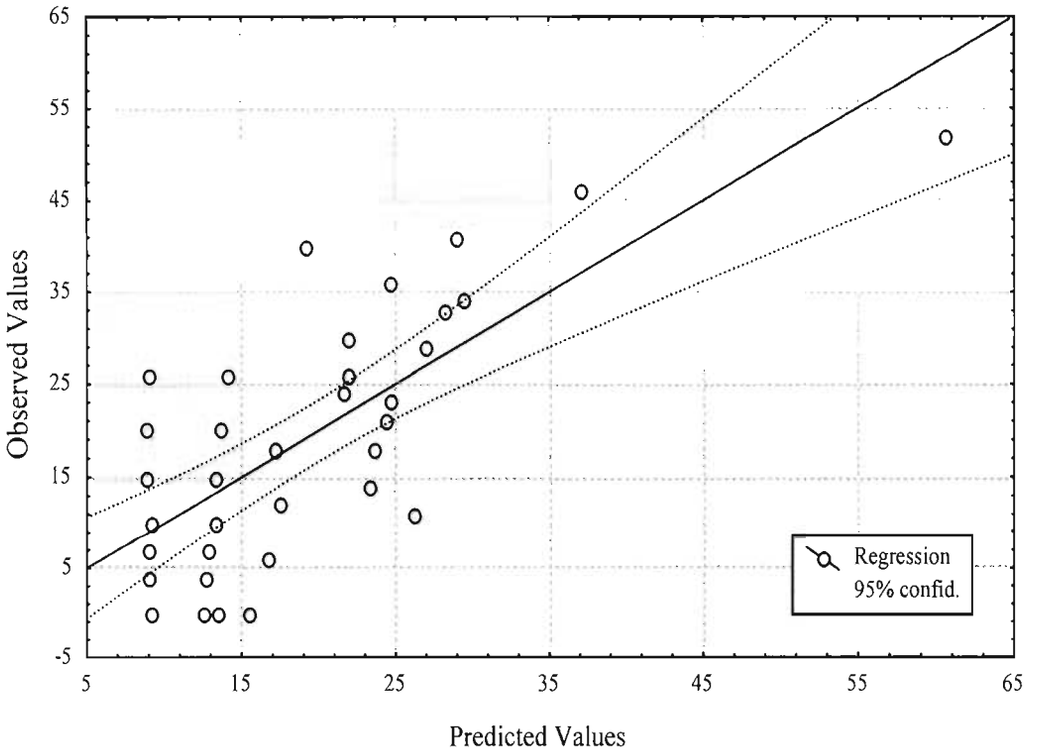


Figure 32: Predicted versus observed shelf-life values in weeks for palm-olein oil as determined by Model 10. The dependant variable is the shelf-life.

4.1.3.2 Jackknifing

The practical applicability and reliability of the models was tested applying the jackknifing procedure (Hair et al, 1998).

PV and AV based models

The results of the jackknifing are shown in Table 21. Where the "Ideal model" is as described for Model 5, "Practical model" as described for model 6 and the OSI model as described for Model 7.

Table 21: Jackknifing results of three selected predictive models for palm-olein oil that was stored for a period of 52 weeks at 50 °C where the shelf-life of the samples was based on the PV and AV values.

Excluded case	Observed value	Ideal model		Practical model		OSI model	
		Predicted value	Observed minus predicted	Predicted value	Observed minus predicted	Predicted value	Observed minus predicted
1	22	19.3	2.7	18.1	3.9	20.0	2.0
2	6	9.2	-3.2	9.2	-3.2	2.3	3.7
3	6	5.8	0.2	5.0	1.0	-0.69	6.6
4	6	8.4	-2.4	6.9	-0.9	3.8	2.2
5	16	15.3	0.7	16.1	-0.1	9.8	6.2
6	0	4.9	-4.9	2.9	-2.9	8.4	-8.4
7	0	-4.5	4.5	-4.6	4.6	0.4	-0.4
8	0	0.4	-0.4	2.3	-2.3	7.2	-7.2
9	11	7.5	3.5	9.8	1.2	6.6	4.4
10	6	4.1	1.9	3.4	2.6	5.2	0.8
11	3	4.5	-1.5	5.3	-2.3	7.4	-4.4
12	0	-0.3	0.3	0.1	-0.1	4.9	-4.9
Mean			0.1167		0.1250		0.0533
Std error of estimate			2.8006		2.6189		5.1430
95% confidence interval			± 6.2		± 5.8		± 11.3

All of the predicted values of the jackknifing results of the Ideal, Practical and OSI models were within the 95 % confidence level of 6.2, 5.8 and 11.3 weeks, respectively. The observed minus predicted values (errors) of the models is represented graphically in Figure 33. The errors were grouped into 3 categories namely: 0 to ± 2 weeks, ± 2 to ± 4 weeks and more than + 4 weeks and less than – 4 weeks on the x-axis. The percentage cases with an error falling in each category were calculated from the total number of cases, which were 12 in this instance. The total percentage of cases with an error between 0 to ± 2 weeks was 50, 41.7 and 25 % for the Ideal, Practical and OSI models, respectively. The percentage cases with an error between ± 2 to ± 4 were 33.4, 50 and 16.7 % for the three models in the same order, respectively. The percentage cases with an error of more than + 4 weeks or less than – 4 weeks were 16.6, 8.3 and 58.3 % for the three models respectively.

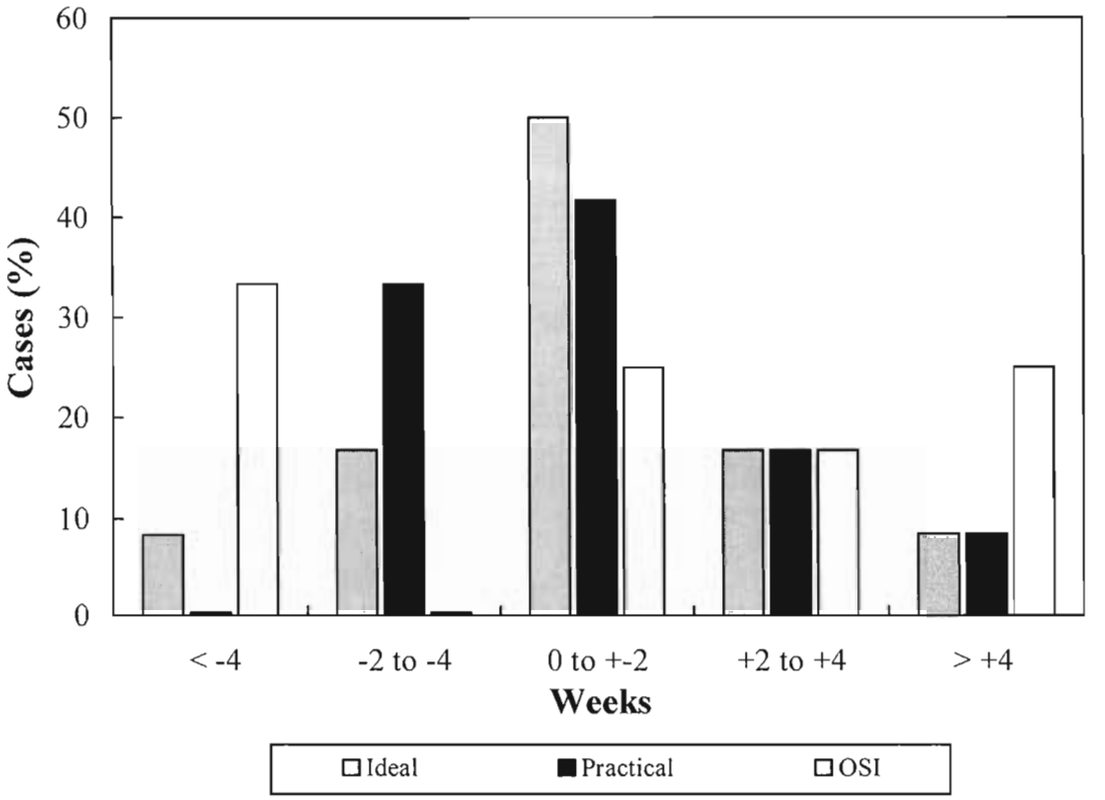


Figure 33: The percentage cases that fall within < - 4, - 2 to - 4, 0 to ± 2, 2 to + 4 and > + 4 weeks when the jackknifing procedure had been applied to the Ideal, Practical and OSI models where the shelf-life was based on PV and AV values.

Sensory based models

The results of the jackknifing on the selected models that were based on sensory evaluation are shown in Table 22.

The 95 % confidence level of prediction for the Ideal model is 7.3 weeks with one case outside the level of confidence and for the Practical model the 95 % level of confidence is 8.3 weeks with one case outside the level of confidence and in the OSI model two cases are on or outside the 95 % confidence level of 21.4 weeks.

Table 22: Jackknifing results of three selected models that were based on the sensory evaluation.

Excluded case	Observed value (weeks)	Ideal model		Practical model		OSI model	
		Predicted value	Observed minus predicted	Predicted value	Observed minus predicted	Predicted value	Observed minus predicted
1	52	58.1	-6.1	56.6	-4.6	73.4	-21.4
2	40	40.5	-0.5	35.6	4.4	18.1	21.9
3	26	29.2	-3.2	25.3	0.7	8.0	18.0
4	26	32.9	-6.9	21.2	4.8	11.9	14.1
5	46	51.9	-5.9	49.6	-3.6	35.9	10.1
6	34	37.7	-3.7	30.1	3.9	29.1	4.9
7	20	15.6	4.4	16.9	3.1	8.1	11.9
8	20	19.0	1.0	15.9	4.1	12.6	7.4
9	41	39.9	1.1	40.0	1.0	28.2	12.8
10	29	31.3	-2.3	28.1	0.9	26.9	2.1
11	15	20.2	-5.2	19.5	-4.5	8.4	6.6
12	15	18.6	-3.6	14.4	0.6	13	2.0
13	36	33.6	2.4	32.9	3.1	24.2	11.8
14	24	30.9	-6.9	18.2	5.8	21.5	2.5
15	10	10.8	-0.8	11.0	-1.0	9.1	0.9
16	10	9.1	0.9	7.0	3.0	13.8	-3.8
17	33	32.8	0.2	34.8	-1.8	27.9	5.1
18	21	20.6	0.4	16.0	5.0	24.5	-3.5
19	7	8.3	-1.3	6.5	0.5	9.2	-2.2
20	7	11.2	-4.2	6.9	0.1	13.6	-6.6
21	30	29.5	0.5	29.0	1.0	21.5	8.5
22	18	15.9	2.1	19.8	-1.8	23.9	-5.9
23	4	5.2	-1.2	11.7	-7.7	9.4	-5.4
24	4	2.9	1.1	10.8	-6.8	13.7	-9.7
25	26	24.4	1.6	29.3	-3.3	21.7	4.3
26	14	10.1	3.9	12.7	1.3	23.7	-9.7
27	0	-0.4	0.4	2.3	-2.3	9.8	-9.8
28	0	-0.7	0.7	3.5	-3.5	14.1	-14.1
29	23	23.6	-0.6	24.5	-1.5	24.8	-1.8
30	11	17.1	-6.1	13.9	-2.9	27.0	-16
31	18	11	7	6.8	11.2	17.1	0.9
32	6	8.4	-2.4	9.0	-3.0	17.2	-11.2
33	12	18	-6	18.5	-6.5	17.7	-5.7
34	0	1.4	-1.4	2.5	-2.5	16.3	-16.3
35	0	8.2	-8.2	-3.4	3.4	14.3	-14.3
Mean			-1.3943		0.0171		-0.3314
Std error of estimate			3.5984		4.0867		10.527
95% confidence interval			± 7.3		± 8.3		± 21.4

The observed minus predicted values (errors) of the models are represented graphically in Figure 34. The percentage cases with an error falling in each category were calculated from the total of 35 cases. The total percentage of cases with an error between 0 to ± 2 weeks was 45.7, 37.1 and 11.4 % for the Ideal, Practical and OSI models, respectively. The percentage cases with an error between ± 2 to ± 4 was 22.9, 34.3 and 31.4 % for the three models in the same order, respectively. The percentage cases with an error of more than + 4 weeks or less than – 4 weeks was 31.4, 14.3 and 74.2 % for the three models respectively.

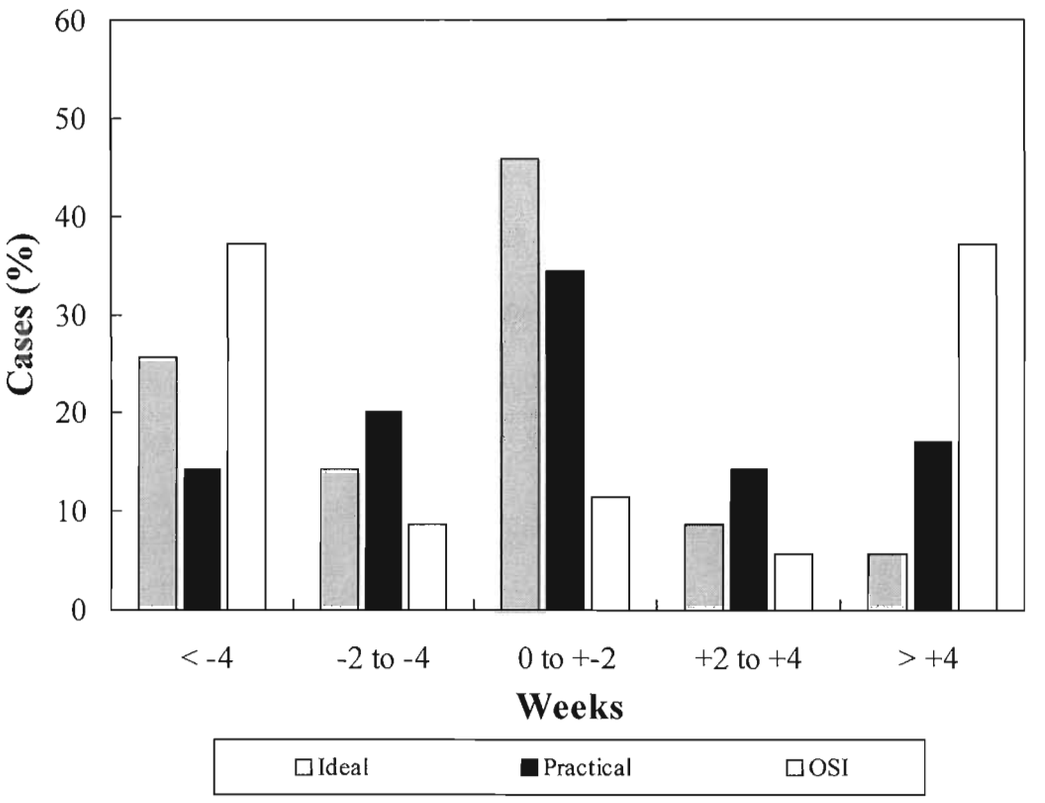


Figure 34: The percentage cases that fall within < - 4, - 2 to - 4, 0 to ± 2, 2 to + 4 and > + 4 weeks when the jackknifing procedure had been applied to the Ideal, Practical and OSI models where the shelf-life was based on the sensory evaluation.

4.2 SUNFLOWER SEED OIL

4.2.1 Composition of oil

The fatty acid composition of the sunflower seed oil that was used for the shelf-life test is given in Table 23. The fatty acid composition of typical sunflower seed oil has been included (Codex Alimentarius Commission, 1997).

Table 23: Fatty acid composition (g/100 g fatty acids) of the sunflower seed oil used and that of a typical sunflower seed oil (Codex Alimentarius Commission, 1997).

Fatty acids	Sunflower seed oil	Typical sunflower seed oil
C14 : 0	0.07	ND*-0.2
C16 : 0	6.23	5.6-7.6
C16 : 1	0.07	ND*-0.3
C18 : 0	5.62	2.7-6.5
C18 : 1	20.18	14.0-39.4
C18 : 2	65.08	48.3-74.0
C18 : 3	0.08	ND*-0.2
C20 : 0	0.36	0.2-0.4
C20 : 1	0.12	ND*-0.2
C22 : 0	0.94	0.5-1.3
C24 : 0	0.25	0.2-0.3

* ND – Non detectable, defined as ≤ 0.05 g/100g fatty acids

The copper, iron, moisture and TBHQ contents are given in Table 24.

Table 24: Copper, iron, moisture and TBHQ content of the sunflower seed oil.

	Copper mg/100g	Iron mg/100g	Moisture g/100g	TBHQ (mg/kg)
Sunflower seed oil	< 0.48	0.17	0.17	Not detected*

* Not detectable defined as ≤ 2.0 mg/kg TBHQ

4.2.2 Shelf-life tests

The four sample treatments were the sunflower seed Control oil, sunflower seed oil with 54 mg/kg TBHQ added, sunflower seed oil with 217 mg/kg TBHQ added and sunflower seed oil with 435 mg/kg TBHQ added.

4.2.2.1 Free fatty acids

The effect of storage at 30°C on the FFA (% oleic acid equivalents) content of the four sunflower seed oil sample treatments is given in Figure 35.

The FFA content of all the samples increased gradually over the 52 weeks. The FFA content of the Control increased from 0.10 % oleic acid at Day 0 to 0.36 % oleic acid at Week 52, which was slightly higher than the samples with TBHQ. The sample with the highest concentration of TBHQ (435 mg/kg) had the lowest FFA value of 0.31 % oleic acid at Week 52.

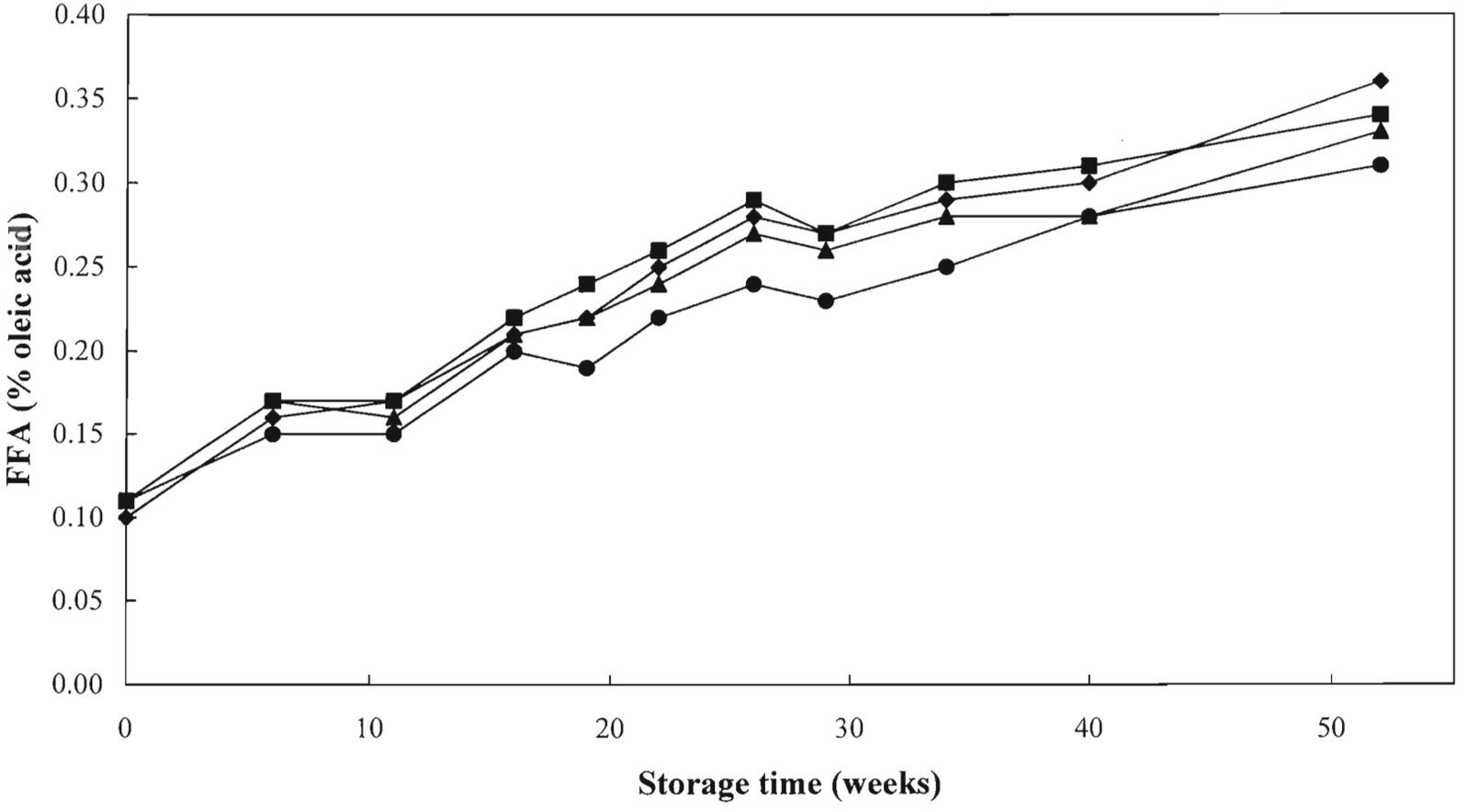


Figure 35: The effect of storage at 30°C for 52 weeks on the free fatty acid (% oleic acid) content of the sunflower seed oil samples: Control (♦); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.2 Peroxide value

The effect of storage at 30°C on the PV of the four sample treatments is given in Figure 36.

The PV of all the samples increased steadily up to Week 34, after which the samples containing TBHQ reached a plateau and the Control appeared to decrease. The rate of increase for the Control sample was highest followed by the sample with 54 mg/kg TBHQ, then 217 mg/kg TBHQ and the slowest rate was the sample with 435 mg/kg TBHQ. The Control sample reached the highest PV at Week 34.

