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The Effect of Storage Conditions on Extra Virgin Olive Oil Quality

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Foreword

The purpose of this study has been to determine the effect of storage conditions, including storage temperature, exposure to oxygen and exposure to light on the oxidative stability and quality of olive oil.

Controlled environment storage is generally difficult to maintain by many olive producers, with olive oil often stored in totally inappropriate settings such as sheds or other convenient locations. Often the storage temperature is high, or there are problems with exposure to light or oxygen which quickly reduces the quality of the oil. This report shows the importance of controlling the temperature, exposure to light and exposure to oxygen on the quality of oil.

Olive oil passes through many situations during the supply chain from the producer to wholesalers to exporters, importers, retailers and consumers. At each of these stages there is a responsibility to maintain the appropriate storage conditions to maintain the quality of the olive oil.

This report also illustrates the importance of determining the quality of olive oil soon after extraction, and monitoring that quality through the different stages of the supply chain in order to deliver the highest quality oil possible to the consumer.

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our Olives R&D program, which aims to:

- provide information which establishes the benefits of Australian olive products
- maintain the current high quality product while improving productivity, profitability and environmental management through all stages of the supply chain
- develop strategies for existing and new olive producers to reduce the effects of climate change and variability
- build an educated, collaborative, innovative and skilled industry workforce and a cost effective, well-funded RD&E program.

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About the Author

Jamie Ayton has been involved in olive oil research since 1997. He was a technical officer for RIRDC projects: DAN197A, Harvest timing; DAN 237A, Optimal olive oil quality; DAN-239A, Compliance to international standards; PRJ-002297, Effect of storage containers on olive oil quality; and joint participant in UCS-19A, Assessment of olives. The research team at the Wagga Wagga Agricultural Institute plays a leading role in national olive industry research having developed substantial expertise in oil chemistry and quality. Mr Ayton works closely with the olive industry through participation in field days, conferences and workshops. He has been a member of the team which has been an AOCS Approved Chemist laboratory continuously since 1993. He has also played a critical role in the team which has maintained accreditation from the International Olive Council (IOC) for the Australian Oils Research Laboratory for the past 11 years. Mr Ayton is also a founding member of the Australian Olive Oil Sensory Panel, located in Wagga Wagga. During the last 5 years, Mr Ayton has published a number of scientific papers on olives and presented at a number of olive workshops and conferences. He has completed a Master of Science degree from the University of Western Sydney. He is a member of the following professional organisations:

- American Oil Chemists Society (AOCS)
- German Fats and Oils Society (DGF)
- Australian Oilseeds Federation (AOF)

Acknowledgments

This study was carried out at the NSW Department of Primary Industries Australian Oils Research Laboratory at Wagga Wagga, NSW. Technical assistance was provided by Miss Janelle Rowland. Organoleptic assessment was carried out by the Australian Olive Oil Sensory Panel in Wagga Wagga. Oils were supplied by Boundary Bend Estate, Bentavoglio Olive Oil and Frankland River Olive Oil.

Abbreviations

AOA	Australian Olive Association
AORL	Australian Oils Research Laboratory
AOCS	American Oil Chemists' Society
1,2-DAGs	1,2-diacylglycerols
DGF	German Society of Fats and Oils
EVOO	extra virgin olive oil
FFA	free fatty acids
GC	gas chromatography
HPLC	high performance liquid chromatography
IOC	International Olive Council
ISO	International Organization of Standardization
NATA	National Association of Testing Authorities
NSW DPI	New South Wales Department of Primary Industries
PPP	pyropheophytin
PV	peroxide value
RIRDC	Rural Industries Research and Development Corporation
TAG	triacylglycerol
UV	ultra violet

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Executive Summary

What the report is about?

This report provides information about the effect of exposure to oxygen, light and storage temperature on the quality of olive oil. A wide range of storage conditions are used to store freshly produced olive oil in Australia, including warehouses, sheds, refrigerated cool rooms as well as other less suitable storage.

Olive oil has been shown to quickly deteriorate when stored under less than ideal conditions. Sometimes growers produce oil with very high quality initially, which quickly deteriorates due to the conditions under which it is stored. The olive oil can deteriorate so much that it can no longer be classified as extra virgin olive oil, at a huge expense to the growers.

This study measured changes in the oil when stored at different temperatures, exposed to the light or kept in the dark, and exposed to oxygen or closed over a three year period. The chemical parameters which are indicative of oxidation and ageing, as well as the sensory characteristics of the oils were monitored and the results are reported here.

Who is the report targeted at?

The report is targeted at olive oil producers, transporters, warehouse operators, importers and exporters, retailers and consumers. It is important to be aware of the risks of improper storage on olive oil quality whether buying, selling, transporting or storing the product. During short term storage olive oil quality may be satisfactory; however adverse storage conditions will quickly cause the oil to deteriorate.

Background

The effect of olive oil storage, and how the oil quality will change over time, is a major consideration for olive oil producers. Storage of olive oil at inadequate temperatures, for example when the temperature is not controlled such as in sheds or warehouses, exposed to light or oxygen is a common practice in the olive industry, especially for small producers without the funds for the adequate storage facilities.

While most producers who wish to sell their olive oil will have the quality of the oil analysed soon after extraction, there is little follow up analysis of the oil after storage.

Incorrect storage, even for a short period of time can reduce the quality of olive oil, sometimes to a level where the oil can no longer be classified as extra virgin.

Aims/Objectives

Due to the high value of extra virgin olive oil compared to other edible oils, it is important the oil is stored in conditions which will maintain the initial quality characteristics. The aim of this project was to study the relationship between the initial olive oil matrix and the oxidative stability of the oil. The

project was designed to include a wide range of oils from different cultivars, growing sites and fruit maturities to encompass the entire range of characteristics present in Australian olive oil.

An additional aim was to study the oils under a number of different storage conditions to compare the effect of storage temperature (cool room, room temperature and heated), oxygen exposure and exposure to light on the oils over three years.

Methods used

Glass bottles were obtained from manufacturers in Australia, which were tinted green, except for those which would store the oil exposed to light, which were clear. Oils with different levels of polyphenols and polyunsaturated fatty acids (the two variables determined to most affect oxidative stability of olive oil) were obtained from bulk suppliers. The oils were analysed upon receival to determine the initial quality and provide a baseline of the quality for the project. The oils were then decanted into bottles and placed in the different storage conditions. The oils were stored in a cool room (15°C), at room temperature (22°C) and at high temperature (37°C). These oils were sparged with nitrogen to remove the influence of oxygen, and kept in the dark to remove the influence of light. The oils were also stored exposed to oxygen, with the lid of the bottle only loosely attached and 10 mls of oil removed each month to ensure the headspace in the bottle contained oxygen. These oils were kept in the dark and at room temperature to remove influences of temperature and light. Finally, the oils were stored in clear bottles which were exposed to both sunlight and artificial light from fluorescent lights. These oils were stored at room temperature and sparged with nitrogen to remove the influences of oxygen and temperature. The oils were analysed after 3, 6, 12, 18, 24 and 36 months storage to determine the changes in oil quality due to the storage conditions. This was an intensive study, with a total of 882 bottles of olive oil used to generate the data (9 oils,7 samples of each oil (one for each test date), 7 storage conditions, in total duplicate).

Each sample was tested for free fatty acids and fatty acid composition to determine changes in the oil composition. Other analyses included peroxide value, total polyphenol content, UV absorbance, α-tocopherol content, total chlorophyll content, colour, pyropheophytin a and 1,2-diacylglycerol content, as well as induction time and organoleptic assessment by a trained, IOC accredited olive oil sensory panel.

Based on these results, predictions can, and have been made, on shelf life of individual olive oils

Results/Key findings

This study confirms the importance of proper storage in maintaining the quality of olive oil. It builds on anecdotal evidence that high storage temperature, exposure to light and exposure to oxygen has a detrimental effect on the quality of olive oil. It is clear from this work that oil should be stored at cool temperatures, away from light and without exposure to oxygen. This should be the case not just in the short term, but throughout the life of the oil which includes during the transport, storage and marketing of the oil as well as when the oil has reached its final destination – that is when it is with the consumer.

Implications for relevant stakeholders

These results are important for the olive oil industry.

Considerable time, effort and money go into producing high quality olive oil, and this can be lost if correct storage procedures are not used to maintain that quality.

The changes in oil quality described in this report will hopefully encourage producers, as well as others in the supply chain of olive oil, to consider the facilities used to store olive oil in order to maintain high quality olive oil.

Olive oil begins to deteriorate from the time of extraction and all methods available to reduce the rate of this deterioration need to be adopted. This project has shown that low storage temperature and the exclusion of oxygen are important factors in maintaining a longer shelf life of olive oil. Exposure to light has also been shown to affect the composition of olive oil and therefore storage conditions which exclude light are also an important factor.

Recommendations

This study has shown that the shelf life of olive oil can be altered dependent on the storage condition which the oils are exposed to. Oils should be stored in closed glass bottles which have been sparged with nitrogen to remove oxygen. Storage at low temperatures, with the exclusion of light will further extend the life of the oil.

The information from this project should be disseminated to the industry to ensure best practice is used when storing olive oil, maintaining the high quality product that Australian olive oil producers are increasingly renowned for. For the first time there is scientific evidence to determine shelf life of olive oil from analytical data.

Olive oil traders, exporters, importers, wholesalers, retailers, transporters and consumers need to be aware of the implications of the conditions they store the olive oil under and to promote this information to ensure the product is of the highest quality possible.

1. Introduction

1.1 Background

Virgin olive oils are known to be more resistant to oxidation than other edible oils because of their natural antioxidant content, particularly polyphenols and relatively low content of polyunsaturated fatty acids (Garcia *et al.*, 2002; Cinquanta *et al.*, 2001; Okogeri and Tasioula-Margari, 2002). Extra virgin olive oil (EVOO) is the highest grade of olive oil and is produced from fresh olives using mechanical extraction processes and without the use of excessive heat, chemical interference or blending with other edible oils.

Olive growers aim to produce olive oil which meets chemical and organoleptic standards and thereby provide good quality material to the market. In most cases oil produced by Australian growers meets those standards when they reach the marketplace, however the length of time before the oil quality deteriorates and loses EVOO characteristics will vary for different oils. To be classified as EVOO the oil must contain less than 0.8% free fatty acid (measured as oleic acid) and the peroxide content must not exceed 20 mEq oxygen/kg of oil, as well as many other components such as UV absorption and fatty acid also meeting established guidelines. Additionally, the oil must not contain anything but mechanically extracted olive oil. Numerous chemical tests have been developed to determine if the oil has been adulterated by the addition of seed oils or if it contains oil which has been extracted by methods other than mechanical extraction. The product must also pass an organoleptic assessment which indicates that there are positive attributes in terms of fruitiness and no defects, such as rancidity, to be classified as EVOO.

It is known storage conditions are a major factor in the shelf life of olive oil, as well as the composition of the oil. Therefore the producer must make predictions based on the oils chemical composition and how the oil will be stored in order to determine what the shelf life for that oil will be and give the product a “use-by” or “best before” date. Predicting the shelf life of the olive oil is a complex process because of the influence of several factors such as temperature, light, oxygen availability, enzymes and microorganisms (Stefanoudaki, 2010).

1.2 Oil stability

The factors affecting olive oil stability have been previously discussed. Light, oxygen and heat reduce the organoleptic and nutritional assets of the product through oxidation. Oxidative stability also varies between oils as a result of cultivar, crop management during fruit development, harvest timing and the seasonal climate and can have a marked influence on fruit quality. Generally, this is related to the composition of the oil and particularly to the level of antioxidants and the degree of polyunsaturation. Higher levels of polyunsaturation results in reduced oxidative stability.

Olive oil contains antioxidants such as polyphenolic compounds and tocopherols, such as vitamin E (α-tocopherol), and these are implicated with nutritional benefits for consumers. In addition, phenolic compounds provide the pungent sensory characteristics in olive oil.

Extending the shelf life of olive oil is important for the benefit of the consumer but also for the long term viability of the producer. Sensory characteristics of olive oil are expected to be of high quality during storage and up to the time of consumption. The assessment of stability is important for the prediction of shelf life and used-by-dates. Measurement of all components such as fatty acid profiles, phenolic content, chlorophyll, and tocopherols, can assist in predicting the stability of oil. New tests, pyropheophytin a and 1,2-diacylglycerols show good potential for predicting and determining olive oil quality and ageing.

1.3 Oil properties

1.3.1 Peroxide value

Oxidation, and the formation of peroxides, occurs during oil extraction and processing and can continue after bottling and during storage. Peroxides are intermediate oxidation products of oil which lead to the formation of a complex mixture of volatile compounds such as aldehydes, ketones, hydrocarbons, alcohols and esters responsible for the deterioration of olive flavours (Pristouri *et al.*, 2010). Peroxides have been shown to occur when oil is exposed to oxygen and/or light, particularly at elevated temperatures.

1.3.2 Ultraviolet absorption

Fatty acids absorb light at particular wavelengths in the UV region and this may be used to determine olive oil quality. Refining causes a change in the configuration of fatty acids and the formation of conjugated dienes and trienes (Angerosa *et al.*, 2006). Increased values of K_{232} and K_{268} in olive oil usually indicate the presence of refined oils. Autoxidation reactions are also associated with conjugation, due to the formation of either carbon-carbon bonds or carbon-oxygen bonds which cause an increase of absorption in the region between 225 and 325nm (Boskou, 1996).

1.3.3 Free fatty acids

Olives contain endogenous lipase enzymes which hydrolyse triacylglycerides (oil molecules) to release free fatty acids (hydrolysis). Although isolated from the oil in intact fruit, if the fruit is damaged prior to harvesting (pests, disease) or stored for extended periods prior to processing, the enzymes react with the triacylglycerols, causing the production of free fatty acids (Pristouri *et al.*, 2010).

1.3.4 Pyropheophytin a

Pyropheophytins are by-products of chlorophyll formed when the pigment structures change as a result of heating or ageing. Chlorophyll converts to pheophytin and ultimately to pyropheophytins. The proportion of pyropheophytin a to the total pheophytins is useful in discriminating fresh oil from oil which has been in long term storage or which has been heated in the refining process (Boskou *et al.*, 2006) (Mailer and Ayton, 2008).

1.3.5 1,2-diaclyglycerol

Diaclyglycerols (DAGs) are formed when a fatty acid is hydrolysed from a triacylglycerol molecule. The resulting DAG is a glycerol moiety and two fatty acids. As oil ages, or undergoes heat treatment, fatty acids can be lost from the 3-position of the triacylglycerol to form 1,2-diacylglycerols. Over time, these molecules equilibrate to form 1,3-diacylglycerols (Fronimaki *et al.*, 2002). The proportions of 1,2 and 1,3 -diacylglycerols reportedly can be used to detect old or damaged oil (Mailer and Ayton, 2008).

1.3.6 Total phenolic content

Phenolics are important minor components in olive oil which, due to the powerful antioxidant effect, contribute to shelf life stability of olive oil (Mailer *et al*, 2005). Phenolics identified in olive oil belong to a number of different classes and inhibit oxygen by a variety of mechanisms based on radical scavenging, hydrogen atom transfer and metal-chelating attributes (Krichene *et al.*, 2010). Phenolic content is greater in immature olives and decreases as the fruit ripens. The maturity of the fruit therefore is closely related to oil stability (Mailer *et al.*, 2002). Phenolic compounds also contribute to the organoleptic qualities of the oil, particularly bitterness and pungency.

1.3.7 α-tocopherol

Tocopherols are fat soluble antioxidants valued for their ability to inhibit oxidation in food. Vitamin E, α-tocopherol, is only synthesized by plants and is an important dietary nutrient for humans. The tocopherol content of food increases storage life by protecting food lipids from autoxidation (Kamal-Eldin and Appelqvist, 1996).

1.3.8 Chlorophyll

Chlorophyll is one of the main contributors to the colour of virgin olive oils. It has been demonstrated that high concentrations of chlorophylls compromise the resistance to oxidation of olive oils exposed to light. Photo-oxidation of oil in the presence of chlorophylls leads to the formation of highly unstable and reactive singlet oxygen that tends to react with the unsaturated fatty acids leading to the formation of hydroperoxides (Caponio *et al.*, 2005).

1.3.9 Colour

Colour is an important sensorial component in the presentation of extra virgin olive oil. Consumers are attracted to green oils, or those rich in colour as it implies freshness and genuineness of the product. Analysis of chromatic coordinates such as L* (luminosity), a* (greenness) and b* (yellowness) give an objective measurement of the colour of the oil (Cerretani *et al.*, 2008).

1.3.10 Fatty acid profile

Fatty acid profile is a description of all of the fatty acids which make up a particular oil. The fatty acid composition is an important measure of quality as the proportions of individual fatty acids determine the physical properties and nutritional value of the oil. The profile describes the structure of the fatty acids, if they are saturated, monounsaturated or polyunsaturated. Olive oil has a characteristic fatty acid profile that distinguishes it from many seed oils.

1.3.11 Induction time

Induction time is a test designed to measure the relative stability of an oil sample. The oil is heated, usually to 110°C, and air is forced through the sample at a set rate (usually 20L air/hour).Under theses conditions, the oxidative process is accelerated and the short chain volatile acids that are produced are captured and measured using a conductivity meter in water. The time taken for a sudden increase in the conductivity due to volatile acids formation is called the induction time (Mateos *et al.*, 2006). The most stable oils resist oxidation and result in longer induction time. Although induction time can be used to compare oils relative stability it cannot be used to precisely represent shelf life, as the conditions in which the oil is stored will have a major influence on shelf life. Induction time can be used to indicate the relative stability of oil when stored under the same conditions (Mailer, *et al*., 2005).

1.3.12 Organoleptic assessment

Extra virgin olive oil, one of the few edible oils which is mechanically extracted and not refined prior to consumption, retains many volatile and polyphenolic compounds which are responsible for the oils typical flavour and aroma. This makes extra virgin olive oils highly appreciated in many countries. Trained tasting panels are able to assess the oils to determine the levels of positive attributes, such as fruitiness, bitterness and pungency. Negative attributes arising due to poor quality fruit, incorrect processing or issues arising during storage, such as rancidity, musty and fusty, can be also be determined by sensory panels. It has been found that aldehydes are mainly responsible for these “off” flavours in olive oil while other volatiles, as well as polyphenolic compounds, have a significant role in determining the complex sensory qualities of olive oil (Kiritsakis, 1998).

2. Aims/Objectives

This study aimed to determine the shelf life of olive oil, in particular the effect of different storage conditions on the chemical and sensory profile of different olive oils.

The initial oil matrix, or make-up, is an important factor in the potential shelf life of an oil. Many factors, such as the level of anti-oxidants and fatty acid composition have a considerable impact on the oils ability to resist oxidation. By determining the effect of different storage conditions on a range of olive oils from all over Australia, growers, processors, wholesalers, retailers and consumers will have a better understanding of the practices required to maintain the quality of their product.

The aims of the project were to:

- study oils under different storage conditions to compare the effect of oxidation, and photoxidation on the stability of oil of different chemical composition. Additionally the oils would be stored at a range of temperatures over three years to determine the effect of storage temperature on the quality of the oil.

- study the relationship between olive oils with different levels of polyunsaturation and different levels of antioxidants with the oxidative stability of the oil and relate this to cultivar and growing conditions.

- determine useful methods for the prediction of a “use-by” or “best-before” date for olive oil.

3. Methodology

3.1 Australian Oils Research Laboratory

This study was carried out at the NSW Department of Primary Industries Australian Oils Research Laboratory at Wagga Wagga. The laboratory staff is experienced in oil quality evaluation with olives and other oil crops. The laboratory is AS/NZS ISO 9001:2000 certified and has ISO 17025 certification through the National Australian Testing Authority (NATA). The AORL has had Approved Chemist status of the American Oil Chemists' Society for many years. The laboratory is also accredited by International Olive Council (IOC), as is the Australian Olive Oil Sensory Panel (AOOSP).

3.2 Samples

3.2.1 Olive oil samples

Extra virgin olive oil samples with a wide range in quality composition were obtained which represent a range of olive oils produced in Australia. As there are many components of the olive oil matrix which contribute to the oxidative stability of the oil, the two main contributors to stability, namely fatty acid composition and polyphenol concentration, were used to select oil samples for this project. Oils with different levels of these components were included to best represent the spectrum of Australian olive oil (Table 3.1).

Table 3.1 Oil composition representing the range of oils produced in Australia, with different potential oxidative stability based on polyphenol content and fatty acid composition

LP,HL Low Polyphenols High Linoleic acid	**LP,ML** Low Polyphenols Mid Linoleic acid	**LP,LL** Low Polyphenols Low Linoleic acid
MP,HL Mid Polyphenols High Linoleic acid	**MP,ML** Mid Polyphenols Mid Linoleic acid	**MP,LL** Mid Polyphenols Low Linoleic acid
HP,HL High Polyphenols High Linoleic acid	**HP,ML** High Polyphenols Mid Linoleic acid	**HP,LL** High Polyphenols Low Linoleic acid

Nine oils were produced, either using single varieties or in some cases blends of a number of varieties to produce the oils used as a basis for this project (Table 3.2)

Table 3.2 Initial total polyphenol and linoleic acid content of olive oils used in this study

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid.

Oil sample	Description	Initial Polyphenol (PP)content	Initial linoleic acid (C18:2) content
LP,HL	Low Polyphenols, High Linoleic acid	103	16.4
LP,ML	Low Polyphenols, Mid Linoleic acid	86	10.2
LP,LL	Low Polyphenols, Low Linoleic acid	89	4.0
MP,HL	Mid Polyphenols, High Linoleic acid	156	17.6
MP,ML	Mid Polyphenols, Mid Linoleic acid	202	11.6
MP,LL	Mid Polyphenols, Low Linoleic acid	245	5.6
HP,HL	High Polyphenols, High Linoleic acid	242	18.9
HP,ML	High Polyphenols, Mid Linoleic acid	322	13.1
HP,LL	High Polyphenols, Low Linoleic acid	392	7.2

Note - Units: total polyphenols - mg/kg oil (as caffeic acid); linoleic acid - % of total fatty acids.

The oils were transferred to dark glass bottles (except for those oils to be exposed to light which were stored in clear glass bottles) and stored in different conditions to evaluate the effect of those conditions on the quality of the oil (Table 3.2). Each sample was poured into 14 bottles for each of the storage conditions (seven storage conditions, in duplicate). At each analysis one bottle was removed from storage for analysis. Therefore, at each stage of analysis a fresh bottle of olive oil was used. Therefore a total of 882 bottles of olive oil were used in this project.

3.2.2 Storage conditions

Temperature – Samples were stored at each of the three storage temperatures, in a cool room at 15°C, at laboratory room temperature (22°C), and in an incubator at 37°C. The purpose of these different storage temperatures was to determine the effect of storage at temperatures on olive oil. As olive oil is stored in a number of different environments depending on the producers' facilities, it was thought these temperatures gave a good range of what may be expected in those facilities which range from cool rooms to sheds, warehouses and other types of storage. Samples were stored in dark glass bottles and placed in a cool-room, the back of a dark cupboard, or in an incubator (depending on the storage conditions applied). Samples were sparged with nitrogen to remove the influence of oxygen.

Figure 3.1 Samples stored in dark bottles, prior to storage in a dark cupboards to exclude light

Oxygen – Samples were stored exposed to oxygen**.** The oil was stored in dark bottles in a dark cupboard at 22°C to remove the influence of temperature and light, and the lid was left loose on the bottle. Once per month approximately 10mL of oil was poured from the bottle, the lid was put back on and the bottle was shaken to introduce the oxygen to the oil. The lid was removed again before the oil was stored.

Light –Samples were stored exposed to light. The samples were stored in clear glass bottles and placed in a position in the laboratory which was exposed to both artificial lighting, as well as sunlight from adjacent windows (Figure 3.2). The samples were sparged with nitrogen at the beginning of the experiment, and stored at room temperature.

Figure 3.2 Samples stored in clear bottles and exposed to light to determine the influence of light exposure on olive oil quality

Table 3.3 Storage conditions used to determine the effect of temperature, oxygen and light on the quality of olive oil

Temperature	15°C	closed	dark
	22°C	closed	dark
	37°C	closed	dark
Oxygen	22°C	open	dark
	22°C	closed	dark
Light	22°C	closed	light
	22°C	closed	dark

3.2.3 Storage time

To determine the effect of the storage conditions on the olive oil quality over time, oils were tested after 3, 6, 12, 18, 24 and 36 months storage. At each of these analysis times, two bottles of the 14 bottles originally stored for each storage condition was removed from storage and the oil was analysed. That oil was then discarded and not used again in the study. A total of 882 bottles of oil were used in this study.

3.3 Methods of analysis

3.3.1 Peroxide value

Peroxide value was determined using the International Standard Organisation method, ISO 3960:2007 (E): Animal and vegetable fats and oils – Determination of peroxide value – Idometric (visual) endpoint determination.

Oil (2.50 g) was dissolved in acetic acid / 2,2,4-trimethylpentane mixture (3:2). To this solution, 0.5 mL of saturated potassium iodide (KI), (70 g KI/40 mL water), was added and shaken for 1 minute. Water (50 mL) was added, followed by approx 0.5 ml of 1 % starch solution (1 g starch/100mL water). The solution was titrated with previously standardised 0.01N sodium thiosulphate ($Na_2S_2O_3$). The volume of titrant was recorded and the peroxide value calculated and reported as mEq of active oxygen/kg oil.

3.3.2 Ultraviolet absorption

Ultraviolet absorption was determined using the International Olive Council method COI/T.20/Doc19/Rev2 (2008): Spectrophotometric investigation in the ultraviolet.

A slight modification to the method was made where a smaller sample weight and solvent volume was used to save on solvent usage (0.1 g oil/10 mL solvent vs. 0.25 g oil/25 mL solvent). This does not have any significant effect on the final result. Oil (0.1g) was weighed into a 10ml volumetric flask and made to volume with 2, 2, 4-trimethylpentane. The absorbance of the oil sample was measured on a double beam spectrophotometer, using 2, 2, 4-trimethylpentane as a reference, at 264, 268 272 and 232 nm. The UV absorbance was then calculated and reported as ΔK, K_{232} and K_{268}.

3.3.3 Free fatty acid

Free fatty acids were determined by the method of the American Oil Chemists Society, AOCS Ca 5a-40 (1998): Free fatty acids

Oil (7.05 g) was dissolved in 50 ml neutralised isopropanol. Two drops of phenolphthalein (1% in ethanol) was added to the solution. The solution was then titrated with 0.1N sodium hydroxide (NaOH), previously standardised against hydrochloric acid (HCl). The volume of titrant was recorded and the results calculated as a percentage of the oil (expressed as oleic acid).

3.3.4 Pyropheophytin a

Pyropheophytin was measured using the International Standards Organisation method, ISO 29841:2009 (E): Vegetable fats and oils – Determination of the degradation products of chlorophyll a and á (pheophytins a, á and pyropheophytins). This method determines the degradation products of chlorophyll a into pheophytin a, pheophytin á and pyropheophytin a.

A miniaturised chromatography column of silica gel 60 at 5% moisture was used to separate the pheophytins. A 5 ml pipette tip was plugged with defatted cotton wool. Silica gel 60 (1.0 g) that had been prepared at 5% moisture was then weighed on top of the cotton wool. The silica layer was then covered with a stopper of cotton wool. Oil (0.6 g) was weighed into a test tube and dissolved in hexane. The solution was transferred to the column with the aid of two 1 mL portions of petroleum ether. The column was washed with two 5 mL portions of eluent (petroleum ether: diethyl ether (90:10)) and extracted under slight vacuum using a solid phase extraction unit. This initial solution was then discarded. The pheophytin portion of the solution was then eluted using two 5 mL portions of acetone. The solution was filtered, transferred to a pear shaped flask and evaporated to dryness using a rotary evaporator. The residual sample was dissolved in 200 µL of acetone and analysed on the HPLC with a mobile phase of water:methanol:acetone (4:36:60), using a Phenomonex Luna 5 µ silica column (250 x 4.60 mm). The separated components were measured at 410 nm using a photodiode array detector and data was analysed with Waters Empower Pro version 5.00. Peak areas were quantified and reported as % pyropheophytin a (of total pheophytins).

3.3.5 1,2-diacylglycerol

Diacylglycerols were determined using the International Standards Organisation method, ISO 29822:2009 (E): Vegetable fats and oils – Isomeric diacylglycerols – Determination of relative amounts of 1,2 and 1,3-diacylglycerols.

A miniaturised chromatography column of silica gel 60 at 5% moisture was used to separate the isomeric diacylglycerols. A 5 ml pipette tip was plugged with defatted cotton wool. Silica gel 60 (1.0 g) that had been prepared at 5% moisture was weighed on top of the cotton wool. The silica layer was then covered with a stopper of cotton wool. Oil (0.1 g) was weighed into a test tube and dissolved in toluene. The solution was transferred to the column with the aid of 1 mL of eluent, (2, 2, 4-trimethylpentane:di-isopropyl ether (85:15). The column was washed with two 3.5 mL portions of eluent and extracted under slight vacuum using a solid phase extraction unit. This initial solution was discarded. The diacylglycerols were eluted using two 3.5 mL portions of diethyl ether. The solution was transferred to a pear shaped flask and evaporated to about 1 mL using a rotary evaporator. The remainder of the solution was evaporated under a gentle stream of nitrogen. Silylation reagent, 3:1:9 - Sylon^{TM} HPT, was added to the flask (200 µL) and allowed to react for at least 20 minutes. After silylation, 1 mL acetone was added and left to stratify for 10 minutes, centrifuged, then transferred to a GC vial. The diacylglycerol profiles were determined by gas chromatography using a SGE BP5 capillary column (30 m, 0.25 mm, 0.25 µm film) and a flame ionisation detector. The column temperature program was 240^{o}C for 1 minute, increased at a rate of 10^{o}C / minute to 320^{o}C

and held for 10 minutes, increased to 340^{o}C at 20^{o}C /min and held for 10 mins. The injector temperature was set at 340^{o}C. The detector temperature was 340^{o}C. Data was analysed using Star® Workstation Chromatography software (version 6.20). Results are expressed as a % 1,2-diacylglycerols (of total 1,2 and 1,3-diacylgylerols).

3.3.6 Total polyphenol content

A modification of the Gutfinger (1981) method, using caffeic acid as the standard, was used to determine total phenolic content.

Oil (10 g) was dissolved in 50 mL hexane and extracted 3 times with 20 mL portions of 80% aqueous methanol. The mixture was shaken for 2 minutes for each extraction. The sample was made to 100 ml with water and left to stand in a dark cupboard overnight. A 1 ml aliquot was transferred to a 10 ml volumetric flask to which 5 mL of water was added. Folin-Ciocalteau reagent (0.5 mL) was then added to the flask and the sample shaken and left for 3 minutes. Saturated sodium carbonate (Na_2CO_3) (1 mL) was added to the sample and shaken. The sample was made to volume with water and allowed to stand in the dark for an hour. The absorption of the sample was read at 725 nm on a spectrophotometer. A series of different concentrations of caffeic acid were prepared and used to produce a standard calibration curve. The standards were prepared and analysed in the same way as the sample solution. Results were expressed as mg of caffeic acid/kg of oil.

3.3.7 α-tocopherol

α-tocopherol was measured using the International Standards Organisation method, ISO-9936:2006 (E): Animal and vegetables fats and oils – Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography.

Oil (2 g) was weighed into a 25 mL volumetric flask, and made up to volume with hexane. The samples were filtered and transferred to HPLC vials. The α-tocopherol concentration was determined by HPLC, with hexane:isopropanol (99:1) as the mobile phase, with a flow rate of 0.9 mL/minute. A Phenomonex Luna 5μ silica column (250 x 4.60 mm) was used. The peaks were measured using a UV detector set at 292 nm. Data was analysed using Waters Empower Pro version 5.00. A calibration curve was used to calculate the α-tocopherol, which was expressed as mg/kg oil.

3.3.8 Chlorophyll

Chlorophyll was measured using the method of the American Oil Chemists Society, Ch 4-91 (AOCS, 1998). The absorbance of the oil sample was measured, using dichloromethane as a reference, at 630, 670 and 710 nm. The chlorophyll content was calculated as described in the method and reported as mg chlorophyll/kg oil.

3.3.9 Colour

The oils were placed in quartz cuvettes and the visible spectra (380-770nm) were recorded using a Varian Cary 1 spectrophotometer. Samples were measured using hexane as a reference. The oils were examined without dilution to avoid colour variation. The oil colour was expressed as L*(luminosity), a* (greenness) and b* (yellowness).

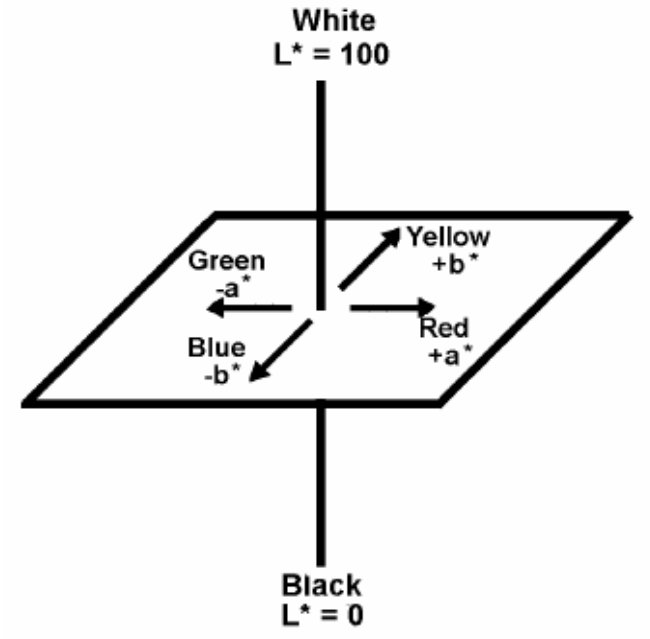

Figure 3.3 Coordinates of chromatic components L*, a* and b*

(Source: Hunterlab, 2012)

3.3.10 Fatty acid profile

Fatty acid methyl esters were prepared using the International Olive Council method COI/T.20/Doc. No 24 - Preparation of the fatty acid methyl esters from olive oil and olive-pomace oil.

