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# Effect of Storage Containers on Olive Oil Quality

RIRDC Publication No. 09/160



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**Rural Industries Research and  
Development Corporation**

# **Effect of Storage Containers on Olive Oil Quality**

by Dr Rodney J. Mailer and Kerrie Graham

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# Foreword

This report assesses the impact of storage in a range of flexible storage containers on extra virgin olive oil quality. The research was conducted by the Australian Oils Research Laboratory (AORL). It was supported by RIRDC and the Australian Olive Association.

The outcome of this study reinforces that the best storage conditions for olive oil is in opaque, impervious and inert containers, stored at cool temperatures. Stainless steel or glass would appear to be the best options for long term storage. Metallised flexible bags used for short term transport may provide reasonable protection. Storage in clear plastic, particularly in the light and at elevated temperatures, is clearly unacceptable and will result in total loss of extra-virgin olive oil quality within weeks and perhaps days. Re-use of these containers would appear to be highly undesirable and would be expected to cause more rapid degradation.

This report, an addition to RIRDC's diverse range of over 1900 research publications, forms part of our New Plants Products R&D program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

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**Peter O'Brien**  
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# About the Author

Dr Rod Mailer, Principal Research Scientist, has been involved in olive research since 1996. He was principal investigator for projects: DAN197A, Harvest timing; DAN 237A, Optimal olive oil quality; DAN-239A, Compliance to international standards and joint participant in UCS-19A, Assessment of olives. His group at Wagga Wagga Agricultural Institute plays a leading role in national olive industry research having developed substantial expertise in oil chemistry and quality. He works closely with the olive industry through participation in field days, conferences and workshops. Dr Mailer is an AOCS Approved Chemist since 1993 and is an honorary member of the Australian Olive Association. During the last 5 years, Dr Mailer has published numerous scientific papers on olives and presented at over 30 olive workshops and conferences. In addition, he has published extensively on canola and other oil products. He has mentored several students and has held the following positions:

- Adjunct Professor, CSU
- President - International Society for Fat Research, Germany
- Chair – World Congress on Fats and Oils and ISF Congress, 2005- 2009.
- President – Groupe Consultatif International de Recherche sur le Colza, Paris 1997-2001
- ISO Fats and Oils Committee, Australian representative TC34/SC 11 & SC 2

# Acknowledgments

This study was carried out at the NSW Department of Primary Industries' "Australian Oils Research Laboratory" at Wagga Wagga, NSW. Particular thanks to Jamie Ayton for assistance. The external cubic containers used for storage of the flexible bags were loaned by CHEP Australia. Companies that supplied flexible bags and containers for this study included CHEP Australia, Entapak Pty Ltd, Mark Crasti and Company Pty Ltd, Schütz DSL (Australia) Pty Ltd and Redisland Marketing Pty Ltd. The Australian Olive Association and the President, Mr Paul Miller, have provided assistance in this research.

# Abbreviations

AOA	Australian Olive Association
AORL	Australian Oils Research Laboratory
AOCS	American Oil Chemists' Society
DAGs	diacylglycerols
DGF	German Society of Fats and Oils
EVOH	Ethylene Vinyl Alcohol
EVOO	extra virgin olive oil
HPLC	high performance liquid chromatograph
IOC	International Olive Council
IUPAC	International Union of Pure and Applied Chemistry
MSHFBA	N-methyl-N-(trimethyl-silyl)-hepta-fluorobutyramide
NATA	National Association of Testing Authorities
NSW DPI	New South Wales Department of Primary Industries
PE	polyethylene
PP	pheophytins
PPP	pyropheophytin
RIRDC	Rural Industries Research and Development Corporation
RP HPLC	reverse phase high performance liquid chromatography
USDA	United States Department of Agriculture

# Contents

Foreword .....	iii
About the Author .....	iv
Acknowledgments.....	iv
Abbreviations.....	v
Executive Summary .....	ix
<b>1.Introduction .....</b>	<b>1</b>
1.1 Background.....	1
1.2 Oil Stability .....	2
1.3 Oil Properties .....	2
1.3.1 Peroxide Value .....	2
1.3.2 Free Fatty Acids .....	2
1.3.3 Total Phenolic Content.....	2
1.3.4 Induction Time .....	2
1.3.5 $\alpha$ -tocopherol.....	3
1.3.6 Ultraviolet Absorption.....	3
1.3.7 Chlorophyll .....	3
1.3.8 Pyropheophytin .....	3
1.3.9 Diacylglycerol .....	3
1.3.10 Fatty Acid Profile .....	3
<b>2. Aims/Objectives .....</b>	<b>4</b>
<b>3. Methodology .....</b>	<b>4</b>
3.1 Australian Oils Research Laboratory.....	4
3.2 Samples.....	4
3.2.1 Apparatus .....	4
3.2.2 Sample collection .....	7
3.3 Limitations .....	7
3.4 Methodology .....	9
3.4.1 Peroxide Value .....	9
3.4.2 Free Fatty Acids .....	9
3.4.3 Total Phenolic Content.....	9
3.4.4 Induction time .....	9
3.4.5 $\alpha$ -Tocopherol.....	10
3.4.6 Ultraviolet absorption.....	10
3.4.7 Chlorophyll .....	10
3.4.8 Pyropheophytin .....	10
3.4.9 Diacylglycerol .....	10



3.4.10 Fatty acid profiles.....	11
<b>4. Results .....</b>	<b>12</b>
4.1 Peroxide value.....	12
4.2 Free Fatty Acids.....	13
4.3 Phenolic content.....	14
4.4 Induction time .....	15
4.5 $\alpha$ -Tocopherol.....	16
4.6a Specific extinction coefficient.....	17
4.6b Specific extinction coefficient (continued).....	18
4.7 Chlorophyll .....	19
4.8 Pyropheophytin.....	19
4.9 Diacylglycerol.....	20
4.10 Fatty acid profile.....	21
<b>5. Discussion of Results .....</b>	<b>22</b>
5.1 Peroxide value.....	22
5.2 Free fatty acids.....	22
5.3 Total polyphenols .....	22
5.4 $\alpha$ -tocopherol .....	22
5.5 UV absorption.....	24
5.6 Pyropheophytin a.....	24
5.7 1,2-Diacylglycerols.....	24
<b>6. Implications.....</b>	<b>25</b>
<b>7. Recommendations .....</b>	<b>25</b>
<b>8. Author References.....</b>	<b>26</b>
<b>9. References .....</b>	<b>27</b>
<b>9. References .....</b>	<b>27</b>
<b>10. Appendices .....</b>	<b>28</b>