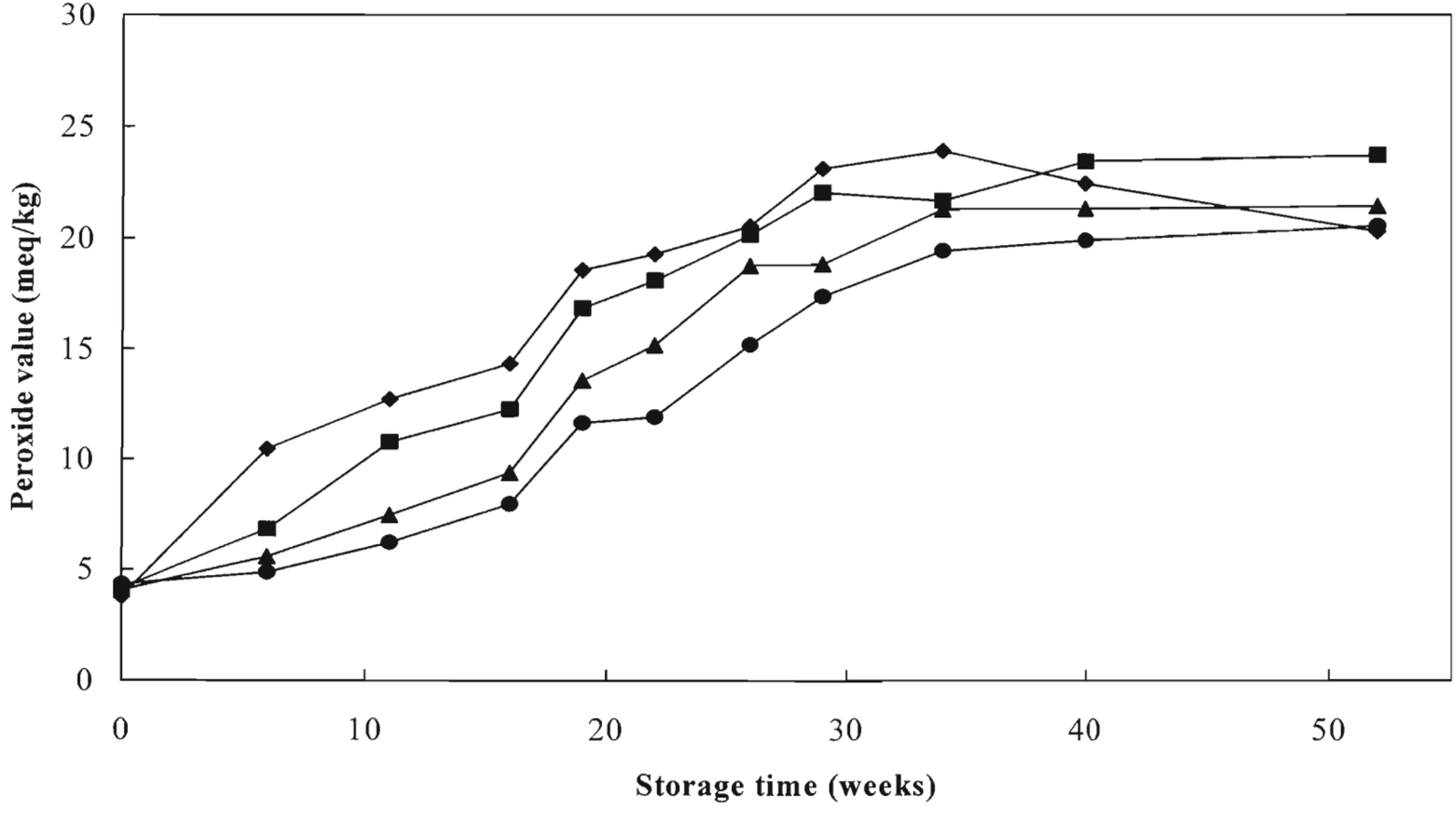


Figure 36: The effect of storage at 30°C for 52 weeks on the peroxide value (meq/kg) of the sunflower seed oil samples: Control (♦); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.3 Anisidine value

The effect of storage at 30°C on the AVs of the four samples is given in Figure 37.

There was an increase in AV for all the samples. The AV for the Control was the highest. The AV for the Control appeared to remain constant from Week 40 onwards. The increase in AV for the sample with 54 mg/kg TBHQ was slightly higher than for the samples with 217 and 435 mg/kg. The AV of the samples containing 217 and 435 mg/kg TBHQ increased at very similar rate with little difference in their values. It appears that the samples containing TBHQ reached a plateau after Weeks 22–26.

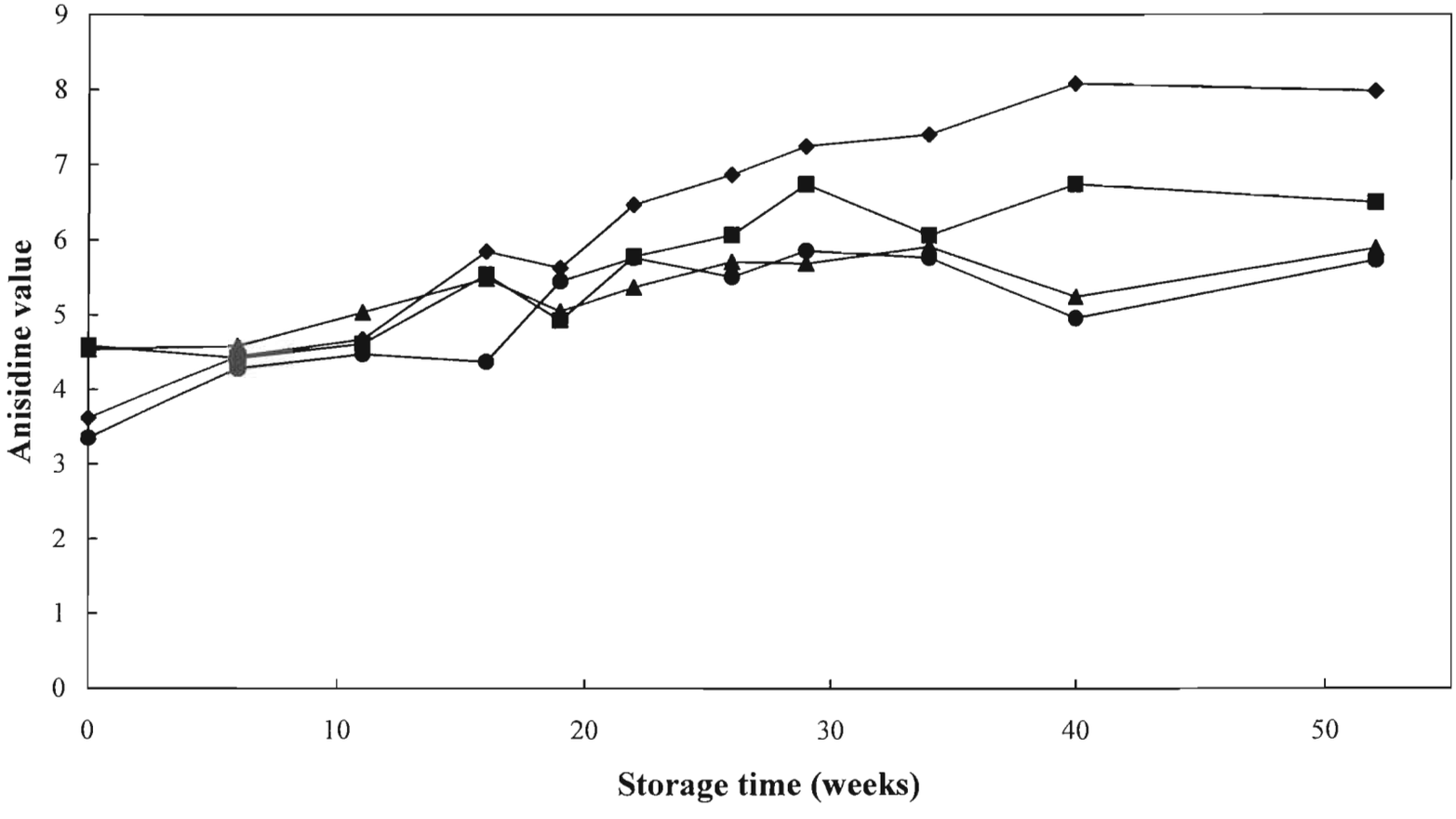


Figure 37: The effect of storage at 30°C for 52 weeks on the anisidine value of the sunflower seed oil samples: Control (♦); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.4 Totox value

The effect of storage at 30°C on the Totox value of the four samples is given in Figure 38.

The Totox value (2PV + AV) of all the samples increased during the storage period. The increase in Totox value of the Control was the highest followed in order by lower levels in the 54, 217 and 435 mg/kg TBHQ containing samples respectively. The values of the Control sample appeared to decrease after Week 34, whereas the values for the TBHQ containing samples seemed to reach a plateau after Week 34.

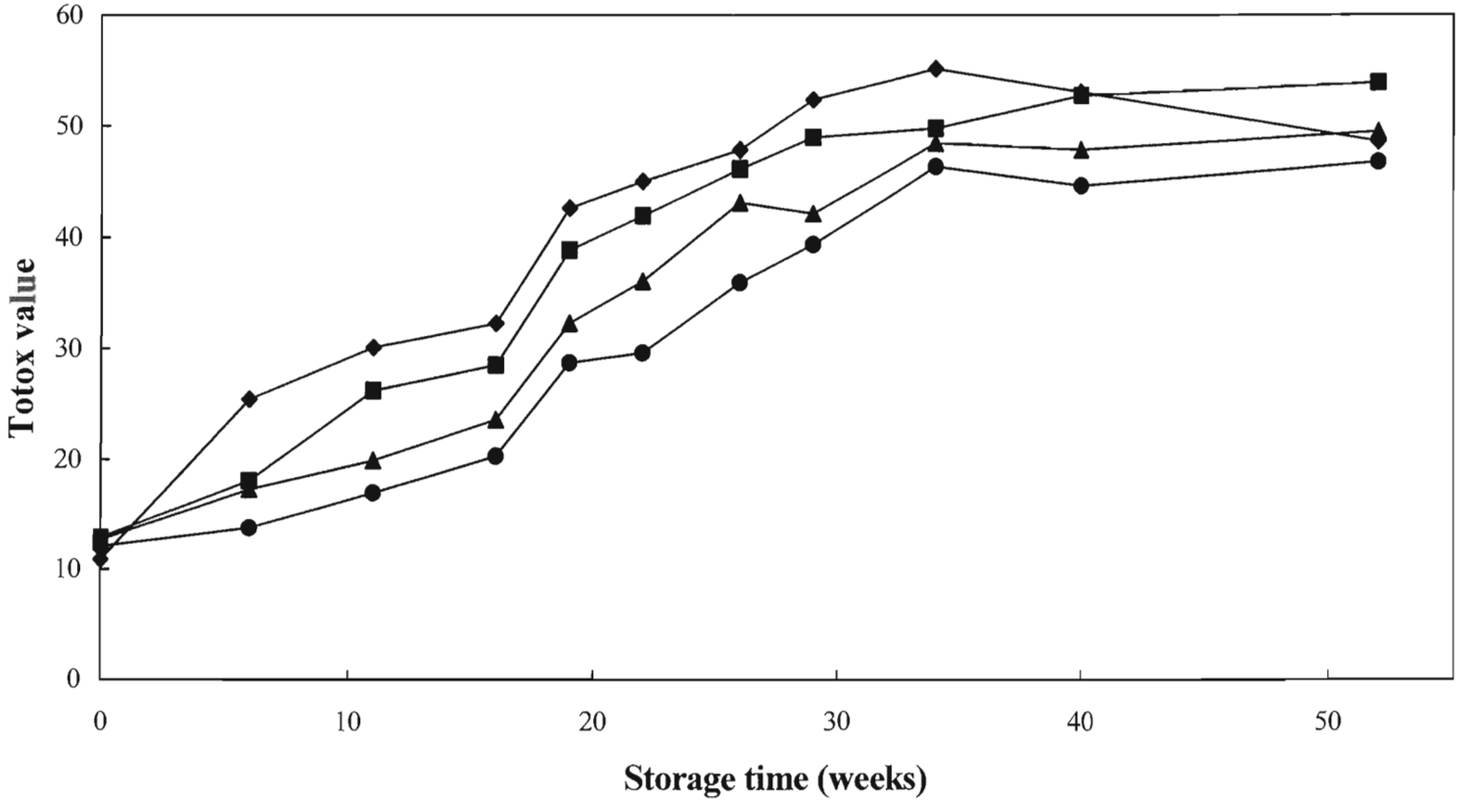


Figure 38: The effect of storage at 30°C for 52 weeks on the Totox value of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.5 Oxidative stability Index

The effect of storage at 30°C on the OSI conducted at 100°C of the four treatments is given in Figure 39.

There were pronounced differences in the curves and oxidative stability values of the four sample treatments. The Control had the lowest values. The oxidative stability of the Control was at 9.1 h at time 0 storage and decreased slowly to 6.7 h at Week 52. The higher the concentration of TBHQ in the samples, the longer the oxidative stability was. The oxidative stability of the sample with 54 mg/kg TBHQ was slightly better than the Control sample. The oxidative stability started at 16.6 h and decreased to 7.9 h at Week 52. The sample with 217 mg/kg TBHQ had double the stability of the 54 mg/kg TBHQ. At time 0 it was 32.1 h and decreased to 15.5 h at Week 52. The sample with 435 mg/kg had values of about 10 h longer than the sample with 217 mg/kg TBHQ. It started at 43.5 h and decreased to 27.6 h at Week 52. The initial decrease in time 0 to Week 11 was more rapid for the samples with 217 and 435 mg/kg TBHQ and was followed by a more gradual decrease in oxidative stability values.

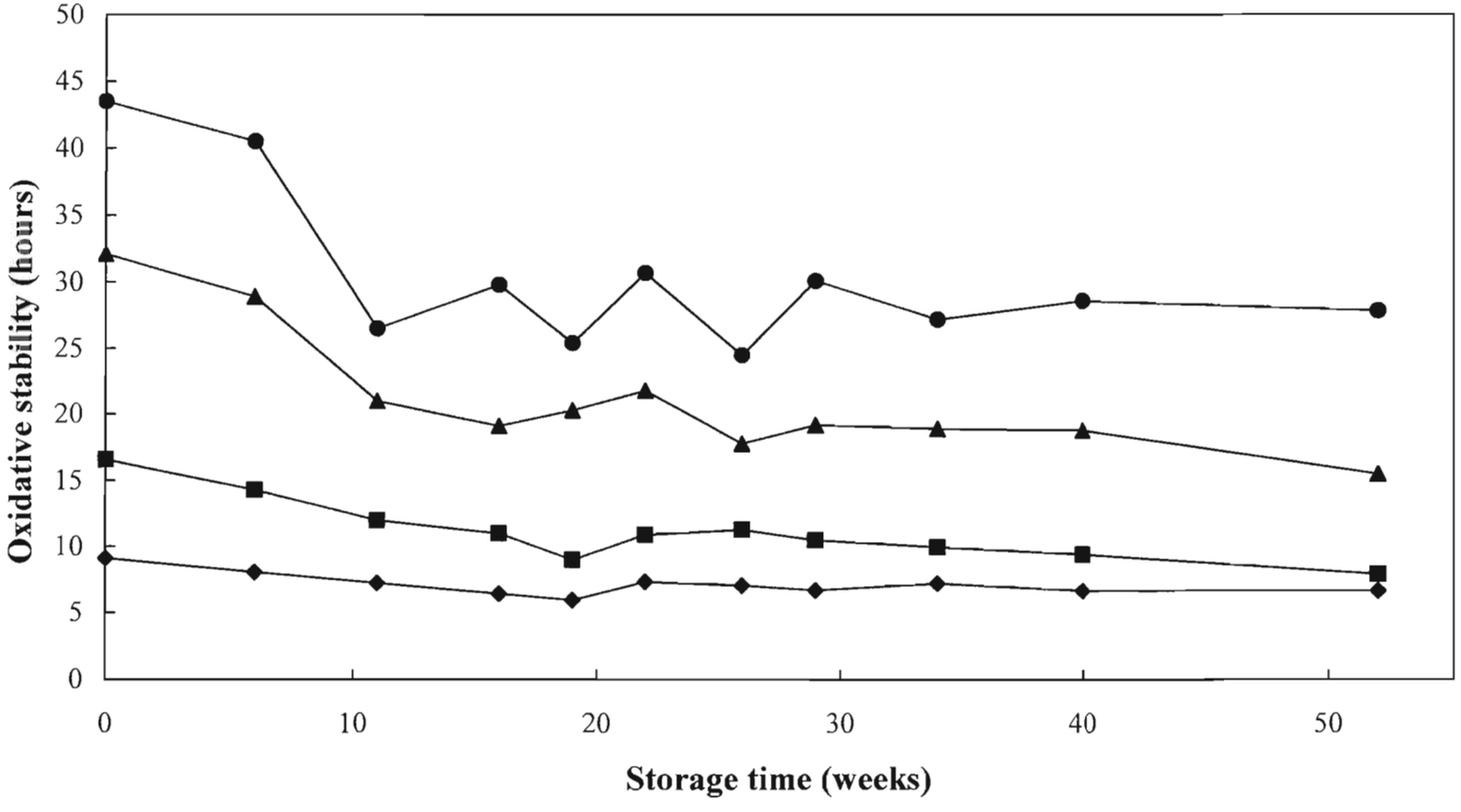


Figure 39: The effect of storage at 30°C for 52 weeks on the oxidative stability (hrs) conducted at 100° C of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.6 Tocopherols

Total tocopherols

The effect of storage at 30°C for a period of 52 weeks on the total tocopherol content of the four treatments is given in Figure 40.

The total tocopherol content in all the samples decreased only slightly over the 52-week storage period. The sample with the highest concentration of TBHQ (435 mg/kg), had slightly higher values after 52 weeks than the other samples, 41.6 mg/100 g compared to 38.4, 38.9 and 38.8 mg/100 g for the Control, 54 mg/kg and 217 mg/kg TBHQ containing samples, respectively.

The change in total and individual tocopherol content (mg/100 g) from the values obtained at Day 0 and after 52 weeks of storage is given in Table 25.

Table 25: Total and individual tocopherols (mg/100 g) of sunflower seed oil after 52-week storage period at 30°C.

Tocopherols	**Tocopherol content (g/100 g)**				
	Day 0	**Week 52**			
		Control	54 mg/kg TBHQ	217 mg/kg TBHQ	435 mg/kg TBHQ
Total tocopherols	47.2	38.4	38.9	38.8	41.6
Alpha-tocopherol	46.2	36.6	37.1	37.0	39.8
Beta-tocopherol	0.5	1.34	0	0	0
Gamma-tocopherol	0.9	1.8	1.7	1.8	1.8

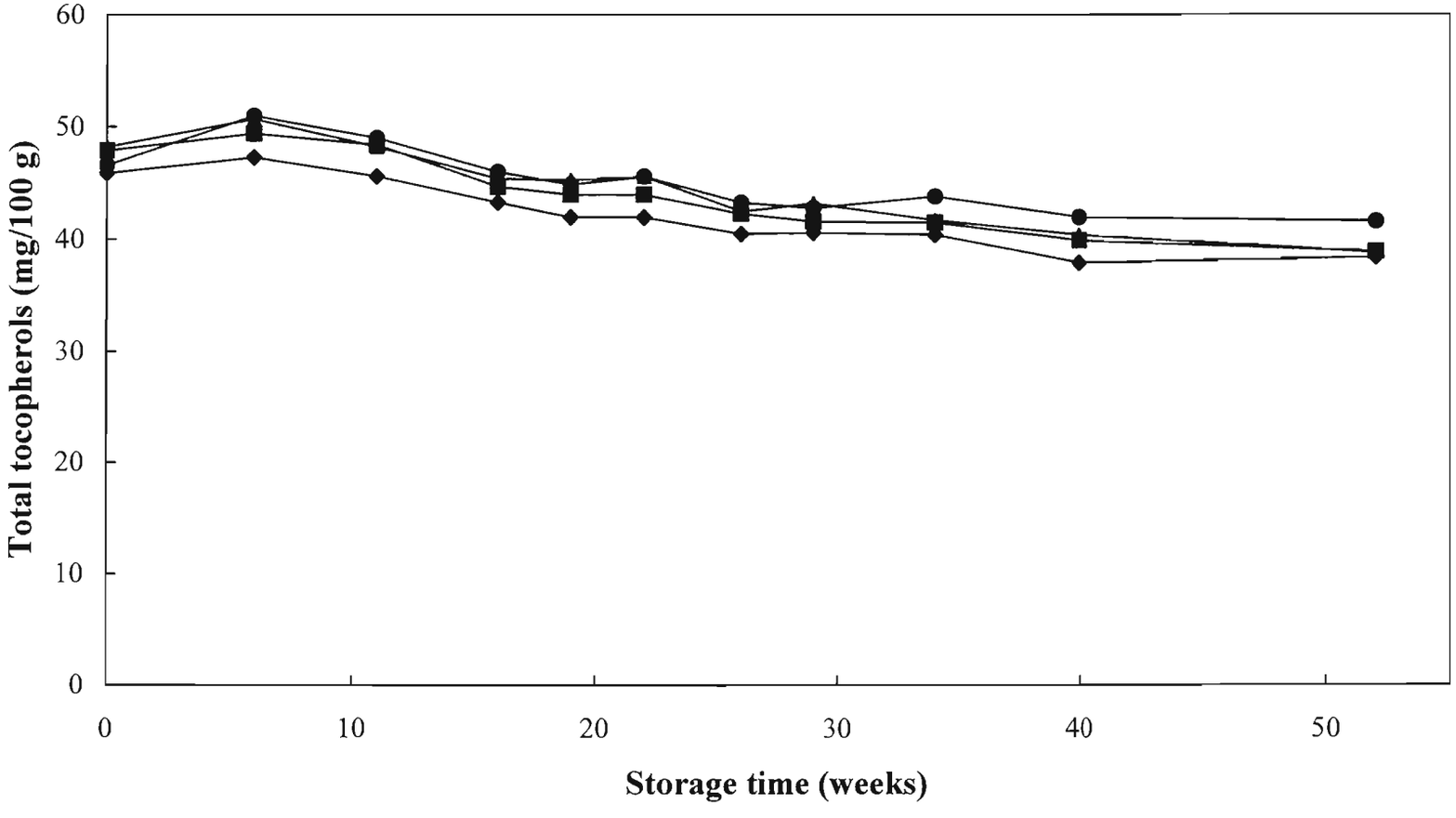


Figure 40: The effect of storage at 30°C for 52 weeks on the total tocopherol content (mg/100 g) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

Alpha-tocopherol

The effect of storage at 30°C for a period of 52 weeks on the alpha-tocopherol content of the four treatments is given in Figure 41.