Oil (0.1 g or 5 drops) was dissolved in 2 mL heptane. The sample was mixed and 0.2 ml of 2N methanolic potassium hydroxide was added. The sample was mixed for 30 seconds, covered and left until the two phases separated (30 minutes). The upper heptane layer was then transferred to GC vials. The fatty acid profiles were determined by gas chromatography using a SGE BPX70 capillary column (30 m, 0.25 mm, 0.25 µm film) and a flame ionisation detector. The column temperature program was 185^{o}C for 8 minutes and then increased at 10 ^{o}C / minute to a final temperature of 220 ^{o}C and held for 3 minutes. The injector temperature was set at 250 ^{o}C with a split ratio of 1:50. The detector temperature was 260 ^{o}C. Results are expressed as a percentage of the total fatty acids.

3.3.11 Induction time

A Metrohm® 743 Rancimat was utilised to determine the induction time of the oil.

Oil (2.50 g) was weighed and placed in a heating block at 110^{o}C . Air was forced through the sample at 20 L / hour. Volatile components which develop as a result of oxidation are captured in water and measured using a conductivity metre. As the oil oxidised, the changes in the conductivity were recorded at regular intervals by the Rancimat. The point of inflection on the curve recorded when the oil had lost the ability to resist oxidation was reported as induction time in hours.

3.3.12 Organoleptic assessment

Organoleptic assessment, or sensory analysis of the olive oil samples, was performed by a trained, accredited panel using the International Olive Oil method COI/T.20/Doc15/Rev3 (2010): Method for the organoleptic assessment of virgin olive oil. Samples were placed in blue tinted glasses and heated to 28 ^{o}C. Trained olive oil tasters (8-12 tasters each session), assessed the oils, recorded the level of positive and negative attributes, and the median results were calculated. Results were expressed as fruitiness, bitterness, pungency and rancidity.

4. Results and discussion

This project has studied the influence of temperature, oxygen and light on the oxidative stability of olive oil. At the commencement of the project, nine oils were obtained with different levels of polyphenols and linoleic acid as described previously. As olive oil is a complex product made up of many different components, this led to oils with very different matrices. The initial result for each component for each oil is shown in Table 4.1. Some of these oils, especially those high in linoleic acid (LP,HL; MP,HL and HP,HL) were already showing signs of degradation as indicated by the relatively high peroxides and K_{232}values of the oils at the beginning of the project.

Due to the large amount of data produced, graphs and tables have been used to best represent the trends and results for each of the components for each of the oils. In some cases, results for low polyphenols, high linoleic acid oil (LP,HL)(least stable), mid polyphenols, mid linoleic acid oil (MP,ML) (mid stability) and high polyphenol, low linoleic acid oil (HP,LL) (most stable) have been shown in graphs to give an uncluttered representation of the results. Other graphs show the initial results for each oil, followed by the level of the components after 36 months storage, in order to give an overview of what happened with each oil over the entire time period of analysis.

The effect of each of these variables on extra virgin olive oil quality is described in the following sections:

4.1 Effect of storage temperature

4.2 Effect of exposure to oxygen

4.3 Effect of exposure to light

This study's objective was to determine the effects of different storage conditions on the quality of olive oil over time. The oils were subjected to different temperatures, exposure to oxygen and exposure to light to determine the effect these had on oil with different composition, and therefore different abilities to cope with the conditions.

The oil was analysed at the initial bottling and then placed in the different storage conditions. The oil was then tested at regular intervals to determine the effect the storage conditions had on the quality of the oil. This report describes the changes that occurred.

It was shown that even the oils with the greatest potential to resist oxidation or degradation is susceptible to loss of quality when place in extreme storage conditions. This information is important to the industry as during the life of an oil, many different people are responsible for the storage of the oil, and any adverse storage conditions will have a detrimental effect on the oil. Many producers house oil in sheds on farms, the oil is subject to temperature, light and possibly oxygen during transport, and the oil is potentially exposed to these conditions all the way along the supply chain in warehouses, retail outlets as well as when the consumer has possession of the oil. Samples have been received in the Australian Oil Research Laboratory for analysis which far exceeded the acceptable limits for extra virgin olive oil, and in many cases this could be attributed to poor storage.

In this study in each case the storage parameter being tested was isolated so only that extreme could have an effect. For example, the sample exposed to different temperature were sparged with nitrogen to remove the effect of oxygen, as well as being stored in the dark to remove the effect of light. The oils exposed to oxygen were kept in the dark and at room temperature, while those exposed to light were sparged with nitrogen and kept at room temperature.

In addition to the information gained about the storage conditions on the quality of olive oil in this project, this project was an opportunity to investigate the methods used to define oil freshness and

quality. Peroxide value, UV absorbance and free fatty acids are common tests used to evaluate the quality of olive oil, as is organoleptic assessment. Other analyses, such as fatty acid composition, phenolic compounds and α-tocopherol were carried out to show the changes in these parameters due to the deterioration in quality of the oil.

The Australian Olive Oil Standards, AS5264-2011, includes a limit for pyropheophytin a and 1,2-diacylglycerol concentrations in olive oil. These limits are in place to indicate the freshness and authenticity of the olive oil. As these analyses are relatively new, and exclusive to the Australian standard at this time, this project was a good opportunity to observe the changes in these parameters under different storage conditions and evaluating their usefulness in determining olive oil quality.

Analyses carried out on each of the oils in the different storage conditions are discussed in detail below. It is shown in this study that if the oil is kept in a cool environment, away from light and oxygen, the quality of the oil can be maintained for extended periods of time, in some cases 18 months or more.

Table 4.1 Initial composition of extra virgin olive oils used in this study

		LP,HL	LP,ML	LP,LL	MP,HL	MP,ML	MP,LL	HP,HL	HP,ML	HP,LL
Peroxide value	**meQ oxygen/kg oil**	16 ± 1	12 ± 0	5 ± 1	15 ± 1	12 ± 0	7 ± 1	14 ± 2	12 ± 0	8 ± 2
UV Absorbance	**K232**	2.05 ± 0.01	1.69 ± 0.01	1.20 ± 0.01	2.01 ± 0.00	1.77 ± 0.02	1.47 ± 0.00	1.98 ± 0.01	1.84 ± 0.00	1.62 ± 0.00
	K268	0.09 ± 0.01	0.08 ± 0.00	0.07 ± 0.00	0.10 ± 0.00	0.11 ± 0.01	0.11 ± 0.00	0.11 ± 0.02	0.14 ± 0.00	0.14 ± 0.01
FFA	**% of oleic acid**	0.53 ± 0.03	0.36 ± 0.01	0.10 ± 0.02	0.44 ± 0.03	0.32 ± 0.02	0.17 ± 0.01	0.31 ± 0.01	0.30 ± 0.06	0.26 ± 0.02
Pyropheophytin a	**% of total pheophytins**	3.9 ± 0.0	2.8 ± 0.1	2.3 ± 0.2	3.0 ± 0.0	2.7 ± 0.0	2.8 ± 0.0	3.0 ± 0.1	2.8 ± 0.1	2.9 ± 0.2
1,2-diacylglycerols	**% of total diacylglycerols**	61.8 ± 0.0	72.8 ± 0.0	80.2 ± 0.0	70.5 ± 0.0	74.4 ± 0.0	74.0 ± 0.0	76.8 ± 0.0	78.9 ± 0.0	80.7 ± 0.0
Total Polyphenols	**mg/kg oil (as caffeic acid)**	103 ± 4	86 ± 3	89 ± 0	156 ± 5	202 ± 0	245 ± 4	242 ± 8	322 ± 2	392 ± 5
α- tocopherol	**mg/kg oil**	249 ± 4	248 ± 10	277 ± 8	206 ± 7	241 ± 5	274 ± 5	155 ± 4	232 ± 10	290 ± 7
Total chlorophyll	**mg/kg oil**	3.0 ± 0.1	3.8 ± 0.1	4.4 ± 0.0	2.7 ± 0.1	5.5 ± 0.2	8.4 ± 0.1	2.3 ± 0.1	7.2 ± 0.2	12.4 ± 0.0
Colour	**L***	92.0 ± 0.4	89.8 ± 0.5	88.4 ± 0.3	91.7 ± 0.4	87.8 ± 0.2	84.7 ± 0.2	92.1 ± 0.2	86.0 ± 0.1	81.7 ± 0.6
	a*	-12.6 ± 0.1	-11.6 ± 0.1	-10.1± 0.04	-12.4 ± 0.0	-10.0 ± 0.0	-7.3 ± 0.0	-12.2 ± 0.0	-8.5 ± 0.1	-5.5 ± 0.1
	b*	75.7 ± 0.7	98.8 ± 0.2	113.6 ± 0.2	79.3 ± 0.4	109.7 ± 0.4	122.9 ± 0.2	83.0 ± 0.1	117.2 ± 0.2	126.0 ± 0.7
Fatty acid composition % of total fatty acids	**Sat**	18.0 ± 0.0	16.9 ± 0.0	15.8 ± 0.0	17.4 ± 0.0	16.0 ± 0.0	14.7 ± 0.0	16.5 ± 0.0	15.0 ± 0.0	13.5 ± 0.0
	Mono	64.8 ± 0.0	72.1 ± 0.0	79.4 ± 0.0	64.1 ± 0.0	71.6 ± 0.0	78.9 ± 0.0	63.6 ± 0.1	71.1 ± 0.0	78.5 ± 0.0
	Poly	17.2 ± 0.0	11.0 ± 0.0	4.8 ± 0.0	18.5 ± 0.0	12.4 ± 0.0	6.4 ± 0.0	19.9 ± 0.0	13.9 ± 0.0	8.0 ± 0.0
Induction time	**(hours)**	7.2 ± 0.1	12.9 ± 0.0	28.4 ± 0.2	9.9 ± 0.1	17.1 ± 0.2	30.6 ± 0.1	12.1 ± 0.0	18.9 ± 0.1	32.1 ± 0.4
Organoleptic assessment	**Fruitiness**	3.95	3.85	5.00	5.30	5.10	5.00	5.50	5.90	5.10
	Bitterness	1.80	1.90	2.05	3.00	3.50	4.45	4.50	4.80	6.00
	Pungency	1.55	1.20	2.05	3.50	3.50	5.00	4.00	5.65	6.35
	Rancidity	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: Sat – Saturated fatty acids, Mono – monounsaturated fatty acids, Poly - polyunsaturated fatty acids.

LP,HL: Low polyphenols, High linoleic acid, LP,ML: Low polyphenols, Mid linoleic acid, LP,LL: Low polyphenols, Low linoleic acid, MP,HL: Mid polyphenols, High linoleic acid, MP,ML: Mid polyphenols, Mid linoleic acid, MP,LL: Mid polyphenols, Low linoleic acid, HP,HL: HIgh polyphenols, High linoleic acid, HP,ML: High polyphenols, Mid linoleic acid, HP,LL: High polyphenols, Low linoleic acid

4.1 Effect of storage temperature

4.1.1 Peroxide value

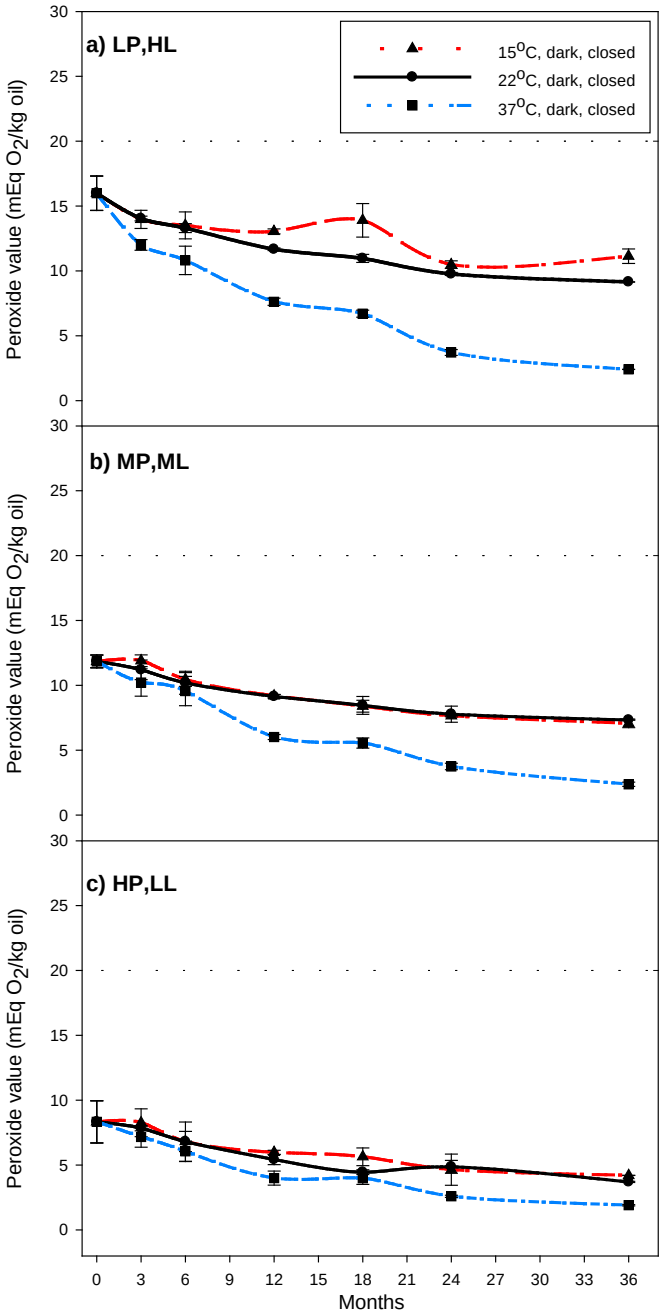

Figure 4.1 Effect of various storage temperatures on peroxide value of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

The peroxide value for each of the oils at the initial analysis was below the Australian and IOC standard of 20 mEq oxygen/kg oil. The oil matrix had an influence on the initial peroxide value of the oils, with the low linoleic oils (LP,LL; MP,LL and HP,LL) having the lowest initial peroxide value, and the high linoleic oils (LP,HL; MP,HL and HP, HL having the highest initial values.

The peroxide value of all samples declined over time, even at 15°C (Figure 4.1). For example oil LP,HL was initially 16 mEq oxygen/kg oil, which decreased to 11 mEq oxygen/kg oil when stored at 15°C for 36 months.

The peroxide value was significantly affected by storage temperature. In all cases the oils stored at higher temperatures declined at a more rapid rate that at the lower temperatures. Oil LP,HL, mentioned above, which had a peroxide value of 11 mEq oxygen/kg oil when stored at 15°C for 36 months, had a peroxide value of 2 mEq oxygen/kg oil when stored at 37°C for 36 months (Figure 4.2).

Peroxide value is commonly used as a measure of olive oil quality. Eventually peroxides convert to secondary oxidation products and the peroxide value will decline. A low peroxide value is therefore not verification that the oil is good quality.

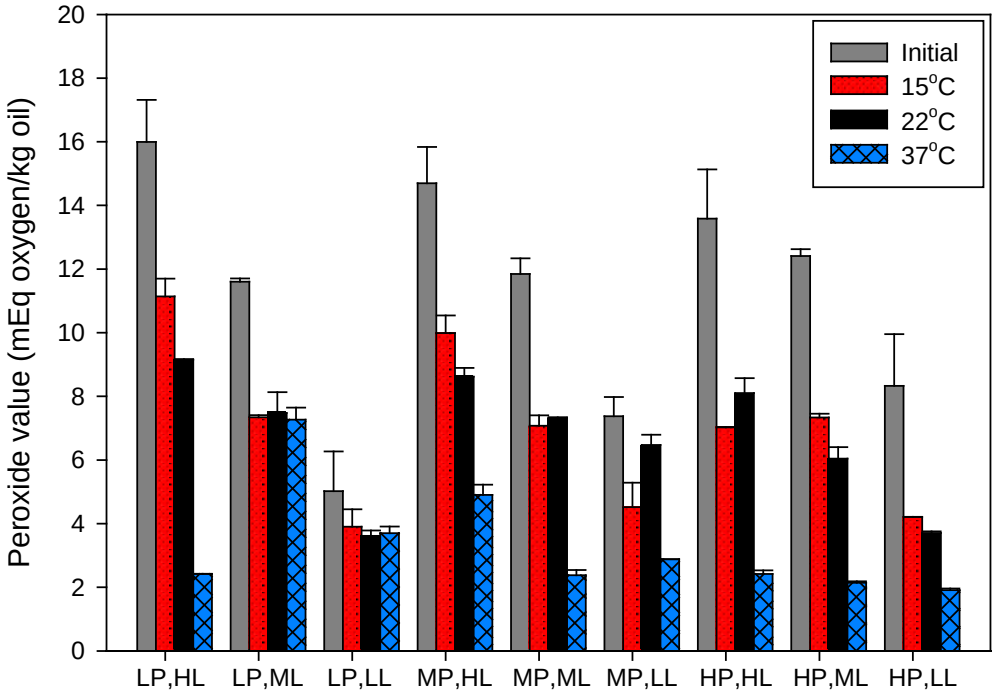

Figure 4.2 Peroxide value at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

Storage temperature had a significant effect on the peroxide value of all oils regardless of the oils matrix. There was a decrease of the peroxide value of all oils, with the decrease more rapid in oils stored at higher temperatures. Due to the impermeability to oxygen of the container, the oxygen available is depleted and therefore further peroxide production is avoided. Higher temperature allows the oxidation reactions to occur at a faster rate, therefore the samples stored at 37°C showed a quicker decline in the peroxide value of the oil. These results are in agreement with those of Gutierrez and Fernandez (2002a).

4.1.2 UV absorbance – K_{232}

Storage temperature had little effect on the UV absorbance at 232nm (K_{232}) of the oils in this study after 36 months of storage. At no time was the IOC or Australian standard limit of 2.5 exceeded. Some oils did show a slight increase in the K_{232} level after 36 months storage, such as oils LP,ML; MP,HL; MP,ML and MP,LL. However these changes were not significant and this trend was not consistent with the other oils. In those oils which were affected, temperature did seem to have an effect, with the oils stored at higher temperature showing a slightly higher K232 value (Figure 4.3).

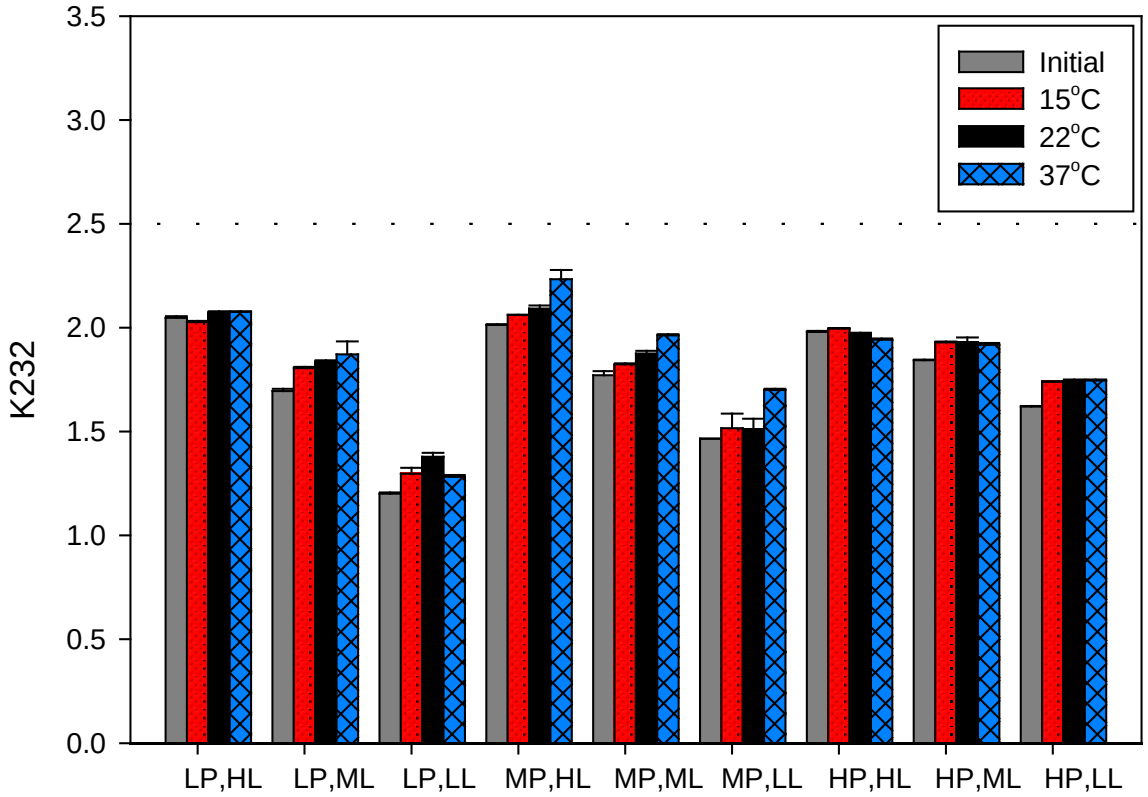

Figure 4.3 K_{232} after initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

K_{232} is related to the formation of hydroperoxides, carboxylic acids, conjugated dienes and conjugated trienes. These compounds are formed during the process of lipid oxidation (Allouche *et al.*, 2007). As shown in Section 4.1.1, the peroxide value of the oils decreased as the oxygen available was depleted. Therefore the amount of compounds measured at 232nm was seen to remain relatively constant.

4.1.3 UV absorbance K_{268}

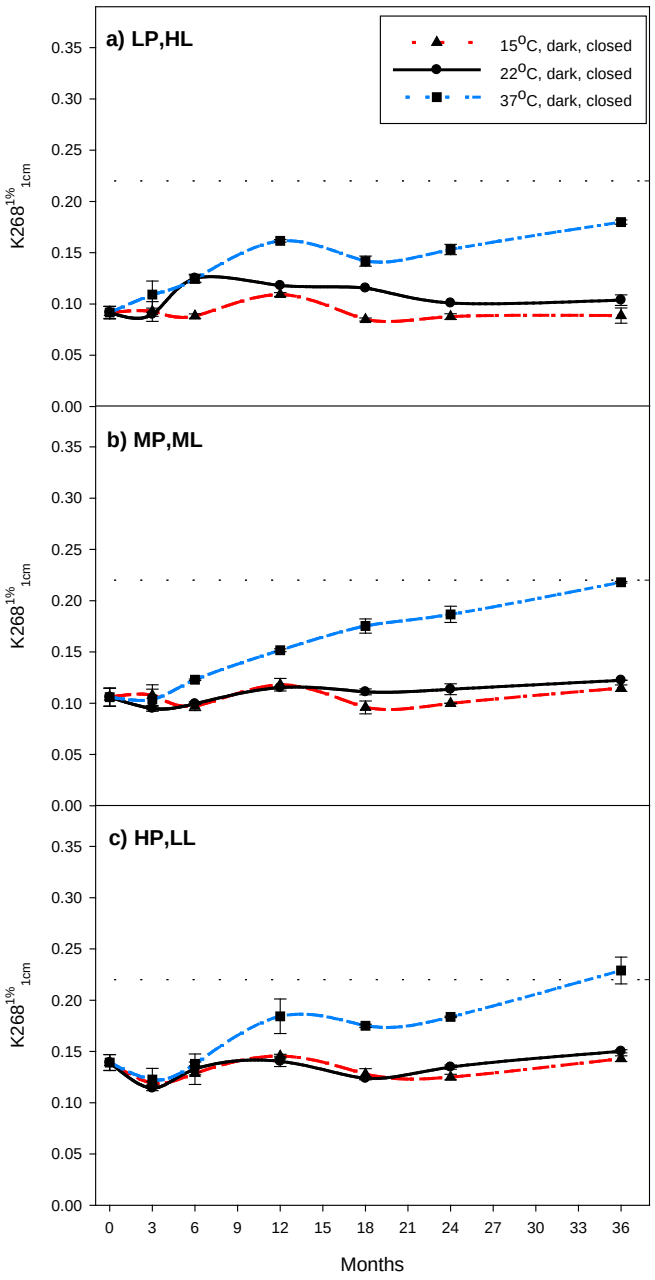

Figure 4.4 Effect of various storage temperatures on K_{268} of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

K_{268} is a measure of secondary oxidation products formed in olive oil during the oxidation process (Bilancia *et al.*, 2007). When the oils were initially analysed, all of the oils were well below the IOC/Australian limit of 0.22 (Figure 4.5). However, after 36 months storage at different temperatures, some significant changes had occurred. In most cases, storage at 15°C and 22°C for 36 months led to little or no change, for example oil LP,HL which had a K_{268} value of 0.09 initially and was unchanged after 36 months storage at 15°C, while the same oil stored at 22°C had increased to 0.10 after 36 months storage. The same oil stored at 37°C, however, increased to 0.18 after 36 months storage

(Figure 4.4). All oils, regardless of original matrices, showed the same trend. Oils MP,ML; HP,ML and HP,LL exceeded the allowable limit for K_{268} when stored at 37°C for 36 months.

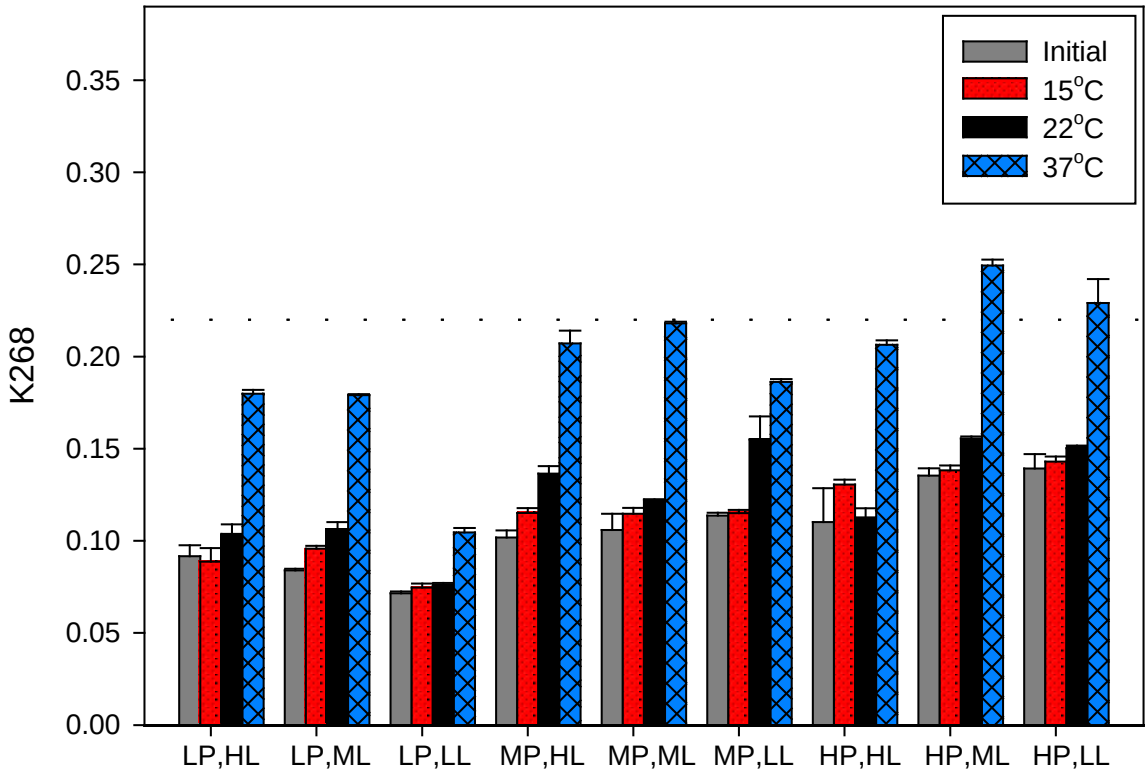

Figure 4.5 K_{268} at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

K_{268} remained relatively constant for the oils stored at 15° and 22°C, however oils stored at 37°C increased significantly over the storage period, with some oils exceeding the IOC/Australian limit by the end of the study. This increase in the absorption at 268nm can be explained by the transformation of the peroxides already formed into other products such as aldehydes and ketones (Allouche *et al.*, 2007).

4.1.4 Free fatty acids

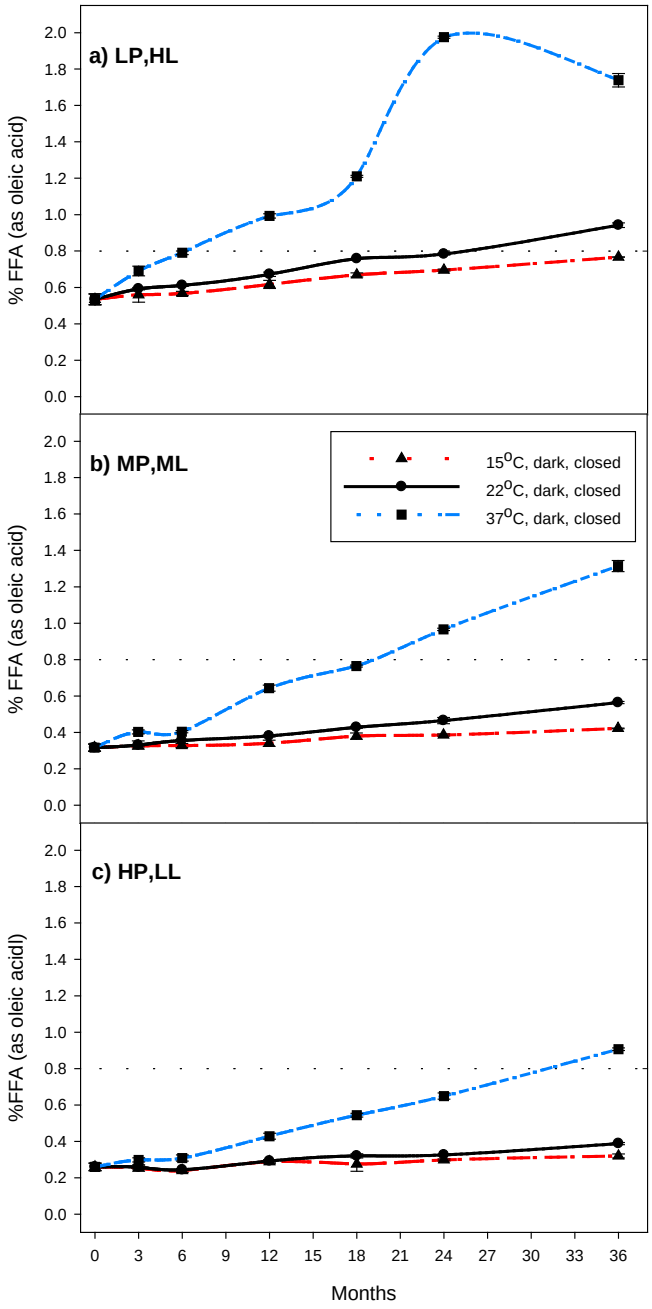

Figure 4.6 Effect of various storage temperatures on free fatty acid content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Free fatty acids were significantly affected by the temperature at which the oil was stored. Figure 4.6 shows the %FFA of the oils stored at 15°C increased only slightly throughout the analysis period, while the oils stored at 22°C showed a slightly greater increase and the oils at 37°C increased significantly. The initial %FFA content also had an influence on the increase in %FFA. Oil LP,HL which was initially 0.53% FFA increased to 1.74% after 36 months storage at 37°C, whereas oil

LP,LL, which had an initial FFA content of 0.10% increased to only 0.46% after 36 months storage at 37°C (Figure 4.7).

Importantly, after 36 months storage at 37°C, seven of the nine oils studied exceeded the IOC/Australian standard limit of 0.8% FFA. Oil LP,HL exceeded the limit after just 6 months storage at 37°C, while oil HP,LL reached the limit after 36 months. Oil LP,HL was the only oil which exceeded the limit when stored at 22°C, which occurred after 24 months storage at that temperature. None of the oils stored at 15°C exceeded the limit (Figure 4.7).

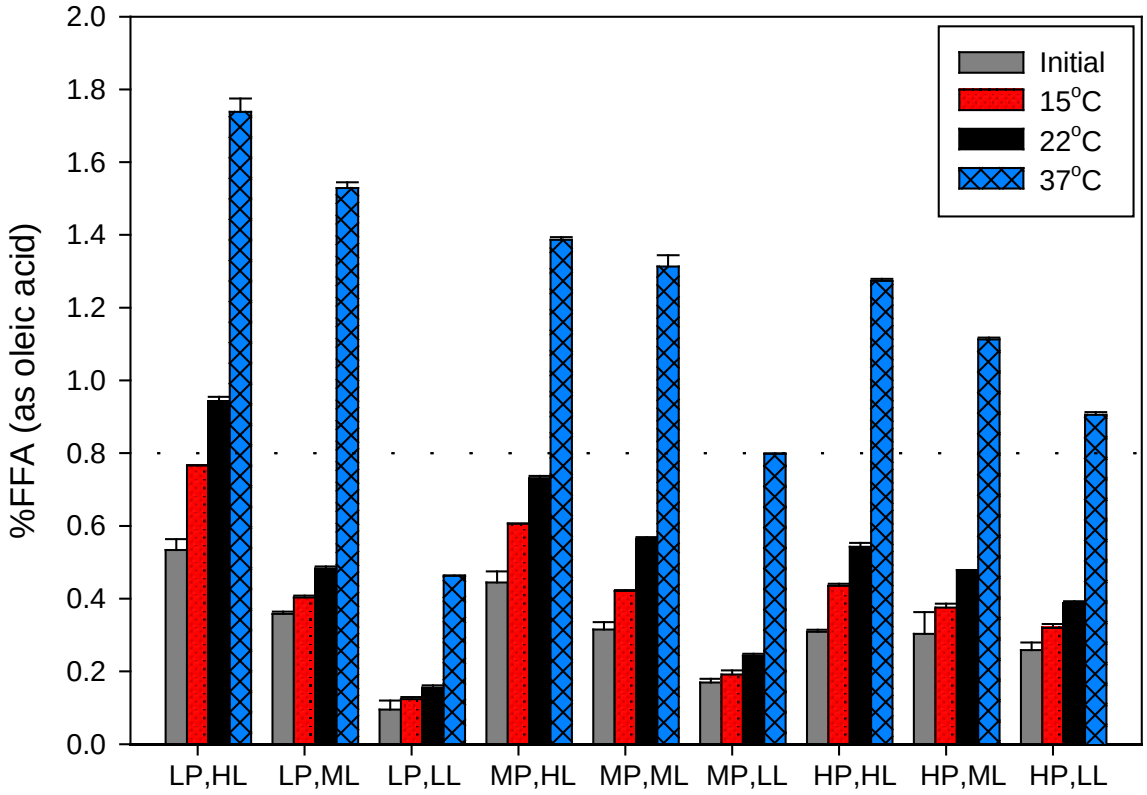

Figure 4.7 Percentage of FFA at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

Free fatty acids occur in olive oil due to hydrolysis while the fruit is still on the tree (pests, disease, ageing) and after the fruit has been harvested but prior to processing, especially if the fruit is stored for long periods. However hydrolysis continues to occur in the oil even after extraction due to the presence of lipase enzymes in the oil (Pereira *et al.*, 2002). The presence of fatty acids also leads to the formation of more fatty acids in the oil; that is it acts as a catalyst for further production of free fatty acids. As can be seen from Figures 4.6 and 4.7, the lower the initial free fatty acid content, the slower the accumulation of free fatty acids is, especially at high storage temperatures. This information is important for producers as oil which conforms to the olive oil standards when bottled can, in a relatively short period of time, become oil which must be classified at a lower grade.