## Tables

Table 3.1	Description of each of 12 containers supplied by various manufacturers including various polymers and volume of the container.....	5
Table 3.2	Gas transmission rates of various polymers @ 80% R.H.....	6
Table 5.1	Container description showing gas transmission rates and peroxide value after four months storage – sorted from lowest to highest peroxide value .....	23
Table 10.1	Comparison of peroxide value, polyphenol content, induction time and $\alpha$ tocopherols of oil stored in 12 containers for 4 months.....	28
Table 10.2	Comparison of oil specific extinction coefficient $K_{232nm}$ and $K_{268nm}$ , when stored in various containers for between 0 months and 4 months.....	29
Table 10.3	Comparison of containers between 0 months and 4 months for free fatty acids, chlorophyll, pyropheophytins and DAGS.....	30
Table 10.4	Comparison of fatty acid profiles at 0 months and 4 months .....	31

## Figures

Figure 3.1	Original cylinder containing a metallic lined collapsible bag with 1000 L extra virgin olive oil. ....	7
Figure 3.2	Examples of the range of plastic containers being tested including Ecobulk tank, metallised bags, 4 litre bottle .....	8
Figure 4.1	Effect of 12 alternative types of storage material on the peroxide value of olive oil stored for 16 weeks .....	12
Figure 4.2	Effect of 12 alternative types of storage material on free fatty acids of olive oil stored for 16 weeks .....	13
Figure 4.3	Effect of 12 alternative types of storage material on phenolic content of olive oil stored for 16 weeks. ....	14
Figure 4.4	Effect of 12 alternative types of storage material on induction time of olive oil stored for 16 weeks .....	15
Figure 4.5	Effect of 12 alternative types of storage material on $\alpha$ -Tocopherol of olive oil stored for 16 weeks. ....	16
Figure 4.6a	Effect of 12 alternative types of storage material on $K_{232nm}$ of olive oil stored for 16 weeks .....	17
Figure 4.6b	Effect of 12 alternative types of storage material on $K_{268nm}$ of olive oil stored for 16 weeks .....	18
Figure 4.7	Effect of 12 alternative types of storage material on chlorophyll content of olive oil stored for 16 weeks .....	19
Figure 4.8	Effect of 12 alternative types of storage material on pyropheophytin content of olive oil stored for 16 weeks .....	20
Figure 4.9	Effect of 12 alternative types of storage material on DAGS content of olive oil stored for 16 weeks .....	21

# Executive Summary

## ***What the report is about***

This report describes the effects of short term storage of olive oil in plastic containers and the influence of this storage on oil quality. Most oil producers are aware that olive oil must be stored in opaque storage containers constructed of inert material such as glass or stainless steel. Often containers labelled as “Food Grade”, generally designed for use with aqueous material, are utilised for oil storage and transport. Additionally, where some plastics are recommended for oil storage they are often substituted by oil producers with cheaper alternatives. These containers are generally 1000 L capacity and will hold over \$6,000 of oil product. It is essential that oil producers know the consequences of using plastic containers when storing or transporting this valuable product.

## ***Who is the report targeted at?***

The report is targeted at those who might need to store or transport edible oil, in particular olive oil. This will include farmers and oil producers, wholesale and retail merchants, importers and exporters and transport companies. It is important not only for the seller, but also the purchaser, to be aware of the risks of improper storage of the product. Oil may be satisfactory in short term storage and during initial evaluation but will age quickly in the incorrect containers.

## ***Background***

Some samples of olive oil submitted to the Australian Oils Research Laboratory (AORL) for testing as extra virgin olive oil were found to contain very high levels of peroxide. Further investigation found the oil had been stored in plastic vessels, in the sun for several weeks.

Information obtained from members of the industry indicated that these storage vessels were commonly used for storage and transport of high quality olive oil as well as for other types of edible oil. The manufacturers of these containers provided the AORL with a range of product specifications, showing that some containers were recommended for oil storage and some were not. Despite this, it appears that oil producers will often use the lowest cost containers despite the risk of product quality deterioration.

Oil producers will often use the lowest cost containers despite the risk of product quality deterioration.
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The AOA and RIRDC were asked to support an investigation of temporary storage containers to determine which materials, if any, was suitable for this purpose. Suppliers of the containers were also asked to assist by supplying a range of containers of different materials for evaluation. A Technical Officer was employed to carry out the study, sampling oil from each of the containers on a regular basis and monitoring the reduction in quality. After four months sampling and testing the research was terminated as most of the oil had reached an unacceptable level of quality and could no longer be described as extra virgin olive oil.

## ***Aims/Objectives***

The aim was to provide to the industry a description of the effects of plastic storage containers on the quality of oil stored in them. It was designed to help those who need to store and transport oil to make decisions about what type of containers to use and under what conditions they should be used. Although this project was carried out in a shed with no temperature regulation, it is expected that others may also store oil under these conditions.

Based on the outcomes of this study, it will be important to disseminate the results of this work for the benefits of growers and others to help them preserve the high quality of Australian olive oil by following the best storage principles.

### **Methods used**

The project was carried out with funding from RIRDC and the AOA. Storage containers constructed of a range of materials were contributed by several suppliers for evaluation. High quality extra virgin olive oil (1000 L) was purchased from Tatiara Olive Processing in South Australia and transported to Wagga Wagga by road transport. The oil was transferred from stainless steel tanks to a metallised plastic container and transported to Wagga Wagga overnight. The oil was decanted into the sample containers within 48 hours to minimise any quality damage to the oil in the transported container. Oil testing of the oil at this initial period indicated that it was still of high quality at the commencement of the project.