The decrease in alpha-tocopherol in all the samples during the storage period appeared very similar to the decrease of the total tocopherols. The sample containing 435 mg/kg TBHQ once again had slightly higher values of alpha-tocopherol 39.8 mg/100 g at Week 52 compared to the 36.6, 37.1 and 37.0 mg/100 g alpha-tocopherol values of the Control and the 54 and 217 mg/kg TBHQ containing samples, respectively.

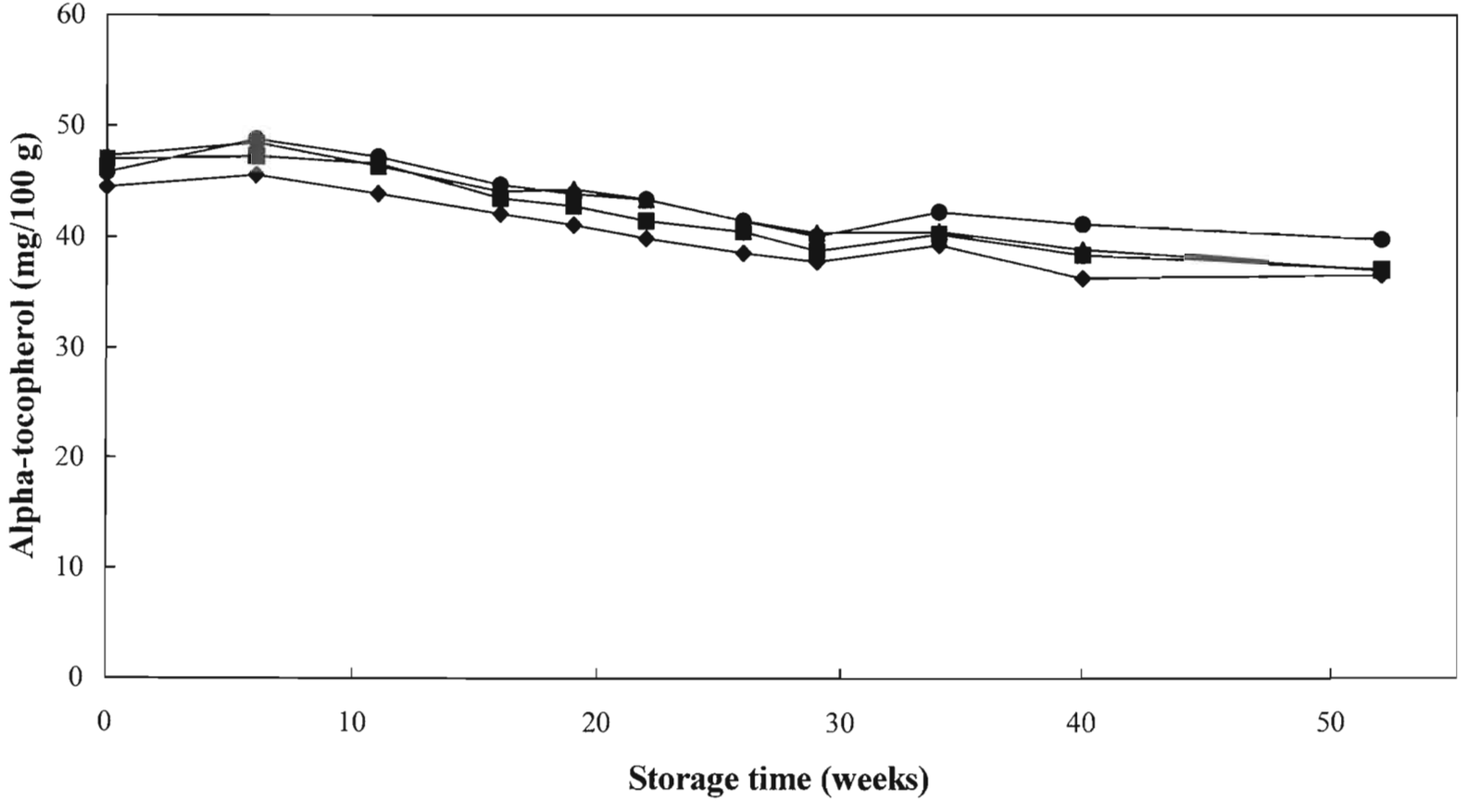


Figure 41: The effect of storage at 30°C for 52 weeks on the alpha-tocopherol content (mg/100 g) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

Beta-tocopherol

The effect of storage at 30°C for a period of 52 weeks on the beta-tocopherol content of the four treatments is given in Figure 42.

Low values of beta-tocopherol were obtained for all the samples during the storage period. The values showed no pattern and fluctuated in a narrow range of 0-1.35 mg/100g.

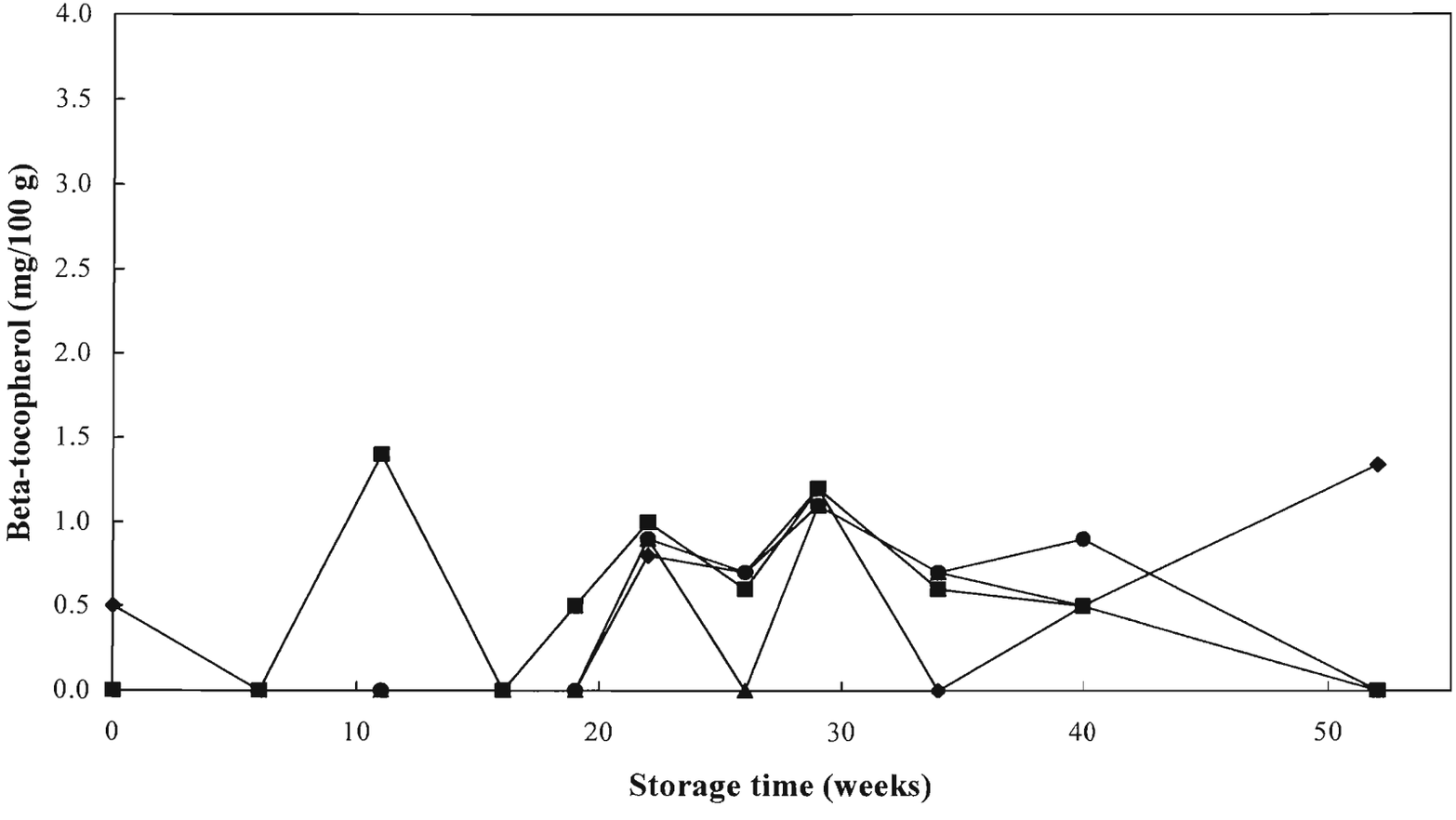


Figure 42: The effect of storage at 30°C for 52 weeks on the beta-tocopherol content (mg/100 g) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

Gamma-tocopherol

The effect of storage at 30°C for a period of 52 weeks on the gamma-tocopherol content of the four treatments is given in Figure 43.

The gamma-tocopherol content was also low and fluctuated between 0.9-2.2 for all of the sample values during the storage period. The variations are due to sampling and analytical error and no conclusion about increases or decreases would be possible.

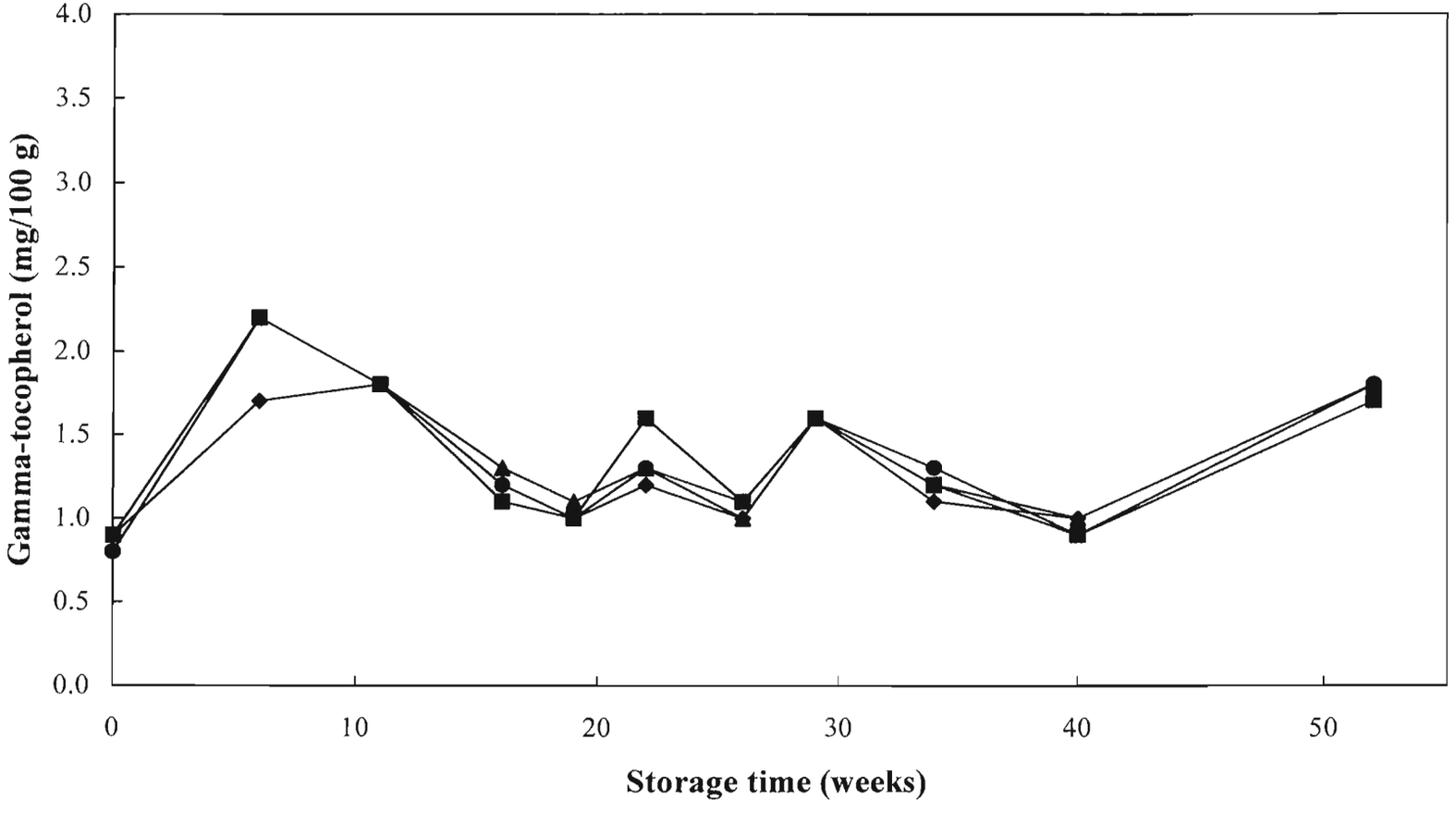


Figure 43: The effect of storage at 30°C for 52 weeks on the gamma-tocopherol content (mg/100 g) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.7 Conjugated dienes and triene values

The effect of storage at 30°C for a period of 52 weeks on the conjugated diene and triene values of the four treatments are given in Figures 44 and 45, respectively.

Conjugated dienes (UV 232 nm)

For all samples there was only a very slight increase in CV during storage. The values of the Control appeared to be higher than the values of the samples containing TBHQ. The values for the 54 mg/kg TBHQ sample were lower than the Control and slightly higher than the 217 and 435 mg/kg TBHQ samples. The 435 mg/kg sample appeared to have the lowest values.

Conjugated trienes (UV 268 nm)

The conjugated triene value did not appear to have increased markedly in any of the samples during the storage period.

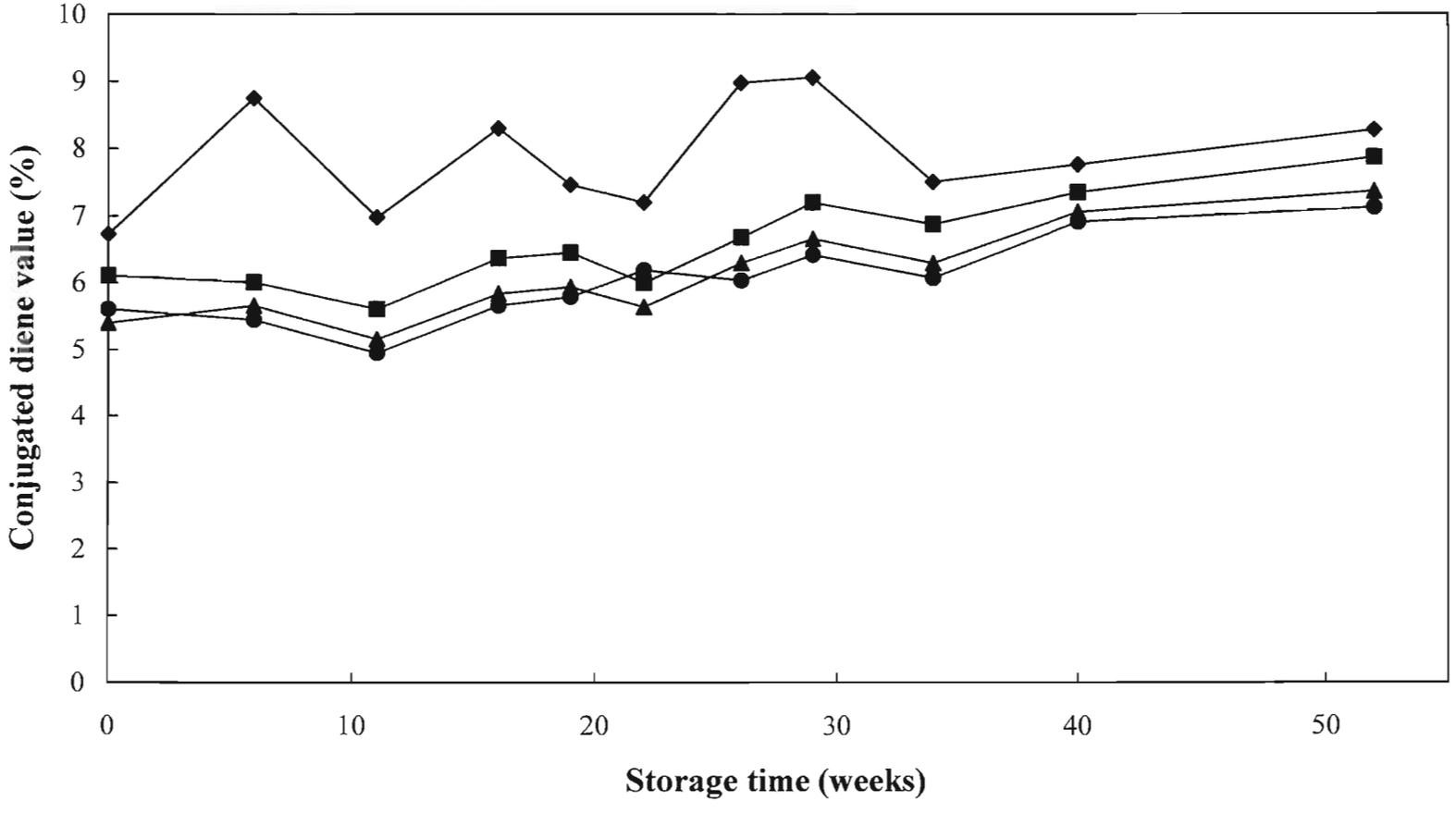


Figure 44: The effect of storage at 30°C for 52 weeks on the conjugated diene value (%) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

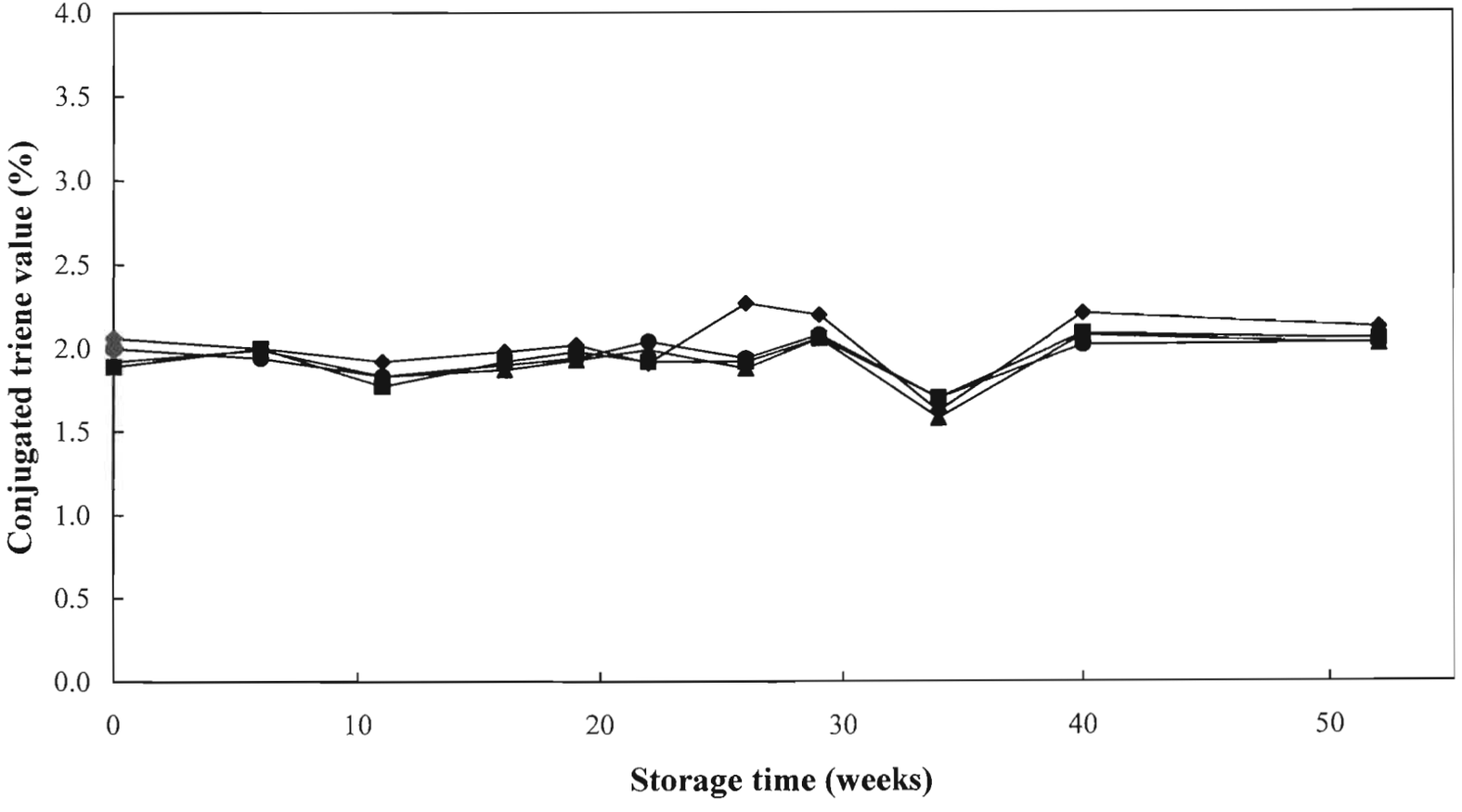


Figure 45: The effect of storage at 30°C for 52 weeks on the conjugated triene value (%) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.8 Iodine value

The effect of storage at 30°C on the IV on the four treatments is given in Figure 46. The IV hardly changed during the storage period with a range of 131.1 (lowest value obtained) and 132.2 (highest value obtained) between the samples. No clear trend or difference between samples took place seen.