4.1.5 Pyropheophytin a

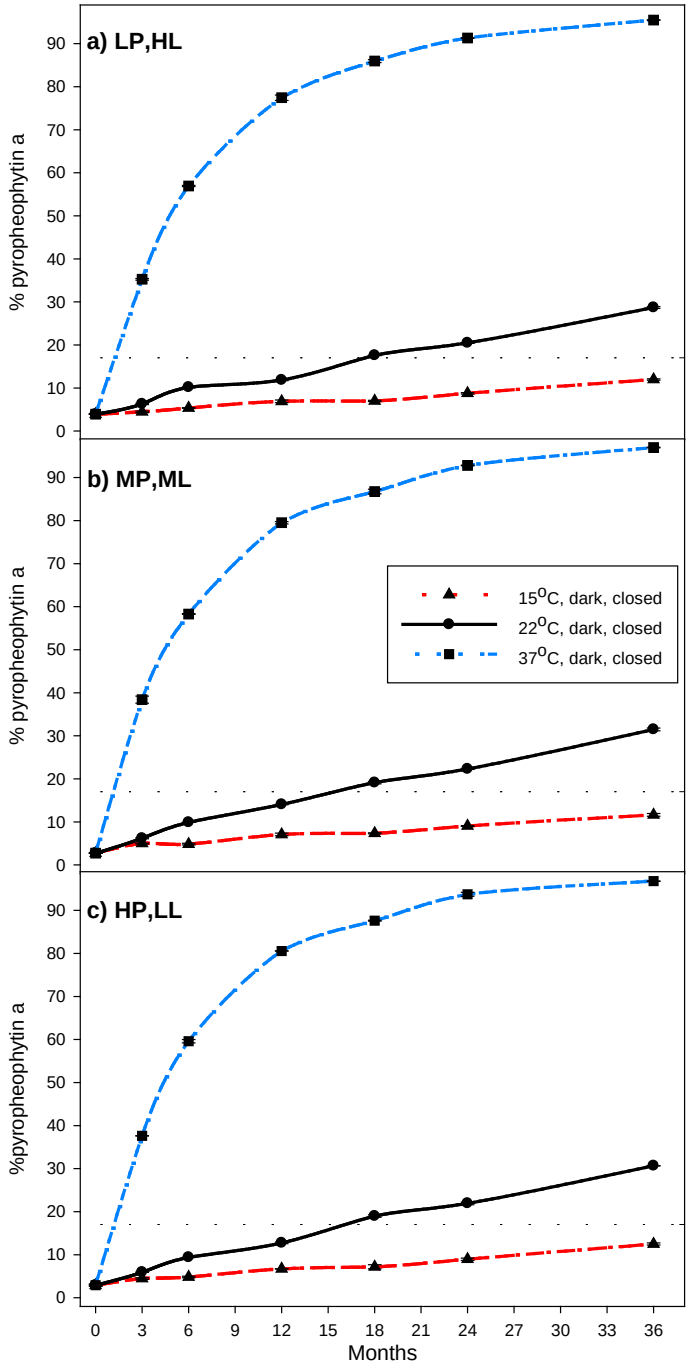

Figure 4.8 Effect of various storage temperatures on pyropheophytin a content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

The initial pyropheophytin a content of the oils used in this project were all relatively low, with oil LP,HL having the highest initial pyropheophytin a content at 3.9%, indicating the freshness of the oils used in this project .

Storage temperature had a significant impact on the pyropheophytin a content of the oil. The pyropheophytin a content of the oils increased at an almost identical rate dependent on the storage temperature, regardless of the other components in that oils matrix (Figure 4.8). For example, oils LP,HL and HP,LL are the least similar oils used in this project (LP,HL had low polyphenols, high linoleic acid, HP,LL had high polyphenols, low linoleic acid). After 36 months at 15°C, the pyropheophytin a content of oil LP,HL was 12.0% compared to 12.5% for oil HP,LL. At 22°C, oil LP,HL was 28.7% after 36 months storage compared to 30.7% for oil HP,LL and at 37°C oil LP,HL was 95.5% compared to 96.8% for oil HP,LL (Figure 4.8).

The pyropheophytin a content of all oils used in this study exceeded the Australian standard limit of ≤17% when stored at 22°C or 37°C (Figure 4.9). At 22°C the oils exceeded the limit after approximately 18 months storage for all oils, while each of the oils stored at 37°C exceeded the limit after less than 3 months storage. None of the oils stored at 15°C exceeded the limit during the 36 months storage.

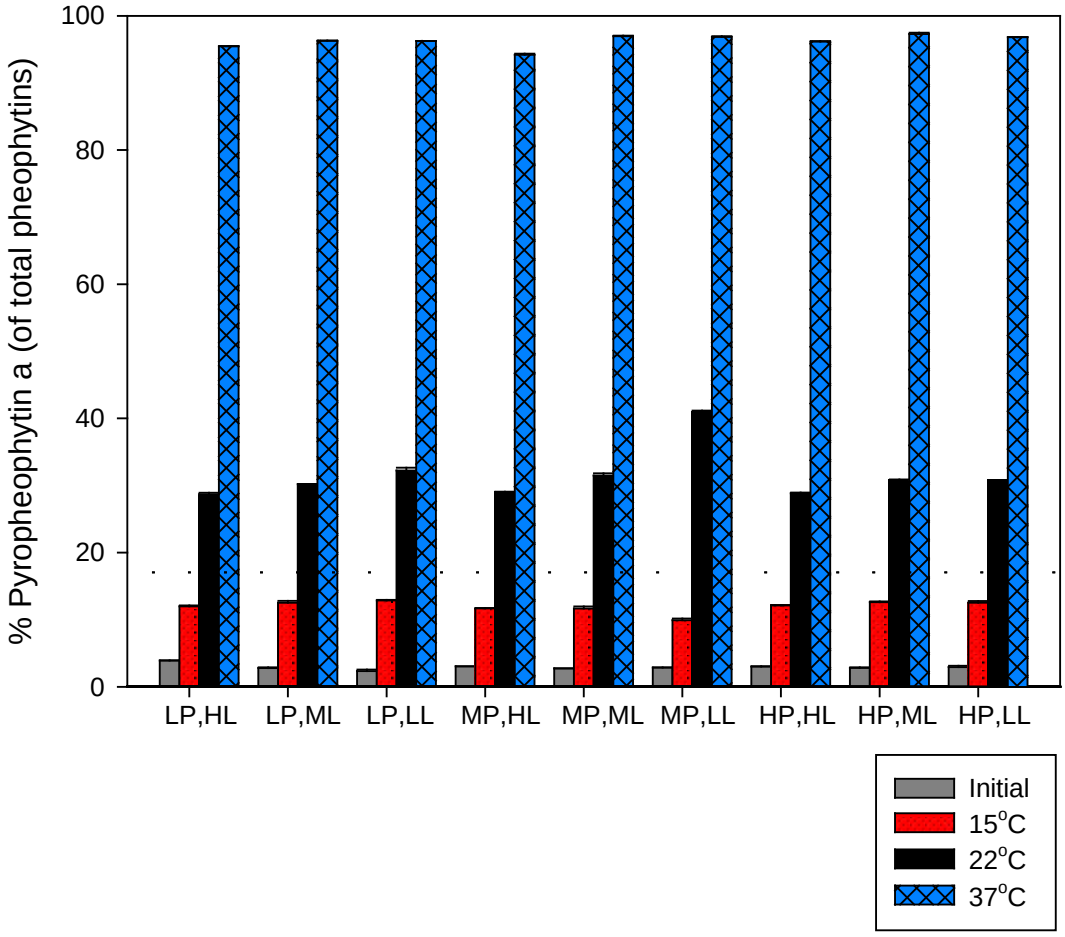

Figure 4.9 Pyropheophytin a concentration at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

Pyrophoephytins in olive oil are formed due to breakdown of chlorophyll pigments. This reaction begins as soon as the oil is extracted from the fruit. The pigments break down due to a process which involves the decarbomethoxylation of chlorophyll and pheophytins to form pyropheophytins (Hornero-Mendez *et al.*, 2005).

In fresh oil, the levels of pyropheophytins are low, as can be seen from the initial values found in the oils used in this project (Figures 4.8 and 4.9).The pyropheophytin a content continued to increase throughout the analysis period, only tapering off once the level reached about 90%.These results are interesting for a number of reasons. Each oil gave an almost identical result, regardless of the initial pyropheophytin content, and also regardless of the composition of the other components of the oil such as fatty acid composition or antioxidants available. These results indicate that pyropheophytin a content will increase gradually as an oil ages, however any increase in the storage temperature for that oil will have a significant effect on the rate of this reaction, leading to a very rapid increase in the production of pyropheophytin a from pheophytin a. These results indicate that the measurement of pyropheophytin a is a very good test for indicating degradation of olive oil, although changes can be due to storage temperature or time.

4.1.6 1,2-diacylglycerols

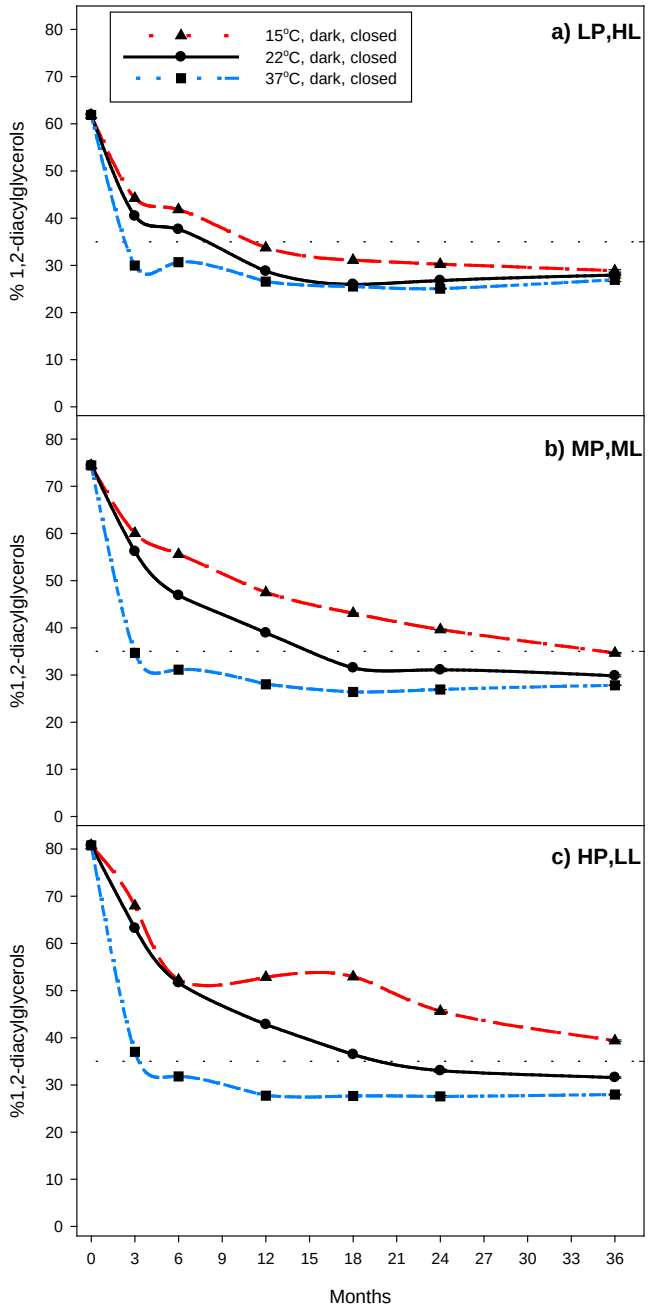

Figure 4.10 Effect of various storage temperatures on %1,2-diacylglycerol content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

The initial free fatty acid content of the oils had a significant effect on the initial 1,2-DAG contents. Oil LP,LL showed an initial %FFA content of 0.1%, the lowest of all of the oils in the study while oil LP,HL had the highest initial %FFA of 0.53%. The same oils had initial 1,2-DAG contents of 80.2% and 61.8% respectively which were the highest and lowest values recorded for 1,2-diacylglycerols (Figure 4.11).

Storage temperature had a significant effect on the 1,2-DAG content of the oils in this study. Each oil showed a significant initial decline for the first 3 months of storage; however the rate of decline was greater in the oils stored at 37°C than those stored at the lower temperatures. The rate of decline then slowed for the remainder of the storage period, with those oils stored at 15°C declining at the slowest rate (Figure 4.10).

The oils stored at 37°C quickly declined to a level below the Australian standard limit (≥35%), with five of the nine oils (Oils LP,HL; LP,ML; MP,HL; MP,ML and HP,HL) below 35% 1,2-DAGs after just three months storage This is due to the isomerisation from 1,2 to 1,3-DAGs occurring at a faster rate as the storage temperature increased. These findings are very similar to those of other researchers (Perez-Camino *et al.*, 2001, Cossignani *et al.*, 2007).

Oils MP,LL; HP,ML and HP,LL took a little more than 6 months to fall below the 35% limit, while only oil LP,LL was above the limit at this time. Oil LP,LL fell below the limit after 12 months storage at 37°C. As mentioned, the oils stored at 22°C declined at a slower rate with most oils exceeding the limit between 6 months (oils LP,HL and MP,HL) and 24 months (oil MP,LL). Only oil LP,LL (43.7%) did not exceed the limit during the entire 36 months storage period. The 1,2-DAG content of the oils stored at 15°C declined at the slowest rate (Figure 4.10), with oil LP,HL exceeding the limit most quickly, after just 12 months of storage. Oils LP,ML; MP,HL; MP,ML and HP,HL exceeded the limit for 1,2-DAG content between 18 and 24 months of storage at 15°C, while oils LP,LL; MP,LL; HP,ML and HP,LL did not exceed the limit.

The 1,2-DAG content of the oils stored at 37°C seemed to reach an equilibrium after the initial rapid decrease with most oils declining quickly to a level of about 27 to 30% 1,2 DAGs, and then maintaining that level for the remainder of the storage period (Figure 4.10)

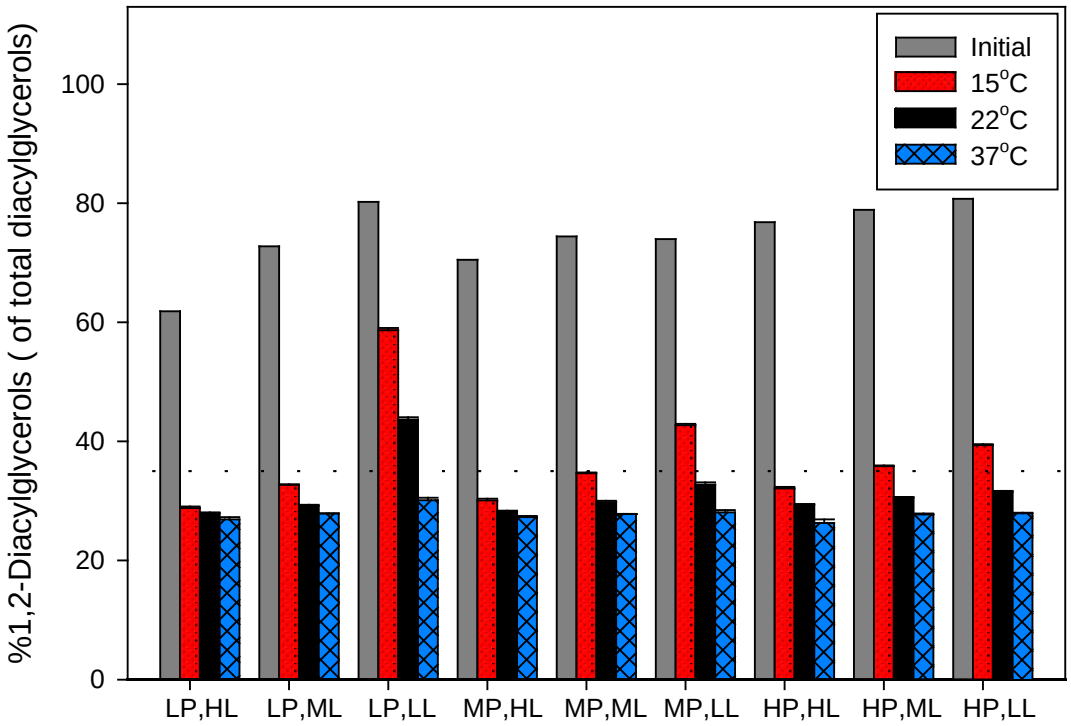

Figure 4.11 Percentage of 1,2-diacylglycerols at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

The 1,2-diacylglycerols (DAGs) in olive oil are generated during the biosynthesis or hydrolysis of triacylglycerols (TAGs). While total DAGs were not measured in this project, it can be deduced that the total DAG content of acidic olive oils is relatively high, as hydrolysis is the primary cause of DAG accumulation during oil storage. As mentioned previously, the ratio of 1,2-DAGs to total DAGs is high in fresh oil, while the ratio of 1,3 DAGs increases (and 1,2-DAGs subsequently decreases) due to isomerisation as the oil ages or is subject to unfavourable storage conditions (Shimizu *et al.*, 2008).

While the 1,2-DAG content is useful for assessing the aging or "freshness" of the oil, other factors may be important such as the initial free fatty acid content of the oil as well as the storage conditions, especially the temperature at which the oil is stored as these variables have a significant impact on the 1,2-DAG content of olive oil.

4.1.7 Total polyphenols

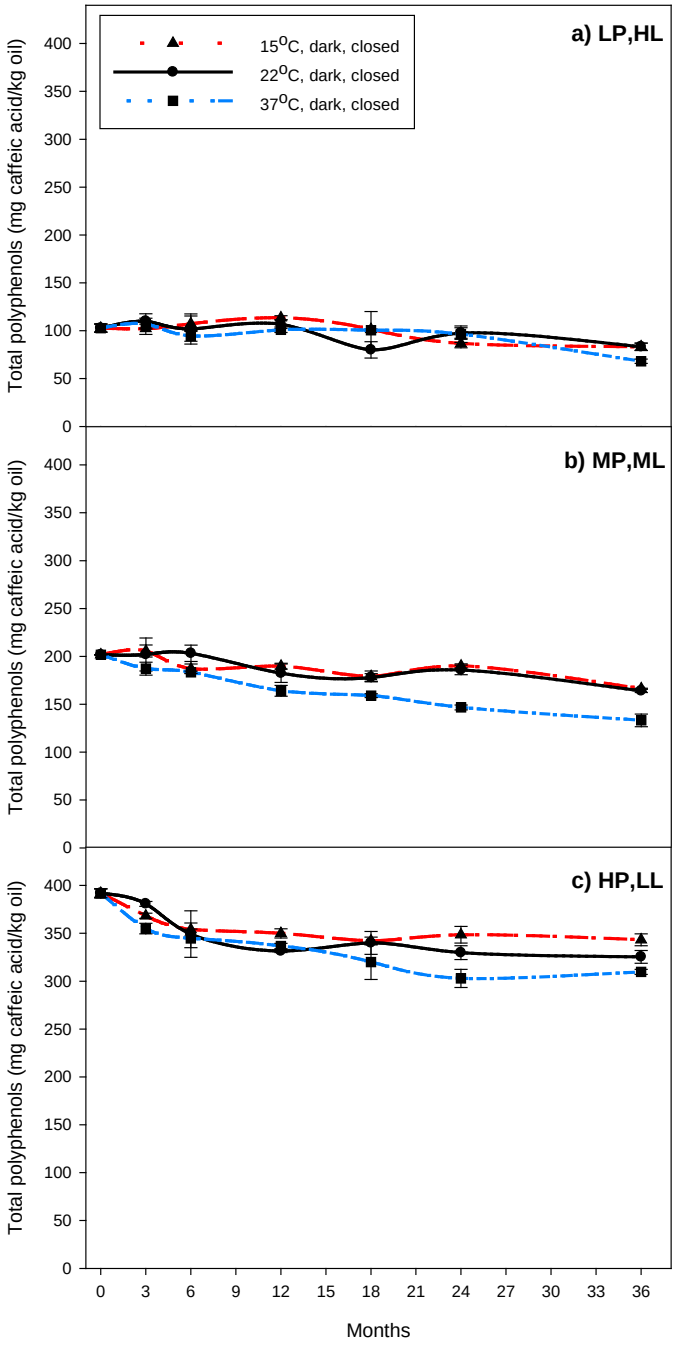

Figure 4.12 Effect of various storage temperatures on total polyphenol content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Regardless of the storage temperature, the polyphenol content decreased over time (Figure 4.12). For example the total polyphenol content of oil MP,ML decreased from 202 mg/kg initially to 166 mg/kg after 36 months at 15°C.

Storage temperature had a slight, but significant effect on the total polyphenol content of the oils in this study. The same oil (MP,ML) decreased to 164 mg/kg at 22°C and 133 mg/kg after 36 months storage at 37°C.

The percentage loss of total polyphenol over 36 months ranged from 0% to 19% of the initial polyphenol content for the oils stored at 15°C and 22°C. The oils stored at 37°C lost between 21% and 35% of the initial polyphenol content after 36 months storage (Figure 4.13).

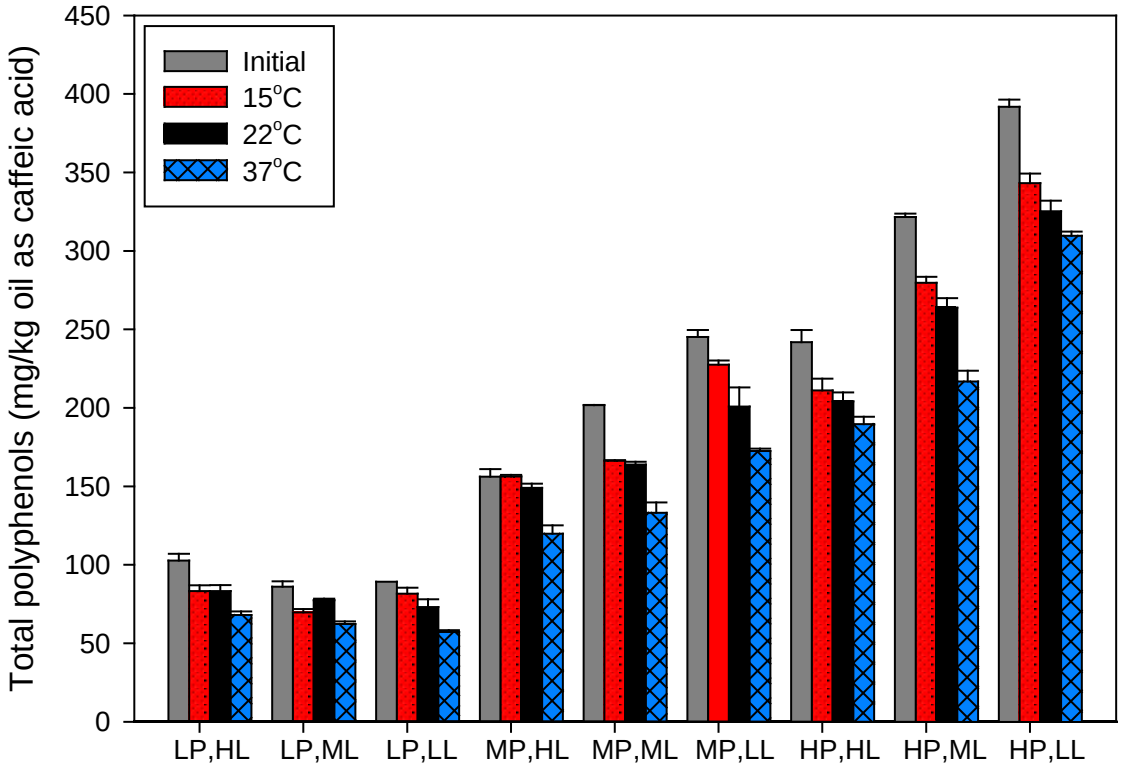

Figure 4.13 Total polyphenol content at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

Polyphenols have multiple roles in the oxidation cycle such as acting as free radical scavengers, singlet oxygen quenchers and also inactivating sensitisers. Polyphenols are also susceptible to heat treatment, and will degrade into other compounds if exposed to heat for long periods of time (Lee *et al.*, 2007). In this study, total polyphenols decreased over time due to the heat labile properties of the polyphenolic compounds. Oils exposed to the higher temperatures were shown to lose polyphenol content at a quicker rate and to a greater extent (Figures 4.12 and 4.13) than those stored at lower temperatures. As no further oxygen was made available, as seen by the peroxide value decreasing for the same oils, then the losses incurred are almost solely due to the storage temperature.

4.1.8 α-tocopherol

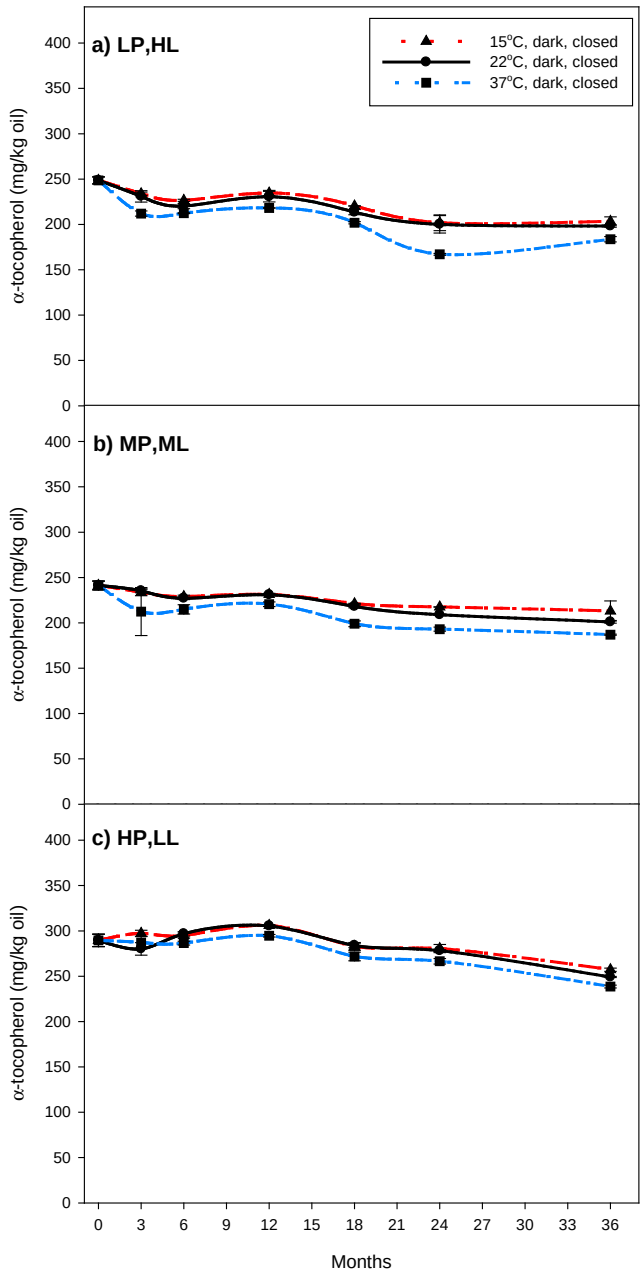

Figure 4.14 Effect of various storage temperatures on α-tocopherol content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

There was no effect of storage temperature on the α-tocopherol content of the oils in this study . The α-tocopherol content of the oils reduced over time regardless of the storage temperature. For example oil MP,ML decreased from 241 mg/kg initially to 213 mg/kg after 36 months storage at 15°C (Figure 4.14).

However the same oil (MP,ML), decreased to 201 mg/kg at 22°C and 187 mg/kg after 36 months storage at 37°C.

The percentage loss of α-tocopherols over 36 months ranged from 1% to 59% of the initial polyphenol content for the oils store at 15°C and 22°C. The oils stored at 37°C lost between 3% and 60% of the initial α-tocopherols content after 36 months storage (Figure 4.15).

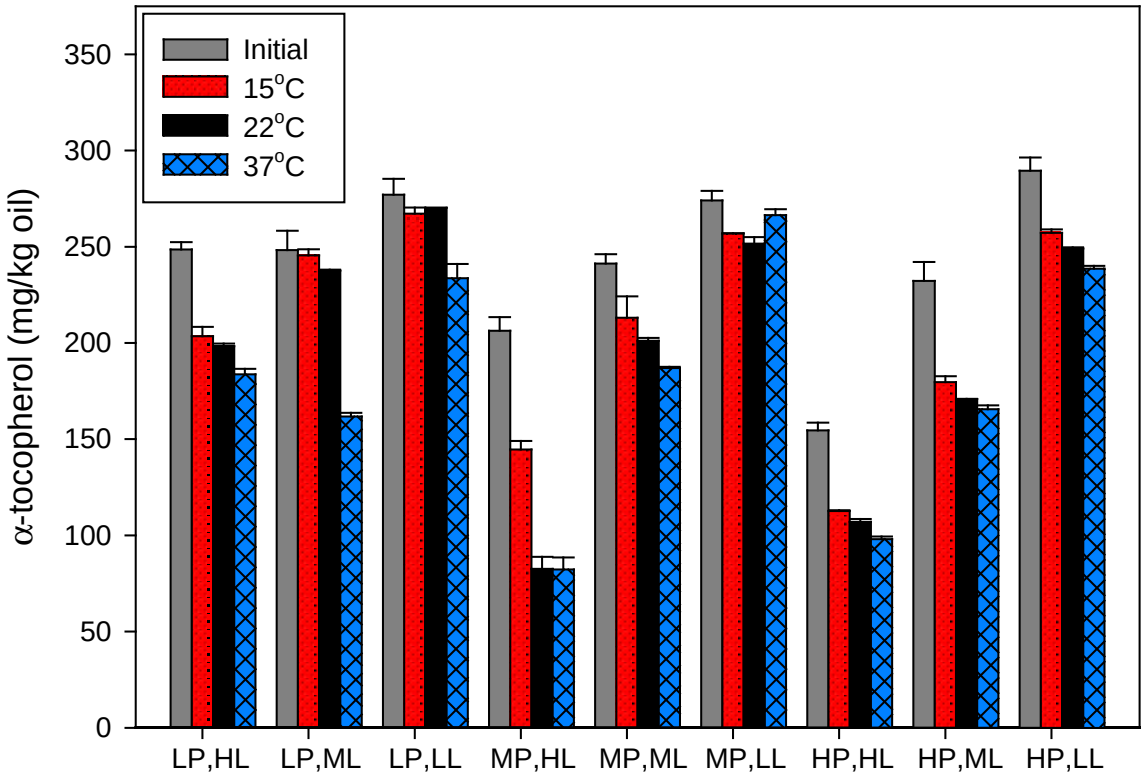

Figure 4.15 α-tocopherol content at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

The α-tocopherol content of the oils in this study showed a slight decline when stored at lower temperatures (15 and 22°C). At 37° storage, the α-tocopherol declined at a slightly greater rate for most oils. As with the reduction in polyphenols, this is probably mainly due to the degradation of the α-tocopherol by the high temperature, as limited oxygen was available for the α-tocopherol to become involved in the oxidation process.

4.1.9 Chlorophyll

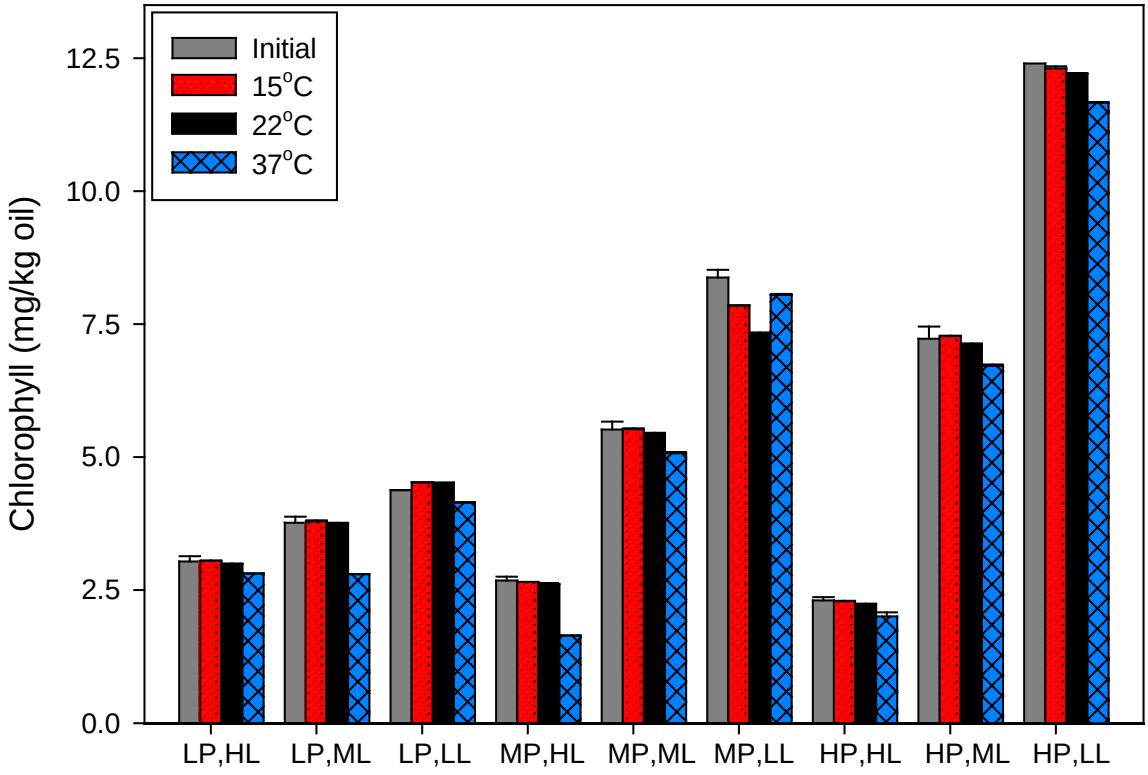

Figure 4.16 Chlorophyll content at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

In this study, the total chlorophyll content of the oils was not significantly affected by the temperature at which the oil was stored, with the chlorophyll content in most oils remaining the same or similar after 36 months storage to the initial content. For example, oil MP,ML had a total chlorophyll content of 5.5 mg/kg initially. After 36 months storage at 15°C the chlorophyll content was still 5.5 mg/kg, at 22°C was 5.4 mg/kg and at 37°C was 5.1 mg/kg.