Samples were taken from the range of containers at 0, 1, 2, 4, 8 and 16 weeks and tested for all of the likely quality parameters that might be used to describe extra virgin olive oil. These tests included peroxide value, free fatty acids, induction time, UV extinction coefficient and other tests. Additionally, new methods, pyropheophytins and diacylglycerols, described by the DGF (German Fat Society) were evaluated against current chemical tests to determine the ability of these new methods to detect changes in oil quality.

### **Results/Key findings**

Optimum storage conditions for olive oil include no contact with oxygen or light and reduced temperatures of around 16°C. It is important to indicate that this experiment was designed as a worst-case scenario and these tests were carried out in a shed, over summer, without temperature control. The containers were stored within four sided enclosures but the top was exposed to filtered light as it often is in commercial situations. As a result, the outcomes would be expected to be more rapid than if the investigation had been carried out at optimum storage conditions. It was not intended to investigate the range of storage conditions used by the industry. Better and worse storage conditions are used in reality and the limited storage in this study may justify further testing in the future for more detailed outcomes.

All of the samples in the range of plastic containers, failed to meet the standards for extra virgin olive oil after only 16 weeks.

All of the samples in the range of plastic containers, failed to meet the standards for extra virgin olive oil after only 16 weeks
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Clearly some materials were considerably better than others. Metallised containers were clearly superior. This may be for two reasons. Firstly the oxygen transfer rates are less than for polyethylene containers but also the containers shield the oil from light. Other tests have shown that light can influence oxidation as much as access to oxygen or elevated temperatures.

### **Implications for relevant stakeholders**

The industry needs to be made aware of these outcomes as a matter of urgency.

Discussions with numerous clients of the AORL indicated that users were not aware of the implications of storing oils in plastic containers. The high cost of olive oil may result in significant losses to merchants. The reputation of the suppliers can also be damaged by supplying good quality oil in containers, where it will lose that quality quickly and reflects on the supplier. The reputation of

the Australian industry could be damaged if oil is exported in unsuitable containers to overseas countries.

Oil is dynamic and changes with time, temperature and exposure to oxygen and light.

Australia produces high quality olive oil which is comparable to, and in some cases exceeds, quality of oil produced in other parts of the world. However, that oil is dynamic and changes with time, temperature and exposure to oxygen and light. Poor storage and transport conditions will have an adverse effect for all of the people involved in producing transporting and selling the product.

### ***Recommendations***

In this study, after eight weeks, eight of the 12 containers had exceeded the maximum peroxide value allowable for extra virgin olive oil. From Table 5.1 on page 23, it can be seen that the four metallic bags had the least change in peroxide value. Low density polyethylene was the least resistant to oxidation whereas the four metallic bags showed best resistance. After four months, even the best metallic liners had reached 16 mEq oxygen and would fail soon after. It is clear from Table 5.1 that the bags with the greatest oxygen transfer rates performed the worst.

Based on these findings, olive oil should be stored only in the recommended flexible metallic containers and only for the absolute minimum time necessary to transport the oil between the supplier and the consumer. Use of clear, porous polyethylene containers is not recommended under any conditions and not even for short periods of time. This will ensure that the quality of the oil does not deteriorate to unacceptable levels.

RIRDC and AOA need to disseminate the details of this study to the industry with some urgency. The AOA has access to a large portion of the industry through its members and the well utilised AOA web page and this data can quickly be made available to those who may be using the wrong material for oil storage.

- Olive oil should be stored only in the recommended flexible metallic containers to transport the oil between the supplier and the consumer.
- Use of clear, porous polyethylene containers is not recommended under any conditions.



# 1.Introduction

## 1.1 Background

Although olive oil has been utilised for thousands of years in the Mediterranean region it is only in recent years that Australia has increased productivity to commercial levels for both domestic and exports markets. Extra virgin olive oil (EVOO) is the highest grade of olive oil. EVOO is produced from fresh olives using only mechanical extraction processes and without the use of excessive heat, chemical interference or blending with other oils. Consumption of EVOO is constantly increasing as consumers begin to appreciate the taste and the apparent health benefits of the product. Studies indicate that the health benefits of EVOO over other grades is due to lower levels of peroxide and free fatty acid and higher levels of antioxidants in the form of polyphenols and tocopherols.

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). 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.

Chemical composition of the olive oil can be influenced by a number of factors including genetic variety, harvesting time of the fruit and storage of the fruit before processing. The storage of the processed oil can also have an affect on the quality of the oil (Mailer 2006). Storage of olive oil is known to be of critical importance in maintaining quality. Kiritsakis (1984) compared glass and plastic bottles in direct and diffused sunlight and found dark glass the best to avoid oxidation. Francesco *et al*, (2005) also studied oil stored in light and dark to show the influences of light in increasing the rate of oxidation. Tsimis and Karakasides (2002) looked at the benefits of tinfoil against various plastic containers (PVC, PET, HDPE) in terms of suitability for export, environmental benefits and protection from sunlight, oxygen humidity and microorganisms. Tin and Tetra-Brik® were found to be superior to glass and plastic (Mendez and Falque 2005) in storage trials over 6 months. Comparison of glass with PET containers for sunflower oil showed plastic to be inferior, especially in the light. No prior scientific surveys were found for large capacity plastic bags and containers proposed for this study.

Samples of olive oil submitted to the Australian Oils Research Laboratory (AORL) for testing as extra virgin olive oil were found to contain very high levels of peroxide. Further investigation found the oil had been stored in a plastic vessel, in the sun for several weeks.

The container was constructed of EVOH material which has an oxygen transfer rate at 25°C of 0.021 cc / m<sup>2</sup> / 24 hour / atm for 1mm of thickness. The client indicated that although this is a common method of storing olive oil, some oil is stored in HDPE containers which are more permeable and have a transfer rate of around 58 cc / m<sup>2</sup> / 24 hour / atm for 1mm of thickness. As indicated by product specification tables on Table 3.2, the gas transmission rates increase significantly from 58 to 111 cc / m<sup>2</sup> / 24 hour / atm for 1mm of thickness with 10°C increase in temperature and therefore deterioration in oil quality would accelerate rapidly.

## 1.2 Oil Stability

Olive oil is subject to change during storage. Oxidation caused by light, oxygen and heat are factors which reduce the organoleptic and nutritional assets of the product. As a result, there is substantial commercial emphasis placed on the chemical and organoleptic stability (shelf life) of EVOO.