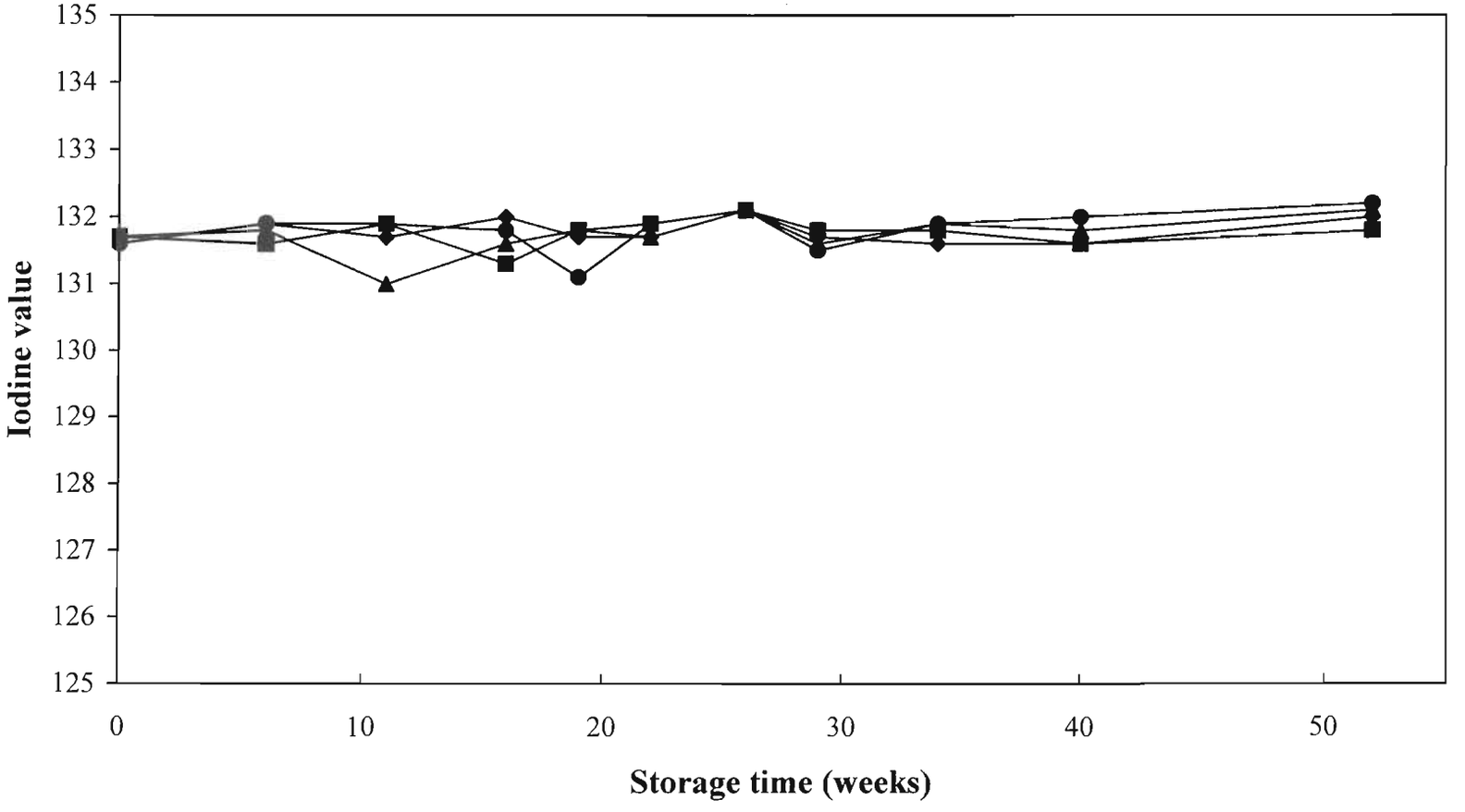


Figure 46: The effect of storage at 30°C for 52 weeks on the iodine value of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.9 Headspace volatile components

Total volatile peak area

The effect of storage at 30°C on the total volatile peak area of the samples is shown in Figure 47. The total volatile peak area increased gradually for all the samples during the storage period. The Control appeared to have the highest peak area throughout the storage trial, although on one occasion at Week 34 it had the same peak area as the 54 mg/kg TBHQ sample followed by a sharp increase to Week 40 and a sharp drop thereafter. The peak areas of the TBHQ containing samples were very similar up to Week 26, after which the 54 mg/kg had slightly higher peak areas than the 217 and 435 mg/kg TBHQ samples. At Weeks 34 and 40 the 217 mg/kg TBHQ sample had higher peak areas than the 435 mg/kg TBHQ sample but at Week 52 the 217 mg/kg TBHQ sample had lower peak areas than that of the 435 mg/kg TBHQ sample.

Hexanal

The effect of storage at 30°C on the hexanal content of the four sample treatments is given in Figure 48. The Control showed the highest values during the storage period and the 435 mg/kg TBHQ sample had the lowest values. After Week 30 the hexanal values reflected the TBHQ levels. The 217 mg/kg TBHQ samples followed with slightly higher values than the 435 mg/kg samples and the 54 mg/kg had slightly higher values than the 217 mg/kg samples. The values appeared to have reached a maximum at Week 40. After Week 40 the values for the Control decreased slightly, the 217 mg/kg sample remained stable and the 54 and 435 mg/kg samples increased slightly.

trans-2-hexenal

The effect of storage at 30°C on the t-2-hexenal content of the four sample treatments is given in Figure 49. Low values of trans-2-hexenal were obtained for all the samples during the storage period. The values showed very little variation between the samples with a fluctuation range of 0-1.9 mg/kg.

trans, trans-2,4-decadienal

The effect of storage at 30°C on the t,t-2,4-decadienal content of the four sample treatments is given in Figure 50. Trans, trans-2,4-decadienal was only detected in the samples from Week 34 and onwards in the storage trial. A considerate amount of

variation was found in the values for all the samples and no pattern could be discerned for or between any of the samples.

Pentanal

The effect of storage at 30°C on the pentanal content of the four sample treatments is given in Figure 51. The pentanal peak area increased during the storage period for all the samples. It appeared that the peak area for the Control sample was higher than for the samples containing TBHQ, although at times the peak area decreased to less or similar values than that of the TBHQ containing samples. The Control and the 54 mg/kg TBHQ values decreased after Week 40. The TBHQ containing samples had similar values up to Week 26, after which the 435 mg/kg sample had the lowest values followed by the 217 mg/kg TBHQ sample and then the 54 mg/kg TBHQ sample. The value of the 54 mg/kg sample decreased at Week 52 to less than the values of the 217 and 435 mg/kg TBHQ samples.

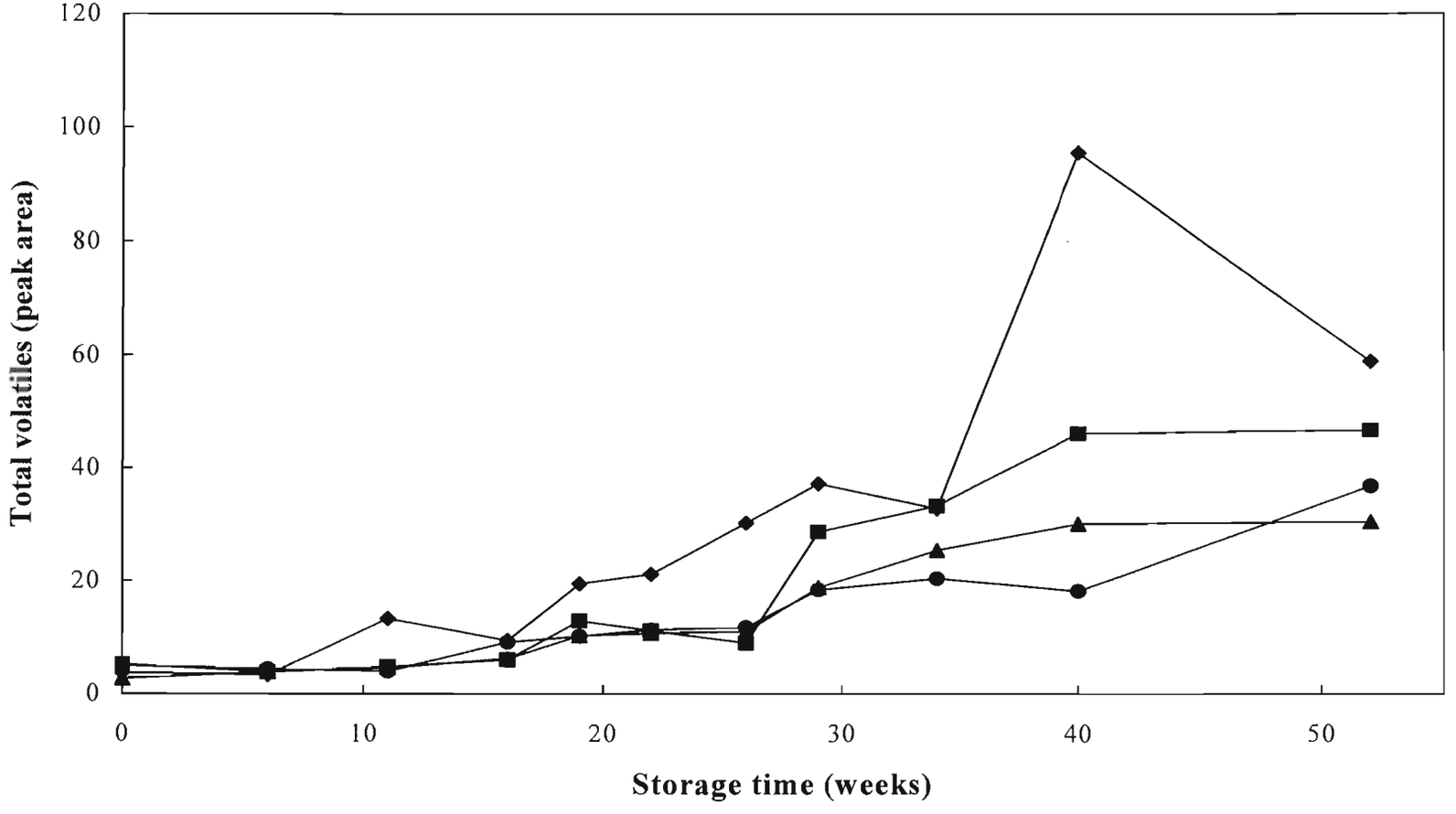


Figure 47: The effect of storage at 30°C for 52 weeks on the total volatile peak area of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

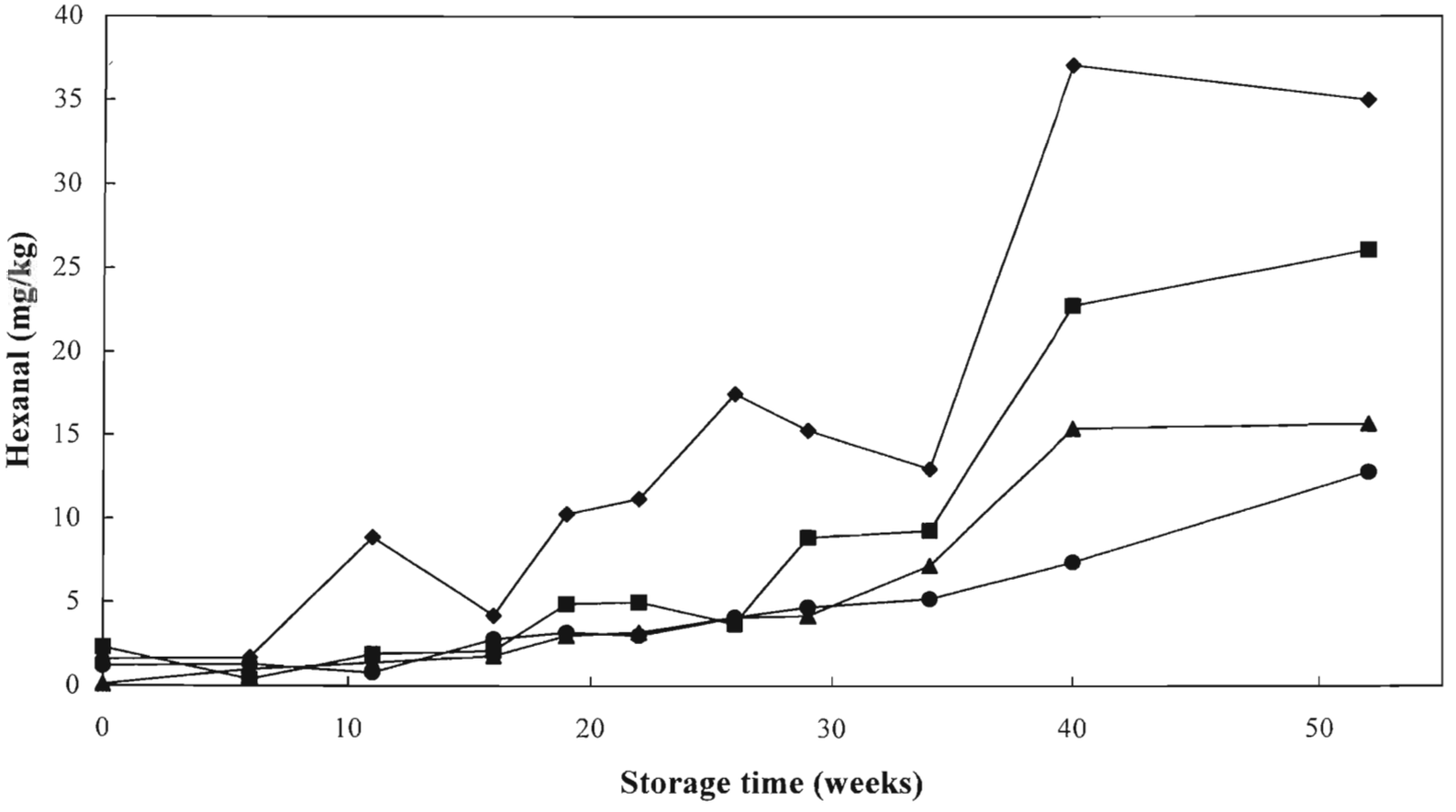


Figure 48: The effect of storage at 30°C for 52 weeks on the hexanal content (mg/kg) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

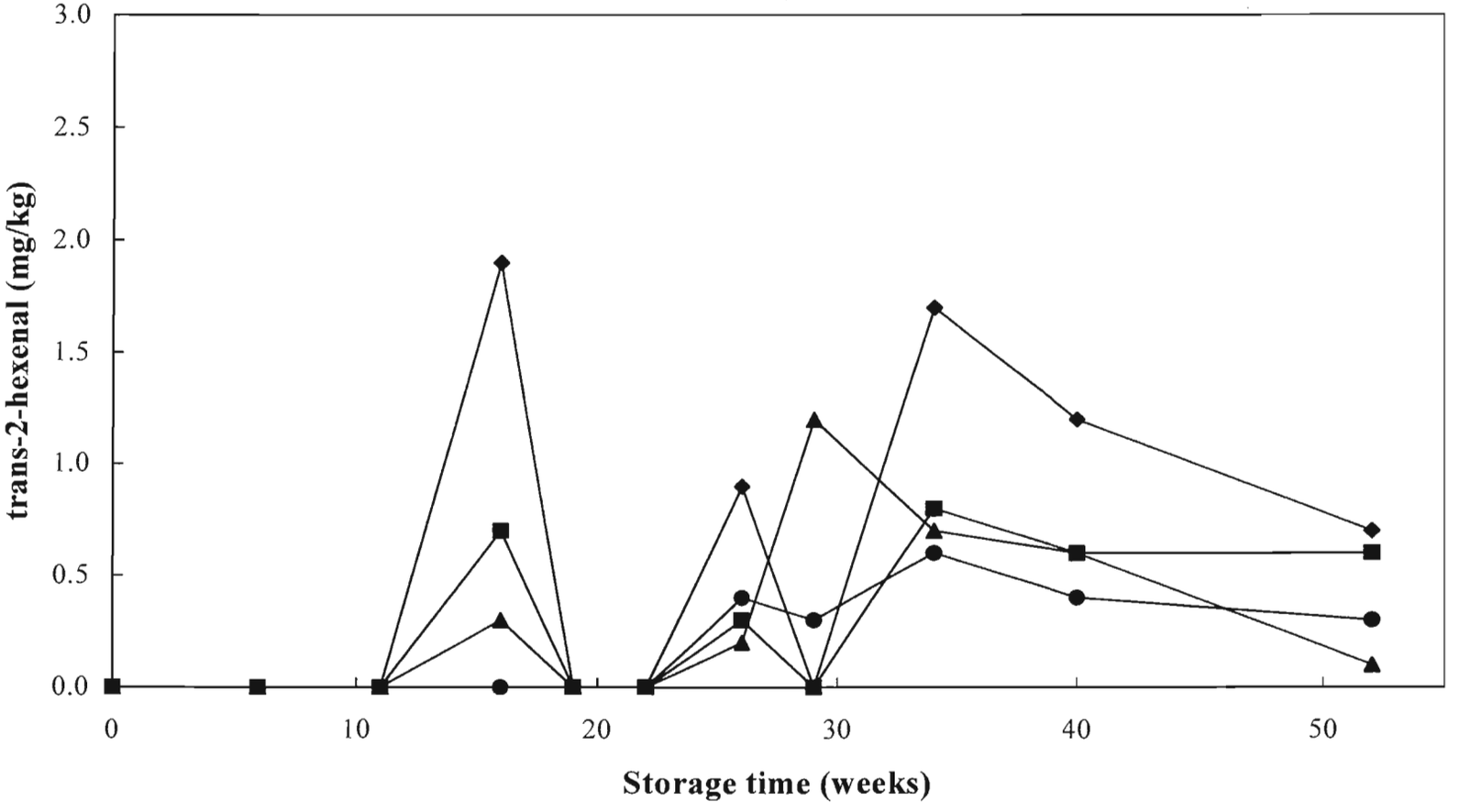


Figure 49: The effect of storage at 30°C for 52 weeks on the trans-2-hexenal content (mg/kg) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

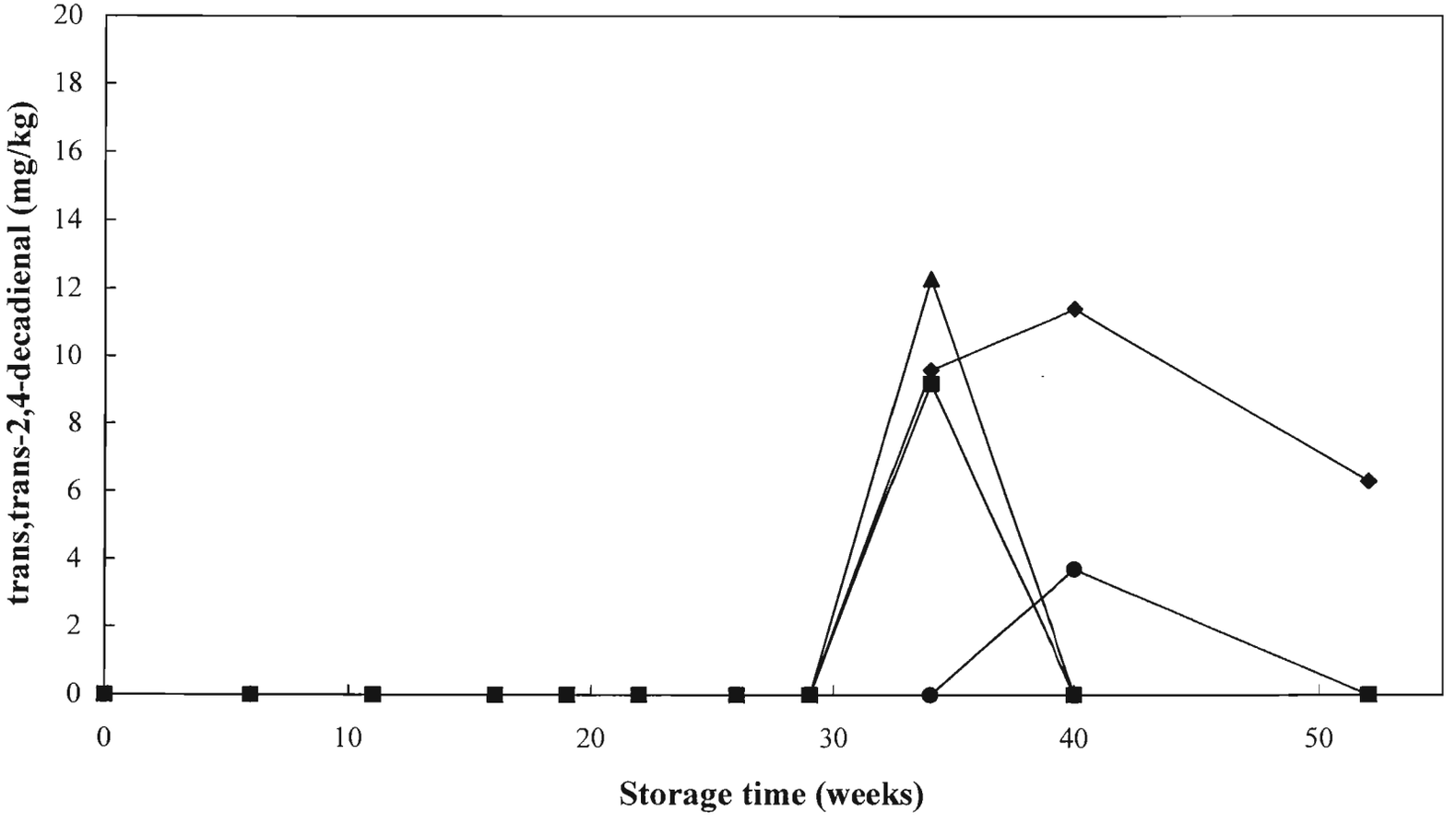


Figure 50: The effect of storage at 30°C for 52 weeks on the trans, trans-2,4-decadienal content (mg/kg) of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