Some oils, such as oil MP,HL, did have a slightly lower total chlorophyll content after 36 months storage at 37°C, however this was not consistent across all of the oils (Figure 4.16).

These results may seem at odds with the results from the analysis of pyropheophytins – however this method measures the total chlorophyll pigments (chlorophyll a, chlorophyll b, pheophytin a, pheophytin b, pheophorbide a and allomerised derivatives) (Gallardo-Guerrero *et al.*, 2005), regardless of the conformation, therefore it is a measurement of chlorophyll and all of its derivatives. This is interesting to note as measuring total chlorophylls will not give the complete overview of the mechanisms occurring during the storage of olive oil.

4.1.10 Colour

Table 4.2 Colour (L*, a*, b) of olive oil samples at initial analysis and after 36 months storage at different temperatures

	Storage	L*	a*	b*
LP,HL	Initial	92.0 ± 0.4	-12.6 ± 0.1	75.7 ± 0.7
	15°C, after 36 months	91.9 ± 0.1	-12.3 ± 0.0	73.1 ± 0.1
	22°C, after 36 months	92.2 ± 0.2	-12.2 ± 0.0	70.8 ± 0.3
	37°C, after 36 months	93.2 ± 0.2	-12.0 ± 0.1	63.5 ± 0.1
LP,ML	Initial	89.8 ± 0.5	-11.6 ± 0.1	98.8 ± 0.2
	15°C, after 36 months	90.1 ± 0.1	-11.4 ± 0.0	97.6 ± 0.1
	22°C, after 36 months	90.3 ± 0.3	-11.6 ± 0.0	96.0 ± 0.3
	37°C, after 36 months	92.1 ± 0.7	-12.9 ± 0.1	97.8 ± 0.7
LP,LL	Initial	88.4 ± 0.3	-10.1 ± 0.0	113.6 ± 0.2
	15°C, after 36 months	88.7 ± 0.3	-10.0 ± 0.0	113.3 ± 0.2
	22°C, after 36 months	89.1 ± 0.4	-10.0 ± 0.1	113.1 ± 0.4
	37°C, after 36 months	90.1 ± 0.4	-11.1 ± 0.0	102.8 ± 0.3
MP,HL	Initial	91.7 ± 0.4	-12.4 ± 0.0	79.3 ± 0.4
	15°C, after 36 months	91.9 ± 0.5	-12.2 ± 0.1	76.8 ± 0.3
	22°C, after 36 months	92.7 ± 0.1	-12.4 ± 0.1	75.1 ± 0.0
	37°C, after 36 months	94.9 ± 0.2	-10.8 ± 0.0	47.0 ± 0.1
MP,ML	Initial	87.8 ± 0.2	-10.0 ± 0.0	109.7 ± 0.4
	15°C, after 36 months	88.4 ± 0.5	-10.2 ± 0.3	107.7 ± 0.2
	22°C, after 36 months	88.2 ± 0.1	-10.1 ± 0.0	105.4 ± 0.1
	37°C, after 36 months	89.1 ± 0.0	-10.7 ± 0.1	96.7 ± 0.2
MP,LL	Initial	84.7 ± 0.2	-7.3 ± 0.0	122.9 ± 0.2
	15°C, after 36 months	85.0 ± 0.3	-8.2 ± 0.1	115.5 ± 0.4
	22°C, after 36 months	86.1 ± 0.1	-9.8 ± 0.0	104.4 ± 0.1
	37°C, after 36 months	86.1 ± 0.3	-7.7 ± 0.0	115.9 ± 0.2
HP,HL	Initial	92.1 ± 0.2	-12.2 ± 0.0	83.0 ± 0.1
	15°C, after 36 months	92.4 ± 0.0	-12.1 ± 0.1	81.0 ± 0.1
	22°C, after 36 months	92.7 ± 0.1	-12.2 ± 0.0	79.0 ± 0.0
	37°C, after 36 months	92.6 ± 0.1	-12.0 ± 0.2	71.6 ± 0.2
HP,ML	Initial	86.0 ± 0.1	-8.5 ± 0.1	117.2 ± 0.2
	15°C, after 36 months	86.1 ± 0.1	-8.4 ± 0.2	114.3 ± 0.2
	22°C, after 36 months	86.5 ± 0.1	-8.5 ± 0.5	112.4 ± 0.2
	37°C, after 36 months	87.5 ± 0.2	-9.5 ± 0.0	104.5 ± 0.1
HP,LL	Initial	81.7 ± 0.6	-5.5 ± 0.1	126.0 ± 0.7
	15°C, after 36 months	81.7 ± 0.6	-5.2 ± 0.0	125.0 ± 0.7
	22°C, after 36 months	82.3 ± 0.1	-5.3 ± 0.0	124.6 ± 0.1
	37°C, after 36 months	82.5 ± 0.6	-5.3 ± 0.4	119.4 ± 0.7

The colour of the oils in this study was measured as L* (luminosity), a* (greenness) and b*(yellowness). As can be seen from Table 4.2, L* and a* were in general not significantly affected by the temperature at which the oil was stored. However b* was significantly affected by storage at

37°C, with the value decreasing significantly. This indicates the colour is slightly less yellow than in the initial oil.

As mentioned previously, the objective measurement of colour is an important component to food production, due to the relationship between the colour attributes and the acceptability of foods by the consumer. The study and interpretation of colour indices in food products is a complex and challenging as there are many variables which can affect the outcome.

These results indicate oils stored at higher temperatures may display a slight change in colour, however those changes would be minor and may be difficult to detect with the naked eye subjectively.

There are many pigments which contribute to the colour of olive oil including chlorophylls and carotenoids. Due to the complex nature of these compounds and the complex interaction with the conditions under which they are stored, further investigation focussing on this area would be needed to elucidate sound conclusions for these changes.

4.1.11 Fatty acid profile

No significant effect of storage temperature on the fatty acid profile of the oils in this study was found (data not shown). After 36 months storage at 15°C, 22°C and 37°C, the saturated, monounsaturated and polyunsaturated fatty acid composition of most of the oils were either exactly the same as the initial analysis or very similar.

While there were definitely changes in the overall structure of the oil, including the triacylglycerol structure (evidenced by the change in DAGs, UV absorbance) as well as some of the minor components due to the storage temperature, there was no significant change observed in the overall fatty acid profile of the oil. As the method used for this determination is qualitative only, the actual changes in the amount of each fatty acid present are not observed. However these results indicate that the changes which are occurring do so at a consistent rate – that is, storage temperature does lead to the breakdown of the individual fatty acid, but at a consistent rate therefore the proportion of fatty acids does not alter, similar to findings by other researchers (Mendez and Falque, 2007).

4.1.12 Induction time

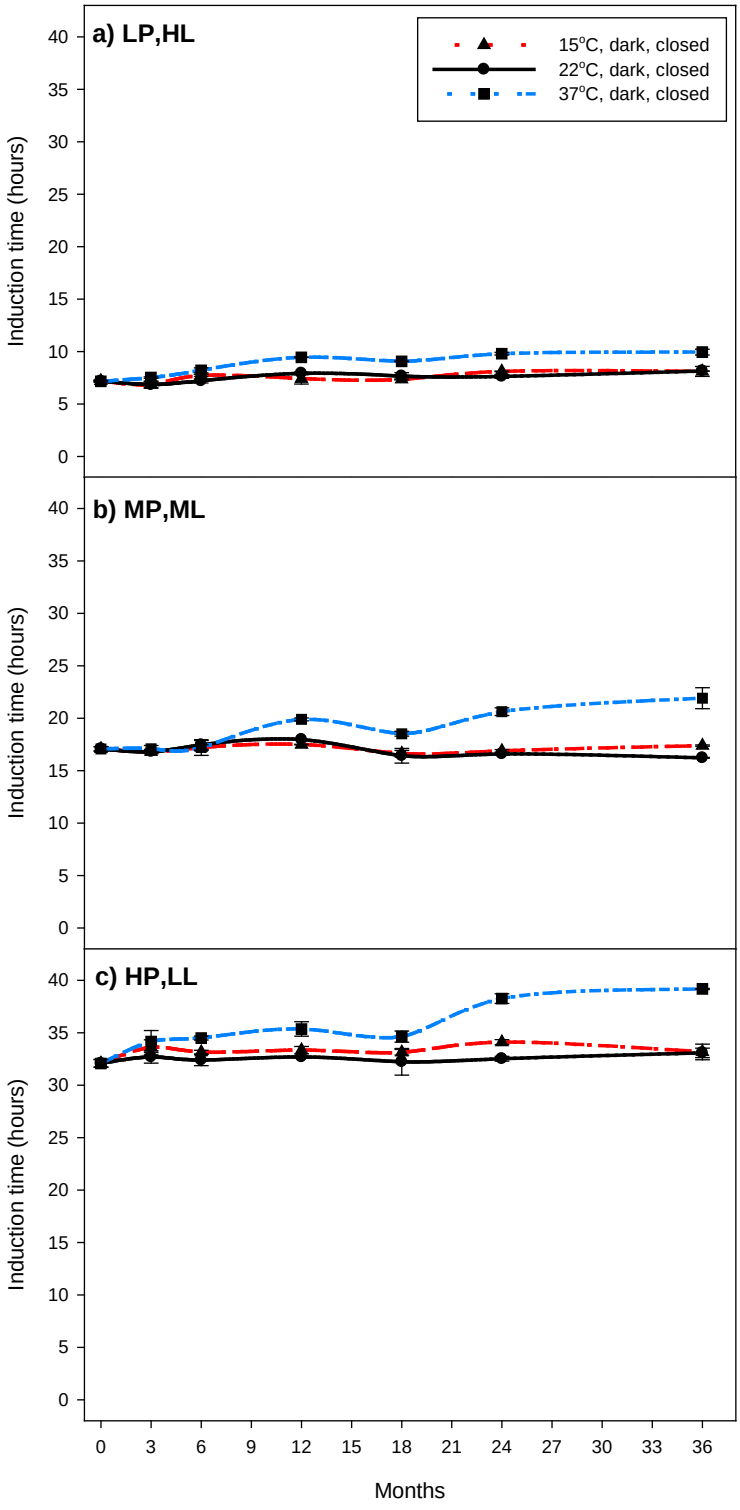

Figure 4.17 Effect of various storage temperatures on induction time of different types of olive oil stored for 36 months

LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

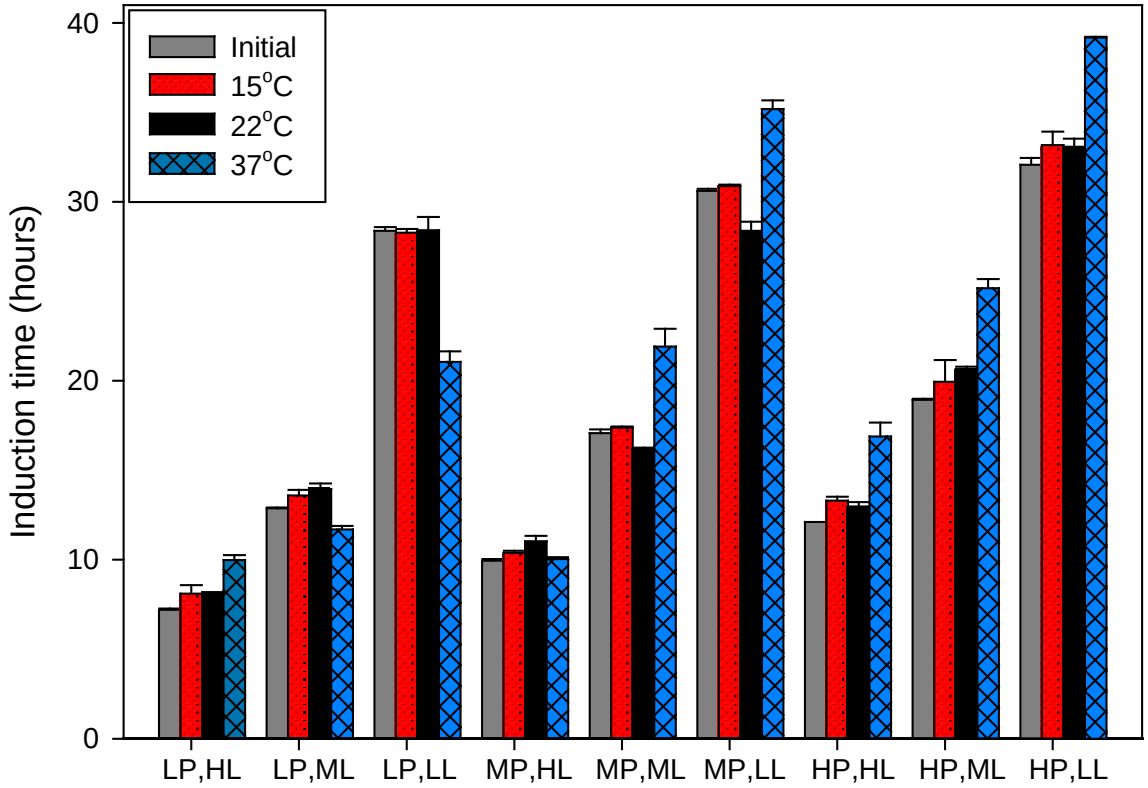

Figure 4.18 Induction time at initial analysis and after 36 months storage at various temperatures for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

The induction time of the oils was significantly affected by the temperature at which the oil was stored, with the induction time increasing at the highest storage temperature. The oils stored at 15°C and 22°C did not vary significantly after 36 months storage. For example oil MP,ML had an initial induction time of 17.1 hours and after 36 months storage at 15°C the induction time was 17.4 hours. The same oil (MP,ML) stored at 22°C had an induction time of 16.2 hours after 36 months while the oil stored at 37°C had an induction time of 21.9 hours (Figure 4.18). The induction time increased in the oils stored at 37°C almost immediately after the storage conditions were imposed (Figure 4.17).

This occurred as the oils stored at 37°C had a much lower peroxide value than those stored at the other temperatures. While total polyphenols did decrease in the oils stored at the higher temperatures, the fatty acid composition did not change, and the oxygen already in the oil was minimal in these oils, which led to the a longer induction time in the oils stored at the higher temperature.

4.1.13 Organoleptic assessment

The sensory profile of olive oil is influenced by many compounds, including volatile and polyphenolic compounds. When olive oil is fresh, the majority of these compounds give rise to pleasant flavours and aromas, however as the oil ages these compounds can deteriorate into compounds which have unpleasant flavour and aroma characteristics.

In the oils studied in this project, temperature did have a significant effect on the organoleptic assessment of the olive oil. The sensory profile of the oils stored at 15°C were generally seen to change only slightly during the storage period, with some of the oils maintaining the extra virgin status (median fruitiness >0, median defects = 0). This is suppoprted by the data from the chemical analysis which showed, generally, that oils stored at 15°C showed little change in polyphenol content over the storage period, as well as the measure of secondary oxidation products, K_{268}, also remaining relatively stable.

Generally, the oils with the lowest polyphenol content showed a presence of rancidity during the 36 months of storage at 15°C. Oils LP,HL; LP,ML; LP,LL and MP,HL were classified as rancid after between 18 and 36 months of storage at 15°C. Oils MP,ML; MP,LL; HP,HL; HP,ML and HP,LL did not show any sign of rancidity during the 36 months storage at 15°C (Figure 4.19).

For the oils stored at 22°C, rancidity was found to be present in seven of the oils (Oil LP,HL; LP,ML; LP,LL; MP,HL; MP,LL; HP,HL; HP,ML) between 18 and 36 months storage with the oils with the lowest initial polyphenol content being the first oils to show sign of rancidity. Oils MP,ML and HP,LL were not classified as rancid during the 36 months storage at 22°C (Table 4.3).

Storage at 37°C had the greatest influence on the presence of rancidity in the oils in this study. All oils were deemed to be rancid after 36 months storage at 37°C (Figure 4.19). This generally occurred after a short storage period, between 6 and 18 months after the initial analysis. The rancidity increased significantly during the storage period, with some of the oils reaching the lampante classification after 36 months of storage, meaning the oil is inedible unless refined.

The oils stored at higher temperatures, especially the oils stored at 37°C showed significant changes. Oils quickly lost the extra virgin status as the oils were deemed too rancid by the sensory assessors very early in the storage period. The positive attributes fruitiness, bitterness and pungency declined at the same time. These results are confirmed by the chemical analysis with the secondary oxidation products measured by K_{268} increasing in these oils, while the polyphenol content decreased at the same time.

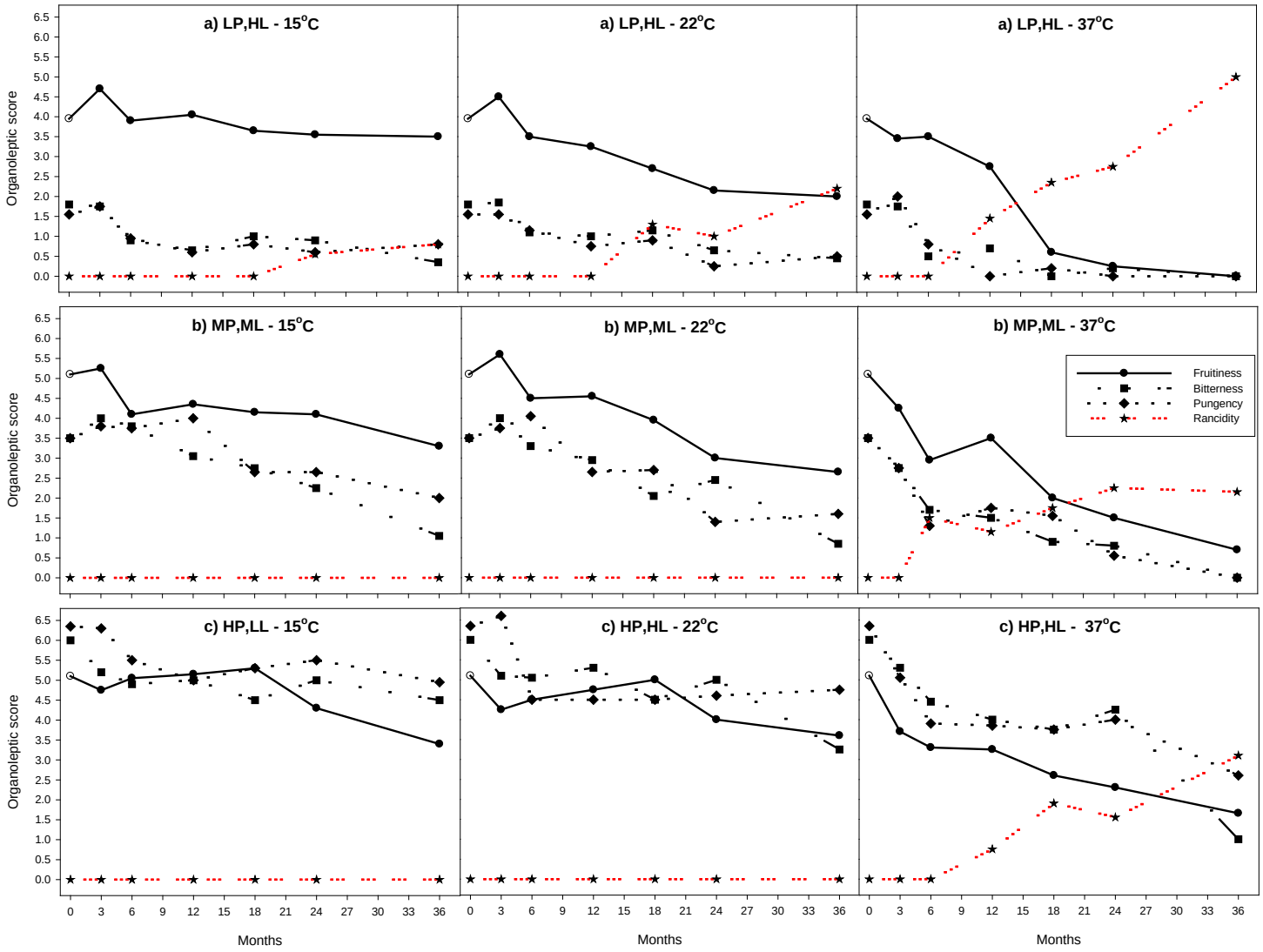

Figure 4.19 Organoleptic assessment of olive oils

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Table 4.3 Organoleptic assessment of olive oil samples at initial analysis and after 36 months storage at different temperatures

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

	Storage	Fruitiness	Bitterness	Pungency	Rancidity
LP,HL	Initial	3.95	1.80	1.55	0.00
	15°C, after 36 months	3.50	0.35	0.80	0.80
	22°C, after 36 months	2.00	0.45	0.50	2.20
	37°C, after 36 months	0.00	0.00	0.00	5.00
LP,ML	Initial	3.85	1.90	1.20	0.00
	15°C, after 36 months	3.00	0.75	0.50	0.85
	22°C, after 36 months	2.75	0.75	0.75	0.75
	37°C, after 36 months	0.00	0.00	0.00	6.00
LP,LL	Initial	5.00	2.05	2.05	0.00
	15°C, after 36 months	4.00	0.75	1.25	0.75
	22°C, after 36 months	3.25	0.70	0.80	0.50
	37°C, after 36 months	0.70	0.20	0.20	3.00
MP,HL	Initial	5.30	3.00	3.50	0.00
	15°C, after 36 months	3.10	1.00	0.80	0.70
	22°C, after 36 months	1.25	0.75	0.40	2.65
	37°C, after 36 months	1.00	0.00	0.00	4.00
MP,ML	Initial	5.10	3.50	3.50	0.00
	15°C, after 36 months	3.30	1.05	2.00	0.00
	22°C, after 36 months	2.65	0.85	1.60	0.00
	37°C, after 36 months	0.70	0.00	0.00	2.15
MP,LL	Initial	5.00	4.45	5.00	0.00
	15°C, after 36 months	3.85	2.35	2.10	0.00
	22°C, after 36 months	3.00	1.15	1.45	0.25
	37°C, after 36 months	0.75	0.35	0.90	3.00
HP,HL	Initial	5.50	4.50	4.00	0.00
	15°C, after 36 months	3.00	1.30	2.25	0.00
	22°C, after 36 months	4.00	1.40	2.30	1.35
	37°C, after 36 months	1.25	0.35	0.45	2.50
HP,ML	Initial	5.90	4.80	5.65	0.00
	15°C, after 36 months	4.05	2.10	3.25	0.00
	22°C, after 36 months	3.75	1.85	2.40	0.50
	37°C, after 36 months	1.60	0.75	0.65	3.15
HP,LL	Initial	5.10	6.00	6.35	0.00
	15°C, after 36 months	3.40	4.50	4.95	0.00
	22°C, after 36 months	3.60	3.25	4.75	0.00
	37°C, after 36 months	1.65	1.00	2.60	3.10

4.2 Effect of exposure to oxygen

4.2.1 Peroxide value

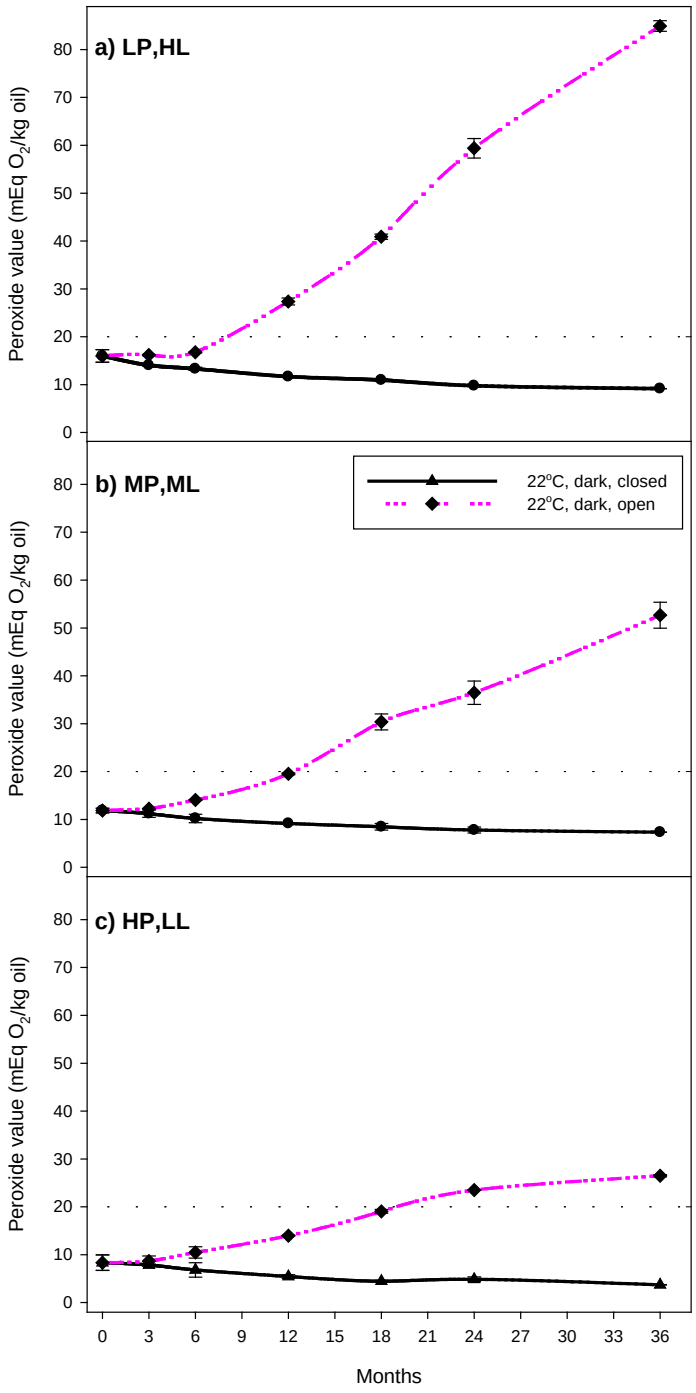

Figure 4.20 Effect of exposure to oxygen on peroxide value of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

The peroxide value for each of the oils at the initial analysis was below the Australian and IOC standard of 20 mEq oxygen/kg oil.

The peroxide values declined in the samples kept closed over the 36 months storage period. For example oil LP,HL decreased from 16 mEq oxygen/kg oil to 9 mEq oxygen/kg oil after 36 months. In contrast, the oils exposed to oxygen showed a drastic increase in the peroxide value. Oil LP,HL, the oil least likely to resist oxidation based on its initial composition, increased from 16 mEq oxygen/kg oil initially to 85 mEq oxygen/kg oil after 36 months storage exposed to oxygen. The oil most able to resist oxygen due to its initial composition, HP,LL, increased from 8 mEq oxygen/kg oil to 26 mEq oxygen/kg oil after 36 months storage exposed to oxygen (Figure 4.20) .

None of the oils kept in closed bottles exceeded the limit of ≤20 mEq oxygen/kg oil. However, all of the bottles which were exposed to oxygen did exceed the level, at varying times due to the composition of the oil (Figure 4.21). The oils with the highest levels of polyunsaturated fatty acids (oils LP,HL; MP,HL and HP,HL) exceeded the standard limit most quickly, after between 6 and 12 months storage exposed to oxygen. The oils with the mid range of polyunsaturated fatty acids (oils LP,ML; MP,ML and HP,ML) exceeded the limit after 6 to 18 months storage, while the oils with the lowest polyunsaturated fatty acid content (oils LP,LL; MP,LL and HP,LL) exceeded the limit after 12-24 months.

The final peroxide value after 36 months was significantly influenced by the fatty acid composition and total polyphenol content.

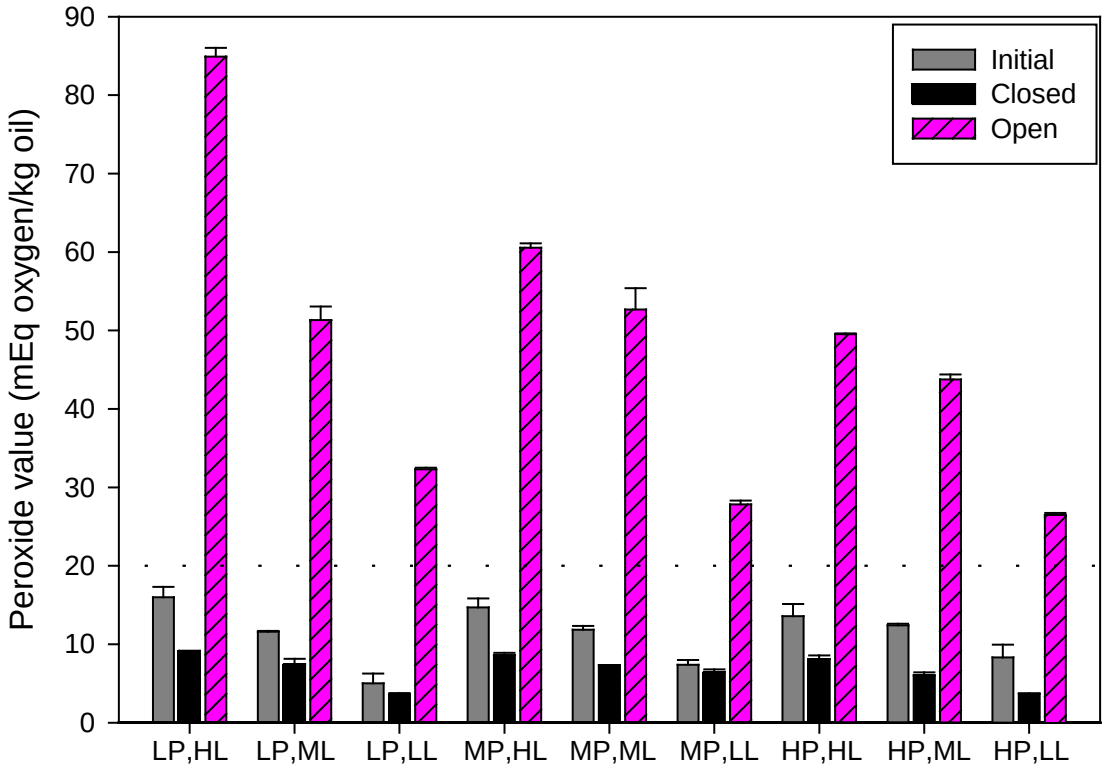

Figure 4.21 Peroxide value at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

The oils exposed to oxygen showed a significant increase in peroxide value. Almost immediately after the oil was stored the peroxide value increased, more slowly for the first few months of storage, followed by a faster increase in the rate of accumulation of the peroxide value for the remainder of the storage period, similar to the results of other researchers (Krichene *et al.*, 2010).

The results are as expected for the oils in open containers as there is no limitation to oxygen availability required for peroxide formation. These results indicate that under the influence of high concentrations of oxygen, olive oil quality quickly deteriorates. It also highlights the importance of correct storage of olive oil with in air tight containers, preferably with oxygen removed using an inert gas such as nitrogen. Also, it clearly shows the influence of fatty acid composition and polyphenol content on the oxidative stability of olive oil.

These results also indicate the usefulness of using peroxide value as a suitable analytical tool for monitoring the progress of oxidation and determining the quality of the olive oil during storage.