The oxidative stability varies considerably between oils as a result of cultivar. Crop management during fruit development, harvest timing and the seasonal climate can also 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 linoleic acid result in reduced oxidative stability.

Antioxidants are predominately polyphenolic compounds but also include tocopherols such as vitamin E and are implicated with nutritional benefits for consumers. In addition, phenolic compounds provide the pungent sensory characteristics in olive oil.

The assessment of stability is important for the prediction of shelf life and used-by-dates. Olive oil is expected to be of high quality during storage and up to the time of consumption. Measurement of all components such as fatty acids profiles, phenolic content, chlorophyll, and tocopherols, can assist to predict the stability of oil.

## 1.3 Oil Properties

### 1.3.1 Peroxide Value

Peroxides are intermediate oxidation products of oil which leads to rancidity and typically occurs when oil is exposed to oxygen and/or light, particularly at elevated temperatures. Oxidation, and the formation of peroxides, occurs during oil extraction and processing and continues after bottling and during storage.

### 1.3.2 Free Fatty Acids

Olives contain endogenous lipase enzymes which hydrolyse triacylglycerides (oil molecules) to release free fatty acids. Although isolated from the oil in intact fruit, fruit damage releases lipases into contact with the oil. Increases in free fatty acids are therefore largely affected by fruit damage, fruit quality, time and temperature of oil extraction from the fruit. This damage occurs prior to the oil being separated from the water and solid portions of the fruit.

### 1.3.3 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). 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).

### 1.3.4 Induction Time

Induction time is a laboratory designed test to cause oxidation of oil at an accelerated rate. It is used to indicate the relative oxidative stability of oil in comparison to others. The process involves passing oxygen through oil at an elevated temperature whilst measuring the increased level of peroxides. The most stable oils resist peroxide formation and result in longer induction time. Although induction time can be used to compare oils to determine relative stability it cannot be used to precisely represent shelf life. This is because 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).

### **1.3.5 $\alpha$ -tocopherol**

Tocopherols are fat soluble antioxidants valued for their ability to inhibit oxidation in food. Vitamin E,  $\alpha$ -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).

### **1.3.6 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. Olive oils with increased values of  $K_{232}$  and  $K_{268}$  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).

### **1.3.7 Chlorophyll**

Chlorophyll is a green pigment and constitutes an important component of olive oil. The amount of chlorophyll pigments in the oil determines the colour intensity. When the fruit is immature, the level of chlorophyll is high but decreases as the fruit ripens. The degree of green colour of the olive oil is dependant on the maturity of the fruit when harvested (Mailer and Ayton, 2008). When the oil is exposed to light, chlorophyll becomes a pro-oxidant and can contribute to oxidative instability (Mailer *et al.*, 2002).

### **1.3.8 Pyropheophytin**

Pyropheophytins are by-products of chlorophyll formed when the pigments structure changes as a result of heating or age. Chlorophyll converts to pheophytin and ultimately to pyropheophytins. The proportion of pyropheophytin to the total pheophytins has been suggested to be useful in discriminating fresh oil from oil which has been in long term storage or which has been heated in the refining process (Mailer and Ayton, 2008).

### **1.3.9 Diacylglycerol**

Diacylglycerols (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 1-position of the triacylglycerol to form 2-3 diacylglycerols. Over time, these molecules equilibrate to form 1-3 diacylglycerols. The proportions of 1-2 and 1-3 diacylglycerols reportedly can be used to detect old or damaged oil (Mailer and Ayton, 2008).

### **1.3.10 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 which distinguishes it from many seed oils.

## 2. Aims/Objectives

The aim of this study is to help growers identify the effects of short term storage in flexible containers which are often used for oil storage. The best storage for extra virgin olive oil (EVOO) is well understood to include an impervious, chemically-inert material, such as glass or stainless steel, and be dark or opaque to limit light absorption. A variety of brands and styles of flexible and rigid containers with various chemical compositions are commonly used to transport and store olive oil. A selection of these was tested over a four month period to determine the influence they have on the quality of EVOO.

The objectives of the report are to provide oil producers, traders and exporters of olive oil a guide to storing oil for short term transport and to help eliminate oil damage and waste due to poor storage techniques.

## 3. Methodology

### 3.1 Australian Oils Research Laboratory

This study was carried out at the new NSW DPI Australian Oils Research Laboratory at Wagga Wagga, New South Wales. The laboratory staff was well experienced in oil research and quality evaluation, with olives and other oil crops. The laboratory is AS / NZS ISO 9001:2000 systems certified and has ISO 17025 certification through the National Australian Testing Authority (NATA). Dr Rodney Mailer is an Approved Chemist of the American Oil Chemists' Society. The AORL is accredited by the International Olive Council (IOC) for chemical and sensory testing of olive oils.

### 3.2 Samples

#### 3.2.1 Apparatus

Twelve plastic storage containers designed for food storage and commonly used to contain oil, were obtained from suppliers within Australia. These containers included seven collapsible bags and 2 rigid plastic containers of 1000 litre capacity. Additionally, two sample bags with a capacity of approximately 50 litres and a 4 litre plastic bottle were submitted for testing. Each of the collapsible bags was housed within a CHEP rigid pallet container with a base and four fold up sides. Although the containers also had a top, this was not utilised and the top of the bags remained open to the light to replicate common storage practices. The container description as given by the suppliers is provided in Table 3.1.

Each of the materials which are used in the bags has a different porosity and oxygen transmission rate. These rates are well known and published in relevant literature. The gas transmission rates of the various polymers at 80% relative humidity are given in Table 3.2. This data has been sourced from the Flextank website, [www.flextank.com.au/technical.htm](http://www.flextank.com.au/technical.htm).

**Table 3.1 Description of each of 12 containers supplied by various manufacturers including various polymers and volume of the container**

No description of the material was provided by the suppliers of containers H and I. The plastic bottle (L) was included as supplementary information although the volume and material is not comparable to the large flexible bags.