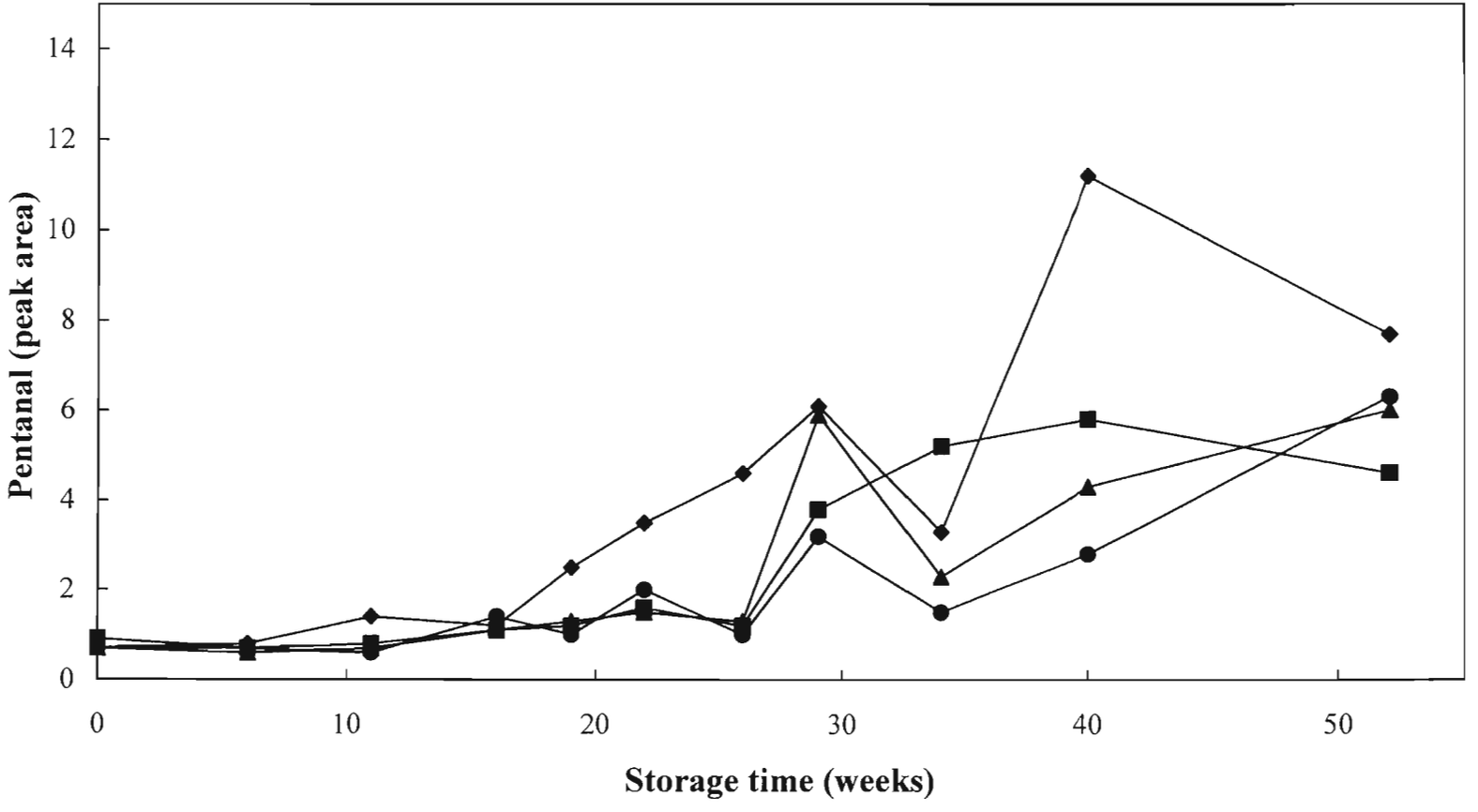


Figure 51: The effect of storage at 30°C for 52 weeks on the pentanal peak area of the sunflower seed oil samples: Control (◆); with 54 mg/kg TBHQ (■); with 217 mg/kg TBHQ (▲) and with 435 mg/kg TBHQ (●).

4.2.2.10 Sensory evaluation

The results of the sensory evaluation conducted by 10 panelists can be seen in Figures 52 and 53 where Figure 52 shows results of Option 1 and Figure 53 the results of Option 2. The four categories as well as the Options 1 and 2 used to evaluate the oils were the same as for the palm-olein oil, see section 4.1.2.10.

According to O'Mahoney (1986) the statistical guideline, would be that if 7 or 8 out of 10 panelists regard the oil to be rancid it would be significant at the 20 % and 10 % level of significance, respectively. This applies for a one-tailed paired-comparison difference test of rancid and fresh oil. For the 5 % level of significance, 9 out of 10 panelists must deem the oil to be rancid.

Control

According to Option 1, all of the panelists judged the oil to be rancid by Week 29. However, according to Option 2 the majority of panelists (8) judged the oil to be rancid by Week 40.

Sample containing 54 mg/kg TBHQ

According to Option 1, all of the panelists judged the oil to be rancid by Week 29. However, according to Option 2 a majority of panelists (6) judged the oil to be rancid by Week 52 although it was not a conclusive judgement.

Sample containing 217 mg/kg TBHQ

According to Option 1, the majority of the panelists (8) judged the oil to be rancid by Week 26 and all the panelists judged the oil to be rancid by Week 52. However, according to Option 2 a majority of panelists (8) judged the oil to be rancid by Week 52.

Sample containing 435 mg/kg TBHQ

According to Option 1, the majority of the panelists (8) judged the oil to be rancid by Week 29 and all the panelists judged the oil to be rancid by Week 52. However, according to Option 2 an unconvincing majority of panelists (7) judged the oil to be rancid by Week 52.

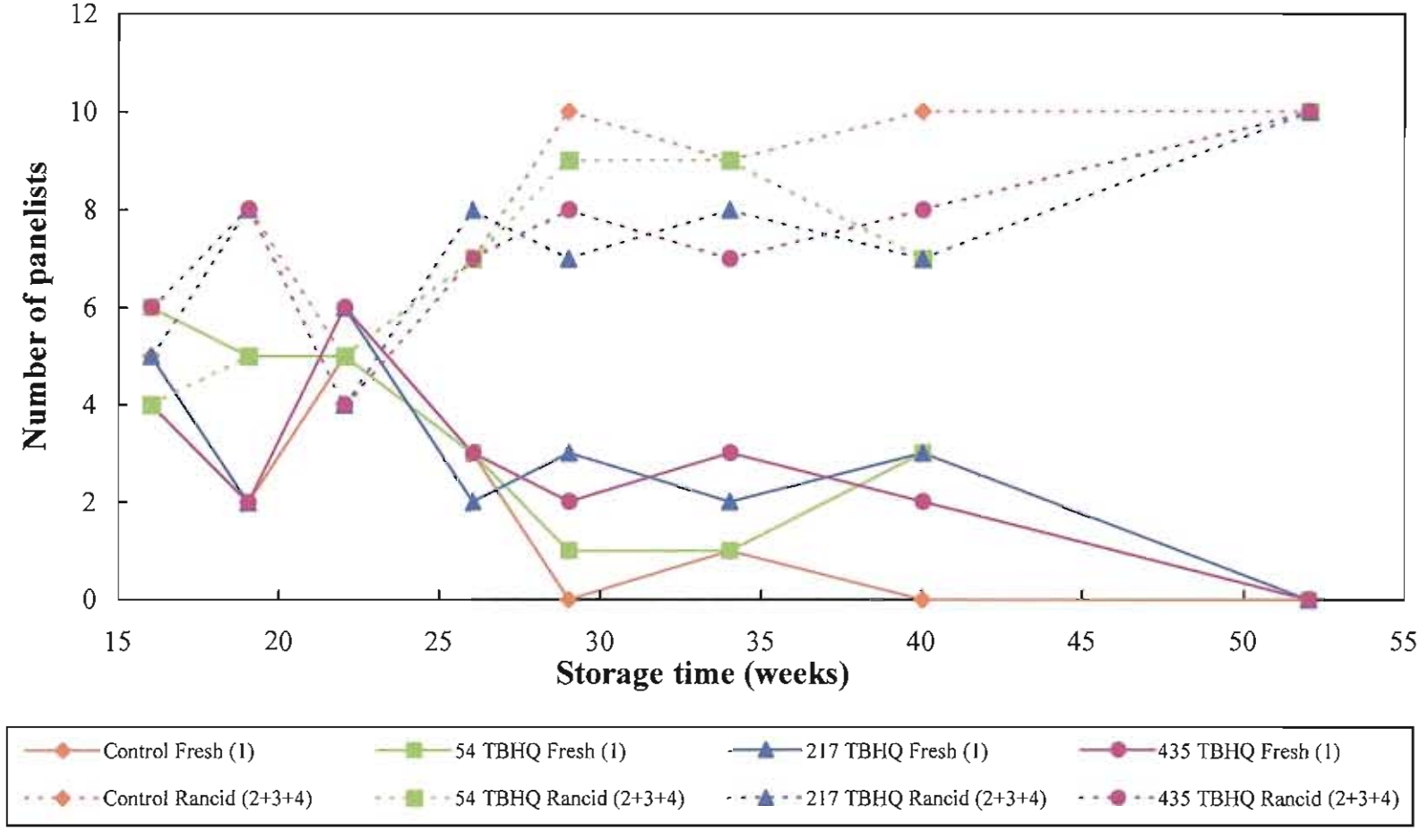


Figure 52: The effect of storage on the sensory quality of sunflower seed oil stored at 30°C for a period of 52 weeks (Option 1).

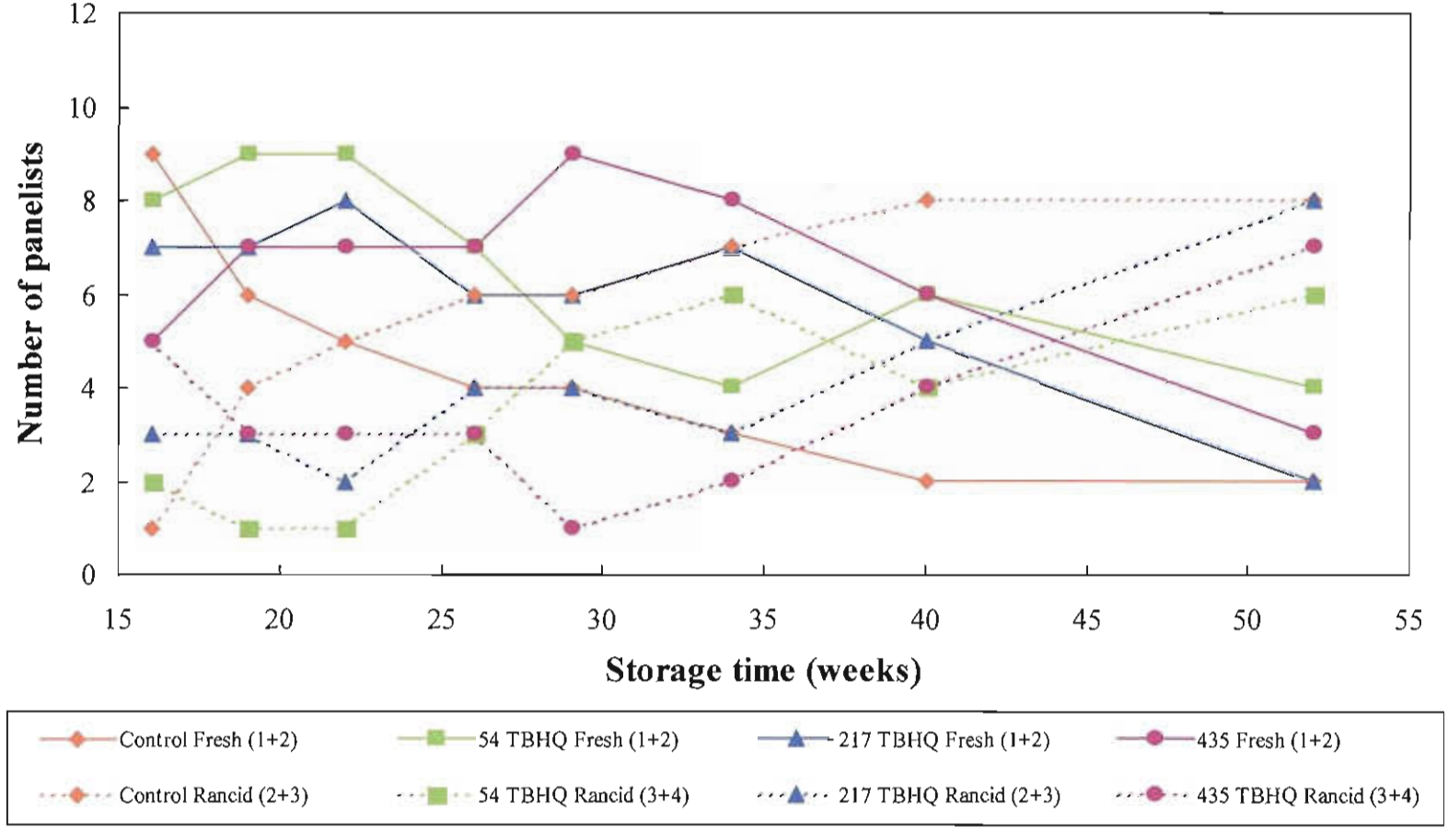


Figure 53: The effect of storage on the sensory quality of sunflower seed oil stored at 30°C for a period of 52 weeks. (Option 2).

A summary of the results is presented in Table 26.

Table 26: Estimation of onset of rancidity time by sensory evaluation and chemical parameters.

Samples	Storage time (weeks) at which oil was deemed to be rancid		
	Sensory evaluation*		Chemical parameters♦
	Option 1 Fresh (1), Rancid (2+3+4)	Option 2 Fresh (1+2), Rancid (3+4)	
Control	29	40	26
54 mg/kg TBHQ	29	>52	26
217 mg/kg TBHQ	26	52	34
435 mg/kg TBHQ	29	>52	52

* A majority of 8 out of 10 sensory panelists deemed the oil rancid

♦ Chemical parameters defined in Section 3.4 Modelling

4.2.3 Modelling

4.2.3.1 Models

Model 1

All the data were used to create Model 1. The results of the variables selected by multiple regression to be used in the model are shown in Table 27. The variables were the following FFA, PV, AV, UV 232 nm, UV 268 nm, OSI, total tocopherols, α-tocopherol, γ-tocopherol, β-tocopherol, total volatile components, hexanal, t-2-hexenal, t,t-2,4-decadienal, pentanal, IV, polyunsaturated fatty acids and TBHQ content.

Table 27: Regression summary for dependant variable: shelf-life of Model 1.

N = 34 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	10.8	10.3	0.303
TBHQ	0.05	0.003	0.000
FFA	-161	12.2	0.000
Pentanal	-2.12	0.526	0.000
Conjugated triene value	18.0	4.96	0.001

Where R^2 = 0.9597

F (4, 29) = 172

Standard error of estimate = 2.93

The graph of the predicted versus the observed values is shown in Figure 54.

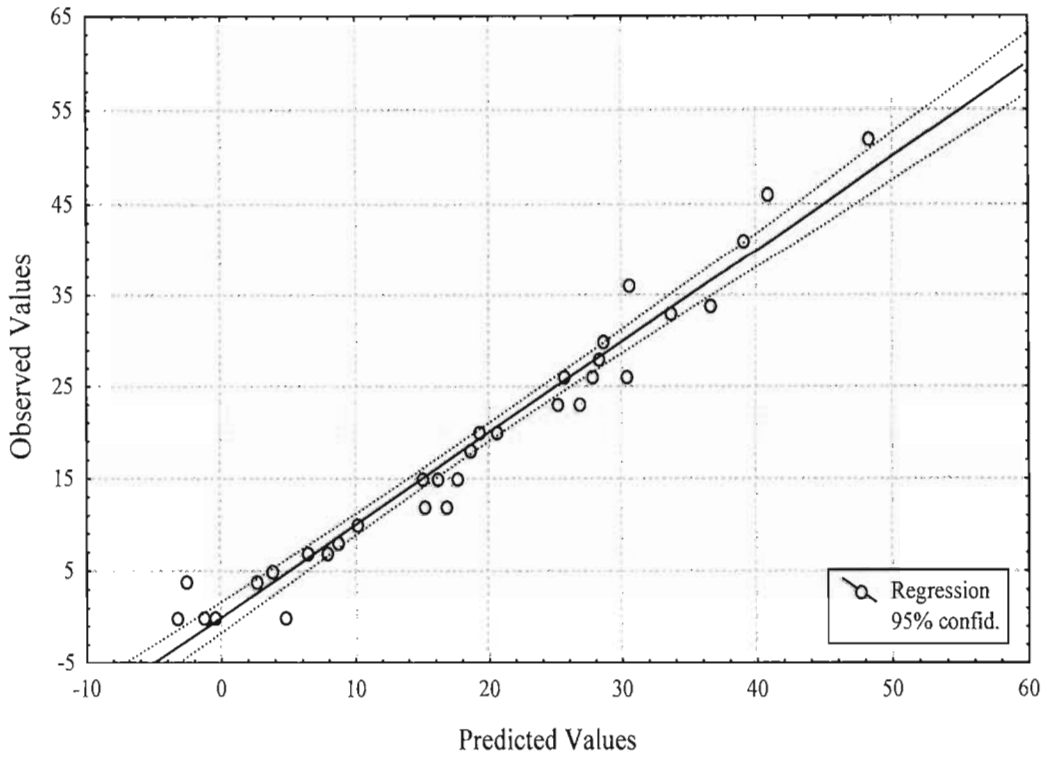


Figure 54: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 1. The dependant variable is the shelf-life.

Model 2

The squares of the values obtained were used to create Model 2. The results of the variables selected by multiple regression to be used in the model are shown in Table 28.

Table 28: Regression summary for dependant variable: shelf-life of Model 2.

N = 34 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	26.7	1.08	0.000
PV^2	-0.014	0.006	0.043
$TBHQ^2$	0.0001	0.000	0.000
FFA^2	-281	40.6	0.000
OSI^2	0.008	0.001	0.000
Total volatile $area^2$	-0.014	0.010	0.000
Hexanal	0.042	0.010	0.000

Where R^2 = 0.9853

F (6, 27) = 301

Standard error of estimate = 1.84

The graph of the predicted versus the observed values is shown in Figure 55.

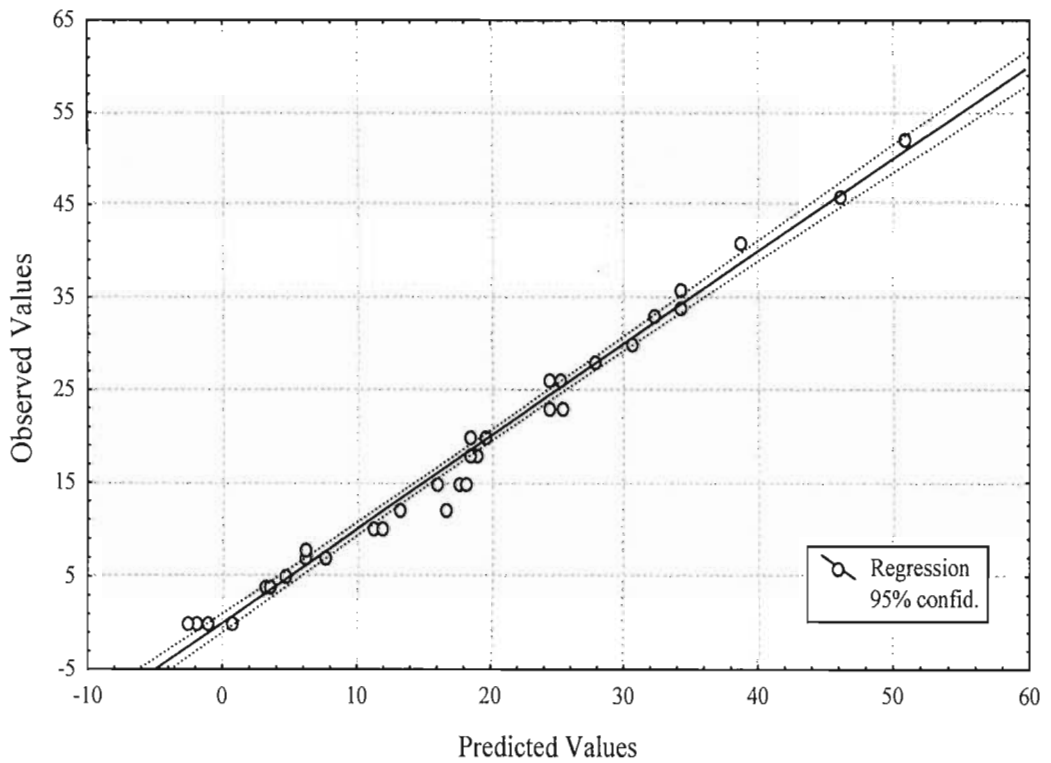


Figure 55: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 2. The dependant variable is the shelf-life.

Model 3

The weighted values were calculated and used to create Model 3. The TBHQ value was not weighted as the values used were the amounts added and thus no standard deviation could be calculated for them. The results of the variables selected by multiple regression to be used in the model are shown in Table 29.

Table 29: Regression summary for dependant variable: shelf-life of Model 3.