4.2.2 UV absorbance K_{232}

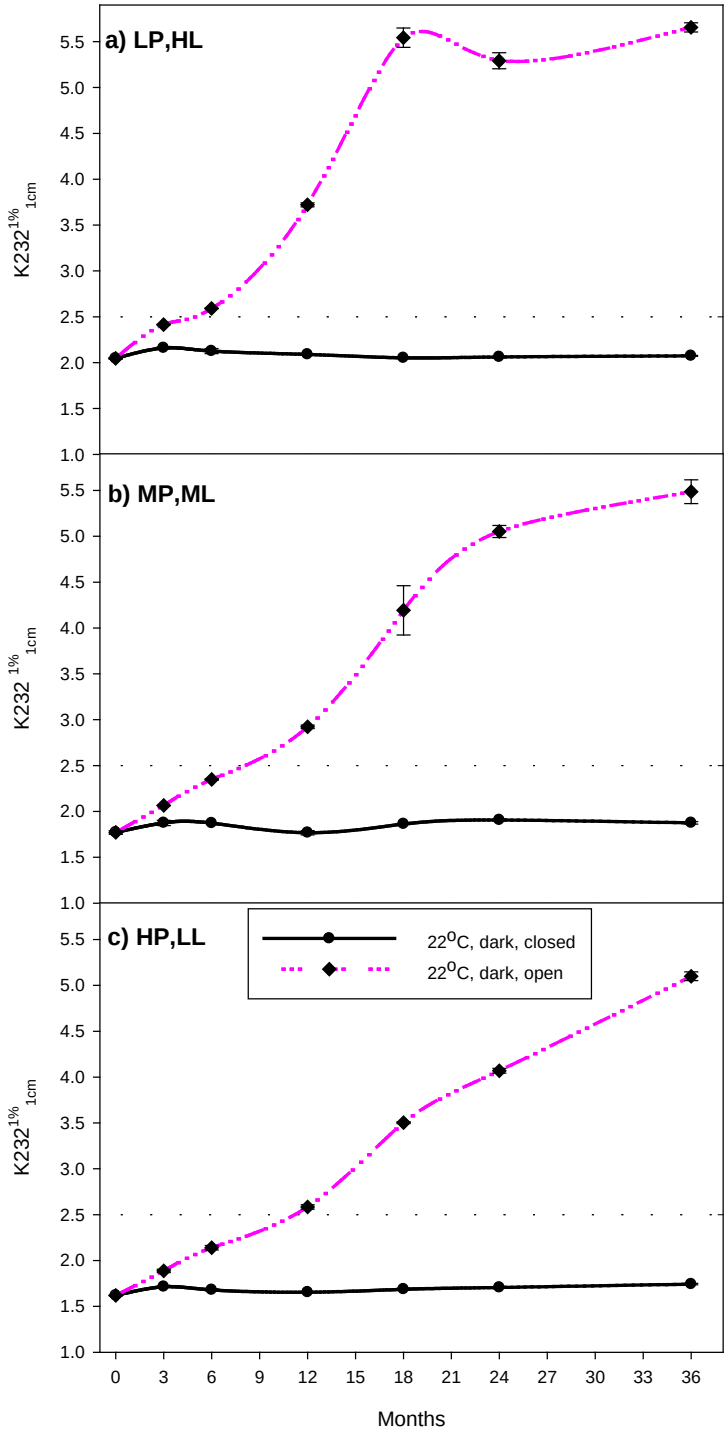

Figure 4.22 Effect of exposure to oxygen on UV absorption K_{232} of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Exposure to oxygen had a significant effect on the UV absorbance K_{232} of the oils in this study. This was expected as K_{232} is a measure of the primary oxidation products in olive oil, therefore the results closely resemble the accumulation of peroxides in the oils when exposed to oxygen. None of the oils which were closed to oxygen showed any change in the K_{232} content. For example the K_{232} of oil LP,HL, the oil least able to resist oxygen because of its initial composition, changed from 2.05 to 2.07 over 3 years of storage when not exposed to oxygen. The K_{232} of the same oil when exposed to oxygen increased to 5.66 after 36 months storage (Figure 4.22 and 4.23).

All of the oils exposed to oxygen exceeded the Australian and IOC limit after (≤2.50) between 3 to 6 months for the less stable oils and 12 to 18 months for the more stable oils.

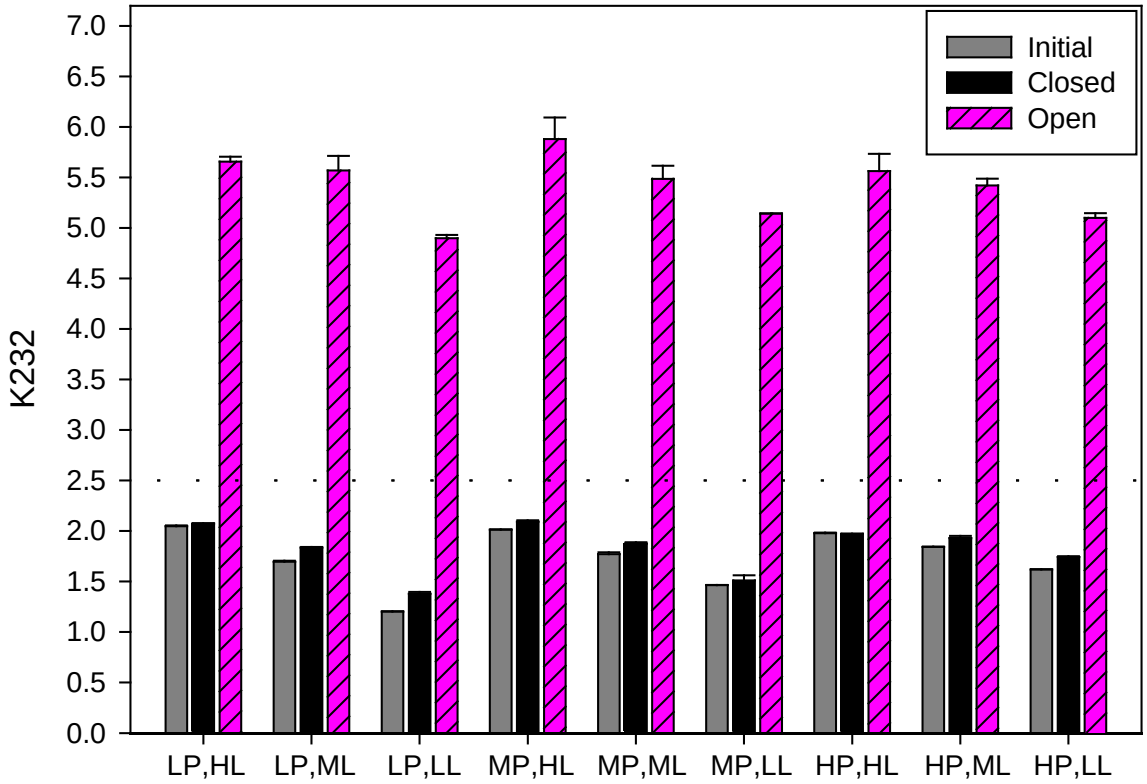

Figure 4.23 K_{232} at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

The level of K_{232} increased almost immediately upon exposure to oxygen, and increased at a slightly faster rate from about 18 months to 36 months of storage. These results indicate that when exposed to oxygen, the oils oxidised very quickly. As mentioned above, K_{232} is a measure of primary oxidation products including hydroperoxides, and these results mirror those seen for the peroxide value.

These results highlight the usefulness of measuring K_{232} as an indicator of the presence of oxidation due to oxygen exposure.

4.2.3 UV absorbance K_{268}

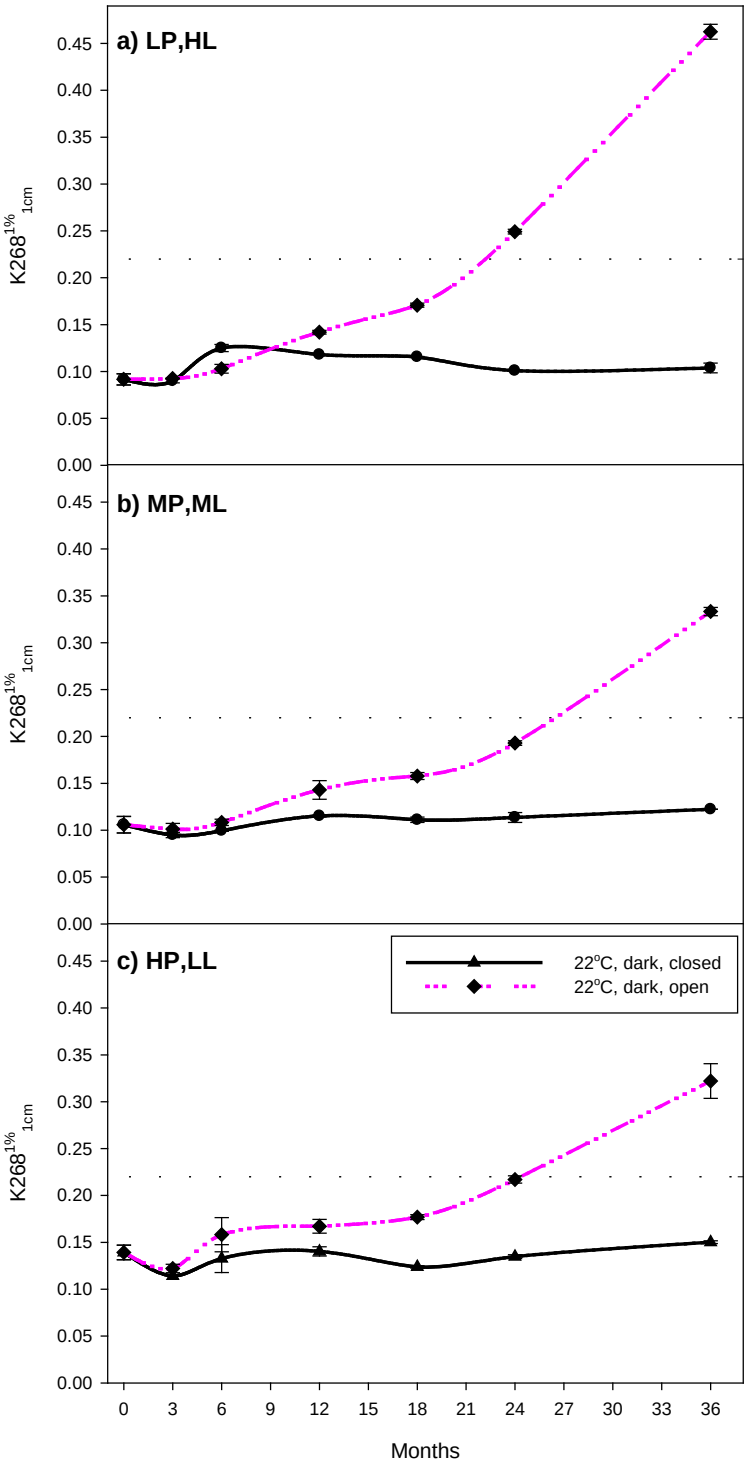

Figure 4.24 Effect of exposure to oxygen on UV absorption K_{268} of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

UV absorbance at 268nm (K_{268}) was significantly affected by exposure to oxygen in the oils in this study. The oils which were not exposed to oxygen remained constant throughout the 36 months storage period (Figure 4.24). The oils exposed to oxygen all showed a similar trend, increasing at a moderate rate until about 18 months of storage, followed by a period of greater accumulation from 18 months until the conclusion of the study after 36 months storage.

All of the oils exposed to oxygen exceeded the Australian and IOC limit of ≤0.22 by the conclusion of the study. Most of the oils exceeded the limit between 24 and 36 months after the beginning of storage when exposed to oxygen. The least stable oil, oil LP,HL, had a final K_{268} level of 0.46, while the remainder of the oils had a final K_{268} level of between 0.29 and 0.35 (Figure 4.25).

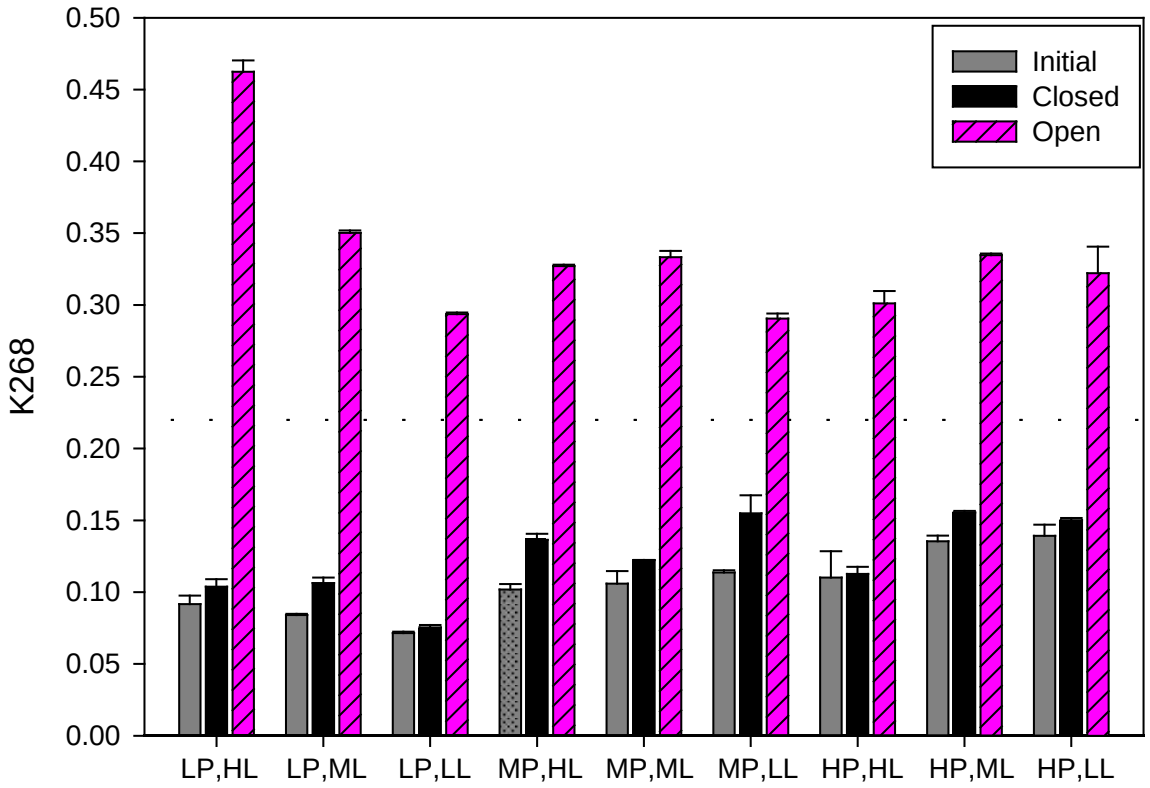

Figure 4.25 K_{268} at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

As primary oxidation products were produced due to the oils exposure to oxygen, as measured at 232nm (K_{232}), they broke down into secondary oxidation products such as aldehydes and ketones which were measured at K_{268}. These results are very similar to those found by other authors (Krichener *et al.*, 2010). As with measurement of UV absorbance at 232 nm (K_{232}), measuring K_{268} is a useful analytical tool for identifying the quality of olive oil which may have been stored when exposed to oxygen.

4.2.4 Free fatty acids

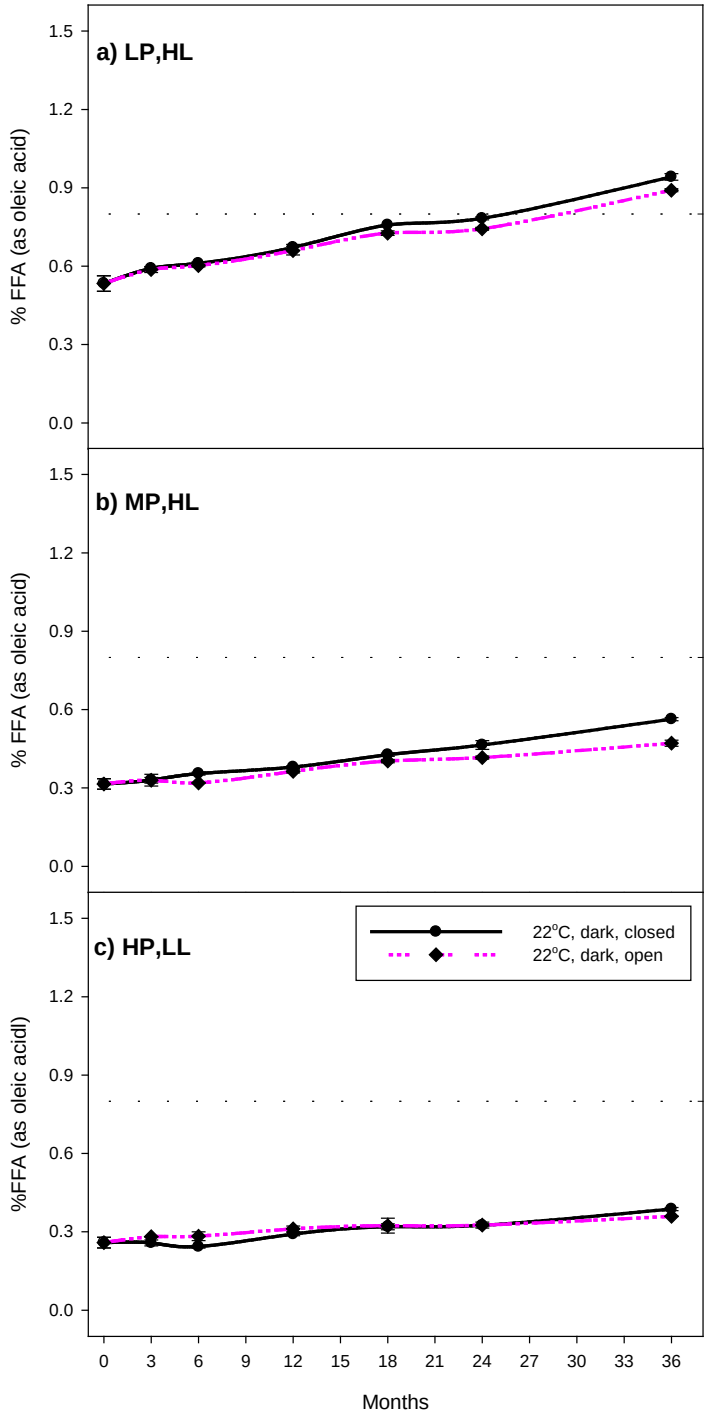

Figure 4.26 Effect of exposure to oxygen on free fatty acid of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Free fatty acids changed over time, increasing at a constant rate in both the closed samples and those exposed to oxygen, however there was no significant difference between the different types of storage (Figure 4.26). Only oil LP,HL, which had the highest initial free fatty acid content exceeded the

Australian and IOC limit (≤0.80% FFA), in both the closed bottle (0.94% FFA) and the sample exposed to oxygen (0.89% FFA) after 36 months storage (Figure 4.27). These results are similar to those found by other researchers (Pristouri *et al.*, 2010).

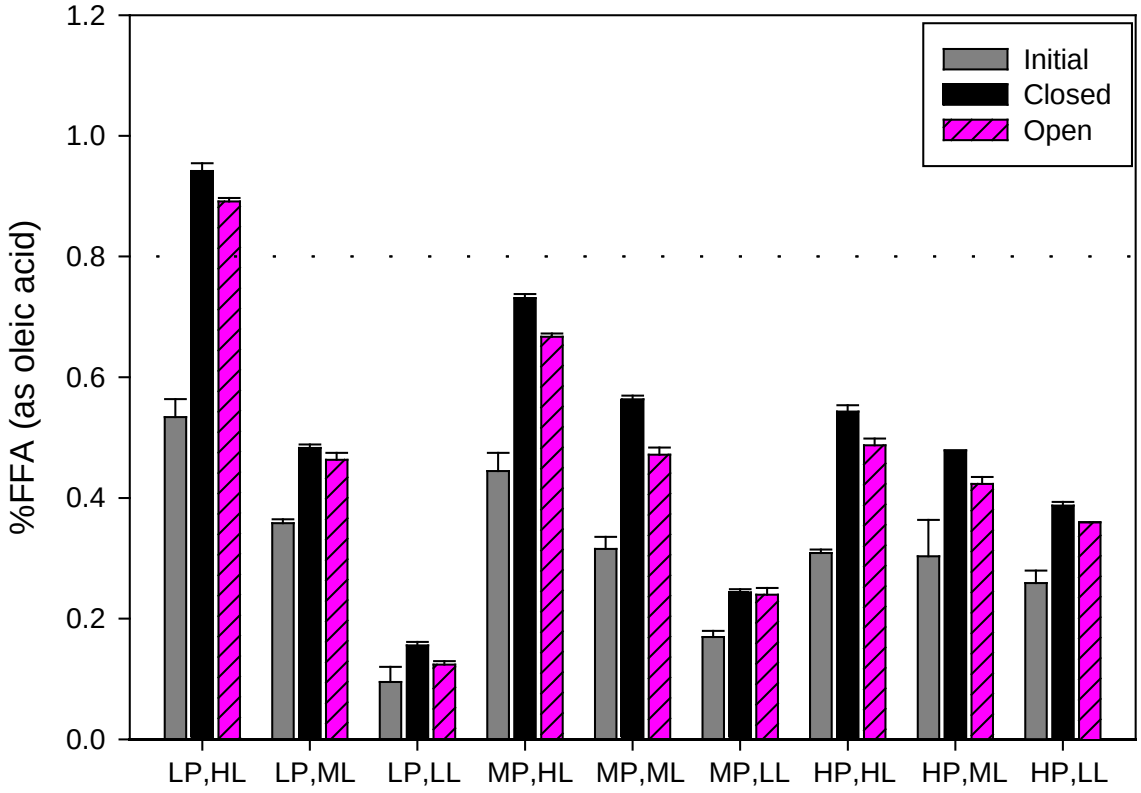

Figure 4.27 Percentage of free fatty acid at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

4.2.5 Pyropheophytin a

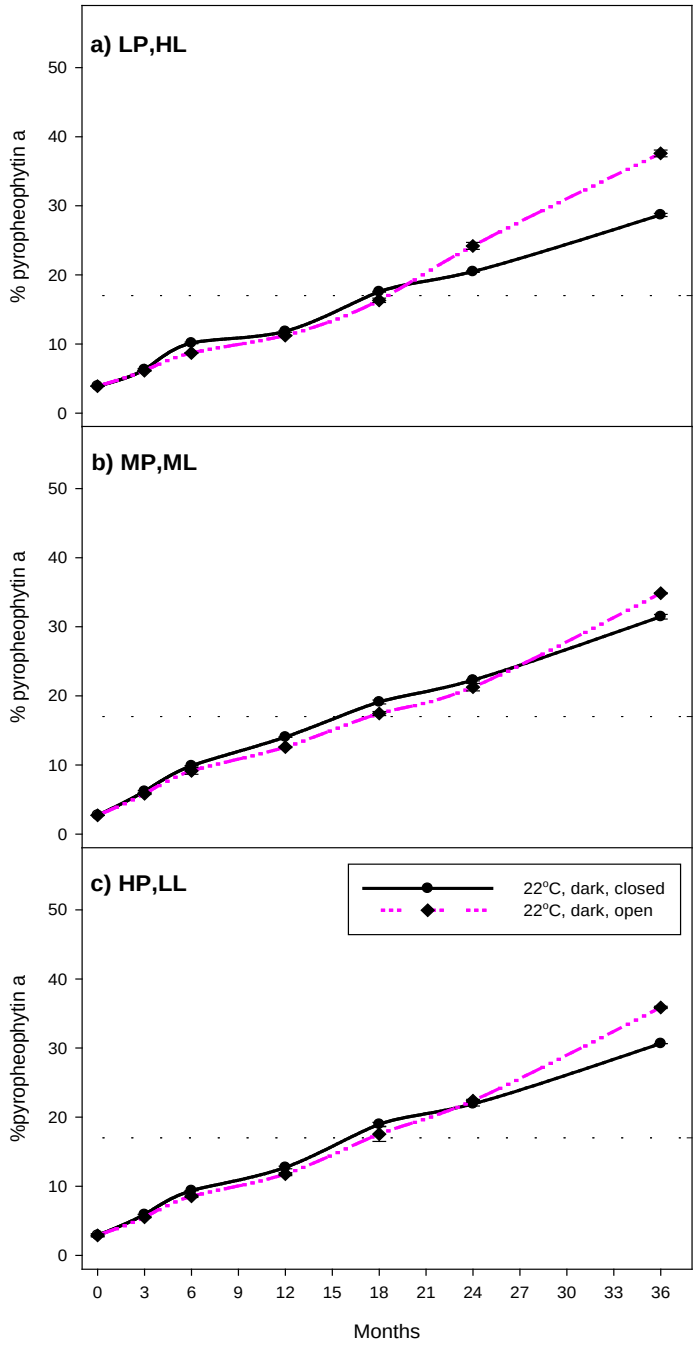

Figure 4.28 Effect of exposure to oxygen on pyropheophytin a content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Pyropheophytin a content increased at a similar rate in both the closed and open bottles for the first 18-24 months, after which time the oils exposed to oxygen increased at a slightly greater rate until the final analysis after 36 months storage. Most oils, regardless of exposure to oxygen, exceeded the Australian limit (≤17%) after about 18 month's storage (Figure 4.28).

There was a significant difference between the final pyropheophytin a content of the open bottles and those which were closed after 36 months storage (Figure 4.29). Generally the oils exposed to oxygen had a higher pyropheophytin a content, for example oil LP,HL which was 28.7% after 36 months closed, while the bottle exposed to oxygen was 37.6%. Only oil MP,LL (40.9% closed, 34.2% open) and oil HP,HL (28.8% closed and 28.2% open) did not follow this trend.

These results were probably due to the action of free radicals on the pigment compounds present in the oil. Polyphenols and α-tocopherols act as antioxidants by reacting with free radicals to help prevent oxidation of the oil. After a period of storage exposed to oxygen (18-24 months), the antioxidants were decreasing due to the reactions with oxygen in the open bottles. Therefore the protective effect from these compounds on the pigments was no longer as strong and the formation of pyropheophytin a continued at a slightly higher rate than those not exposed to oxygen, similar to the findings of Anniva *et al.*, (2006). While the rate of increase was significantly different between those oils exposed to oxygen and those in closed bottles, the effect was not as great as that seen by the influence of temperature on the formation of pyropheophytin a.

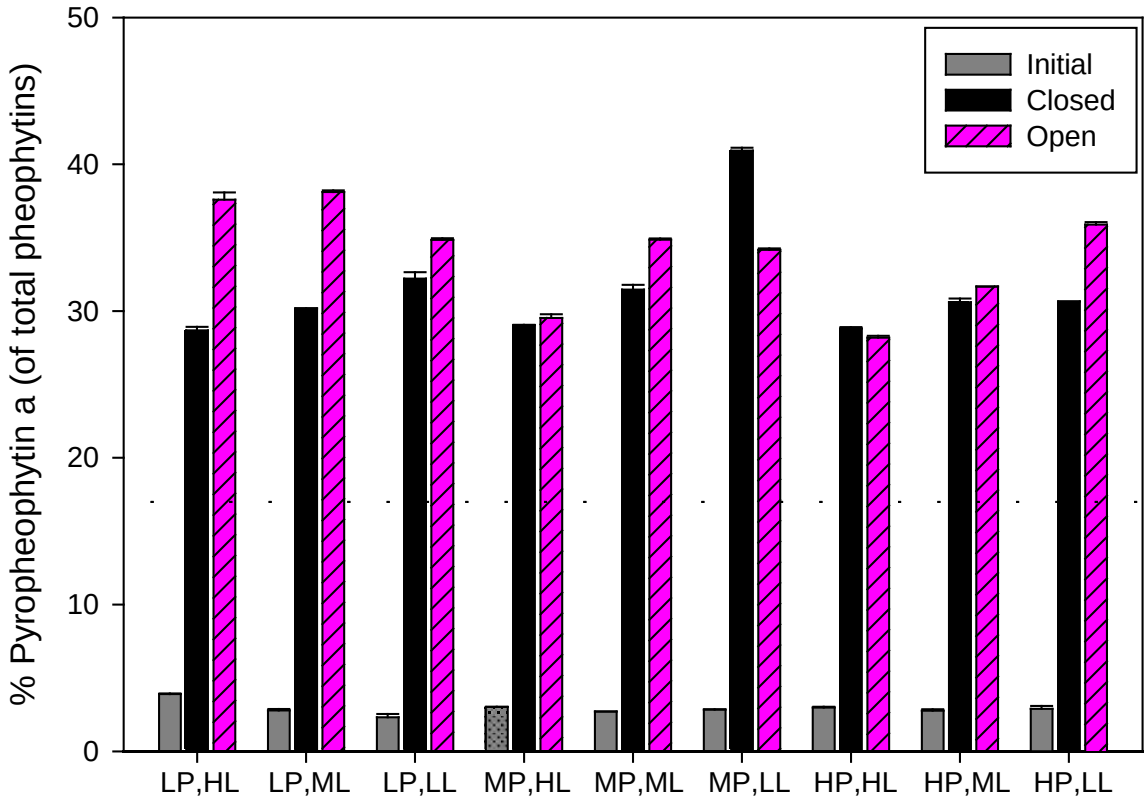

Figure 4.29 Pyropheophytin a concentration at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

4.2.6 1,2-diacylglycerols

Exposure to oxygen had no significant effect on the 1,2-DAG content of the oils in this study (Figure 4.30). As discussed previously (Section 4.1.6), the initial 1,2-DAG content had an impact on the final concentration of 1,2-DAGs with the oils with the highest amount of 1,2-DAGs initially also having the highest levels of 1,2-DAG after 36 months storage (Figure 4.31).

The isomerisation of 1,2-DAGs to 1,3-DAGs was almost identical as the oils aged regardless of exposure to oxygen or not. For example oil MP,ML had a 1,2-DAG concentration of 61.8% initially; after 36 months exposed to oxygen the 1,2-DAG content was 29.8%, the same oil kept closed had a 1,2-DAGs content of 29.8% also. All oils showed the same trend.

Oil LP,LL was the only oil to stay above the Australian Standard limit (≥ 35%) after 36 months storage (Figure 4.31). All other oils were outside the limit at the same time as each other regardless of exposure to oxygen or not. Oils LP,HL; LP,ML; LP,LL and MP,HL were outside the limit after 6-12 months storage, oils MP,ML; HP,HL; HP,ML and HP,LL after 12-24 months and oil MP,LL after 24-36 months.

The changes are due to the isomerisation of 1,2-DAGs to 1,3-DAGs as the oil ages. This reaction is not affected by the presence of oxygen.

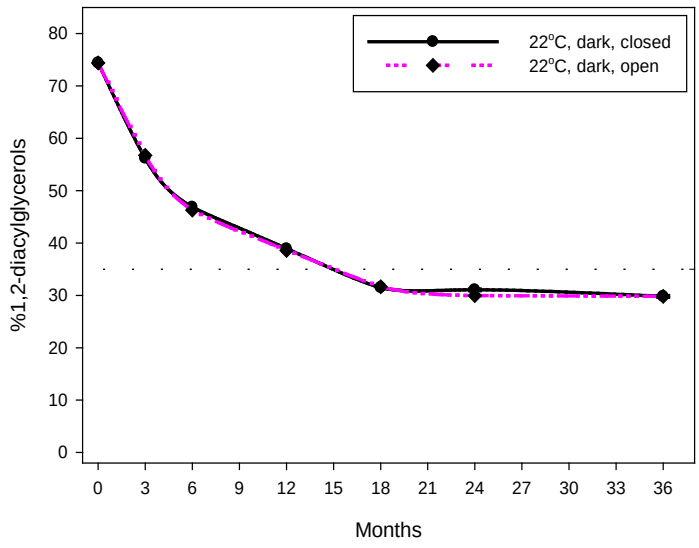

Figure 4.30 Effect of exposure to oxygen on 1,2-diacylglycerol content of different types of MP,ML oil stored for 36 months

MP,ML - Mid polyphenols, Mid linoleic acid. Note - all oils showed similar trends

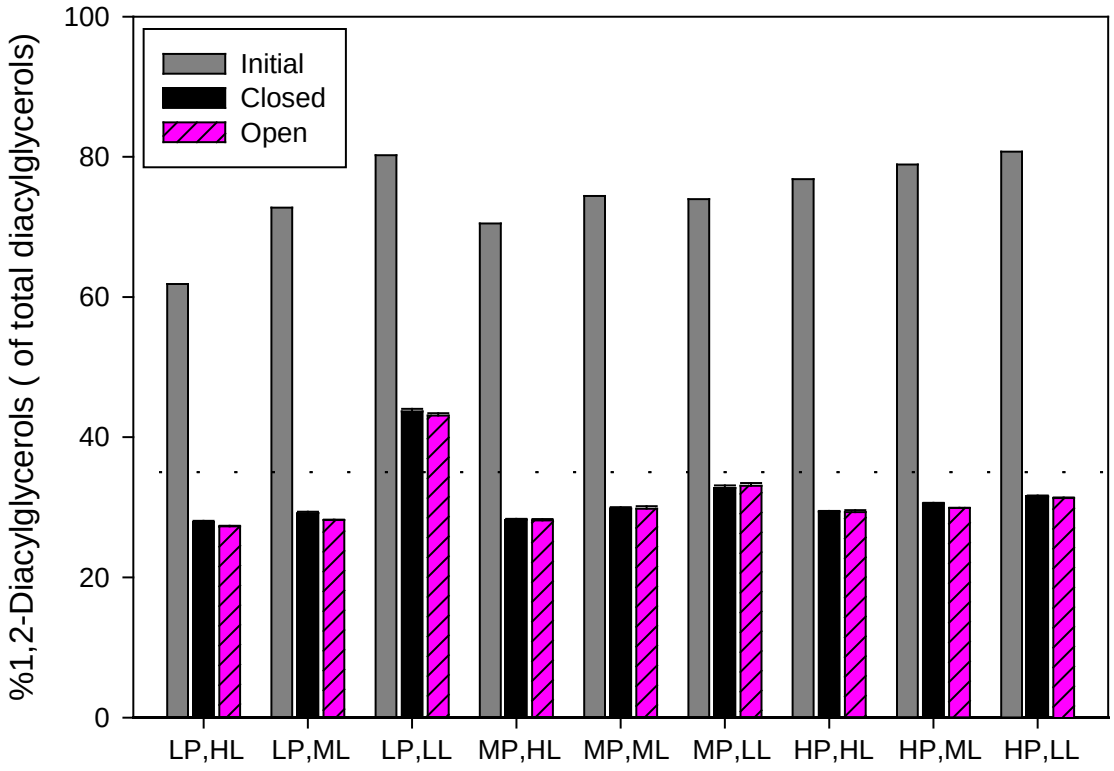

Figure 4.31 1,2-diacylglycerol concentration at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

4.2.7 Total polyphenols

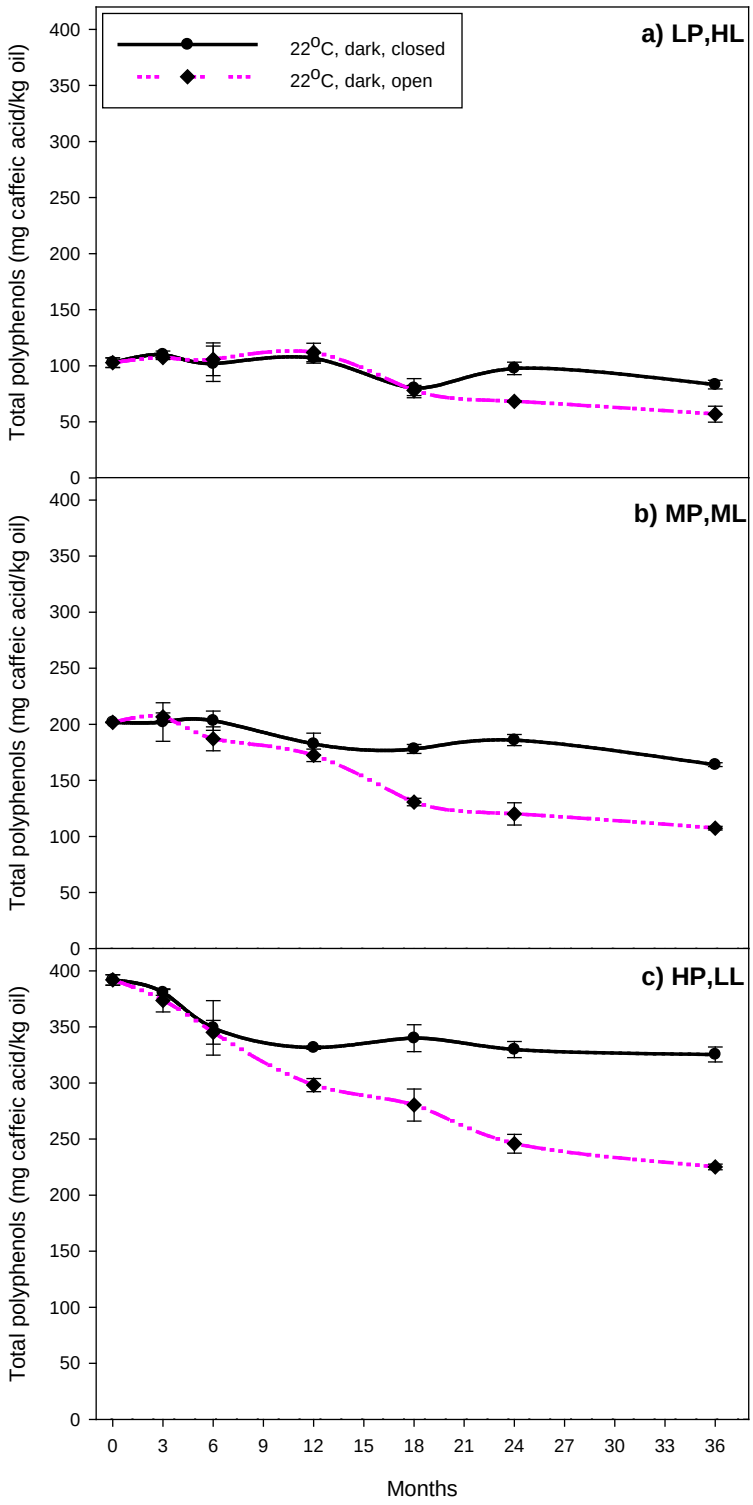

Figure 4.32 Effect of exposure to oxygen on total polyphenol content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Exposure to oxygen had a significant effect on the total polyphenol content of the oils in this study. While the polyphenol content decreased over time in the oils not exposed to oxygen, those which were exposed to oxygen showed a far greater decrease over time (Figure 4.32). Oil MP,ML, when not exposed to oxygen, decreased from 202 mg/kg oil to 164 mg/kg after 36 months; the same oil decreased to 107 mg/kg when exposed to oxygen. All oils showed a similar trend (Figure 4.33).