Sample	Product Description	Product Layers	Volume (L)	Brand
A	Polyethylene Bag Bulk	Low Density Polyethylene	1000	Scholle <sup>1</sup>
B	High Barrier Bag Bulk	Metallised Polyester Laminate Low Density Polyethylene	1000	Scholle <sup>1</sup>
C	Clear Durable Structure Bag Bulk	Nylon Laminate Low Density Polyethylene	1000	Scholle <sup>1</sup>
D	Liner Bag F 1000C N 2P 2/2 CM TTF (Form Fit)	Linear Low Density Polyethylene Ethylene Vinyl Acetate	1000	Scholle <sup>1</sup>
E	Non Barrier Top Fill/Bottom Empty PPP Liner	Linear Polyethylene Hi Flex Polyethylene	1000	Entapak <sup>2</sup>
F	Multipak AMF Liquid Liner	Metallised Tri-Laminate EVOH Co-Extrusion Hi-Flex Polyethylene Linear Polyethylene	1000	Entapak <sup>2</sup>
G	Barrier Top Fill/Bottom Empty MPP Maxicon Liner	Metallised Polyester Laminate Hi Flex Polyethylene Linear Polyester	1000	Entapak <sup>2</sup>
H	Standard Unit for Olive Oil	Clear plastic N/A	50	Crasti <sup>3</sup>
I	Top of the Line Aluminium Foil Barrier	Metallised N/A	50	Crasti <sup>3</sup>
J	Ecobulk MX-EV with additional EVOH Barrier	High Density Polyethylene EVOH with 2 Adhesive Layer	1000	Schütz <sup>4</sup>
K	Ecobulk MX INT – Standard Unit	High Density Polyethylene	1000	Schütz <sup>4</sup>
L	Plastic Storage Container	N/A	4	Redisland <sup>5</sup>

1. Scholle products provided by CHEP Australia, 2. Entapak Pty Ltd, 3. Mark Crasti and Company Pty Ltd, 4. Schütz DSL (Australia) Pty Ltd, 5. Supplementary container provided by Redisland Marketing Pty Ltd.

**Table 3.2 Gas transmission rates of various polymers @ 80% R.H**

(sourced from Flextank website, [www.flextank.com.au/technical.htm](http://www.flextank.com.au/technical.htm))

Polymer	Oxygen cc of O <sub>2</sub> per sqm per 24hr per atm for 1mm of thickness		Carbon Dioxide cc of CO <sub>2</sub> per sqm per 24hr per atm for 1mm of thickness		Nitrogen cc of N <sub>2</sub> per sqm per 24hr per atm for 1mm of thickness	
	25°C	35°C	25°C	35°C	25°C	35°C
<b>EVOH Barrier</b> (i.e. "EVAL F")	0.021	0.051	0.075	0.16	.002	.005
<b>PVDC Barrier</b> ("Saran")	0.058	0.17	0.43	-	.005	-
<b>Oriented Nylon 6</b> ("Cryovac")	0.64	1.3	2.6	-	0.27	-
<b>Unoriented Nylon</b> (food/dairy pouch)	1.6	3.1	-	-	-	-
<b>Oriented PET</b> (i.e. bottle grade)	0.90	2.0	7.6	-	0.18	-
<b>Unoriented PET</b> (un-metallized)	1.8	4.0	15	-	-	-
<b>Metallized PET</b> (25-50µ for wine)	~2.0	-	-	-	-	-
<b>H.D. Polyethylene</b> (S.G. 0.96)	58	111	-	-	-	-
<b>M.D Polyethylene</b> (S.G. 0.935)	117	190	-	-	-	-
<b>L.D. Polyethylene</b> (S.G. 0.92)	215	289	-	-	-	-
<b>Polystyrene</b> (high impact)	101	-	-	-	-	-
<b>PVC</b> (unoriented)	6.5	-	-	-	-	-

### 3.2.2 Sample collection

A 1000 litre container of EVOO, purchased from Tatiara Olive Processing, S.A. was delivered by road transport to Wagga Wagga in December 2008. The oil was housed in a metallised flexible bag inside a rigid and opaque cylinder. A polythene spike tap was used to access the sealed bag and the oil was dispensed from the container using a polythene pipe and tap with connectors.



**Figure 3.1. Original cylinder containing a metallic lined collapsible bag with 1000 L extra virgin olive oil. A polythene pipe connector was used to dispense the oil.**

The oil was dispensed into each of the seven 1000 litre collapsible bags, two rigid containers, and the 4 litre plastic bottle. Each container was then flushed with nitrogen to remove oxygen and the container was sealed. A sample from the original container was immediately taken to the laboratory for analysis to set a baseline for the quality of the original product.

Throughout the study, samples were collected from all of the containers including the original cylinder to determine changes in oil quality. The oil was initially analysed for oil quality at the time of receipt (9<sup>th</sup> December) and again after one week, two weeks, four weeks and eight weeks. A final subsample was taken after 16 weeks (14<sup>th</sup> April). After sampling each container was flushed with nitrogen to retard oxidation. The samples remain in storage for possible further analysis in the future.

### 3.3 Limitations

There are some important limitations to this study. The research leader points out that these conditions were not ideal storage conditions for olive oil. Oil producers and merchants are advised to store oil in the dark in temperature cooled facilities of around 16-18°C. Despite these recommendations, it is well known that oil is often stored in warehouses and sheds at ambient temperature and sometimes exposed to light. Large, semi-rigid containers are often housed in metal cages and exposed to light.



**Figure 3.2 Examples of the range of plastic containers being tested including Ecobulk tank, metallised bags, 4 litre bottle**

Additionally, it is recommended that the containers be filled to capacity to avoid contact with oxygen. Due to the design of the experiment, the majority of the containers were 1000 L capacity. At a cost of \$6 per litre, and limitations of the budget, it was not possible to fill 12 containers of 9105 L capacity.

The 1000 L containers were each loaded with 100 L of oil. Other containers were loaded with similar proportions of oil. The containers were then charged with nitrogen. Each time the container was opened, a sample was taken and the bag was recharged with nitrogen before resealing.

All containers, except semi flexible rigid containers, were stored within CHEP five sided pallet containers. The lids were left off to allow the same access of light as the semi rigid containers.