N = 34 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	10.8	10.3	0.303
TBHQ	0.052	0.003	0.000
Weighted FFA	-1.30	0.098	0.000
Weighted pentanal	-3.48	0.863	0.000
Weighted conjugated triene value	2.24	0.619	0.001

Where R^2 = 0.9597
F (4, 29) = 172
Standard error of estimate = 2.93

The graph of the predicted versus the observed values is shown in Figure 56.

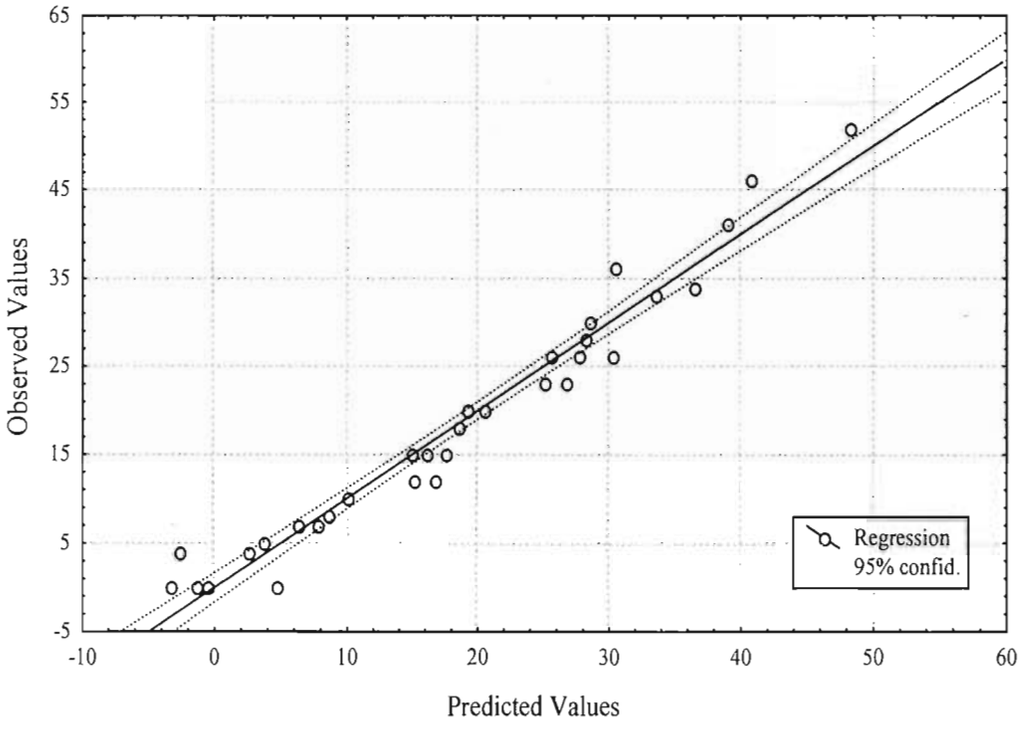


Figure 56: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 3. The dependant variable is the shelf-life.

Model 4

The calculated weighted values of the squares of the data were used to create Model 4. The TBHQ value was not weighted as the values used were the amounts added and thus no standard deviation could be calculated for them. The results of the variables selected by multiple regression to be used in the model are shown in Table 30.

Table 30: Regression summary for dependant variable: shelf-life of Model 4.

N = 34 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	26.7	1.08	0.000
Weighted PV^2	-0.004	0.002	0.043
$TBHQ^2$	0.00009	0.000	0.000
Weighted FFA^2	-2.26	0.327	0.000
Weighted OSI^2	0.006	0.001	0.000
Weighted total volatiles $area^2$	-0.189	0.033	0.000
Weighted $hexanal^2$	0.170	0.040	0.000

Where	R^2	=	0.9853
	F (6, 27)	=	301
	Standard error of estimate	=	1.84

The graph of the predicted versus the observed values is shown in Figure 57.

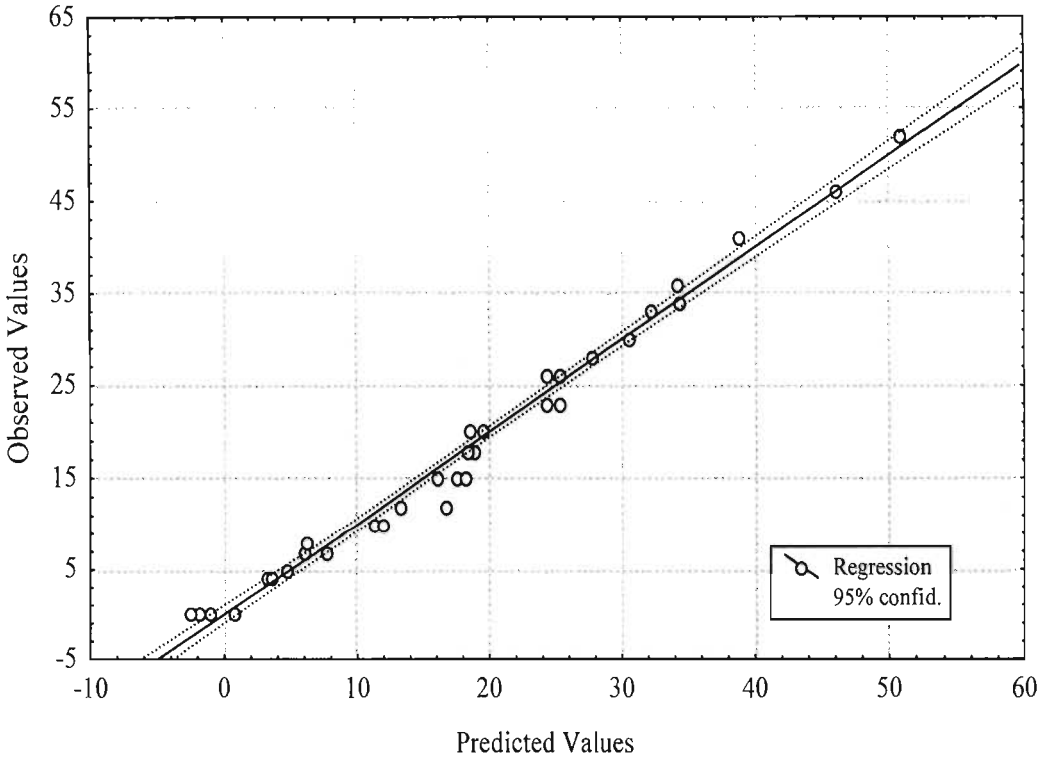


Figure 57: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 4. The dependant variable is the shelf-life.

Model 5

The values obtained and their squares were used to create Model 5. The results of the variables selected by multiple regression to be used in the model are shown in Table 31.

Table 31: Regression summary for dependant variable: shelf-life of Model 5.

N = 34 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	29.1	1.12	0.000
PV	-0.489	0.154	0.004
$TBHQ^2$	0.0001	0.000	0.000
FFA^2	-249	38.6	0.000
OSI^2	0.007	0.001	0.000
Total volatiles $area^2$	-0.015	0.002	0.000
$Hexanal^2$	0.044	0.009	0.000

Where R^2 = 0.9875

F (6, 27) = 355

Standard error of estimate = 1.69

The graph of the predicted versus the observed values is shown in Figure 58.

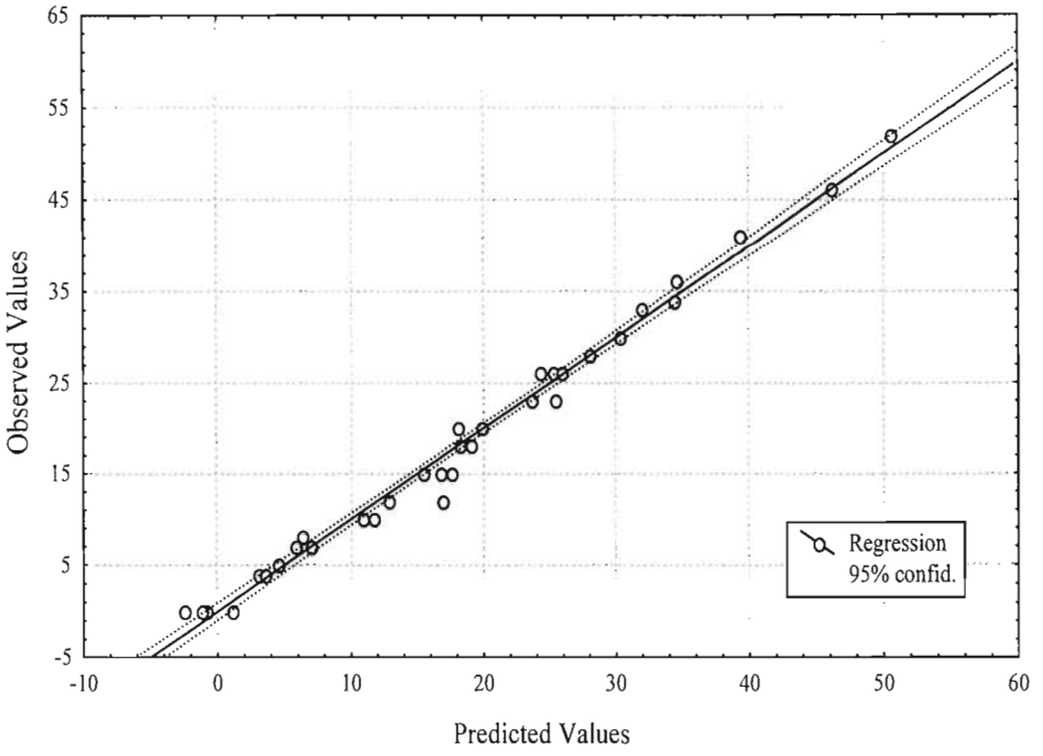


Figure 58: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 5. The dependant variable is the shelf-life.

Model 6

The values obtained and their squares, excluding the TBHQ value, were used to create Model 6. The results of the variables selected by multiple regression to be used in the model are shown in Table 32.

Table 32: Regression summary for dependant variable: shelf-life of Model 6.

N = 34 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	26.6	1.93	0.000
OSI^2	0.007	0.001	0.000
FFA^2	-249	38.6	0.000
Pentanal	-1.23	0.653	0.070

Where R^2 = 0.9233

F (3, 30) = 120

Standard error of estimate = 3.98

The graph of the predicted versus the observed values is shown in Figure 59.

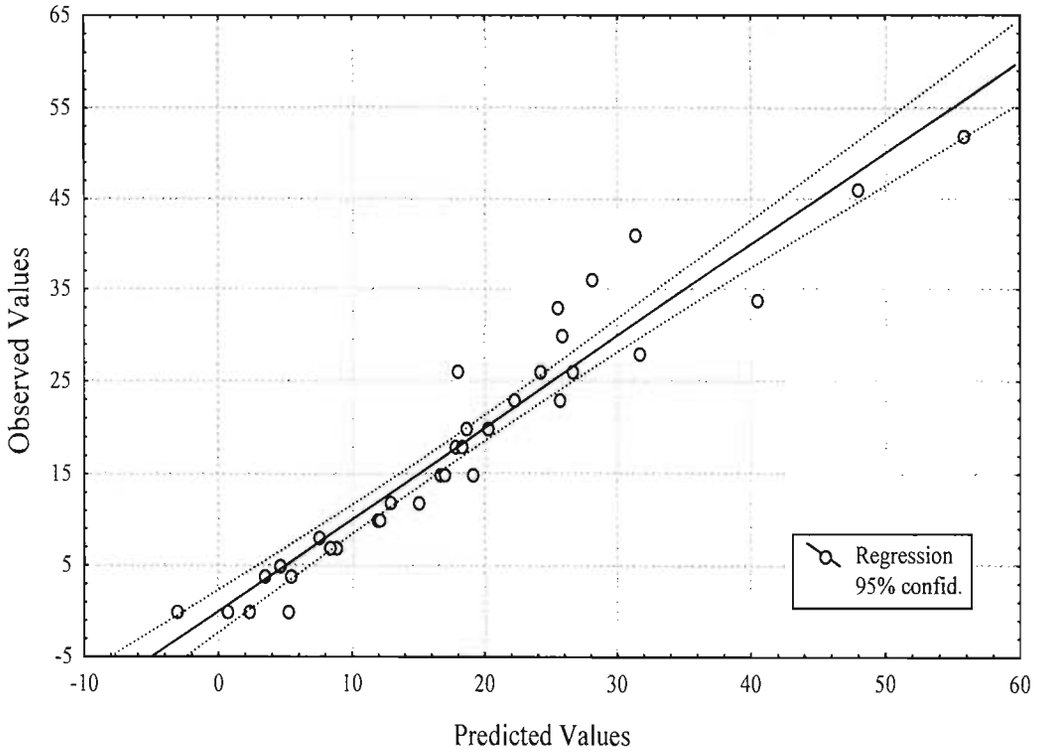


Figure 59: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 6. The dependant variable is the shelf-life.

Model 7

Model 7 is the simple model that was created with the values of simple methods of analyses and their squares. The simple methods of analyses are FFA, PV, OSI and conjugated diene/triene. The results of the variables selected by multiple regression to be used in the model are shown in Table 33.

Table 33: Regression summary for dependant variable: shelf-life of Model 7.

N = 34 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	11.5	8.65	0.194
OSI^2	0.018	0.002	0.000
FFA^2	-757	211	0.001
FFA	-160	86.4	0.074

Where R^2 = 0.9231
F (4, 26) = 120
Standard error of estimate = 3.98

The graph of the predicted versus the observed values is shown in Figure 60.

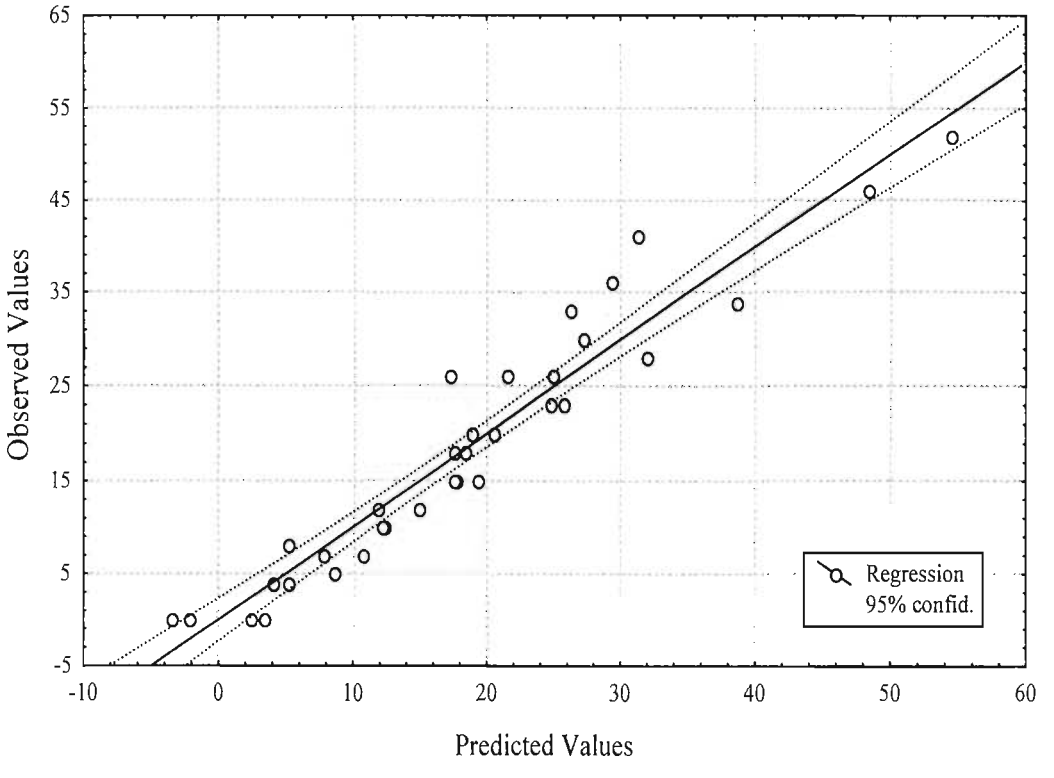


Figure 60: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 7. The dependant variable is the shelf-life.

Model 8

The OSI value and its square were the only variables used to create Model 8. The multiple regression results are shown in Table 34.

Table 34: Regression summary for dependant variable: shelf-life of Model 8.

N = 34 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	11.2	6.56	0.099
OSI	-0.401	0.669	0.553
OSI^2	0.031	0.015	0.047

Where R^2 = 0.5483
F (2, 31) = 18.8
Standard error of estimate = 9.50

The graph of the predicted versus the observed values is shown in Figure 61.

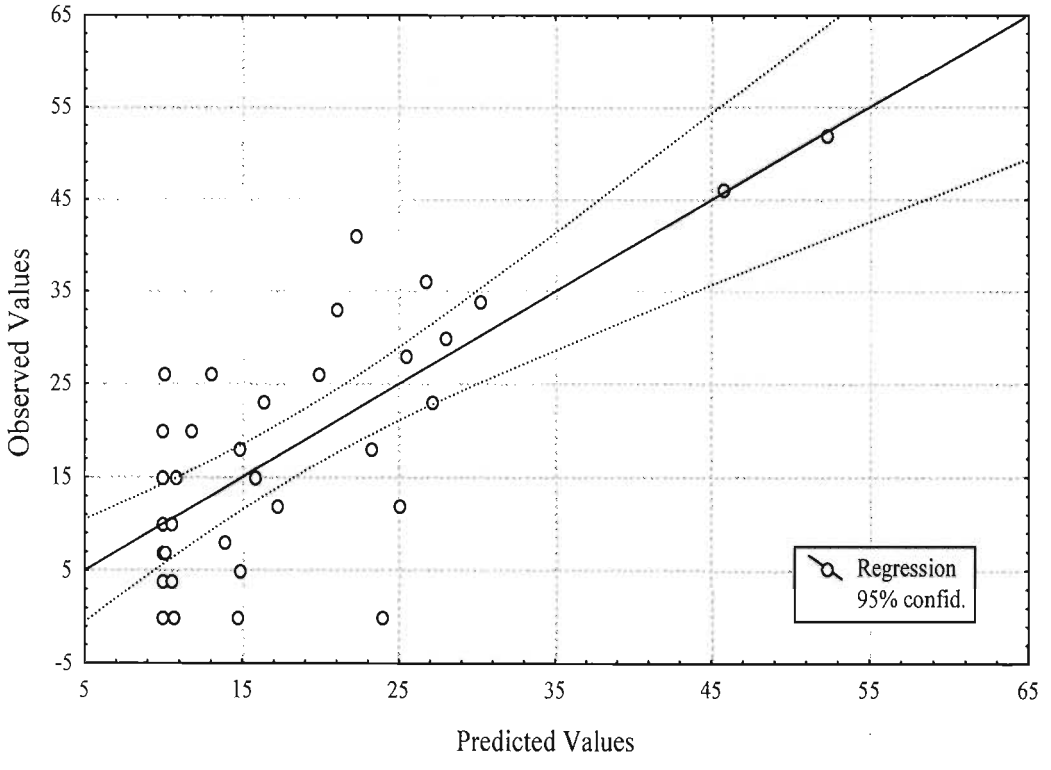


Figure 61: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 8. The dependant variable is the shelf-life.

Model 9

Model 9 is based on the sensory evaluation (Option 1) and the values obtained and their squares were used to create model. Results of the variables selected by multiple regression are shown in Table 35.

Table 35: Regression summary for dependant variable: shelf-life of Model 9.

N = 31 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	26.3	5.08	0.000
FFA	-125	30.5	0.001
TBHQ	-0.076	-12.8	0.000
PV	-0.558	0.131	0.000
OSI^2	0.005	0.009	0.000
$TBHQ^2$	0.0001	0.000	0.000
Pentanal	-0.686	0.253	0.013
Alpha-$tocopherol^2$	0.017	0.006	0.007
FFA^2	184	76.5	0.025
Total $tocopherols^2$	-0.008	0.004	0.059

Where R^2 = 0.9930

F (9, 21) = 338

Standard error of estimate = 0.921

The graph of the predicted versus the observed values is shown in Figure 62.

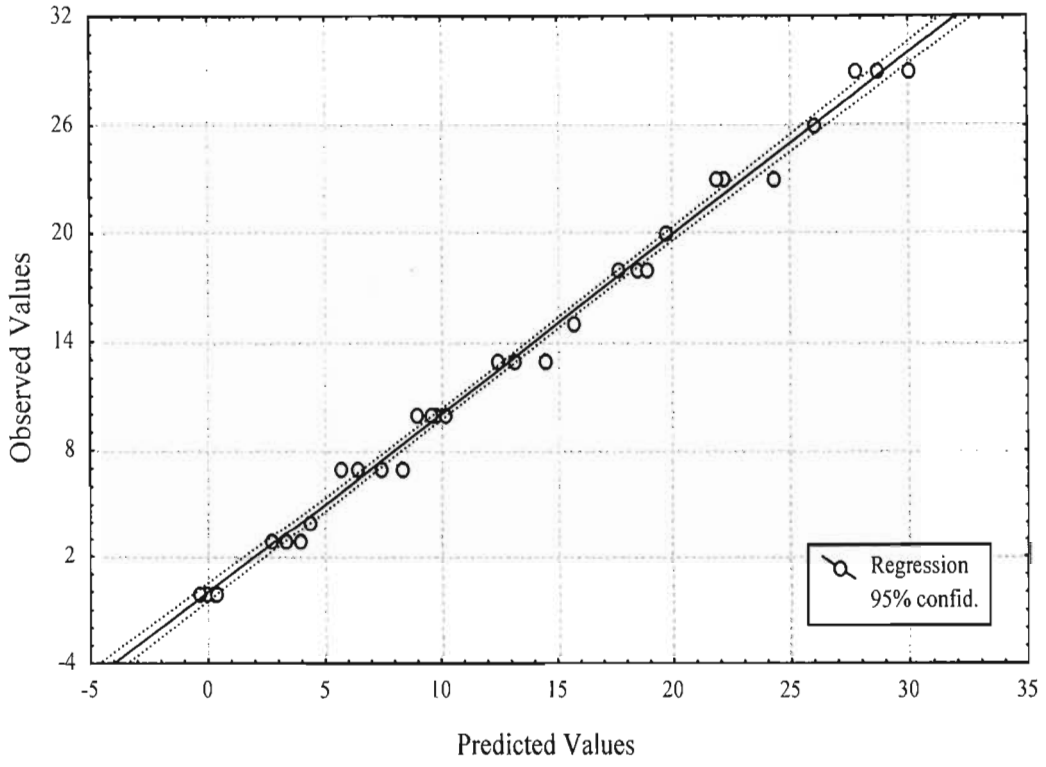


Figure 62: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 9. The dependant variable is the shelf-life.