The percentage loss of total polyphenols over 36 months ranged from 4.6% to 19% of the initial polyphenol content for the oils not exposed to oxygen. Those oil which were exposed to oxygen lost between 34.8% and 52.1% of the initial polyphenol content.

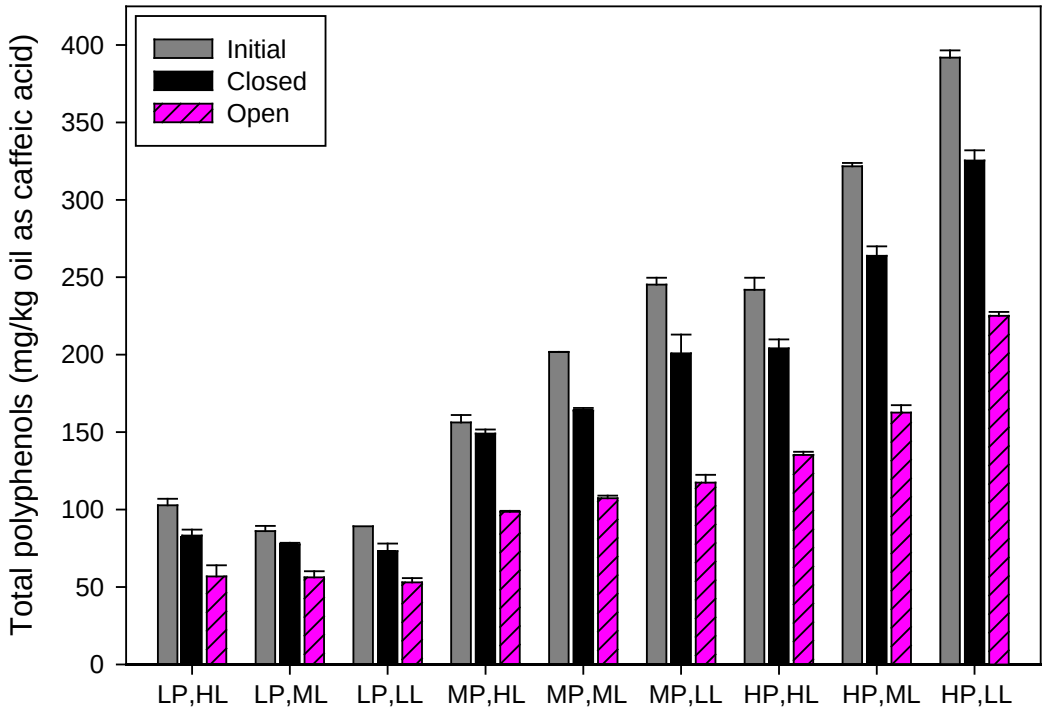

Figure 4.33 Total polyphenol concentration at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

As already mentioned, phenolic compounds are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation (Morello *et al.*, 2004). In this study total polyphenol content of the oils decreased significantly in all oils when exposed to oxygen. As the phenolic compounds are used in the oxidation reaction, the levels are seen to decrease in the oils exposed to oxygen. The initial lag phase (the slower rate of increase) in the peroxide value, K_{232} and K_{268} values in the oils exposed to oxygen in this study can be attributed to the antioxidant properties of the polyphenols present. As the radicals accumulated and the anti-oxidants were consumed in the reaction, the polyphenol content decreased. The phenolic compounds also have a role in the sensory attributes of olive oil, and the loss of these compounds in the oxidation reaction will be discussed further.

4.2.8 α-tocopherol

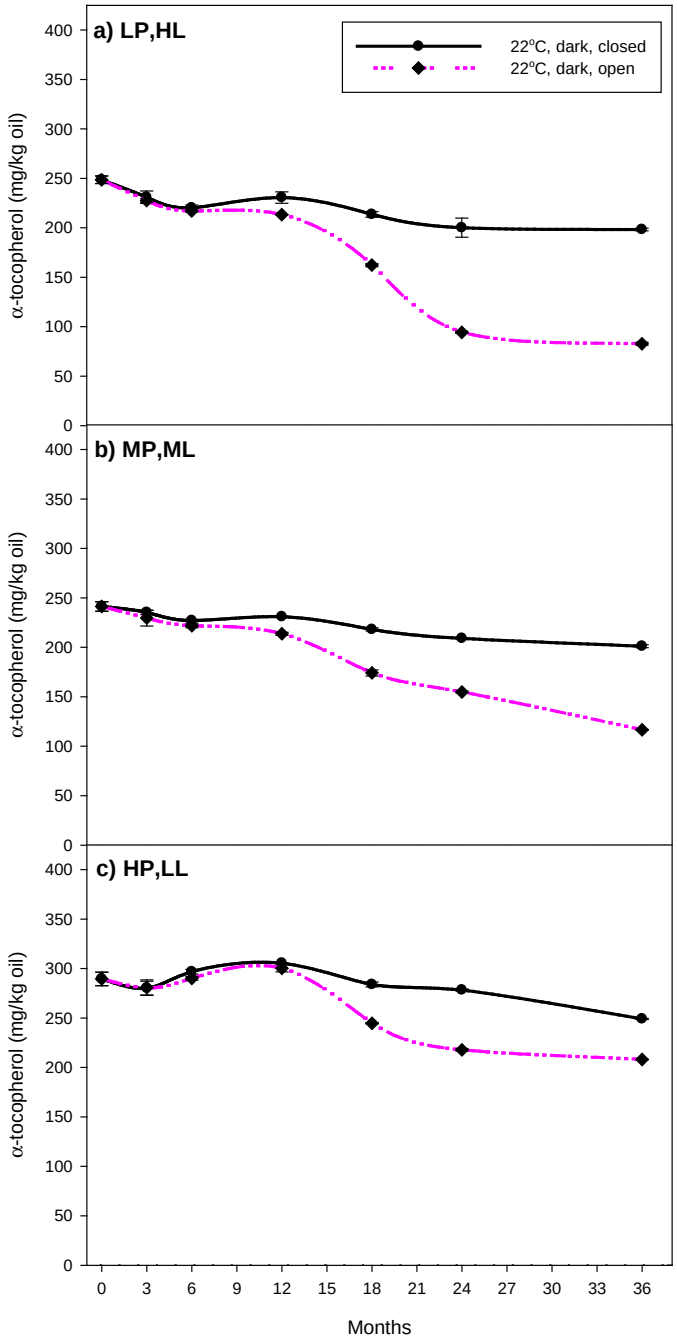

Figure 4.34 Effect of exposure to oxygen on α-tocopherol content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Oxygen exposure was shown to have a significant effect on the α-tocopherol content of the oils. The α-tocopherols decreased slowly in the oils not exposed to oxygen with oil MP,ML decreasing from 241 mg/kg initially to 201 mg/kg after 36 months storage. However when exposed to oxygen the oils decreased at a similar rate to those not exposed for approximately 12 months and then decreased at a

far more rapid rate (Figure 4.34). Oil MP,ML, when exposed to oxygen decreased from 241 mg/kg to 117 mg/kg after 36 months storage (Figure 4.35).

In terms of percentage loss of α-tocopherols, the samples exposed to oxygen lost between 28.2% and 70.0% of the initial α-tocopherol concentration over 36 months storage. Those not exposed to oxygen lost between 2.4% and 30.7% of the total initial α-tocopherol content, except for oil MP,HL which had an unusually high loss at 59.9%.

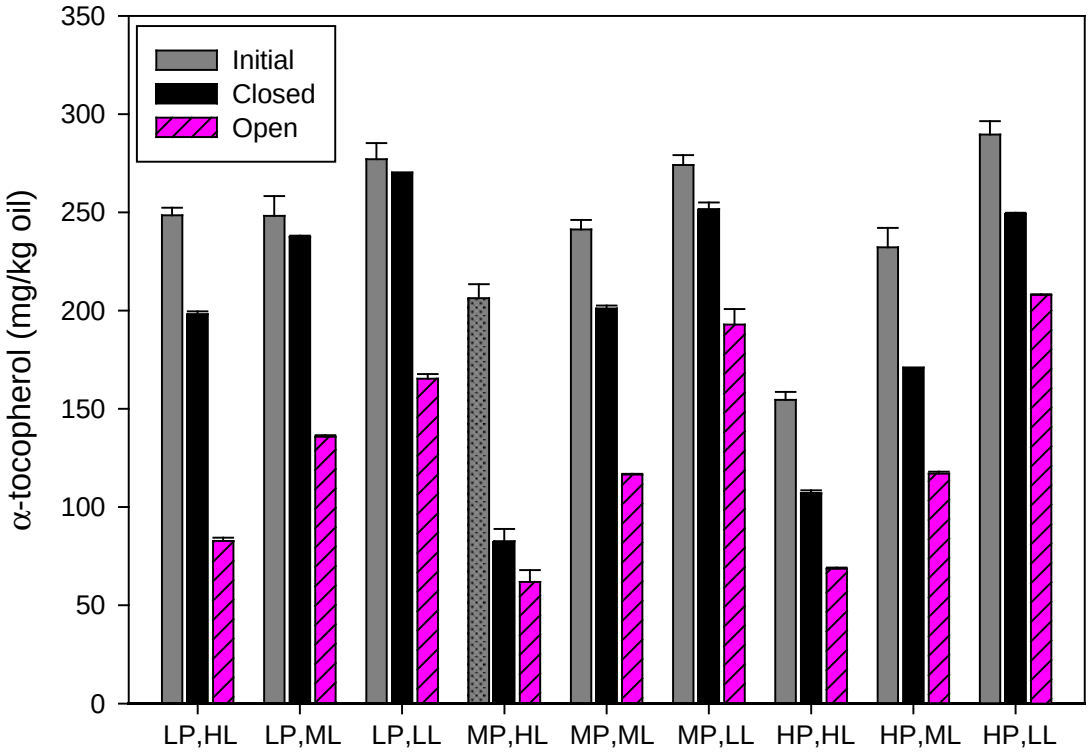

Figure 4.35 α-tocopherol concentration at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

As expected, the degradation rate of α-tocopherol was different for the two storage conditions studied (closed and opened bottles). Those oils not exposed to oxygen decreased at a slow, linear rate for the entire storage period. The α-tocopherol content of the oils which were exposed to oxygen in this study followed a similar trend to the total polyphenol content. In most cases the α-tocopherol content did not decrease substantially for the first 6-12 moths of storage exposed to oxygen. Following this initial lag phase, the α-tocopherol decreased significantly. This mirrored the progress of oxidation as measured by peroxide value, which clearly shows the direct role of α-tocopherol as an anti-oxidant. These trends are very similar to those measured by other researchers (Krichene *et al.*, 2010). Tocopherols are able to transfer hydrogen atoms to lipids during the oxidation process, resulting in tocopheroxy radicals. Tocopheroxy radicals are more stable than lipid peroxy radicals and ultimately slow down the oxidation rate (Choe and Min, 2006).

4.2.9 Chlorophyll

The chlorophyll content of the oils not exposed to oxygen did not change greatly during the 36 months storage period. For example oil MP,ML had a chlorophyll content of 5.5 mg/kg at the beginning of the study and 5.4 mg/kg after 36 months storage. When the oils were exposed to oxygen, there was no effect for 24 months, and then a gradual decline in the chlorophyll content over the last 12 months of storage. On exposure to oxygen, Oil MP,ML was still 5.2 mg/kg after 24 months storage, which decreased to 4.9 mg/kg after 36 months. Other oils showed greater decreases, such as oil HP,LL which decreased from 12.4 mg/kg to 11.1 mg/kg after 36 months exposure to oxygen. The same oil when not exposed to oxygen decreased from 12.4 mg/kg to 12.2 mg/kg over 36 months storage (Figure 4.36). Oil MP,LL was the only oil that did not follow this trend with the oil exposed to oxygen decreasing from 8.4 mg/kg to 7.5 mg/kg; the same oil not exposed to oxygen decreased to 7.3 mg/kg.

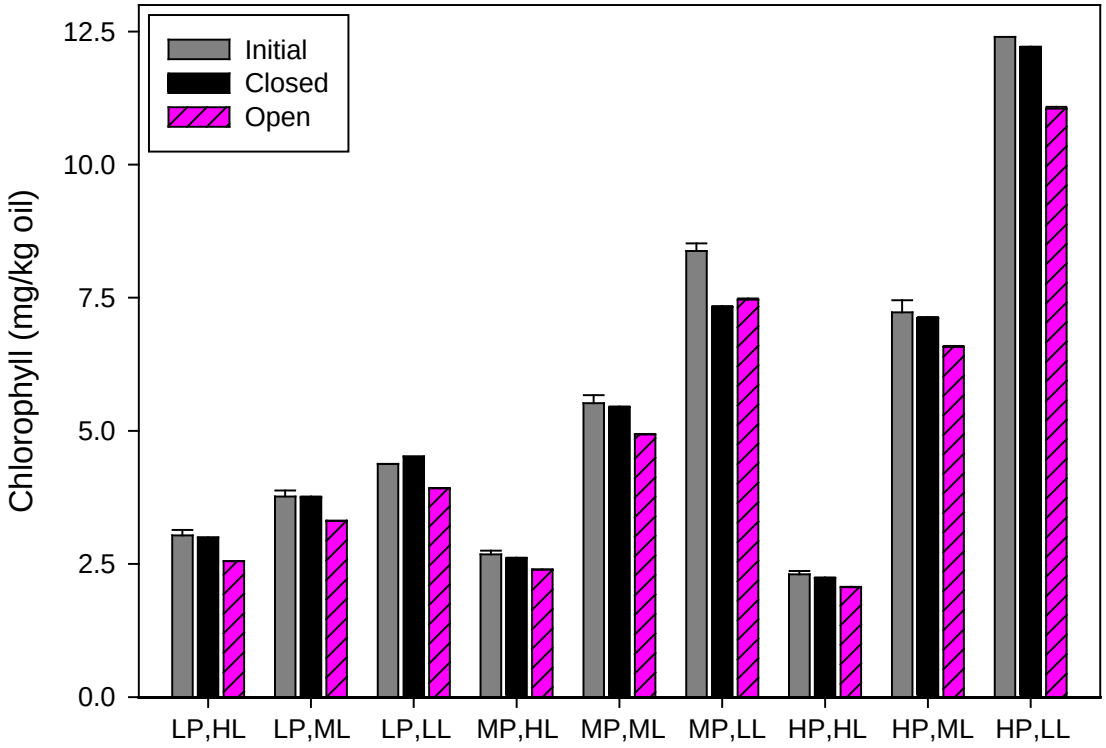

Figure 4.36 Chlorophyll concentration at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

These results are in contradiction to other studies, which concluded that chlorophylls did degrade due to oxidation however no explanation was given for this (Gutierrez *et al.*, 2002b). It is known that chlorophylls act as antioxidants in the dark, which may explain why they decreased slightly after 24 months storage. In this study, until this time (18-24 months storage) the chlorophyll compounds may have been protected by the antioxidants present in the oil (polyphenols and α-tocopherol). Once the antioxidants started to be consumed at a more rapid rate as discussed earlier (Sections 4.2.7 and 4.2.8), this protective effect may have been lessened and therefore the chlorophyll compounds were involved in the oxidation process, indicated by the slight decrease in the last 12 months of analysis.

4.2.10 Colour

Table 4.4 Effect of exposure to oxygen on colour (L*, a*, b) of olive oil samples at initial analysis and after 36 months storage

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

	Storage	L*	a*	b*
LP,HL	Initial	92.0 ± 0.4	-12.6 ± 0.1	75.7 ± 0.7
	closed, 22°C, after 36 months	92.2 ± 0.2	-12.2 ± 0.0	70.8 ± 0.3
	open, 22°C, after 36 months	95.7 ± 0.6	-9.3 ± 0.1	27.4 ± 0.4
LP,ML	Initial	89.8 ± 0.5	-11.6 ± 0.1	98.8 ± 0.2
	closed, 22°C, after 36 months	90.3 ± 0.3	-11.6 ± 0.0	96.0 ± 0.3
	open, 22°C, after 36 months	92.1 ± 0.4	-12.8 ± 0.0	72.2 ± 0.3
LP,LL	Initial	88.4 ± 0.3	-10.1 ± 0.0	113.6 ± 0.2
	closed, 22°C, after 36 months	89.1 ± 0.4	-10.0 ± 0.1	113.1 ± 0.4
	open, 22°C, after 36 months	89.6 ± 0.1	-11.3 ± 0.1	99.4 ± 0.1
MP,HL	Initial	91.7 ± 0.4	-12.4 ± 0.0	79.3 ± 0.4
	closed, 22°C, after 36 months	92.7 ± 0.1	-12.4 ± 0.1	75.1 ± 0.0
	open, 22°C, after 36 months	93.7 ± 0.0	-11.7 ± 0.0	51.1 ± 0.0
MP,ML	Initial	87.8 ± 0.2	-10.0 ± 0.0	109.7 ± 0.4
	closed, 22°C, after 36 months	88.2 ± 0.1	-10.1 ± 0.0	105.4 ± 0.1
	open, 22°C, after 36 months	89.4 ± 0.2	-11.9 ± 0.0	84.1 ± 0.0
MP,LL	Initial	84.7 ± 0.2	-7.3 ± 0.0	122.9 ± 0.2
	closed, 22°C, after 36 months	86.1 ± 0.1	-9.8 ± 0.0	104.4 ± 0.1
	open, 22°C, after 36 months	86.2 ± 0.0	-9.4 ± 0.0	109.6 ± 0.0
HP,HL	Initial	92.1 ± 0.2	-12.2 ± 0.0	83.0 ± 0.1
	closed, 22°C, after 36 months	92.7 ± 0.1	-12.2 ± 0.0	79.0 ± 0.0
	open, 22°C, after 36 months	94.1 ± 0.1	-12.3 ± 0.1	59.4 ± 0.1
HP,ML	Initial	86.0 ± 0.1	-8.5 ± 0.1	117.2 ± 0.2
	closed, 22°C, after 36 months	86.5 ± 0.1	-8.5 ± 0.5	112.4 ± 0.2
	open, 22°C, after 36 months	88.5 ± 0.2	-11.2 ± 0.0	90.3 ± 0.2
HP,LL	Initial	81.7 ± 0.6	-5.5 ± 0.1	126.0 ± 0.7
	closed, 22°C, after 36 months	82.3 ± 0.1	-5.3 ± 0.0	124.6 ± 0.1
	open, 22°C, after 36 months	83.4 ± 0.3	-7.3 ± 0.0	115.0 ± 0.3

Exposure to oxygen had a significant effect on the chromic co-ordinates (colour) of the oils in this study. Luminosity (L*) increased between 1.2 and 3.7 units when exposed to oxygen, while those not exposed to oxygen increased between 0.2 and 1.4 units (Table 4.4).

"Greenness" (a*) was generally the same or very similar after 36 months storage not exposed to oxygen (except for oil MP,LL which did decrease - the same oil which did not follow the trend for chlorophyll content). However exposure to oxygen saw this co-ordinate decrease by between 0.1 and 2.9 units. Some oils increased slightly – oil LP,HL by 3.3 units and oil MP,HL by 0.7 units.

Yellowness (b*) decreased between 0.5 and 4.9 units when not exposed to oxygen and 11 and 48.3 units when exposed to oxygen. Again oil MP,LL did not follow this trend, decreasing 18.5 units in the closed bottle and 13.3 units when exposed to oxygen.

Pristouri *et al.*, (2010) found that there was no influence of oxidation on the chromatic coordinates after 12 months storage which is different to these findings. There are numerous compounds responsible for the colour in olive oil. As chlorophyll changed only slightly in the oils exposed to oxygen (Section 5.9), changes in some other compounds may be responsible for the changes observed here such as carotenoids, xanthophylls and lutein, which is beyond the scope of this project.

4.2.11 Fatty acid profile

Table 4.5 Effect of exposure to oxygen on fatty acid profile of olive oil samples at initial analysis and after 36 months storage

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

	Storage	Sat	Mono	Poly
LP,HL	Initial	18.0 ± 0.0	64.8 ± 0.0	17.2 ± 0.0
	nil oxygen, 22°C, after 36 months	17.9 ± 0.1	64.9 ± 0.0	17.3 ± 0.0
	oxygen, 22°C, after 36 months	18.1 ± 0.0	66.0 ± 0.0	15.9 ± 0.0
LP,ML	Initial	16.9 ± 0.0	72.1 ± 0.0	11.0 ± 0.0
	nil oxygen, 22°C, after 36 months	17.1 ± 0.0	72.0 ± 0.0	11.0 ± 0.0
	oxygen, 22°C, after 36 months	16.7 ± 0.0	73.2 ± 0.0	10.1 ± 0.0
LP,LL	Initial	15.8 ± 0.0	79.4 ± 0.0	4.8 ± 0.0
	nil oxygen, 22°C, after 36 months	15.4 ± 0.0	79.8 ± 0.0	4.8 ± 0.0
	oxygen, 22°C, after 36 months	15.5 ± 0.0	80.2 ± 0.0	4.3 ± 0.0
MP,HL	Initial	17.4 ± 0.0	64.1 ± 0.0	18.5 ± 0.0
	nil oxygen, 22°C, after 36 months	17.4 ± 0.0	64.0 ± 0.0	18.5 ± 0.0
	oxygen, 22°C, after 36 months	17.4 ± 0.0	64.9 ± 0.0	17.7 ± 0.0
MP,ML	Initial	16.0 ± 0.0	71.6 ± 0.0	12.4 ± 0.0
	nil oxygen, 22°C, after 36 months	15.9 ± 0.0	71.5 ± 0.0	12.6 ± 0.0
	oxygen, 22°C, after 36 months	16.0 ± 0.0	72.3 ± 0.0	11.7 ± 0.0
MP,LL	Initial	14.7 ± 0.0	78.9 ± 0.0	6.4 ± 0.0
	nil oxygen, 22°C, after 36 months	14.8 ± 0.0	78.9 ± 0.0	6.3 ± 0.0
	oxygen, 22°C, after 36 months	14.7 ± 0.0	79.3 ± 0.0	5.9 ± 0.0
HP,HL	Initial	16.5 ± 0.0	63.6 ± 0.1	19.9 ± 0.0
	nil oxygen, 22°C, after 36 months	16.6 ± 0.0	63.5 ± 0.0	19.8 ± 0.0
	oxygen, 22°C, after 36 months	16.6 ± 0.0	63.9 ± 0.0	19.4 ± 0.0
HP,ML	Initial	15.0 ± 0.0	71.1 ± 0.0	13.9 ± 0.0
	nil oxygen, 22°C, after 36 months	15.0 ± 0.0	71.2 ± 0.0	13.9 ± 0.0
	oxygen, 22°C, after 36 months	15.3 ± 0.0	71.5 ± 0.0	13.2 ± 0.0
HP,LL	Initial	13.5 ± 0.0	78.5 ± 0.0	8.0 ± 0.0
	nil oxygen, 22°C, after 36 months	13.6 ± 0.0	78.4 ± 0.0	8.0 ± 0.0
	oxygen, 22°C, after 36 months	13.6 ± 0.0	78.6 ± 0.0	7.7 ± 0.0

Note: Sat - Saturated fatty acids, Mono – Monounsaturated fatty acids, Poly – Polyunsaturated fatty acids.

Saturated fatty acids in the olive oils in this study were generally unaffected by exposure to oxygen, with most oils having the same or similar saturated fatty acid content after 36 months storage as they did initially (Table 4.5).

Monounsaturated fatty acids, in particular oleic acid (C18:1), increased by between 0.1% and 1.9% when the oils were exposed to oxygen, dependent on the initial fatty acid composition and the

antioxidants present in the oil. For example oil LP,HL, with the lowest polyphenol content and the highest polyunsaturated fatty acid content showed the greatest increase in monounsaturated fatty acid content. The monounsaturated fatty acid content did not significantly change in the samples not exposed to oxygen.

Polyunsaturated fatty acids decreased in the oils exposed to oxygen, between 0.3 and 1.3%. Almost all of the change in the polyunsaturated fatty acid content can be attributed to the breakdown of linoleic acid (C18:2). Those oils not exposed to oxygen did not change significantly.

In the study of Morello *et al.*, (2004), an increase in the oleic acid contents in commercial olive oil of the Arbequina cultivar was observed, similar to the results found in this study. This occurred because oils with greater unsaturation (polyunsaturates) oxidised more quickly than those with less double bonds (monounsaturates). As the degree of unsaturation increased, the production of peroxides and primary oxidation compounds formed at a greater rate. This is very well illustrated in Figure 4.21, as the oils with the highest polyunsaturated level (Oils LP,HL; MP,HL and HP,HL) showing the greatest increase in the formation of peroxides, and those oils with the lowest level of polyunsaturates (Oils LP,LL; MP,LL and HP,LL) showed the least increase.

4.2.12 Induction time

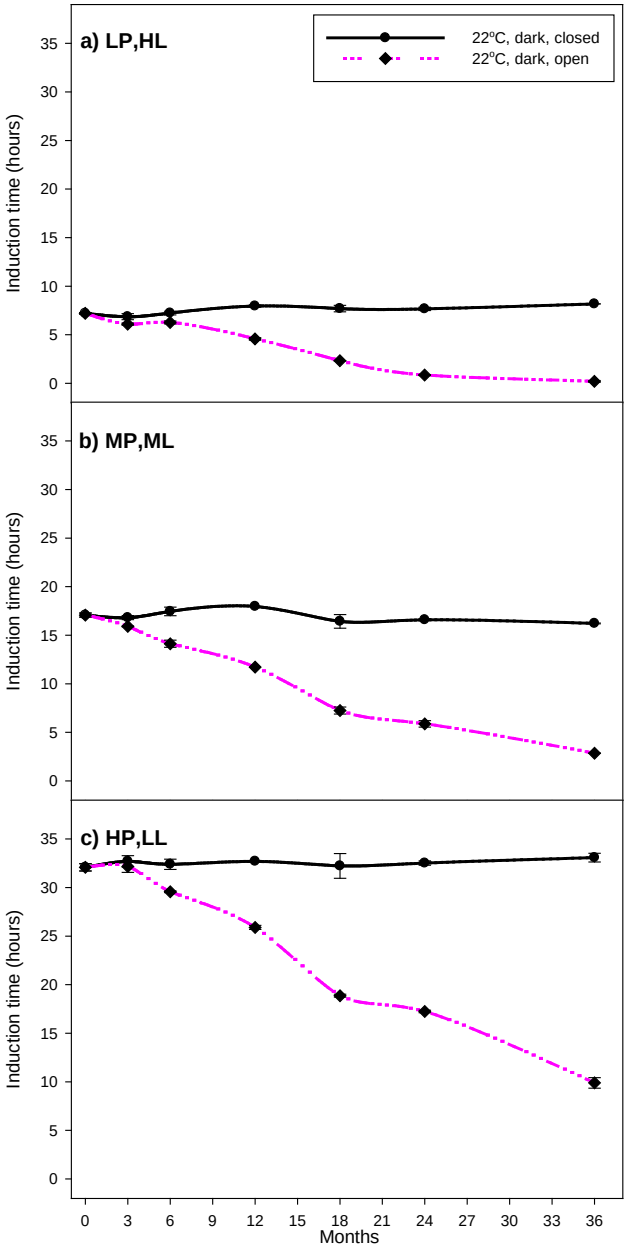

Figure 4.37 Effect of exposure to oxygen on induction time of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Induction time did not change significantly when the oils were not exposed to oxygen during the 36 months storage period. For example, oil MP,ML changed from 17.1 hours initially to 16.2 hours, while oil HP,LL changed from 32.1 to 33.1 hours (Figure 4.37).

However, when exposed to oxygen oil MP,ML decreased from 17.1 hours to 2.9 hours after 36 months exposure, while oil HP,LL decreased from 32.1 to 9.9 hours. Oil LP,HL, which had an initial induction time of 7.2 hours decreased to just 0.2 hours after 36 months storage exposed to oxygen (Figure 4.38).

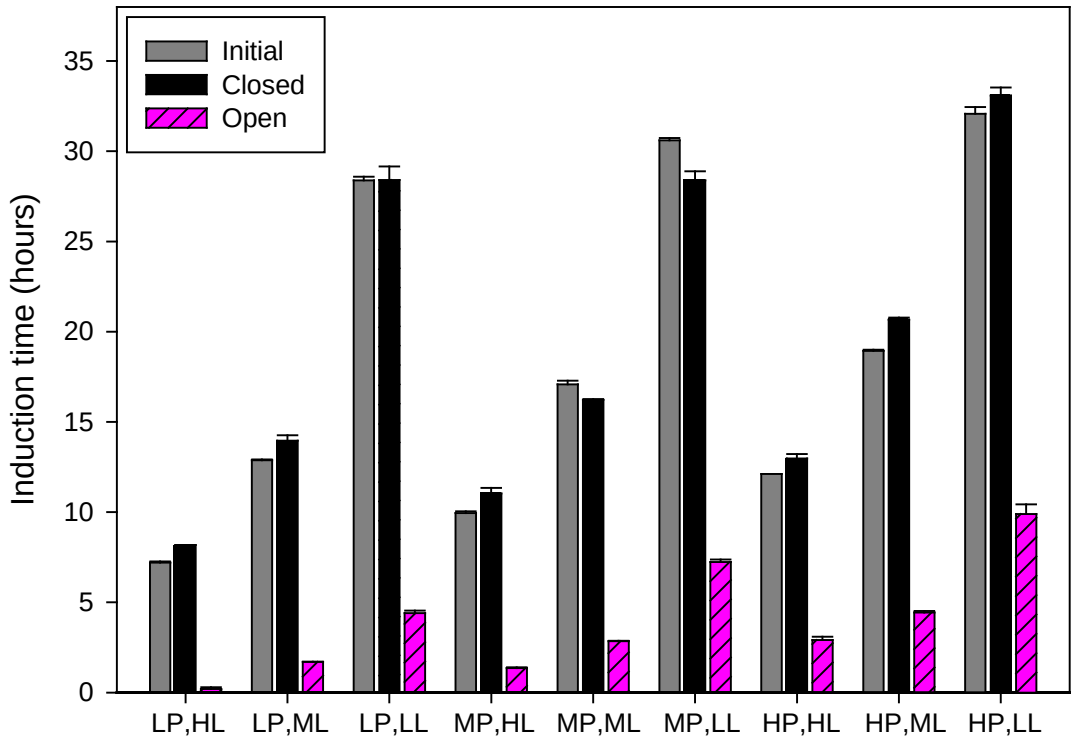

Figure 4.38 Induction time at initial analysis and after 36 months, closed and exposed to oxygen for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

These results are due to a number of factors. As described in detail already, the oils which were exposed to oxygen showed that oxidation is proceeding at a rapid rate. Antioxidants such as polyphenol and α-tocopherol were depleted as the oils were exposed to oxygen over time, while at the same time peroxides and then secondary oxidation products were formed. As a consequence the oil were less able to resist the oxidation, and therefore oxidation progressed at an accelerated rate. At each analysis time, the elevated peroxide value and the already formed oxidation products in the oil are immediately measured by the Rancimat, reducing the induction time of the oil. These results show the importance of reducing the exposure of oils to oxygen, as it greatly reduces the shelf life of the oil.