The study was to compare polymer types. As a result, other conditions have been neutralised to avoid unfair comparisons. The study was done in a closed shed at ambient temperature. As the study commenced in December, reasonably high temperatures were experienced during the life of the study.

Despite the limitations, it is considered that the comparisons between container materials are relevant. The large number of tests carried out provides a clear understanding of the changes taking place. Future studies are recommended utilising different storage conditions of light and dark, cooled and ambient temperatures. This project indicates the reduced number of bags which may be included in this future study and bags which should not be used at all.

## **3.4 Methodology**

### **3.4.1 Peroxide Value**

Peroxide value was determined using the International Union of Applied Chemistry method, 2-501 (IUPAC 1992). Oil (2.50 g) was dissolved in acetic acid/chloroform mixture (3:2). To this solution, 1 mL of saturated potassium iodide (KI), (70 g KI/40mL water), was added and shaken for 1 minute. The solution was then placed in the dark for 5 minutes. Water (75 mL) was added, followed by 2 drops of starch solution (2.5 g starch/100mL water). The solution was titrated with previously standardised 0.01N sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ). The volume of titrant was recorded and the peroxide value calculated and reported as mEq of active oxygen/kg oil.

### **3.4.2 Free Fatty Acids**

Free fatty acids were determined by the method of the American Oil Chemists Society, Ca 5a-40 (AOCS 1998). Oil (approximately 7.05 g) was dissolved in neutralised isopropanol (50 mL). Two drops of phenolphthalein (1% in ethanol) was added to the solution. The solution was the 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).

### **3.4.3 Total Phenolic 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 hexane (50 mL) 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. An aliquot (1 mL) was transferred to a 10 ml volumetric flask to which 5 mL of water was added. Folin-Ciocalteu reagent (0.5 mL) was then added to the flask and the sample shaken and left for 3 minutes. Saturated sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) (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. Known solutions 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 are expressed as mg of caffeic acid/kg of oil.

### **3.4.4 Induction time**

A Metrohm 743 Rancimat was utilised to determine the induction time of the oil. Oil (about 2.50 g) was weighed and placed in a block temperature of 110 °C and airflow of 20 L / hour. Volatile components which develop as a result of oxidation are measured. When the oil begins to oxidise, the change is recorded on the Rancimat. These results were reported as induction time in hours.

### 3.4.5 $\alpha$ -Tocopherol

$\alpha$ -tocopherol was measured using the International Standards Organisation method, ISO-9936 with slight modification. 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  $\alpha$ -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 Phenomenex Luna 5 $\mu$  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  $\alpha$ -tocopherol, which was expressed as mg/kg oil.

### 3.4.6 Ultraviolet absorption

Ultraviolet absorption was determined using the International Olive Council method COIT.20/Doc19/Rev2 (2008). 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 (isooctane). 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 content was then calculated and reported as  $\Delta K$ ,  $K_{232}$  and  $K_{268}$ .

### 3.4.7 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 then calculated as described in the method and reported as mg chlorophyll / kg oil.

### 3.4.8 Pyropheophytin

Pyropheophytin was measured using the German Society of Fats and Oils method, (DGF Draft 4.10.2005). This method determines the degradation products of chlorophyll a into pheophytin a, pheophytin à 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 is then weighed on top of the cotton wool. The silica layer is 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 is 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 set at 35°C. The residual sample was dissolved in 200  $\mu$ L of acetone and analysed on the HPLC with a mobile phase of water:methanol:acetone / 4:36:60, using a Phenomenex Luna 5  $\mu$  silica column (250 x 4.60 mm). The separated components were measured at 410nm using a photodiode array detector and data was analysed with Waters Empower Pro version 5.00. Peak areas were quantified and reported as % pyropheophytins.

### 3.4.9 Diacylglycerol

Diacylglycerols were determined using the German Society of Fats and Oils, DGF standard method C-VI 16(06). 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 then placed 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, (isooctane:diisopropyl 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 at 20°C. The remainder of the solution was evaporated under a gentle stream of nitrogen. Silylation reagent (50 µL 1-Methyl imidazole:1 mL MSHFBA) was added to the flask (200 µL) and allowed to react for at least 20 minutes. After silylation, 1mL acetone is added and left to stratify for 10 minutes before being 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°C for 1 minute, increased at a rate of 10°C / minute to 320°C and held for 10 minutes, increased to 340°C at 20°C /min and held for 10 mins. The injector temperature was set at 340°C. The detector temperature was 340°C. Data was analysed using Star® Workstation Chromatography software (version 6.20). Results are expressed as a percentage of 1, 2 diacylglycerols.

### **3.4.10 Fatty acid profiles**

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°C for 8 minutes and then increased at 10 °C / minute to a final temperature of 220 °C and held for 3 minutes. The injector temperature was set at 250 °C with a split ratio of 1:50. The detector temperature was 260 °C. Data was analysed using Star® Workstation Chromatography software (version 6.20). Results are expressed as a percentage of the total fatty acids