Model 10

Model 10 is based on the sensory evaluation and the values obtained and their squares, excluding TBHQ content, were used to create model. Results of the variables selected by multiple regression are shown in Table 36.

Table 36: Regression summary for dependant variable: shelf-life of Model 10.

N = 31 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	35.7	5.92	0.000
FFA	-187	37.4	0.001
OSI	-1.13	0.118	0.000
OSI^2	0.020	0.003	0.000
PV	-0.723	0.185	0.001
Alpha-tocopherol^2	0.011	0.003	0.000
FFA^2	330	93.1	0.002

Where R^2 = 0.9839

F (6, 24) = 244

Standard error of estimate = 1.32

The graph of the predicted versus the observed values is shown in Figure 63.

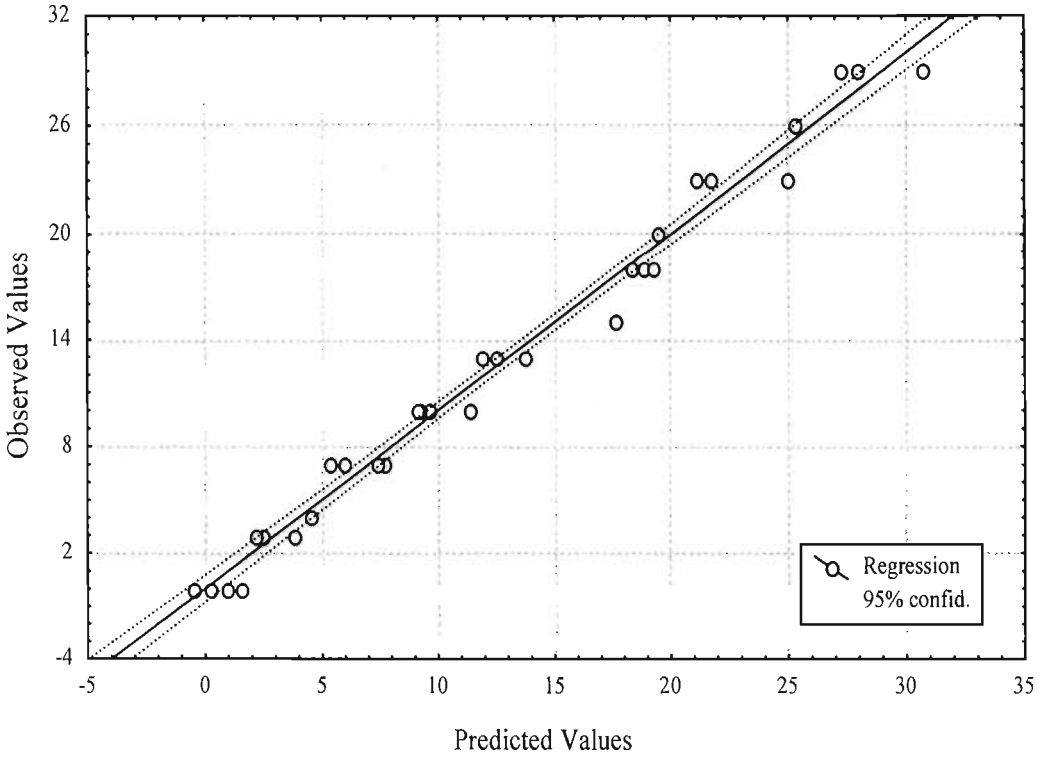


Figure 63: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 10. The dependant variable is the shelf-life.

Model 11

Model 11 is the simple model based on the sensory evaluation that was created with the values of simple methods of analyses and their squares. The results of the variables selected by multiple regression to be used in the model are shown in Table 37.

Table 37: Regression summary for dependant variable: shelf-life of Model 11.

N = 31 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	50.8	1.88	0.000
FFA	-59.3	19.2	0.005
OSI	-1.14	0.156	0.000
OSI^2	0.020	0.003	0.000
PV	-1.15	0.190	0.000

Where R^2 = 0.9691
F (4, 26) = 204
Standard error of estimate = 1.76

The graph of the predicted versus the observed values is shown in Figure 64.

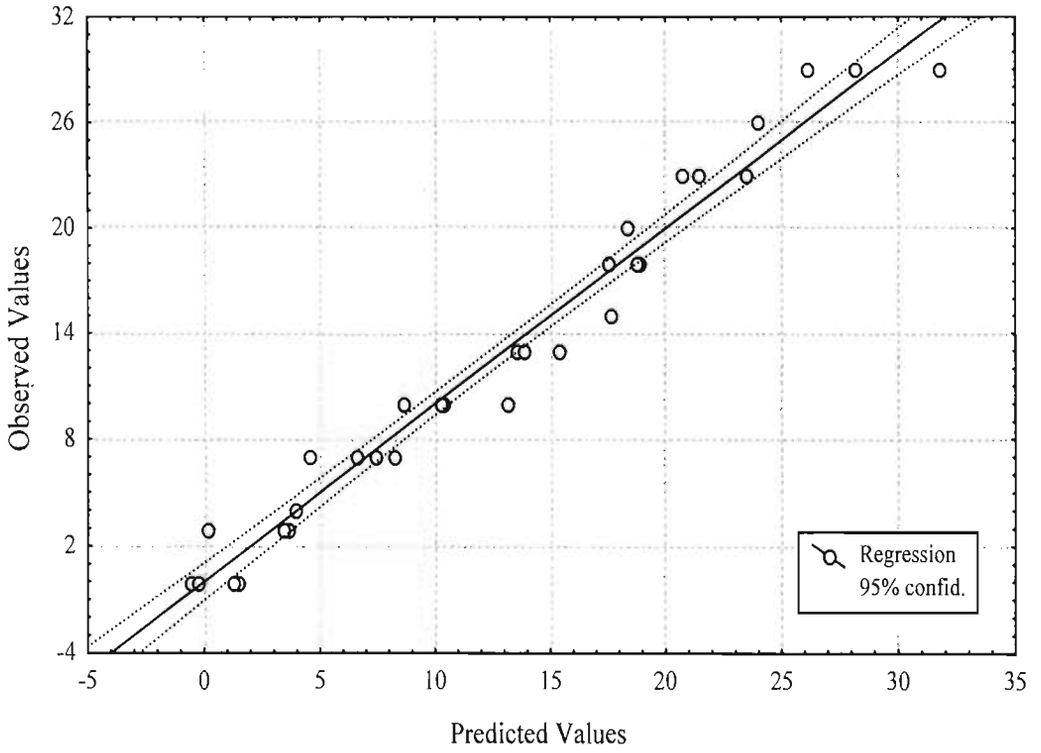


Figure 64: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 11. The dependant variable is the shelf-life.

Model 12

The OSI value and its square were the only variables used to create Model 12, which was based on the sensory evaluation. The multiple regression results are shown in Table 38.

Table 38: Regression summary for dependant variable: shelf-life of Model 12.

N = 31 cases	**B** regression coefficients	Standard error of **B** coefficients	p-level
Intercept	16.4	6.04	0.011
OSI	-0.75	0.650	0.256
OSI^2	0.023	0.014	0.130

Where R^2 = 0.1437
F (2, 31) = 2.35
Standard error of estimate = 8.91

The graph of the predicted versus the observed values is shown in Figure 65.

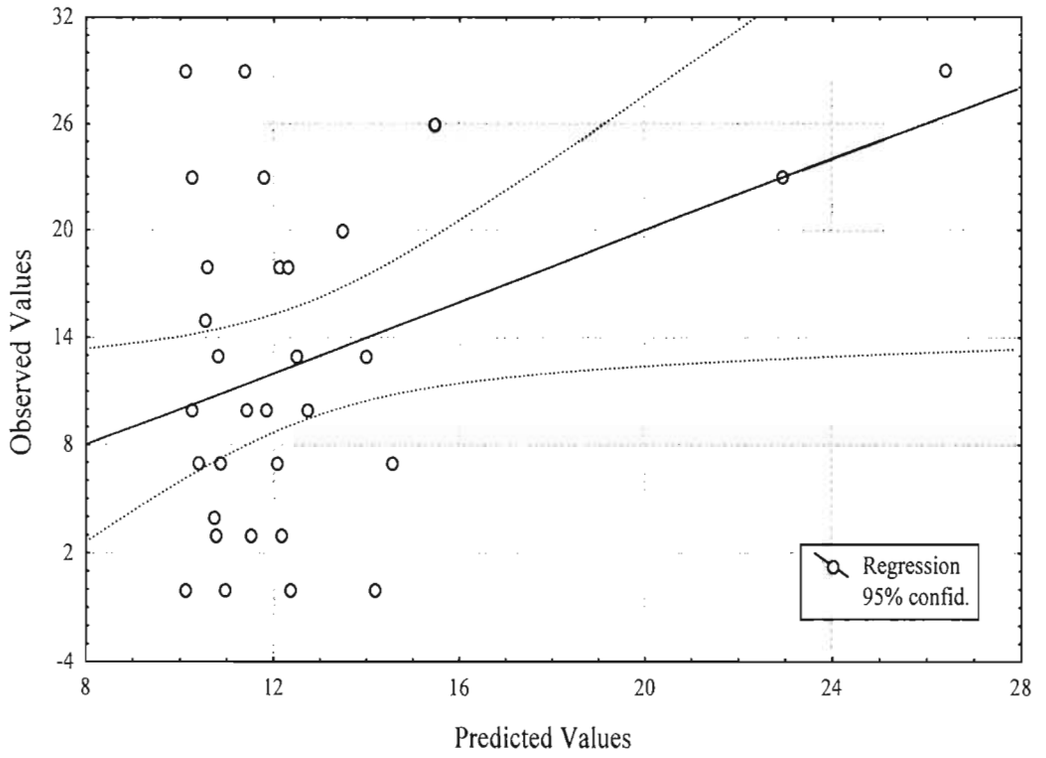


Figure 65: Predicted versus observed shelf-life values in weeks for sunflower seed oil as determined by Model 12. The dependant variable is the shelf-life.

4.2.3.2 Jackknifing

The practical applicability and reliability of the selected models was tested by applying a jackknifing procedure.

Models based on PV and AV

The results of the jackknifing are shown in Table 39 where the Ideal models are as described for Model 5 including TBHQ content and Model 6 excluding TBHQ content, Practical model as described for model 7 and the OSI model as described for Model 8. The number of weeks that would comply with a 95 % confidence level has been calculated for the models. The number of weeks needed for the Ideal model with TBHQ included to comply was 5.5 weeks and 1 case of the jackknifing results was found to be outside the limit. For the Ideal model excluding TBHQ it was 8.5 weeks with 2 cases found to be outside the limit. The Practical model had to comply with 8.7 weeks and 2 cases of the jackknifing results were outside, whereas the OSI model had to comply with 19.9 weeks with 2 cases found to be outside the limit.

The observed minus predicted values (errors) of the models is represented graphically in Figure 66. The errors were grouped into 3 categories, namely: 0 to ± 2 weeks, ± 2 to ± 4 weeks and more than + 4 weeks and less than – 4 weeks on the x-axis. The percentage cases with an error falling in each category were calculated from the total number of cases, which were 34 in this instance, and a frequency diagram was drawn. The total percentage cases with an error between 0 to ± 2 weeks were 70.6, 47.0, 29.4 and 14.7 % for the Ideal + TBHQ, Ideal – TBHQ, Practical and OSI models, respectively. The percentage cases with an error between ± 2 to ± 4 were 23.5, 26.5, 44.1 and 14.7 % for the four models in the same order, respectively. The percentage cases with an error of more than + 4 weeks or less than – 4 weeks was 5.9, 26.5, 26.4 and 70.6 % for the four models, respectively.

Table 39: Jackknifing results of four selected models that were based on the PV and AV values.

Excluded case	Observed shelf-life	Ideal model incl. TBHQ		Ideal model excl. TBHQ		Practical model		OSI model	
		Predicted shelf-life	Observed minus predicted	Predicted shelf-life	Observed minus predicted	Predicted shelf-life	Observed minus predicted	Predicted shelf-life	Observed minus predicted
1	26	25.0	1.0	23.6	2.4	18.7	7.3	8.9	17.1
2	26	25.9	0.1	26.7	-0.7	24.6	1.4	12.4	13.6
3	34	34.5	-0.5	41.2	-7.2	39.8	-5.8	29.8	4.2
4	52	49.2	2.8	58.0	-6.0	55.9	-3.9	52.5	-0.5
5	20	17.7	2.3	18.5	1.5	18.7	1.3	9.0	11.0
6	20	19.9	0.1	20.2	-0.2	20.5	-0.5	11.4	8.6
7	28	28.1	-0.1	31.9	-3.9	32.3	-4.3	25.2	2.8
8	46	46.1	-0.1	48.6	-2.6	49.0	-3.0	45.5	0.5
9	15	17.2	-2.2	16.8	-1.8	18.0	-3.0	9.3	5.7
10	15	17.9	-2.9	19.3	-4.3	19.9	-4.9	10.6	4.4
11	23	25.5	-2.5	25.6	-2.6	25.9	-2.9	16.0	7.0
12	41	38.9	2.1	30.6	10.4	30.6	10.4	21.1	19.9
13	10	10.9	-0.9	12.0	-2.0	12.6	-2.6	9.8	0.2
14	10	11.9	-1.9	12.1	-2.1	12.4	-2.4	10.5	-0.5
15	18	19.3	-1.3	17.7	0.3	18.3	-0.3	14.6	3.4
16	36	34.2	1.8	27.6	8.4	28.7	7.3	26.0	10.0
17	7	7.0	0.0	8.9	-1.9	10.9	-3.9	10.3	-3.3
18	7	5.7	1.3	8.3	-1.3	7.7	-0.7	10.3	-3.3
19	15	15.5	-0.5	17.1	-2.1	17.7	-2.7	15.8	-0.8
20	33	31.7	1.3	25.1	7.9	25.8	7.2	20.2	12.8
21	4	2.9	1.1	3.3	0.7	5.2	-1.2	10.5	-6.5
22	4	3.6	0.4	5.4	-1.4	4.0	0.0	10.8	-6.8
23	12	12.8	-0.8	15.1	-3.1	15.0	-3.0	17.4	-5.4
24	30	30.3	-0.3	25.4	4.6	26.9	3.1	27.8	2.2
25	0	-3.6	3.6	-3.7	3.7	-2.5	2.5	11.0	-11.0
26	0	-1.6	1.6	0.9	-0.9	-4.2	4.2	11.1	-11.1
27	8	6.2	1.8	7.4	0.6	5.0	3.0	14.1	-6.1
28	26	26.3	-0.3	17.1	8.9	16.7	9.3	19.5	6.5
29	5	4.4	0.6	4.2	0.8	8.8	-3.8	15.4	-10.4
30	23	23.8	-0.8	22.0	1.0	24.9	-1.9	27.4	-4.4
31	0	-3.4	3.4	5.8	-5.8	3.8	-3.8	15.4	-15.4
32	18	18.2	-0.2	18.2	-0.2	17.6	0.4	23.5	-5.5
33	12	18.1	-11.8	13.0	-1.0	11.8	0.2	25.8	-13.8
34	0	4.4	-4.4	3.4	-3.4	3.6	-3.6	25.4	-25.4
Mean			-0.182		-0.097		-0.018		-0.009
Std error of estimate			2.7099		4.1961		4.2956		9.8010
95 % confidence interval			± 5.5		± 8.5		± 8.7		19.9

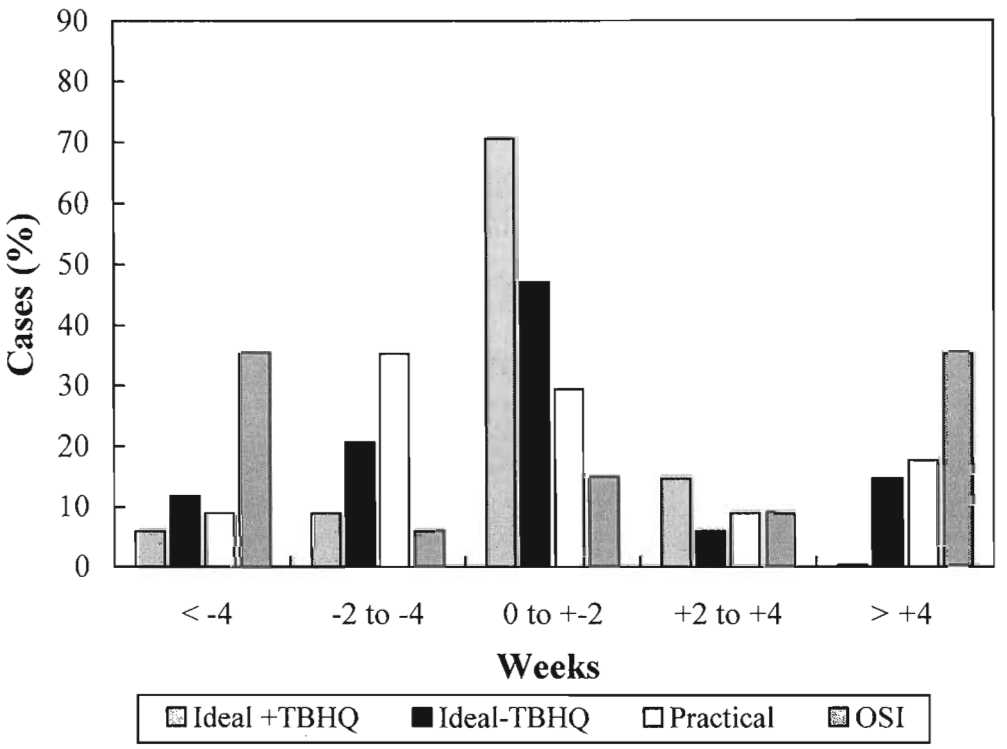


Figure 66: Percentage cases within each week category of the jackknifing results of the four models based on PV and AV values that was grouped into 3 categories namely: 0 to ± 2 weeks, ±2 to ±4 weeks and more than + 4 weeks and less than - 4 weeks.

Models based on sensory evaluation.

The results of the jackknifing are shown in Table 40 where the Ideal models is as described for Model 9 including TBHQ content and Model 10 excluding TBHQ content, Practical model as described for Model 11 and the OSI model as described for Model 12.

The same 95 % confidence level was applied to these models with the following results: the Ideal model including TBHQ had 6.1 weeks limits with one case not complying, the Ideal model excluding TBHQ had to comply within 3.5 weeks and 2 cases did not comply, the Practical model had to fall within 4.0 weeks and no cases were found to be outside the limits and the OSI model had to fall within 19.0 weeks with one case found to be outside the 95 % limit.

Table 40: Jackknifing results of four selected models that were based on the sensory evaluation.

Excluded case	Observed shelf-life	Ideal model incl. TBHQ		Ideal model excl. TBHQ		Practical model		OSI model	
		Predicted shelf-life	Observed minus predicted	Predicted shelf-life	Observed minus predicted	Predicted shelf-life	Observed minus predicted	Predicted shelf-life	Observed minus predicted
1	29	31.6	-2.6	32.8	-3.8	32.7	-3.7	10.2	18.8
2	29	32.7	-3.7	26.4	2.6	25.4	3.6	8.9	20.1
3	26	40.8	-14.8	24.9	1.1	23.4	2.6	14.4	11.6
4	29	25.6	3.4	26.3	2.7	27.1	1.9	23.2	5.8
5	23	21.8	1.2	21.4	1.6	21.1	1.9	10.8	12.2
6	23	21.4	1.6	20.6	2.4	20.2	2.8	9.6	13.4
7	20	19.6	0.4	19.3	0.7	18.0	2.0	12.9	7.1
8	23	25.6	-2.6	26.7	-3.7	23.7	-0.7	22.9	0.1
9	18	18.5	-0.5	18.4	-0.4	19.0	-1.0	11.5	6.5
10	18	19.1	-1.1	19.1	-1.1	17.5	0.5	10.2	7.8
11	15	15.9	-0.9	17.9	-2.9	17.9	-2.9	10.2	4.8
12	18	17.3	0.7	19.4	-1.4	18.8	-0.8	11.8	6.2
13	13	14.9	-1.9	13.8	-0.8	15.7	-2.7	12.4	0.6
14	13	13.1	-0.1	12.4	0.6	13.7	-0.7	10.7	2.3
15	10	9.5	0.5	11.8	-1.8	13.7	-3.7	10.2	-0.2
16	13	12.1	0.9	11.5	1.5	13.9	-0.9	14.1	-1.1
17	10	10.2	-0.2	9.6	0.4	10.4	-0.4	13.1	-3.1
18	10	8.7	1.3	9.2	0.8	8.5	1.5	11.5	-1.5
19	7	7.5	-0.5	7.7	-0.7	7.4	-0.4	10.6	-3.6
20	10	9.3	0.7	9.0	1.0	10.3	-0.3	12.0	-2.0
21	7	6.9	0.1	5.8	1.2	6.5	0.5	12.5	-5.5
22	7	5.2	1.8	5.1	1.9	4.3	2.7	11.0	-4.0
23	4	4.4	-0.4	4.6	-0.6	3.9	0.1	11.2	-7.2
24	7	8.5	-1.5	7.4	-0.4	8.4	-1.4	15.2	-8.2
25	3	3.5	-0.5	4.0	-1.0	3.6	-0.6	13.1	-10.1
26	3	4.9	-1.9	2.2	0.8	-0.3	3.3	11.1	-8.1
27	0	0.3	-0.3	1.1	-1.1	-0.7	0.7	10.8	-10.8
28	3	2.5	0.5	2.0	1.0	3.4	-0.4	12.2	-9.2
29	0	-0.1	0.1	1.9	-1.9	1.8	-1.8	13.7	-13.7
30	0	-0.6	0.6	0.2	-0.2	-0.4	0.4	11.5	-11.5
31	0	0.59	-0.6	-0.9	0.9	1.9	-1.9	15.4	-15.4
Mean			-0.7		0.0		0.0		0.1
Std error of estimate			2.9952		1.6989		1.9716		9.3022
95 % confidence interval			± 6.1		± 3.5		± 4.0		19.0

The observed minus predicted values (errors) of the models is represented graphically in Figure 67. The percentage cases with an error falling in each category were calculated from the total of 31 cases. The total percentage cases with an error between 0 to ± 2 weeks were 83.8, 80.6, 70.9 and 19.4 % for the Ideal + TBHQ, Ideal – TBHQ, Practical and OSI models, respectively. The percentage cases with an error between ± 2 to ± 4 were 12.9, 19.4, 29.0 and 12.9 % for the four models in the same order, respectively. The percentage cases with an error of more than + 4 weeks or less than – 4 weeks was 3.2, 0, 0 and 67.8 % for the four models respectively.