4.2.13 Organoleptic assessment

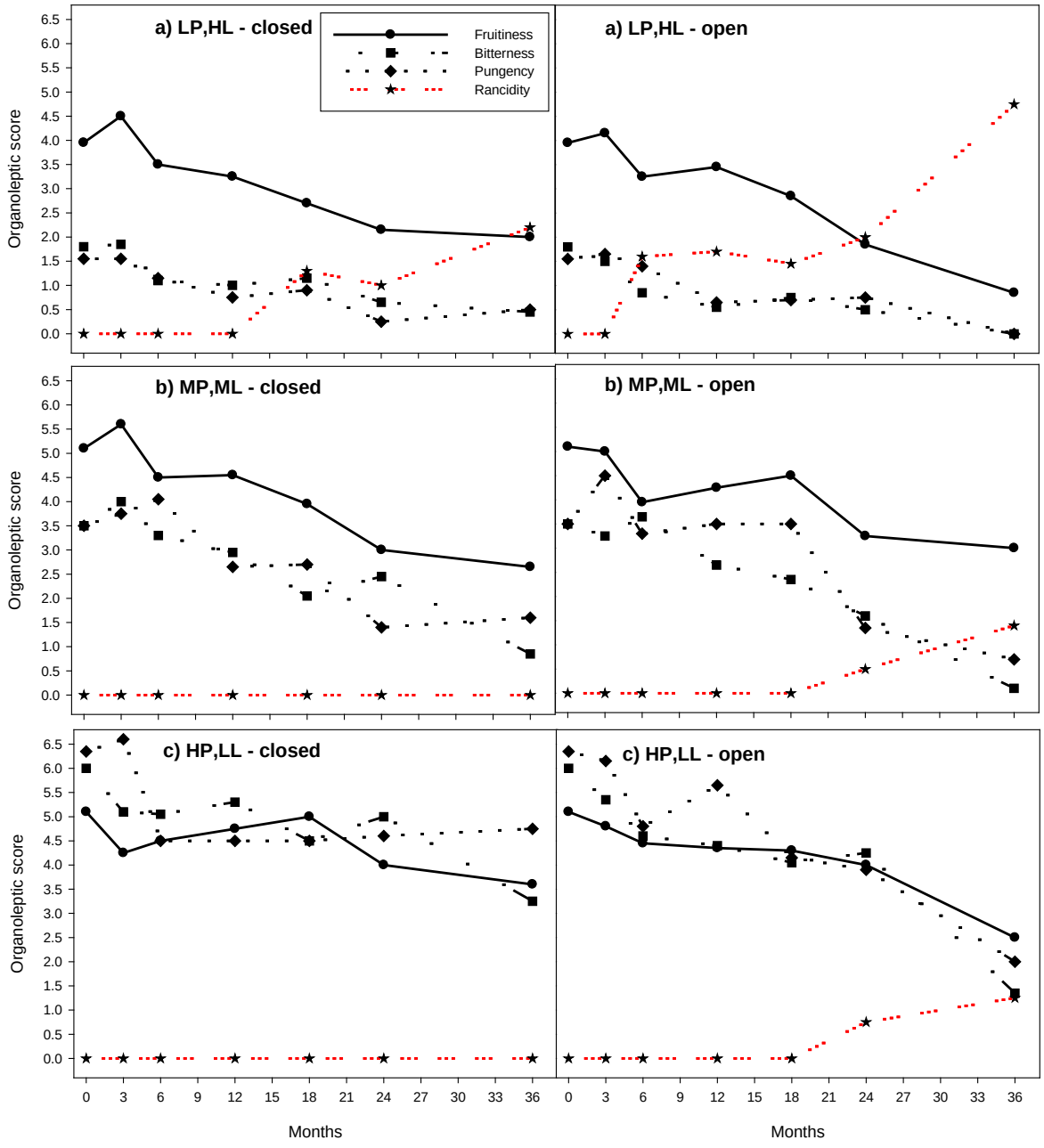

Figure 4.39 Organoleptic assessment of olive oils over 36 months, closed and exposed to oxygen

a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

The results from this project show that exposure to oxygen had a significant effect on the sensory profile of the oils. The oil with the least stability, Oil LP,HL, almost immediately showed signs of rancidity when exposed to oxygen while the same oil when not exposed to oxygen was found to maintain its extra virgin olive oil status for 12 months. The decrease in the polyphenol content also had an affect of the positive attributes found in the oil, with fruitiness, bitterness and pungency decreasing at a faster rate when the oil was exposed to oxygen.

Exposure to oxygen had a significant effect on the sensory quality of the oils in this study. Oils exposed to oxygen all showed signs of rancidity earlier than those in closed bottles, with the less stable oils (oils LP,HL and LP,ML) rancid after only 6 months storage exposed to oxygen. The same oils kept in closed bottles did not show rancidity until after 12 months of storage. Some of the more stable oils such as oil HP,LL, did not show any rancidity for the entire 36 months storage when not exposed to oxygen, however the same oils were rancid between 18 and 24 months of storage when exposed to oxygen (Figure 4.39).

All of the oils showed a greater degree of rancidity after 36 months storage when exposed to oxygen (Table 4.6)

In general, the positive attributes of fruitiness, bitterness and pungency decreased in all samples, however the samples exposed to oxygen saw these attributes decrease at a greater rate and to a greater extent.

As oils oxidise, hydroperoxides are formed which further decompose into secondary oxidation products. It is these secondary oxidation products which are mainly responsible for the rancid attribute that is characteristic of oxidised oil. These compounds which produce the undesirable characteristics include aldehydes, ketones, alcohols, hydrocarbons and esters.

A number of the oils studied did not develop rancidity for the entire analysis period when kept in closed bottles, and the same oils were rancid if exposed to oxygen. This data confirms the importance of correct storage condition in maintaining the extra virgin status of olive oil.

Table 4.6 Organoleptic assessment of olive oil samples at initial analysis and after 36 months, closed and exposed to oxygen

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

	Storage	Fruitiness	Bitterness	Pungency	Rancid
LP,HL	Initial	3.95	1.80	1.55	0.00
	closed, 22°C, after 36 months	2.00	0.45	0.50	2.20
	open, 22°C, after 36 months	0.85	0.00	0.00	4.75
LP,ML	Initial	3.85	1.90	1.20	0.00
	closed, 22°C, after 36 months	2.75	0.75	0.75	0.75
	open, 22°C, after 36 months	2.25	0.25	0.70	2.20
LP,LL	Initial	5.00	2.05	2.05	0.00
	closed, 22°C, after 36 months	3.25	0.70	0.80	0.50
	open, 22°C, after 36 months	2.75	0.20	0.25	2.50
MP,HL	Initial	5.30	3.00	3.50	0.00
	closed, 22°C, after 36 months	1.25	0.75	0.40	2.65
	open, 22°C, after 36 months	2.40	0.40	0.70	3.45
MP,ML	Initial	5.10	3.50	3.50	0.00
	closed, 22°C, after 36 months	2.65	0.85	1.60	0.00
	open, 22°C, after 36 months	3.00	0.10	0.70	1.40
MP,LL	Initial	5.00	4.45	5.00	0.00
	closed, 22°C, after 36 months	3.00	1.15	1.45	0.25
	open, 22°C, after 36 months	2.95	0.90	1.25	0.60
HP,HL	Initial	5.50	4.50	4.00	0.00
	closed, 22°C, after 36 months	4.00	1.40	2.30	1.35
	open, 22°C, after 36 months	2.90	0.75	1.00	2.35
HP,ML	Initial	5.90	4.80	5.65	0.00
	closed, 22°C, after 36 months	3.75	1.85	2.40	0.50
	open, 22°C, after 36 months	3.15	0.80	1.10	3.15
HP,LL	Initial	5.10	6.00	6.35	0.00
	closed, 22°C, after 36 months	3.60	3.25	4.75	0.00
	open, 22°C, after 36 months	2.50	1.35	2.00	1.25

4.3 Effect of exposure to light

4.3.1 Peroxide value

The peroxide value of the oils stored in the dark decreased at a linear rate throughout the storage period. For example oil MP,ML decreased from 12 mEq oxygen/kg oil initially to 7 mEq oxygen/kg oil after 36 months storage. However the same oil stored exposed to light decreased from 12 mEq oxygen/kg oil to 8 mEq oxygen/kg oil after 18 months, increased to 10 mEq oxygen/kg oil at 24 months and then decreased to 6 mEq oxygen/kg oil after 36 months exposed to light. All oils followed a similar pattern, that is the oil in the dark decreased linearly, while the sample in the light deviated around the values found in the oil kept in the dark (Figure 4.40). None of the oils (dark or exposed to light) exceeded the Australian and IOC limit (≤20 mEq oxygen/kg oil) at any stage.

These results are similar to those of Caponio *et al.*, (2005), although that study was for 12 months only.

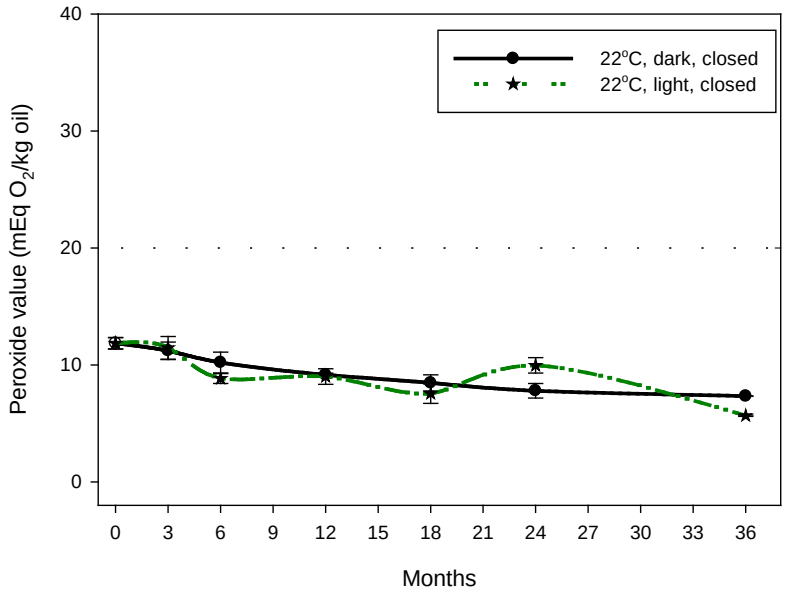

Figure 4.40 Effect of exposure to light on peroxide value of olive oil stored for 36 months

MP,ML: Mid polyphenols, Mid linoleic acid olive oil. All other oils showed similar patterns.

4.3.2 UV absorbance K_{232}

Exposure to light had little effect on the K_{232} of the olive oil. All oils showed a similar pattern, with K_{232} generally constant throughout the storage period, and no significant difference between the storage conditions (Figure 4.41).

These results could indicate that primary oxidation products were not produced in the oils exposed to light. However on closer investigation of the influence of light on secondary oxidation products, (K_{268}), this could not be the case. It is more likely that in the presence of light, oxidation products were quickly converted to secondary oxidation products.

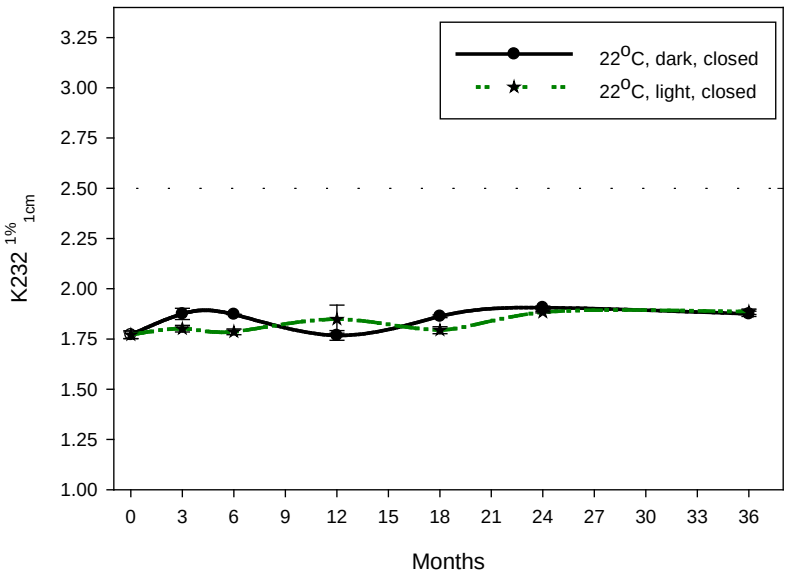

Figure 4.41 Effect of exposure to light on K_{232} of olive oil stored for 36 months

MP,ML: Mid polyphenols, Mid linoleic acid olive oil. All other oils showed similar patterns.

4.3.3 UV absorbance K_{268}

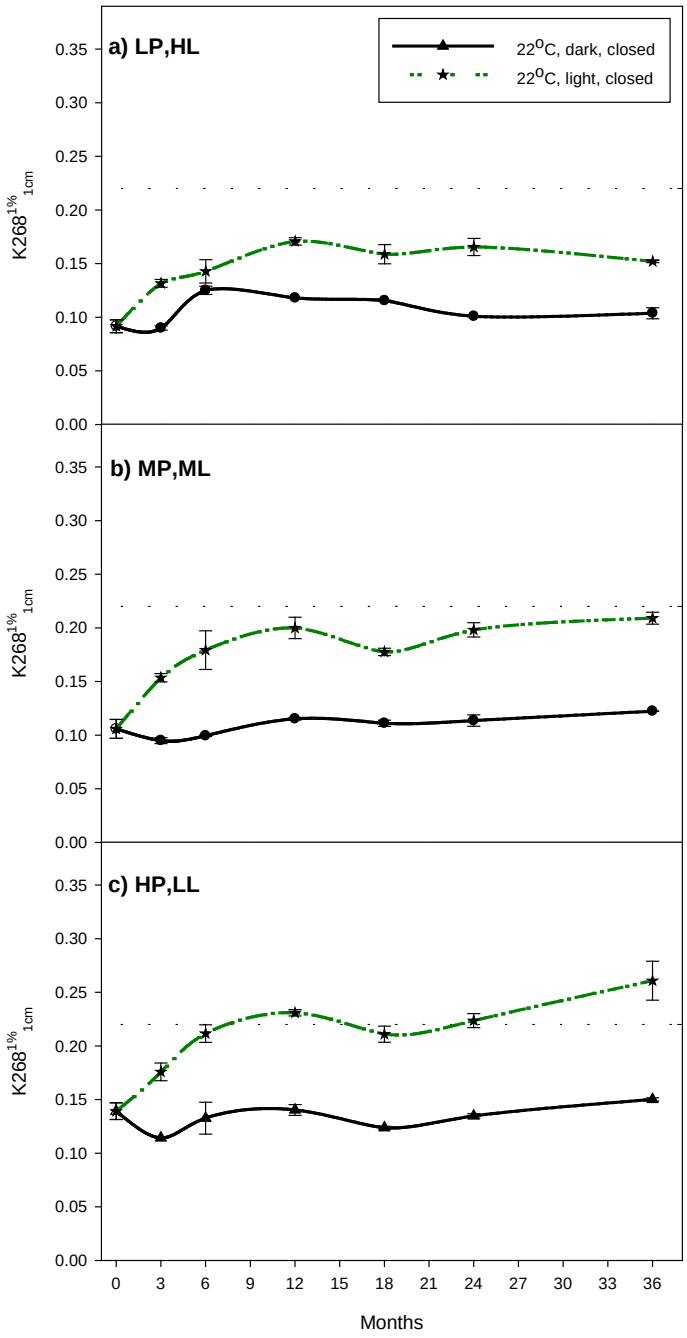

Figure 4.42 Effect of exposure to light on K_{268} of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Exposure to light had a significant effect on the UV absorbance at 268nm (K_{268}). Almost immediately after storage conditions were imposed, the K_{268} increased in all oils at a greater rate from 0 to 6 months storage, followed by little deviation until about 24 months storage, after which there was another slight increase (Figure 4.42). As an example oil MP,ML, when stored in the dark, increased from 0.11 to 0.12 over the storage period. The same oil when exposed to light increased from 0.11 to 0.21 during the storage period.

Only Oil HP,LL exceeded the IOC and Australian limit (≤ 0.22) when exposed to light (Figure 4.43)

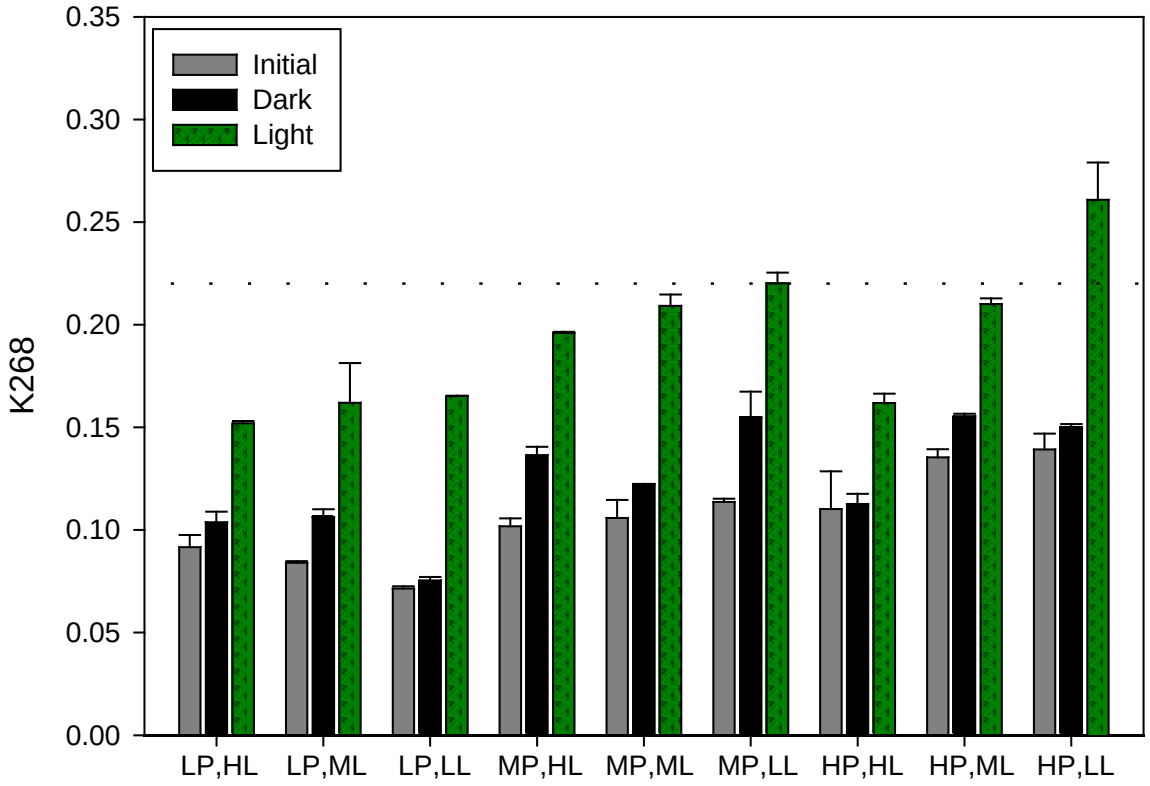

Figure 4.43 **K_{268} at initial analysis and after 36 months, closed and exposed to light for different types of olive oil**

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

These results indicate that in the samples exposed to light, degradation of the primary oxidation compounds was more rapid than those in the dark. These results are almost identical to those of Caponio *et al.*, (2005) and Bilancia *et al.*, (2007). This result confirms the catalytic effect that light has on the propagation of oxidation in olive oil. It also indicates the slight antioxidant effect of chlorophyll in samples kept in the dark.

4.3.4 Free Fatty Acids

Exposure to light had little effect on free fatty acid content of the oils in this study. The %FFA increased gradually and at a linear rate regardless of storage conditions. When stored in the dark, the %FFA of Oil MP,ML increased from 0.32 to 0.56%, while the same oil stored exposed to light increased to 0.58% FFA (Figure 4.44). All oils showed similar a pattern.

These results are in agreement with those of Bilancia *et al.*, (2007) and Caponio *et al.*, (2005).

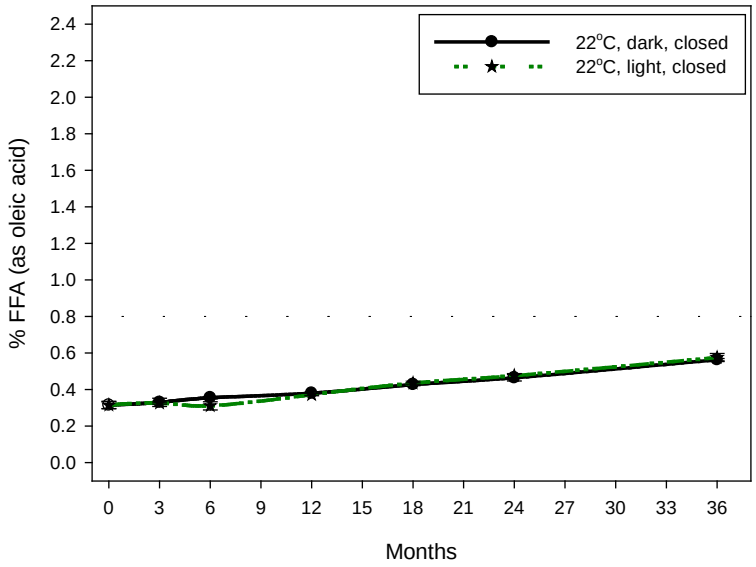

Figure 4.44 Effect of exposure to light on free fatty acid concentration of olive oil stored for 36 months

MP,ML: Mid polyphenols, Mid linoleic acid olive oil. All other oils showed similar patterns.

4.3.5 Pyropheophytin a

Exposure to light had a significant effect on the measurement of pyropheophytin a in this study. After the initial analysis pyropheophytin a was only detectable in oil (HP,LL) after three months storage. At the next analysis point (6 months) pyropheophytin a was undetectable in this oil also. In the oils exposed to light, not only was pyropheophytin a not detected – none of the usual peaks were observed (pheophytin a, pheophytin a` and pyropheophtyin). This is due to the complete breakdown of chlorophylls and all of its derivatives when exposed to light.

This is an important result as the measurement of pyropheophytin a in olive oil is used as an indication of aging, and the Australian standard indicates a maximum level of 17%. The total absence of any detectable amounts of pigments, including pyropheophytin a after just 3 months in most oils in this study indicates that this measurement cannot be used in isolation to interpret the status of an oil. If pyropheophytins are totally absent from oils this would be a good indicator of the improper storage of oil (exposed to light). It may be useful to use this information in tandem with the measurement of K_{268} to indicate the exposure of oil to light. If pyropheophytin a and the other compounds (pheophytins) usually seen in chromatograms from this analysis are absent, and the K_{268} value is high it is likely that at some point the oil being analysed was exposed to light.

4.3.6 1,2-diacylglycerols

No significant difference was observed in the percentage of 1,2-DAGs between the oils kept in the dark and those exposed to light, as observed by other researchers (Caponio *et al.*, 2005)

4.3.7 Total polyphenols

Exposure to light had no significant influence on the polyphenol content of the oils in this study. All oils showed a decrease in the polyphenol content over time. For example, Oil MP,ML decreased from 202 mg/kg to 164 mg/kg when stored in the dark compared to a decrease to 150 mg/kg when stored in the dark (Figure 4.45). All oils showed very similar results. These results are in agreement with those of other researchers (Caponio *et al.*, 2005)

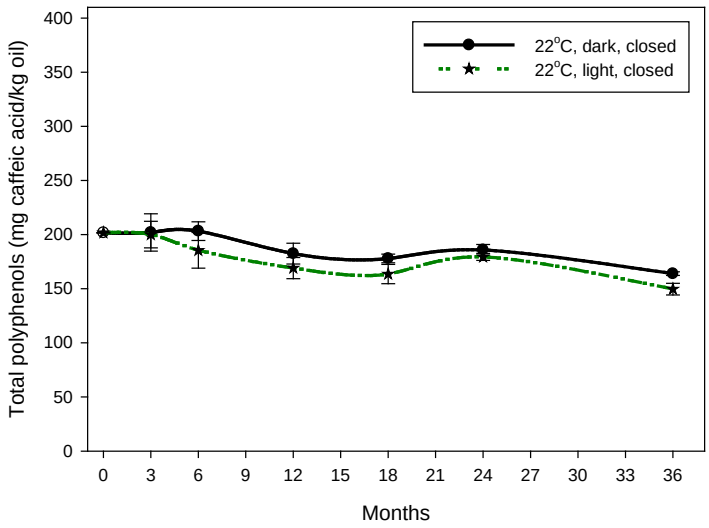

Figure 4.45 Effect of exposure to light on total polyphenol concentration of olive oil stored for 36 months

MP,ML: Mid polyphenols, Mid linoleic acid olive oil. All other oils showed similar patterns.

4.3.8 α-tocopherol

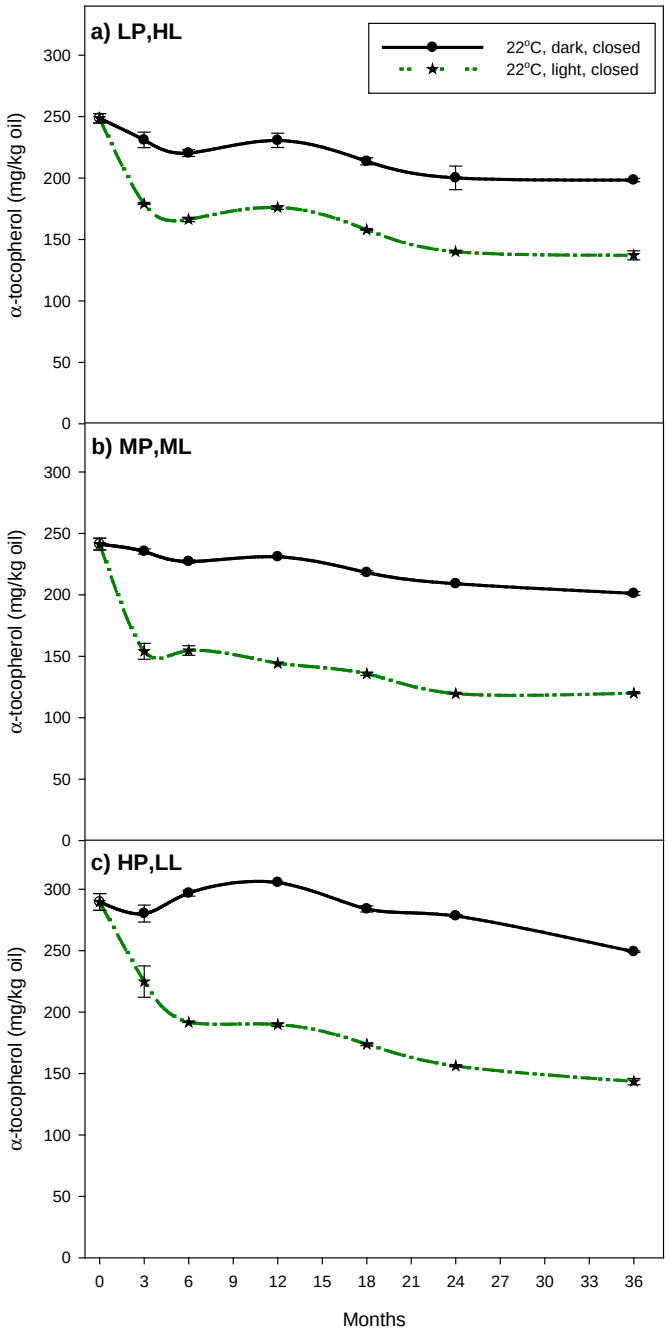

Figure 4.46 Effect of exposure to light on α-tocopherol content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid.

Exposure to light had a significant effect on the α-tocopherol content of the oils in this study. Immediately after the oils were stored, those exposed to light declined at a rapid rate for the first 6 months of storage. Following this, the α-tocopherol content decreased at a far slower rate. All oils showed a similar pattern. As an example, Oil MP,ML had an initial α-tocopherol content of 241 mg/kg, which decreased to 155 mg/kg after 6 months storage exposed to light. From the six months storage time until the final analysis point at 36 months storage, the α-tocopherol content only dropped

another 35 mg/kg to 120 mg/kg. All oils exposed to light showed a similar pattern. The samples stored away from light showed a general linear decline. Oil MP,ML decreased from 241 mg/kg to 201 mg/kg after 36 months storage (Figures 4.46 and 4.47).

When exposed to light tocopherols act as both electron donors, which slow oxidation, and electron acceptors which quench singlet oxygen, the consequence of which is the inhibition of photo-oxidation (oxidation due to light). Singlet oxygen formed during photo-oxidation is 1000-1500 times more reactive that the triplet oxygen taking part in oxidation due to oxygen (Caponio *et al.*, 2005). Therefore there is a greater decrease in tocopherols in the samples exposed to light. These results suggest that in the presence of light oil is protected from oxidation mainly by tocopherols and that polyphenols only have a secondary role. The results from this study are in complete agreement with those from other studies (Caponio *et al.*, 2005; Bilancia *et al.*, 2007).