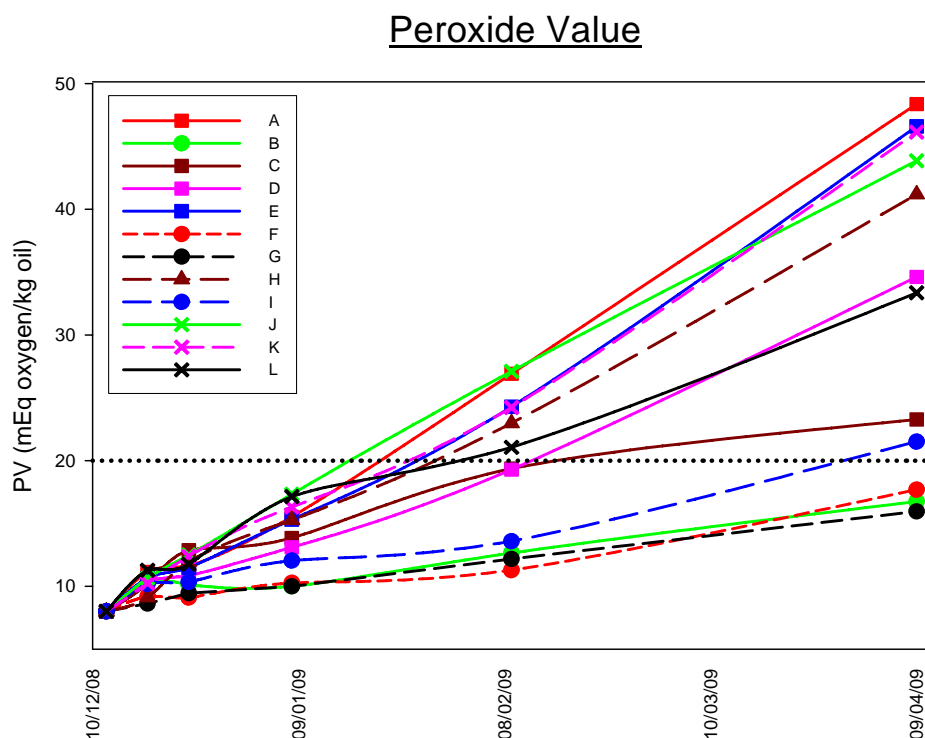
# 4. Results

## 4.1 Peroxide value

The initial peroxide value of the oil purchased was 8 mEq oxygen / kg of oil, well within the IOC standard of <20 mEq. Peroxide value is known to increase with light and oxygen and it was expected that the most porous, clear bags would oxidise most quickly. This was clearly the outcome as illustrated in Figure 4.1, with A (LDPE), E (PE), J (HDPE), K (HDPE) and H (unknown) exceeding the IOC limit within only eight weeks. Containers D (LDPE / EVA) and L (bottle) were also quickly passed the limits of > 20 mEq within 9-10 weeks.

Samples C (Nylon/LDPE) and I (Aluminium foil) also increased in PV and were just over the specification after 16 weeks.

Samples B, F and G, remained within specification over the test period although all had moved from 8 mEq / kg to around 17 mEq / kg within that time.



**Figure 4.1 Effect of 12 alternative types of storage material on the peroxide value of olive oil stored for 16 weeks**

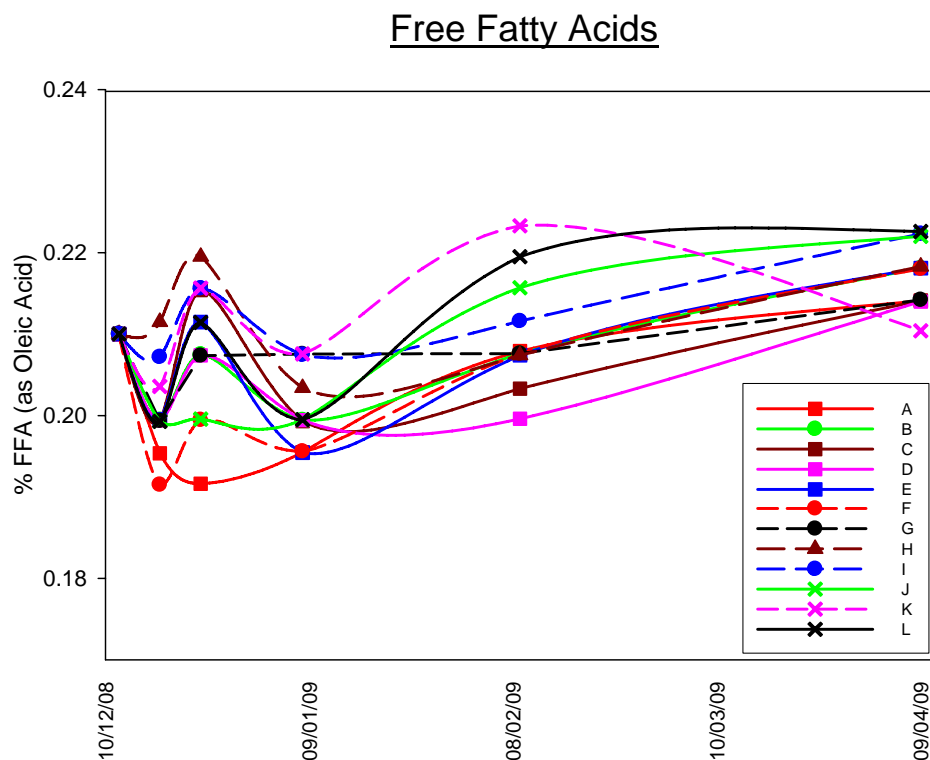
Only three of the 12 containers (B, F and G – which were all metallised PE) maintained oil quality within specification over the period. Perhaps the only surprise in his outcome was the aluminium foil barrier which exceeded 20 mEq but is shown by technical tables as having zero oxygen transfer rate. Regardless, the superior containers in all cases were the metallised PE bags.

Oil in low density polyethylene bags doubled in PV after 4 weeks and reached almost 50 mEq oxygen after 16 weeks, indicating that it is totally unsatisfactory for even short term storage or transport.

Peroxides are the primary oxidation products as the oil deteriorates and PV is commonly used to measure oil quality. Eventually peroxides convert to secondary oxidation products and the peroxide value will fall.

## 4.2 Free Fatty Acids

Free fatty acids are influenced by endogenous enzymes within olive fruit. The enzymes are not soluble in the oil and once the oil is extracted and the water and solid layers removed, free fatty acids are expected to remain constant. This is the case for the samples under test. The oil was clear and free of water or solids. Therefore it would be free of lipase enzyme.



**Figure 4.2 Effect of 12 alternative types of storage material on free fatty acids of olive oil stored for 16 weeks**

The variation seen within the samples is negligible and may indicate analytical error. In this case free fatty acids within the original sample were 0.21% and after 16 weeks ranged from 0.21 to 0.22%.