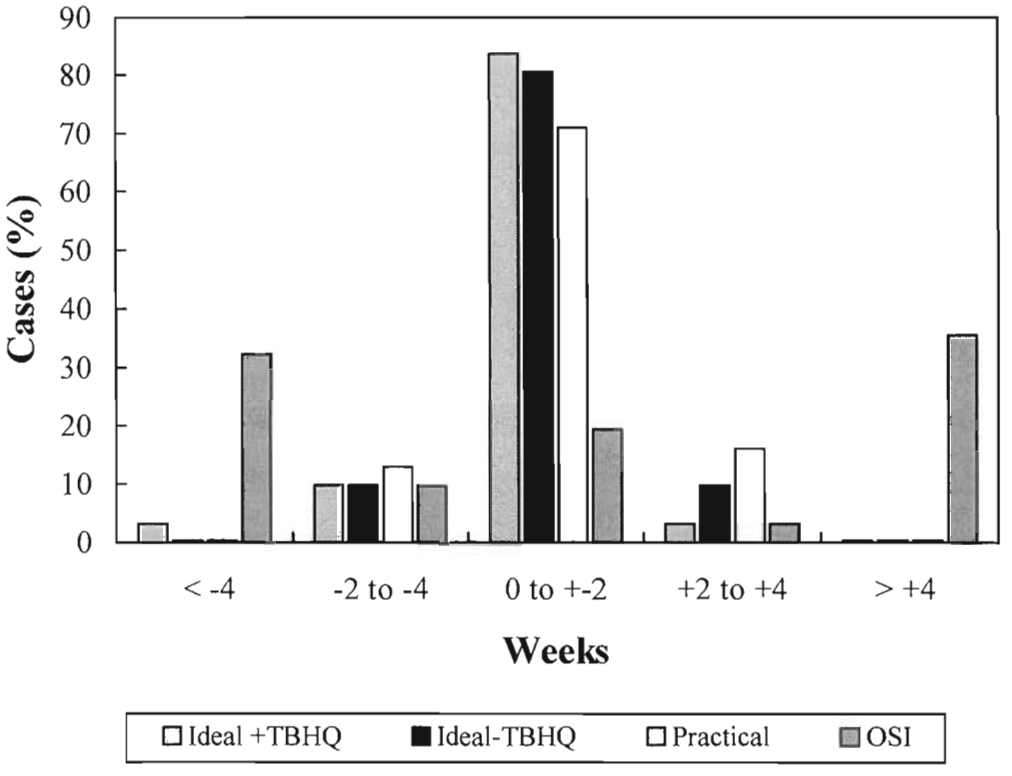


Figure 67: Percentage cases within each week category of the jackknifing results of the four models based on sensory evaluation that was grouped into 3 categories namely: 0 to ± 2 weeks, ±2 to ±4 weeks and more than + 4 weeks and less than - 4 weeks.

4.3 STATISTICAL ANALYSIS

The treatments were compared statistically for each parameter to determine if the trends differed significantly from each other.

4.3.1 Palm-olein oil

Best fitting equations (response for variable versus storage time), either linear regression lines ($y = a + bx$) or quadratic curves ($y = a + bx + cx^2$) were fitted to the curves of each treatment and the intercepts (a) and slopes (b) of the linear regression lines and the intercepts (a), b and c coefficients of the quadratic curves were pair-wise subjected to the t-test at the 99 % confidence level. In the cases where no result is shown a line or curve could not be fitted to the sample curve as no values were obtained. The results for the intercepts of the linear and quadratic equations fitted to the data are shown in Table 41. The results for the slopes (b) of variables that had linear regression lines fitted are shown in Tables 42, whereas the results for the b and c coefficients of quadratic curves fitted to the data are in Table 43.

Statistical analysis of trans-2-hexenal was not included as the values of all the samples showed very little change with a fluctuation of t-2-hexenal content between 0-2.3 mg/kg in total.

The symbols used in the tables have the following meanings:

S	=	significantly different at 99 % confidence level
NS	=	not significantly different at 99 % confidence level
-	=	no values obtained for a sample treatment and it was therefore not possible to fit a regression line or quadratic curve
↑	=	indicates significantly higher absolute values for the coefficient of the first named treatment than the second named treatment
↓	=	indicates significantly lower absolute values for the coefficient of the first named treatment than the second named treatment

Table 41: t-test results at 99 % confidence level of intercepts (a) for each comparison of the different treatments of palm-olein oil.

Variable	Comparisons of treatments					
	Control versus 0.035 mg/kg copper	Control versus 0.17 mg/kg copper	Control versus 0.69 mg/kg copper	0.035 mg/kg versus 0.17 mg/kg copper	0.035 mg/kg versus 0.69 mg/kg copper	0.17 mg/kg versus 0.69 mg/kg copper
FFA	NS	NS	NS	NS	NS	NS
PV	S ↑	S ↑	S ↑	NS	S ↑	NS
AV	S ↓	S ↓	S ↓	S ↓	NS	S ↑
Totox	S ↑	S ↑	S ↑	NS	NS	NS
OSI	NS	S ↑	S ↑	S ↑	S ↑	S ↑
Total tocopherols	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
Alpha tocopherol	S ↑	S ↑	S ↑	S ↑	S ↑	-
Alpha tocotrienol	S ↑	S ↑	S ↑	S ↑	S ↑	NS
Gamma tocotrienol	S ↑	S ↑	S ↑	S ↑	S ↑	NS
Delta tocotrienol	NS	S ↑	S ↑	S ↑	S ↑	NS
CV 232 nm	S ↑	NS	S ↑	NS	NS	NS
CV 268 nm	S ↓	S ↓	S ↓	NS	NS	NS
IV	NS	NS	NS	NS	NS	NS
Total volatiles	NS	NS	NS	NS	S ↓	S ↓
Hexanal	S ↑	S ↑	S ↑	NS	NS	NS
t,t-2,4-decadienal	-	-	-	NS	NS	NS
Pentanal	S ↓	S ↓	S ↓	NS	S ↓	S ↓

The results showed that the majority of the intercepts differed statistically from each other, the exceptions being the curves of the FFA and IV variables, where the intercepts of all the sample treatment comparisons did not differ significantly from each other.

Table 42: t-test results at 99 % confidence level of the slopes (b) for each comparison of the different treatments that has been subjected to linear regression curves of palm-olein oil.

Variable	Comparisons of treatments					
	Control versus 0.035 mg/kg copper	Control versus 0.17 mg/kg copper	Control versus 0.69 mg/kg copper	0.035 mg/kg versus 0.17 mg/kg copper	0.035 mg/kg versus 0.69 mg/kg copper	0.17 mg/kg versus 0.69 mg/kg copper
FFA	S ↑	S ↑	S ↑	NS	S ↑	S ↑
PV	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
AV	S ↓	S ↓	S ↓	S ↓	S ↓	S ↑
OSI	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
Total tocopherols	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
Alpha tocopherol	S ↑	S ↑	S ↑	S ↑	-	-
Alpha tocotrienol	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
Gamma tocotrienol	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
Delta tocotrienol	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
CV 232 nm	S ↑	S ↑	S ↑	S ↓	NS	S ↑
CV 268 nm	S ↓	S ↓	S ↓	S ↓	S ↑	S ↑
IV	S ↑	S ↑	S ↑	NS	S ↓	S ↓
Total volatiles	S ↑	S ↑	S ↓	S ↓	S ↓	S ↓
Hexanal	S ↑	S ↑	S ↑	NS	S ↓	S ↓

The t-test results of the b and c coefficients of variables that had quadratic curves fitted to them are shown in Table 43.

Table 43: t-test results of comparisons of b and c quadratic coefficients of the treatments of palm-olein oil.

Variable	Coefficient	Comparisons of treatments					
		Control versus 0.035 mg/kg copper	Control versus 0.17 mg/kg copper	Control versus 0.69 mg/kg copper	0.035 mg/kg versus 0.17 mg/kg copper	0.035 mg/kg versus 0.69 mg/kg copper	0.17 mg/kg versus 0.69 mg/kg copper
Totox	b	S ↑	S ↑	S ↑	S ↓	S ↑	S ↑
	c	S ↑	S ↑	S ↑	S ↓	S ↑	S ↑
t,t-2,4-	b	-	-	-	S ↓	S ↑	S ↑
decadienal	c	-	-	-	S ↓	S ↑	S ↑
Pentanal	b	S ↓	S ↓	S ↓	S ↓	S ↓	S ↓
	c	S ↓	S ↓	S ↓	S ↓	S ↓	S ↓

Most of the slopes of the sample treatment comparisons of the variables differed significantly from each other. The slopes of 5 sample treatment comparisons of the FFA, CV 232, IV and hexanal variables were significantly different from each other and only one treatment comparison of each variable mentioned was not significantly different (0.035 versus 0.17 mg/kg copper for FFA, 0.035 versus 0.69 mg/kg copper for CV 232 nm, 0.035 versus 0.17 mg/kg copper for IV and 0.035 versus 0.17 mg/kg copper for hexanal). The dissimilarity between the different slopes of the treatments can be gauged from the graph of each variable.

4.3.2 Sunflower seed oil

Best fitting equations, either linear regression lines ($y = a + bx$) or quadratic curves ($y = a + bx + cx^2$) were fitted to the curves of each treatment and the intercepts (a) and slopes (b) of the linear regression lines and the intercepts (a), b and c coefficients of the quadratic curves were pair-wise subjected to the t-test at the 99 % confidence level. The results for the intercepts of the linear and quadratic equations fitted to the data are shown in Table 44. The results for the slopes (b) of variables that had linear regression lines fitted are shown in Tables 45 whereas the results for the b and c coefficients of quadratic curves fitted to the data are in Table 46.

Statistical analysis of beta-tocopherol, trans-2-hexanal and trans,trans-2,4-decadienal was not included as the values of all the samples showed very little change with broad fluctuations within the low values obtained.

The symbols used in the tables have the same meaning as in palm-olein oil.

Table 44: t-test results at 99 % confidence level of intercepts (a) for each comparison of the different treatments of sunflower seed oil.

Variable	Comparisons of treatments					
	Control versus 54 mg/kg TBHQ	Control versus 217 mg/kg TBHQ	Control versus 435 mg/kg TBHQ	54 mg/kg versus 217 mg/kg TBHQ	54 mg/kg versus 400mg/kg TBHQ	217 mg/kg versus 435 mg/kg TBHQ
FFA	NS	NS	NS	NS	S ↑	NS
PV	NS	NS	S ↑	NS	S ↑	NS
AV	NS	NS	NS	NS	NS	NS
Totox	NS	NS	S ↑	NS	NS	NS
OSI	S ↓	S ↓	S ↓	S ↓	S ↓	S ↓
Total tocopherols	NS	NS	S ↓	NS	NS	NS
Alpha tocopherol	NS	S ↓	S ↓	NS	NS	NS
Gamma tocopherolsl	NS	NS	NS	NS	NS	NS
CV 232 nm	S ↑	S ↑	S ↑	S ↑	S ↑	NS
CV 268 nm	NS	NS	NS	NS	NS	NS
IV	NS	NS	NS	NS	NS	NS
Total volatiles	NS	NS	NS	NS	NS	NS
Hexanal	NS	S ↑	S ↑	NS	NS	NS
Pentanal	NS	NS	NS	NS	NS	NS

The results showed that none of the intercepts of the sample treatment comparisons of the following variables AV, gamma-tocopherol, CV 268, IV, total volatiles and pentanal, differed statistically from each other. All the intercepts of the sample treatments of OSI differed significantly from each other and the CV 232 had 5 significant differences in the

sample treatments intercepts with only 217 mg/kg TBHQ versus 435 mg/kg TBHQ being not significantly different. The remainder of the curves for the variables FFA, PV, Totox, total tocopherols and hexanal, had one or two of the intercepts of the sample treatment comparisons that differed significantly from each other.

Table 45: t-test results at 99 % confidence level of the slopes (b) for each comparison of the different treatments that has been subjected to linear regression curves of sunflower seed oil.

Variable	Comparisons of treatments					
	Control versus 54 mg/kg TBHQ	Control versus 217 mg/kg TBHQ	Control versus 435 mg/kg TBHQ	54 mg/kg versus 217 mg/kg TBHQ	54 mg/kg versus 435 mg/kg TBHQ	217 mg/kg versus 435 mg/kg TBHQ
FFA	S ↓	S ↑	S ↑	S ↑	S ↑	S ↑
AV	S ↑	S ↑	S ↑	S ↓	S ↑	S ↑
Total tocopherols	S ↓	S ↓	S ↓	S ↓	S ↓	S ↓
Alpha tocopherol	S ↓	S ↓	S ↓	S ↓	S ↓	S ↓
Gamma tocopherol	S ↓	S ↓	S ↓	NS	NS	NS
CV 232 nm	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
CV 268 nm	S ↑	S ↑	S ↑	S ↑	NS	S ↓
IV	S ↑	S ↑	S ↓	NS	S ↓	S ↓
Total volatiles	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
Pentanal	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑

Most of the slopes of the sample treatment comparisons of the variables differed significantly from each other. The slopes of 5 sample treatment comparisons of the CV 268 and IV variables were significantly different from each other and only one treatment comparison for each variable mentioned was not significantly different (54 versus 435 mg/kg TBHQ for CV 268 nm and 54 versus 217 mg/kg TBHQ for IV). In the gamma-tocopherol variable slopes of the Control versus the TBHQ samples differed significantly from each other, whereas the samples containing TBHQ did not differ significantly from each other. The dissimilarity between the different slopes of the treatments can be gauged from the graph of each variable.

Table 46: t-test results of comparisons of b and c quadratic coefficients of the treatments of sunflower seed oil.

Variable	Coefficient	Comparisons of treatments					
		Control versus 54 mg/kg TBHQ	Control versus 217 mg/kg TBHQ	Control versus 435 mg/kg TBHQ	54 mg/kg versus 217 mg/kg TBHQ	54 mg/kg versus 435 mg/kg TBHQ	217 mg/kg versus 435 mg/kg TBHQ
PV	b	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
	c	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
Totox	b	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
	c	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
OSI	b	S ↓	S ↓	S ↓	S ↓	S ↓	S ↓
	c	S ↓	S ↓	S ↓	S ↓	S ↓	S ↓
Hexanal	b	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑
	c	S ↑	S ↑	S ↑	S ↑	S ↑	S ↑

All of the coefficients for the slopes of the sample treatment comparisons of the variables differed significantly from each other.

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

$RO^\bullet \rightarrow$ 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

$^3O_2$ = normal triplet oxygen

$^1O_2$ = 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: $\alpha\text{-T} < \alpha\text{-T3} < \beta\text{-T} < \beta\text{-T3} < \gamma\text{-T} < \gamma\text{-T3} < \delta\text{-T} < \delta\text{-T3}$ 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, $\alpha\text{-tocopherol}^2$, 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, $\alpha\text{-tocopherol}^2$	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.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The predictive models obtained by the data set for each oil type show great potential for practical application in the food processing industry. The models at this stage can be described as rudimentary models and still need refining for specific cases. The drastic effect of the pro-oxidant, copper at low concentrations on oxidation of palm-olein, emphasises the importance of insisting on copper levels < 0.01 mg/kg in commercial refined oils. The control of transport and storage conditions, such as the type of containers and pipes, to prevent copper from being picked up is thus imperative to ensure good quality refined oils. The effect copper had on the oils during long-term storage lead to greatly enhanced oxidation, as can be seen by the chemical reactions that occurred. This is evident in the elimination of primary oxidation products as possible predictors for the palm-olein model and the inclusion of secondary oxidation products. Although copper addition complicated the predictive modelling, it provided useful information on the chemical reactions that occur and the course of rancidity with a strong pro-oxidant such as copper, on long term storage. Refining the model on palm-olein oil would entail further shelf-life studies with no addition of copper and storage of different palm-olein oils, which will make provision for variation in oil quality, at two temperatures, e.g. 40°C and 50°C. This will supply information on repeatability and robustness of the prediction models and will enable the calculation of a Q_{10} value that will make it possible to estimate shelf-lives at different temperatures. Generally, only parameters highlighted by the models as predictors should be evaluated, although primary oxidation products will have to be included as well to ensure that the effect that copper had is taken into account. Sensory evaluation will be essential at the time the oil is taken out from storage, since sensory evaluation is the ultimate test for food quality and stability. The relationship between onset of rancidity as determined by sensory evaluation to the concentration of chemical quality parameters measured at that time is especially useful. This is particularly relevant for the interaction of volatile oxidation products and typical flavour descriptors.

The addition of the antioxidant, TBHQ to sunflower seed oil provided important information on the prolonged effect of antioxidants. Antioxidant content emerged as an important predictor of shelf-life as it has a clear effect on oil stability. For modelling

purposes it has to be decided whether to determine the actual antioxidant content in the oil or to rely on values that had been added initially to the oil. However, the decision which antioxidant value to use in the model depends on the even dispersion of antioxidant in the oil and the magnitude of decrease in TBHQ content during storage and how the decrease during storage would effect prediction. Should the specified initial antioxidant level be known and where laboratories are unable to perform confirmation analyses, experience gained by this study suggest that the specified antioxidant level can be used in the predictive model. Since no analysis to determine the extent of decrease in TBHQ during storage was done in this study it is not possible to judge which value would be best to use for modelling purposes. As the sunflower seed oil models indicate, primary oxidation products, such as PV, are preferred as predictors to secondary oxidation products. For refining of the sunflower seed oil model, additional shelf-life studies are needed with different sunflower seed oil samples and storage at least two temperatures to eliminate the effect of variation between samples and determine shelf-life at specific temperatures. Storage at an ambient temperature such as 27°C in combination with storage at 37°C is needed, as 27°C would be a more representative storage temperature of sunflower seed oil for temperate local conditions. Sensory evaluation will be needed, as discussed. The important parameters to be studied have been highlighted by the modelling, thereby doing away with the need for an extended shelf-life study that included monitoring all 9 parameters determined.

Notably in the models for both oil types, it turned out that FFA and OSI were selected as predictors of shelf-life of oil. The importance of FFA as predictor was unexpected as FFA is normally associated with hydrolytic rancidity and not oxidative rancidity, but it appears that small changes in hydrolytic rancidity have an important effect on oil shelf-life. A contributing factor to the selection of FFA as predictor could be that only one oil sample was used for both oil types, which could have emphasised small increases in FFA. More oil samples would show greater variations in FFA and the emphases on small FFA changes might not be so noticeable.

The importance of OSI has been highlighted, as it was included in all the models. There has always been interest around in correlating accelerated oxidation tests such as OSI by itself with ambient shelf-life. This study showed that OSI predicated the actual shelf-life of the oils poorly. It appears that various factors are involved in long-term oil stability,

such as FFA formation, that would not necessarily be measured by OSI. Although OSI is one of the most important variables selected for shelf-life prediction in a Practical model, it needs to be used in combination with other parameters such as FFA, PV and possibly conjugated triene value. The applicability of AV for modelling purposes will have to be verified by additional shelf-life studies where the pro-oxidant is excluded.

It was clear, as expected, that monounsaturated oils are more stable than polyunsaturated oils. The Control of both oil types obtained a shelf-life of 22 and 26 weeks, respectively bearing in mind that the monounsaturated oil was stored at 50°C and the polyunsaturated oil at 30°C. Sensory evaluation of the Control of both oil types clearly showed a greater stability in the monounsaturated oil with a shelf-life of 52 weeks when compared to polyunsaturated oil with a shelf-life of 26 weeks. However, with the storage conditions and additives used, oxidation in the monounsaturated oil was perceived in the secondary oxidation products, whereas in the polyunsaturated oil oxidation was encountered in the primary oxidation products. It must thus be noted that the selection of parameters for shelf-life prediction of monounsaturated oil could be altered from the ones selected for this study if the oil is stored without the addition of a strong pro-oxidant. This emphasises the need for further studies.

The Practical models developed (based on chemical as well as sensory detection of rancidity) will be applicable for implementation in the food industry to predict the shelf-life of mono- and polyunsaturated oils generally within an error of ± 4 weeks with 95 % confidence interval and within an error of ± 8 weeks in infrequent cases. Refining of the Practical models as suggested above will strengthen the agreement between the chemical and sensory models. The estimation of the shelf-life of oils will provide the food manufacturer the means to do proper planning of production and distribution lines. Less returns will lead to cost saving and reduction in food price inflation.

CHAPTER 7

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