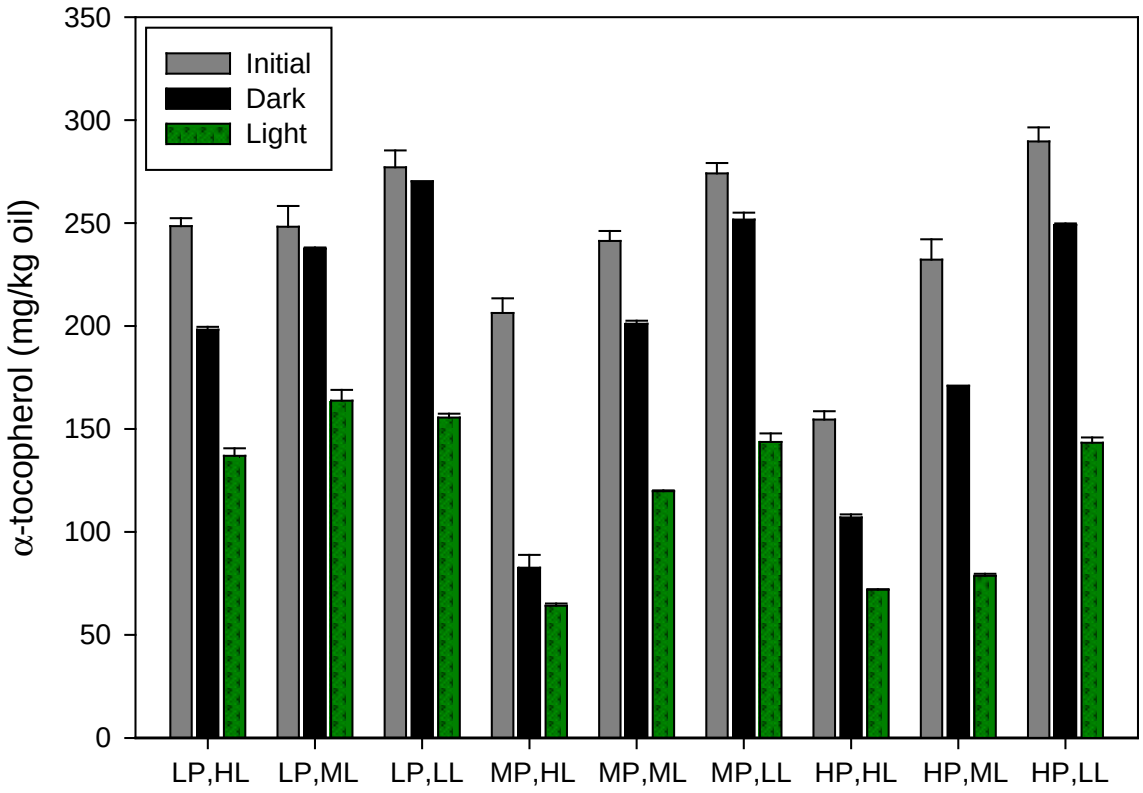

Figure 4.47 α-tocopherol at initial analysis and after 36 months, closed and exposed to light for different types of olive oil

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

4.3.9 Chlorophyll

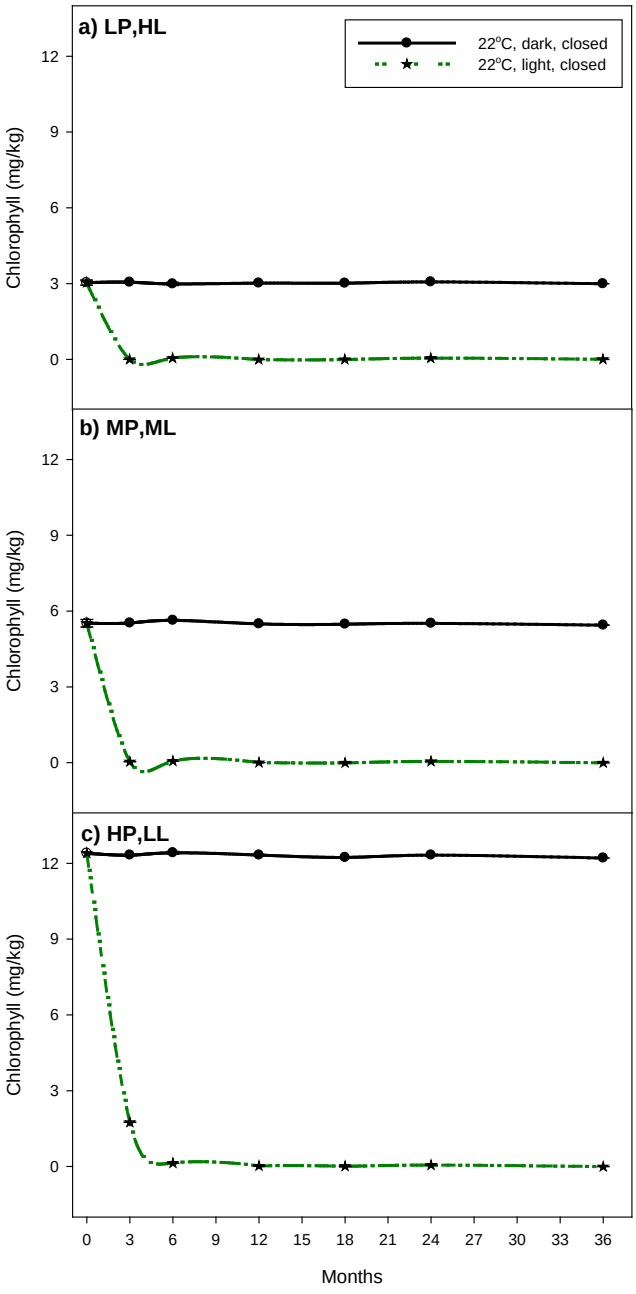

Figure 4.48 Effect of exposure to light on chlorophyll content of different types of olive oil stored for 36 months

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

The levels of chlorophyll in the oil stored in the dark in this study remained almost unchanged during the 36 months storage period. The samples exposed to light showed a drastic decrease in the first 2 months of storage, and in most cases disappeared completely after 6 months storage, regardless of the initial content (Figure 4.48). This occurred because chlorophyll in the oil acts as a sensitiser to produce $^1O_2$ in the presence of light and oxygen, which is highly reactive. The chlorophyll compound is degraded and the singlet produced as part of the reaction consequently participates in the oxidation reaction (Bilancia *et al.*, 2007; Choe and Min, 2006; Caponio *et al.*, 2005)

4.3.10 Colour

Table 4.7 Effect of exposure to light on colour (L*, a*, b) of olive oil samples at initial analysis and after 36 months storage

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

	Storage	L*	a*	b*
LP,HL	Initial	92.0 ± 0.4	-12.6 ± 0.1	75.7 ± 0.7
	dark, 22°C, after 36 months	92.2 ± 0.2	-12.2 ± 0.0	70.8 ± 0.3
	light, 22°C, after 36 months	95.6 ± 0.3	-10.5 ± 0.0	58.0 ± 0.1
LP,ML	Initial	89.8 ± 0.5	-11.6 ± 0.1	98.8 ± 0.2
	dark, 22°C, after 36 months	90.3 ± 0.3	-11.6 ± 0.0	96.0 ± 0.3
	light, 22°C, after 36 months	94.3 ± 0.2	-11.7 ± 0.1	85.0 ± 0.2
LP,LL	Initial	88.4 ± 0.3	-10.1 ± 0.0	113.6 ± 0.2
	dark, 22°C, after 36 months	89.1 ± 0.4	-10.0 ± 0.1	113.1 ± 0.4
	light, 22°C, after 36 months	92.8 ± 0.4	-10.2 ± 0.0	102.6 ± 0.1
MP,HL	Initial	91.7 ± 0.4	-12.4 ± 0.0	79.3 ± 0.4
	dark, 22°C, after 36 months	92.7 ± 0.1	-12.4 ± 0.1	75.1 ± 0.0
	light, 22°C, after 36 months	95.7 ± 0.1	-10.8 ± 0.0	56.6 ± 0.1
MP,ML	Initial	87.8 ± 0.2	-10.0 ± 0.0	109.7 ± 0.4
	dark, 22°C, after 36 months	88.2 ± 0.1	-10.1 ± 0.0	105.4 ± 0.1
	light, 22°C, after 36 months	93.2 ± 0.2	-10.2 ± 0.0	93.9 ± 0.4
MP,LL	Initial	84.7 ± 0.2	-7.3 ± 0.0	122.9 ± 0.2
	dark, 22°C, after 36 months	86.1 ± 0.1	-9.8 ± 0.0	104.4 ± 0.1
	light, 22°C, after 36 months	92.1 ± 0.3	-7.8 ± 0.0	114.7 ± 0.3
HP,HL	Initial	92.1 ± 0.2	-12.2 ± 0.0	83.0 ± 0.1
	dark, 22°C, after 36 months	92.7 ± 0.1	-12.2 ± 0.0	79.0 ± 0.0
	light, 22°C, after 36 months	95.2 ± 0.2	-11.2 ± 0.1	67.9 ± 0.2
HP,ML	Initial	86.0 ± 0.1	-8.5 ± 0.1	117.2 ± 0.2
	dark, 22°C, after 36 months	86.5 ± 0.1	-8.5 ± 0.5	112.4 ± 0.2
	light, 22°C, after 36 months	92.4 ± 0.3	-6.5 ± 0.0	103.5 ± 0.1
HP,LL	Initial	81.7 ± 0.6	-5.5 ± 0.1	126.0 ± 0.7
	dark, 22°C, after 36 months	82.3 ± 0.1	-5.3 ± 0.0	124.6 ± 0.1
	light, 22°C, after 36 months	91.2 ± 0.1	-5.3 ± 0.1	124.1 ± 0.0

Light exposure had a significant effect on the colour of the olive oils in this study (Table 4.7). Luminosity (L*) increased in the oils stored in the light, while those stored in the dark remained stable during the storage period. In the majority of cases a* (greenness) was slightly lower in the oils exposed to light, however this was not seen in all oil as some remained stable as did those stored in the dark. The yellowness (b*) coefficient was significantly affected with the samples exposed to light significantly decreasing, while those in the dark remaining constant throughout the storage period. These results indicate the oil luminosity was slightly higher in the oils stored in the light (more "white"), the oils were slightly less "green" (a*) and significantly less yellow (b*). These results are

similar to those of other researchers (Pristouri *et al.*, 2010). The changes in colour are related to the decomposition of chlorophyll in the photo-oxidation process.

4.3.11 Fatty acid profile

There was no significant difference between the fatty acid composition of the samples stored in the dark and those stored exposed to light (data not shown). These results are similar to the findings of other authors (Mendez and Falque, 2007).

4.3.12 Induction time

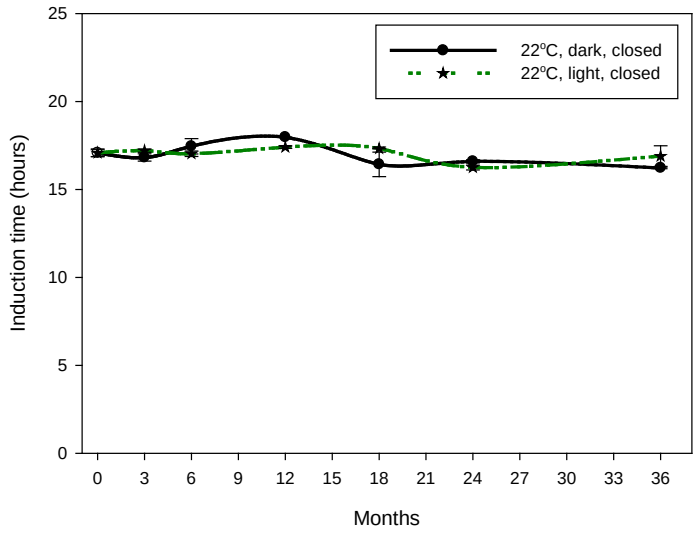

Figure 4.49 Effect of exposure to light on induction time of olive oil stored for 36 months

MP,ML: Mid polyphenols, Mid linoleic acid olive oil. All other oils showed similar patterns.

Induction time was not influenced by exposure to light in the oils in this study. Figure 4.49 shows the typical pattern of the induction times of the oils stored in the different conditions. Oil MP,ML had an initial induction time of 17.1 hours which decreased to 16.2 hours after 36 months storage in the dark. The same oil stored exposed to light decreased to 16.9 hours after 36 months storage. All oils showed the same or similar pattern.

As the fatty acid composition and the total polyphenols were not affected by light, these results are as expected. While there was a higher amount of secondary oxidation compounds K_{268} in the oils exposed to light, this did not influence the induction time, nor did the decreased level of α-tocopherol in the samples exposed to light. Peroxide value of the oils stored in the light was slightly lower than those in the dark, which may have played a role in the induction time at each analysis point.

4.3.13 Organoleptic assessment

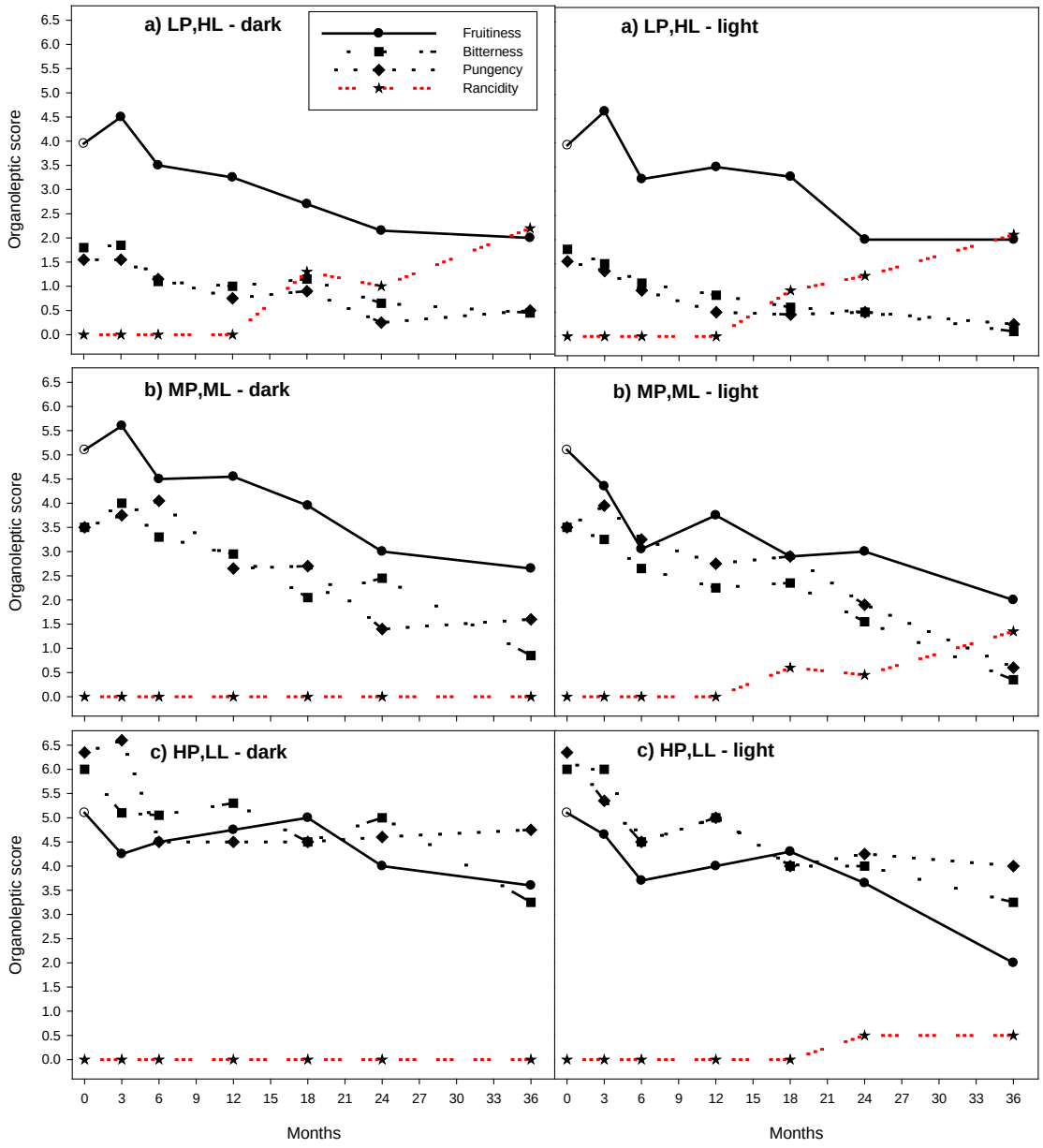

Figure 4.50 Organoleptic assessment of olive oils over 36 months stored in the dark and exposed to light

(a) LP,HL: Low polyphenols, High linoleic acid, (b) MP,ML: Mid polyphenols, Mid linoleic acid, (c) HP,LL: High polyphenols, Low linoleic acid

Exposure to light had a significant effect of the sensory profile of the oils in this, mainly on the negative attribute, rancidity. Rancidity occurred sooner in the storage period in some of the oils exposed to light than those which were stored in the dark. The positive attributes (fruitiness, bitterness and pungency) decreased at a greater rate in the oils stored exposed to light than those in the dark also (Figure 4.50 and Table 4.8).

These results are mainly due to the secondary oxidation products which were present in the samples exposed to light, which impart unpleasant flavours and aromas. High polyphenol levels, as well as the fatty acid composition (low linoleic acid) of the oil provided good resistance to rancidity.

Table 4.8 Organoleptic assessment of olive oil samples at initial analysis and after 36 months closed and exposed to light

LP- Low polyphenols; MP- Mid polyphenols; HP- High polyphenols; HL- High linoleic acid; ML- Mid linoleic acid; LL- Low linoleic acid

	Storage	Fruitiness	Bitterness	Pungency	Rancid
LP,HL	Initial	3.95	1.80	1.55	0.00
	Dark, 22°C, after 36 months	2.00	0.45	0.50	2.20
	Light, 22°C, after 36 months	2.00	0.10	0.25	2.10
LP,ML	Initial	3.85	1.90	1.20	0.00
	Dark, 22°C, after 36 months	2.75	0.75	0.75	0.75
	Light, 22°C, after 36 months	2.50	0.45	0.75	1.50
LP,LL	Initial	5.00	2.05	2.05	0.00
	Dark, 22°C, after 36 months	3.25	0.70	0.80	0.50
	Light, 22°C, after 36 months	2.80	0.60	0.40	1.00
MP,HL	Initial	5.30	3.00	3.50	0.00
	Dark, 22°C, after 36 months	1.25	0.75	0.40	2.65
	Light, 22°C, after 36 months	2.50	0.15	0.70	2.35
MP,ML	Initial	5.10	3.50	3.50	0.00
	Dark, 22°C, after 36 months	2.65	0.85	1.60	0.00
	Light, 22°C, after 36 months	2.00	0.35	0.60	1.35
MP,LL	Initial	5.00	4.45	5.00	0.00
	Dark, 22°C, after 36 months	3.00	1.15	1.45	0.25
	Light, 22°C, after 36 months	3.20	1.50	1.25	0.60
HP,HL	Initial	5.50	4.50	4.00	0.00
	Dark, 22°C, after 36 months	4.00	1.40	2.30	1.35
	Light, 22°C, after 36 months	2.65	0.90	0.85	0.00
HP,ML	Initial	5.90	4.80	5.65	0.00
	Dark, 22°C, after 36 months	3.75	1.85	2.40	0.50
	Light, 22°C, after 36 months	2.50	1.50	1.50	2.50
HP,LL	Initial	5.10	6.00	6.35	0.00
	Dark, 22°C, after 36 months	3.60	3.25	4.75	0.00
	Light, 22°C, after 36 months	2.00	3.25	4.00	0.50

5. Summary of analyses

As the results of this study show, different storage conditions had a significant effect on the quality and shelf life of olive oil. The results of various analytical methods cannot be used in isolation to determine the status of oil. Each storage condition imposed in this study gave distinct patterns which were useful analytical tools for determining the history, as well as the potential shelf life of olive oil. A combination of these results provided detailed information about the oil.

5.1 Storage temperature

Storage temperature had a significant effect on the following measurements:

Peroxide value, K_{268}, free fatty acids, pyropheophytin a, 1,2-diacylglycerols, polyphenols, α-tocopherols, colour, induction time and organoleptic assessment.

Peroxide values decreased significantly when higher storage temperatures were applied. This was important as low peroxide values would not necessarily indicate that an olive oil is high quality. UV absorbance at 268nm (K_{268}), increased significantly when higher storage temperatures were applied, while K_{232} remained relatively stable. Free fatty acids increased in all oils. However the oils exposed to higher storage temperatures increased at a much greater rate than those at lower temperatures.

Pyropheophytin a increased immediately and at a significant rate upon exposure to higher storage temperatures and was a clear indicator of unsatisfactory storage conditions, exceeding the Australian standard limit after only 3 months when stored at 37°C.

However the oils stored at the higher temperatures increased at a much greater rate than those at lower temperatures, with the oils stored at the higher temperatures (22°C and 37°C) exceeding the Australian and IOC limit, with the time taken to exceed the limit varying dependent on the oil.

Pyropheophytin a was also a very useful indicator of aging in oils kept at lower temperatures as there was a constant, linear increase over time which was almost identical in all oils regardless of the initial matrix.

1,2-diacylglycerols were also useful indicators of determining the age of an oil, although the initial free fatty acid content (and therefore 1,2-diacylglycerols content) had an influence on the rate of decline. Storage temperature also had an impact on 1,2-diacylglycerols, with higher storage temperatures causing the level to decrease rapidly, and almost immediately be outside the Australian Standard (>35%).

Polyphenols, α –tocopherols and colour were all slightly, but significantly affected by storage temperature, with higher temperature leading to the greatest changes.

Induction time was shown to increase in oils exposed to higher storage temperatures. This is a significant result from this study, as high induction times do not necessarily indicate high quality oil. Changes in this measurement over time would be indicative of storage conditions, not necessarily the oxidative stability, or shelf life, of the oil.

Storage temperature significantly affected the sensory profile of olive oils. High storage temperature lead to the degradation of positive attributes over time, while the negative attribute, rancidity, developed quickly and to significant levels, reducing the consumer acceptance, and therefore shelf life, of the oil.

5.2 Oxygen exposure

Exposure to oxygen had a significant effect on the following measurements:

Peroxide value, K_{232}, K_{268}, pyropheophytin a, polyphenols, α –tocopherols, chlorophyll, fatty acid profile, induction time, sensory analysis.

Peroxide values and UV absorbance at K_{232} and K_{268} were very good indicators of exposure to oxygen in olive oil. The values for these measurements reacted in a predictable pattern, and were significantly affected almost immediately after exposure. The values exceeded the Australian and IOC limits after varying amounts of time, depending on the oil matrix.

Total polyphenols, α –tocopherols, chlorophyll and fatty acid profile were useful indicators of degradation of the oil due to exposure to oxygen, however initial values for each of these components were required to assess the degradation.

Induction time decreased significantly upon the oils exposure to oxygen, therefore this analysis was a useful indicator of oxidation if measured multiple times during the life of the oil.

Olive oil sensory properties were quickly and significantly degraded when the oil was exposed to oxygen and development of rancidity in the oil was a good indicator that there was a problem with storage.

5.3 Exposure to light

Exposure to light had a significant effect on the following measurements:

K_{268}, pyropheophytin a, α –tocopherols, chlorophyll, organoleptic assessment.

The most significant analysis affected by exposure to light was pyropheophytin a and chlorophyll. In most cases, measurement of the pyropheophytin a levels was a very good indicator of aging in olive oil. However the pigments disappeared completely when oil was exposed to light, therefore the measurement cannot be used to determine the age of the oil.

Organoleptic assessment of the oil was significantly affected by exposure to light. The significant decrease in antioxidants (especially α –tocopherols), as well as the increase in K_{268} (indicating the production of compounds producing unpleasant flavours) were good indicators that the oil had been exposed to light and the quality of the oil has decreased.

5.4 Shelf life

The shelf life of an olive oil was difficult to predict and was dependent on a number of factors including the oil matrix and the storage conditions of the oil. The following table (Table 4.9) gives an indication of the shelf life of different oils (in months).

Table 4.9 Approximate time taken for oils LP,HL, MP,ML and HP,LL to no longer meet the classification criteria for extra virgin olive oil

LP- low polyphenols, MP – mid polyphenols, HP- high polyphenols, HL – high linoleic, ML – mid linoleic, LL – low linoleic

Oil LP,HL								
		PV	UV Absorbance		FFA	Pyro	DAG	Sensory
			K232	K268				
Storage Temperature	15°C	>36	>36	>36	>36	>36	12	18
	22°C	>36	>36	>36	24	18	6	12
	37°C	>36	>36	36	6	3	3	6
Oxygen	Closed	>36	>36	>36	24	18	12	12
	Open	6	6	22	24	18	6	3
Light	Dark	>36	>36	>36	>36	18	12	12
	Exposed	>36	>36	>36	18	*ND	6	12

Oil MP,ML								
		PV	UV Absorbance		FFA	Pyro	DAG	Sensory
			K232	K268				
Storage Temperature	15°C	>36	>36	>36	>36	>36	36	>36
	22°C	>36	>36	>36	>36	18	18	>36
	37°C	>36	>36	36	18	3	3	3
Oxygen	Closed	>36	>36	>36	>36	18	18	>36
	Open	12	9	26	>36	18	18	18
Light	Dark	>36	>36	>36	>36	18	18	>36
	Exposed	>36	>36	>36	>36	*ND	18	12

Oil HP,LL								
		PV	UV Absorbance		FFA	Pyro	DAG	Sensory
			K232	K268				
Storage Temperature	15°C	>36	>36	>36	>36	>36	>36	>36
	22°C	>36	>36	>36	>36	18	18	>36
	37°C	>36	>36	36	30	3	3	6
Oxygen	Closed	>36	>36	>36	>36	18	18	>36
	Open	18	12	24	>36	18	18	18
Light	Dark	>36	>36	>36	>36	18	18	>36
	Exposed	>36	>36	6	>36	*ND	18	18

Note: The number in the box indicates the number of months before the oil is outside the Australian/IOC standard; *ND – not detected after 3 months storage. “>36” indicates oil still within extra virgin olive oil classification limits after 36 months storage.

6. Implications

Storage of olive oil under poor conditions will result in the loss of quality, and therefore value, of the product. This can occur throughout the life of the olive oil, dependent upon the storage conditions of the oil at any time.

Every batch of oil is different. The importance of the matrices of the oil is obvious from this study of oils with different quality, particularly total polyphenols and linoleic acid content. This study provides the best indication so far on how to evaluate shelf life and use by date for olive oil.

The results of this study indicate that the initial matrix of the oil as well as the conditions under which the oil is stored have an influence on the oxidative stability of the oil. This is important information for those involved in the production, storage and sale of olive oil.

This study has shown that some analyses are valuable in illustrating particular things, such as the absence of pyropheophytins upon light exposure, however no single test can identify all issues associated with aging or storage conditions. The results of individual tests need to be carefully evaluated in conjunction with other analyses to evaluate the status of olive oil.

These results will assist the industry to maker important, informed decisions about the storage and transport of olive oil. Many high quality olive oils have been degraded due to lack of knowledge about appropriate storage conditions. Using the information in this report, those responsible for the olive oil will be able to retain the initial high quality of the olive oil.

7. Recommendations

The results of this study showing the effect of storage conditions on the quality of olive oil is important information for the olive oil industry. This project has generated information about the methods used to determine olive oil quality, which will be critical in interpreting the results of analysis of oil stored in certain conditions. Free fatty acids increase during storage of olive oil. However the rate at which they increase is influenced by the storage temperature, with higher temperatures increasing the rate of accumulation. Changes in peroxide value and UV absorbance (K_{232} and K_{268}) are influenced by exposure to oxygen, with total polyphenol and α-tocopherol content also affected. However, peroxide values actually decrease when exposed to higher temperatures, therefore this method of analysis is less useful as an indicator of the quality oil which has been stored at high temperatures. The colour of the oil is affected to a different extent by all of the different storage conditions studied. This is important as the first “sensory” assessment a consumer makes is by assessing the colour of the oil they are going to purchase.

The newer methods incorporated into the Australian standard AS5264-2011, pyropheophytin a and 1,2-diacylglycerol content, were evaluated in this study. The results show the usefulness of these methods for determining the freshness of oils. Both parameters are affected by the storage temperature of the oil. Therefore they are also useful in determining if any heat application has occurred during the life of the oil. The only proviso with the measurement of pyropheophytin a is that when exposed to light the pigments in olive oil, including all of the pyropheophytin a and pheophytins disappear, therefore this analysis would need to be used in conjunction with other analyses to determine the quality of the olive oil. Results from this research can be used to determine use-by dates for olive oil, based on the initial composition of the oil, and the conditions which the oil will be stored under.

This report should be utilised by the Australian olive oil industry to allow all participants in the supply chain of oil to understand the detrimental effects that inappropriate storage can have on olive oil quality.

8. References

Allouche, Y., Jimenez, A., Gaforio, J. J., Uceda, M. and Beltran, G. (2007). "How heating affects extra virgin olive oil quality indexes and chemical composition." *Journal of Agricultural and Food Chemistry* **55**(23): 9646-9654.

Angerosa, F., Campestre, C. and Giansante, L. (2006) Analysis and authentication. In *Olive oil: Chemistry and Technology*. Boskou, D (ed) (second edition). AOCS Press, Champaign. Illinois.

Anniva, C., Grigoriadou, D., Psomiadou, E. and Tsimidou, M. (2006). "Pheophytin a degradation products as useful indices in the quality control of virgin olive oil." *Journal of the American Oil Chemists Society* **83**(4): 371-375.

AOCS (1998). *Official Methods and Recommended Practices of the American Oil Chemists Society*. 5th ed. AOCS Press, Champaign, Illinois.

Bilancia, M.T., Caponio, F., Sikorskab, E., Pasqualonea, A. and Summo, C. (2007). Correlation of triacylglycerol oligopolymers and oxidised triacylglycerols to quality parameters in extra virgin olive oil during storage. *Food Research International* **40** (7): 855-861.

Boskou, D. (1996). Chapter 8. Olive Oil Adulteration. In "*Olive Oil: Chemistry and Technology*" Boskou, D (ed) .AOCS Press, Champaign. Illinois.

Boskou, D., Blekas, G. and Tsimidou M. (2006) Olive oil composition. In "*Olive oil: Chemistry and Technology*, Boskou, D (ed) (second edition). AOCS Press, Champaign. Illinois.

Caponio, F., Bilancia, M.L., Pasqualone, A., Sikorska, E. and Gomes, T. (2005). "Influence of the exposure to light on extra virgin olive oil quality during storage." *European Journal of Food Research and Technology* **221**, 92-98.

Cerretani, L., Moltiva, M.J., Romero, M.P., Bendini, A. and Lercker, G. (2008). "Pigment profile and chromatic parameters of monovarietal virgin olive oils from different Italian cultivars." *European Journal of Food Research and Technology* **226**, 1251-1258.

Choe, E. and Min, D. (2006). "Mechanisms and factors for edible oil oxidation." *Comprehensive reviews in food science and food safety* **5**: 169-186.

Cinquanta, L., Esti, M. and Di Matteo, M. (2001). "Oxidative stability of virgin olive oils." *Journal of the American Oil Chemists Society* **78**(12): 1197-1202.

Cossignani, L., Luneia, R., Damiani, P., Simonetti, M. S., Riccieri, R. and Tiscoenia, E. (2007). "Analysis of isomeric diacylglycerolic classes to evaluate the quality of olive oil inrelation to storage conditions." *European Food Research and Technology* **224**: 379-383.

Fronimaki, P., Spyros, A., Christophoridou, S., and Dais, P. (2002) Determination of diglyceride content in Greek virgin olive oils and some commercial olive oils by employing 31P NMR spectroscopy. *Journal of Agricultural and Food Chemistry* **50**, 2207-2213.

Gallardo-Guerrero, L., Gandul-Rojas, B., Roca, M. and Minguez-Mosquera, M. I. (2005). "Effect of storage on the original pigment profile of spanish extra virgin olive oil." *Journal of the American Oil Chemists Society* **82**(1): 33-39.

Garcia, A., Brenes, M., Romero, C., Garcia, P. and Garrido, A. (2002). "Study of phenolic compounds in virgin olive oils of the Picual variety." *European Food Research and Technology* **215**(5): 407-412.

Gutierrez, F. and Fernandez, J. L. (2002a). "Determinant parameters and components in the storage of virgin olive oil. Prediction of storage time beyond which the oil is no longer of "extra" quality." *Journal of Agricultural and Food Chemistry* **50**(3): 571-577.

Gutierrez, F., Villafranca, M. J. and Castellano, J. M. (2002b). "Changes in the minor components and quality indices of virgin olive oil during oxidation." *Journal of the American Oil Chemists Society* **79**(7): 669-676.

Gutfinger, T. (1981). Polyphenols in olive oils. *Journal of the American Oil Chemists' Society* **62:** 895–898.

Hornero-Mendez, D., Gandul-Rojas, B. and Minguez-Mosquera, M. I. (2005). "Routine and sensitive SPE-HPLC method for quantitative determination of pheophytin a and pyropheophytin a in olive oils." *Food Research International* **38**(8-9): 1067-1072.

Hunterlab, L, a, b Color Space.2012. http://hunterlab.com (accessed February 2, 2012).

Kamal-Eldin, A. and Appelqvist, L.A. (1996). The Chemistry and antioxidant properties of tocopherols and tocotrienols, *Lipid*s **31:** 671-701.

Kiritsakis, A. K. (1998). "Flavor components of olive oil - A review." *Journal of the American Oil Chemists Society* **75**(6): 673-681.

Krichene, D., Allalout, A., Mancebo-Campos, V., Salvador, M. D., Zarrouk, M. and Fregapane, G. (2010). "Stability of virgin olive oil and behaviour of its natural antioxidants under medium temperature accelerated storage conditions." *Food Chemistry* **121**(1): 171-177.

Lee, J., Lee, Y. and Choe, E. (2007). "Temperature dependence of the autoxidation and antioxidants of soybean, sunflower, and olive oil." *European Food Research and Technology* **226**(1-2): 239-246.

Mailer, R.J., Ayton, J. and Conlan, D. (2002). Comparison and evaluation of the quality of thirty eight commercial Australian and New Zealand olive oils. *Advances in Horticultural Sciences.* **16**(3-4): 259–256.

Mailer, R.J., Conlan, D. and Ayton, J. (2005). Olive Harvest: Harvest timing for optimal olive oil quality. *RIRDC publication no. 05/013.* A report prepared for the Rural Industries Research and Development Corporation, Canberra.

Mailer, R.J. and Ayton, J. (2008). A Survey of Australian Olive Cultivars to Determine Compliance with International standards. *RIRDC publication no. 08/167.* A report prepared for the Rural Industries Research and Development Corporation, Canberra.

Mateos, R., Uceda, M., Aguilera, M. P., Escuderos, M. E. and Maza, G. B. (2006). "Relationship of Rancimat method values at varying temperatures for virgin olive oils." *European Food Research and Technology* **223**(2): 246-252.

Mendez, A. and Falque, E. (2007). "Effect of storage time and container type on the quality of extra-virgin olive oil." *Food Control* **18**(5): 521-529.

Morello, J. R., Motilva, M. J., Tovar, M. J. and Paz Romero, M. (2004). "Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction." *Food Chemistry* **85**: 357-364.

Okogeri, O. and Tasioula-Margari, M. (2002). "Changes occurring in phenolic compounds and alpha-tocopherol of virgin olive oil during storage." *Journal of Agricultural and Food Chemistry* **50**(5): 1077-1080.

Pereira, J. A., Casal, S., Bento, A. and Oliveira, M. (2002). "Influence of olive storage period on oil quality of three Portuguese cultivars of Olea europea, Cobrangosa, Madural, and Verdeal Transmontana." *Journal of Agricultural and Food Chemistry* **50**(22): 6335-6340.

Perez-Camino, M. C., Moreda, W. and Cert, A. (2001). "Effects of olive fruit quality and oil storage practices on the diacylglycerol content of virgin olive oils." *Journal of Agricultural and Food Chemistry* **49**(2): 699-704.

Pristouri, G., Badeka, A. and Kontominas, M. G. (2010). "Effect of packaging material headspace, oxygen and light transmission, temperature and storage time on quality characteristics of extra virgin olive oil." *Food Control* **21**(4): 412-418.

Shimizu, M., Kudo, N., Nakajima, Y., Matsuo, N., Katsuragi, Y., Tokimitsu, I. and Barcelo, F. (2008). "Effect of lipase activity and specificity on the DAG content of olive oil from the shodoshima-produced olive fruits." *Journal of the American Oil Chemists Society* **85**(7): 629-633.

Stefanoudaki, E., Williams, M. and Harwood, J. (2010). "Changes in virgin olive oil characteristics during different storage conditions." *European Journal of Lipid Science and Technology* **112**(8): 906-914.

9. Author References

Mailer, R.J., Ayton, J. and Graham, K. (2010). The influence of growing region, cultivar and harvest timing on the diversity of Australian olive oil. *JAOCS* **87:**877-884.

Mailer, R.J. and Ayton, J. (2008). A Survey of Australian Olive Cultivars to Determine Compliance with International standards. *RIRDC publication no. 08/167*. A report prepared for the Rural Industries Research and Development Corporation, Canberra

Ayton, J., Mailer, R.J., Haigh, A., Tronson, D. and Conlan, D. (2007). Quality and oxidative stability of Australian olive oil according to harvest date and irrigation. *Journal of Food Lipids* **14**: 138–156.

Mailer, R.J., Ayton, J. and Conlan, D. (2007). Influence of harvest timing on Olive (*Olea europaea)* oil accumulation and fruit characteristics under Australian conditions. *J Fd Agric & Environ.* **5**(3&4): 58–63

Mailer, R.J., Conlan, D. and Ayton, J. (2005). Olive Harvest: Harvest timing for optimal olive oil quality. *RIRDC publication no. 05/013.* A report prepared for the Rural Industries Research and Development Corporation, Canberra.

The Effect of Storage Containers on Extra Virgin Olive Oil Quality

by Jamie Ayton, Rodney J. Mailer and Kerrie Graham

Publication No. 12/024

This report provides information about the effect of exposure to oxygen, light and storage temperature on the quality of olive oil. Olive oil has been shown to quickly deteriorate when stored under less than ideal conditions. Sometimes growers produce oil with very high quality initially, which quickly deteriorates due to the conditions under which it is stored.

The report is targeted at olive oil producers, transporters, warehouse operators, importers and exporters, retailers and consumers. It is important to be aware of the risks of improper storage on olive oil quality whether buying, selling, transporting or storing the product.

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