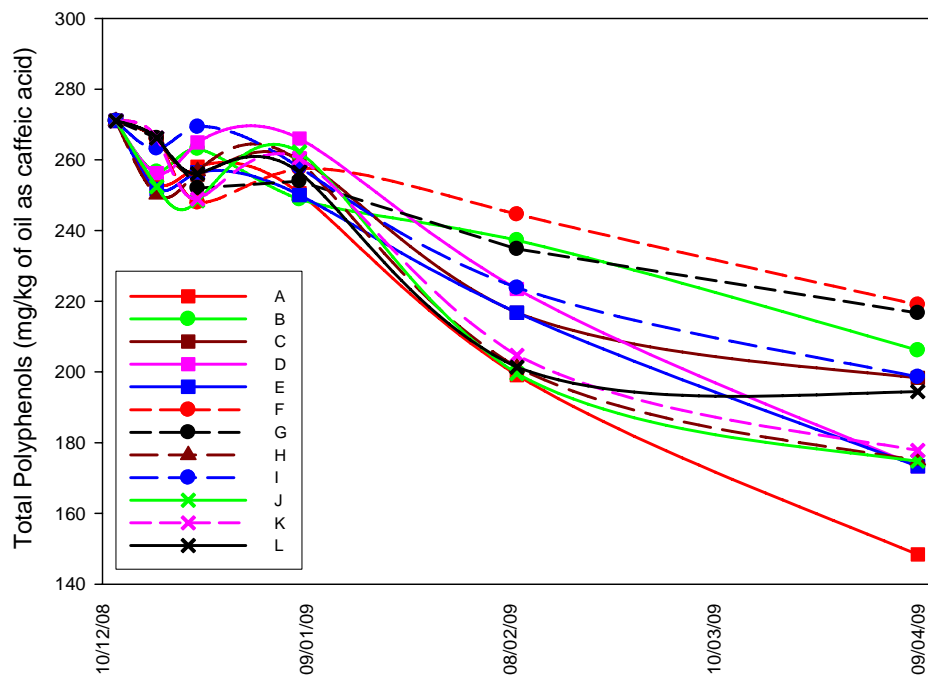
### 4.3 Phenolic content

Phenolic content in olive oil decreases with fruit maturity and continues to decrease after the oil is extracted from the fruit. Early harvested olives will produce oil with higher phenolics than mature fruit. The phenolics act as stabilisers, resisting oxidation and increasing the shelf life of the oil. However, increased levels of oxygen or free radicals will increase the rate at which the antioxidants are utilised and reduce their effectiveness. Total phenolic content of this oil commenced at 270 mg / kg.

In this study, phenolics were diminished most rapidly in sample A, (LDPE). This sample was markedly less effective at protecting the antioxidants than any of the other material, decreasing to 150 mg/kg within 16 weeks. Containers B, F, and G (metallised PE) provided by far the highest protection for the oil maintaining a phenolic content of 220 – 240 mg / kg after 16 weeks although even this is considerably less effective than would be expected in dark storage in glass or stainless steel.

I (aluminium foil), C (Nylon LDPE) and L (bottle) had marginal quality with phenolic levels dropping to around 200 mg/kg. E (PE), H (clear plastic), J (HDPE) and K (HDPE) had some minimal protective quality maintaining a level of around 180 mg/kg. Sample D (LDPE/EVA) was interesting in that it maintained high phenolics for 4 – 8 weeks but eventually was reduced to the level of the PE containers.

Total Polyphenols



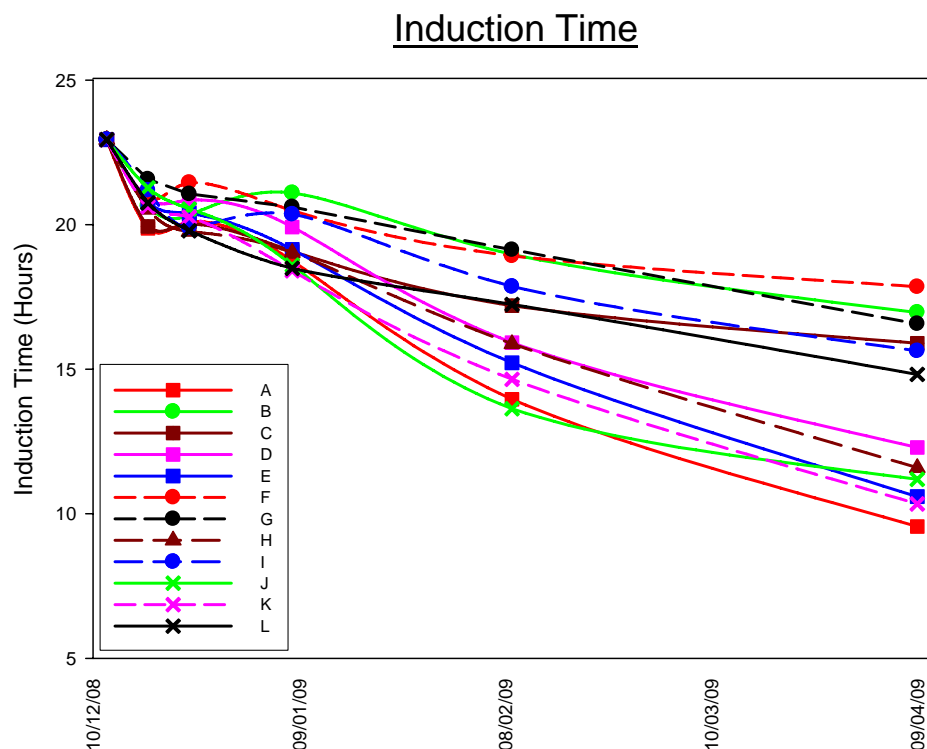
**Figure 4.3 Effect of 12 alternative types of storage material on phenolic content of olive oil stored for 16 weeks.**

These results correlate very closely with peroxide value with A, E, H, J and K having the least protection for the oil and metallised B, F and G being by far the superior packing.

## 4.4 Induction time

The induction time is the period calculated from Rancimat test at which there is a sudden change in conductivity as a result of an increased level of peroxide at a given temperature.

The induction time for the oil as received was 23 hours at 110°C. Induction time has been shown to be highly correlated with phenolic content. Containers B, G and F further endorse this as these were the three containers with the highest phenolic retention and also retain the best induction times. Additionally, A, the worst performing container for peroxide and phenolics also had the shortest induction time with only 8 hours after four months storage. Containers C, I and L were the next best with induction times of 15-17 hours. Containers D, H, J, E and K all decreased to less than 13 hours.



**Figure 4.4 Effect of 12 alternative types of storage material on induction time of olive oil stored for 16 weeks**

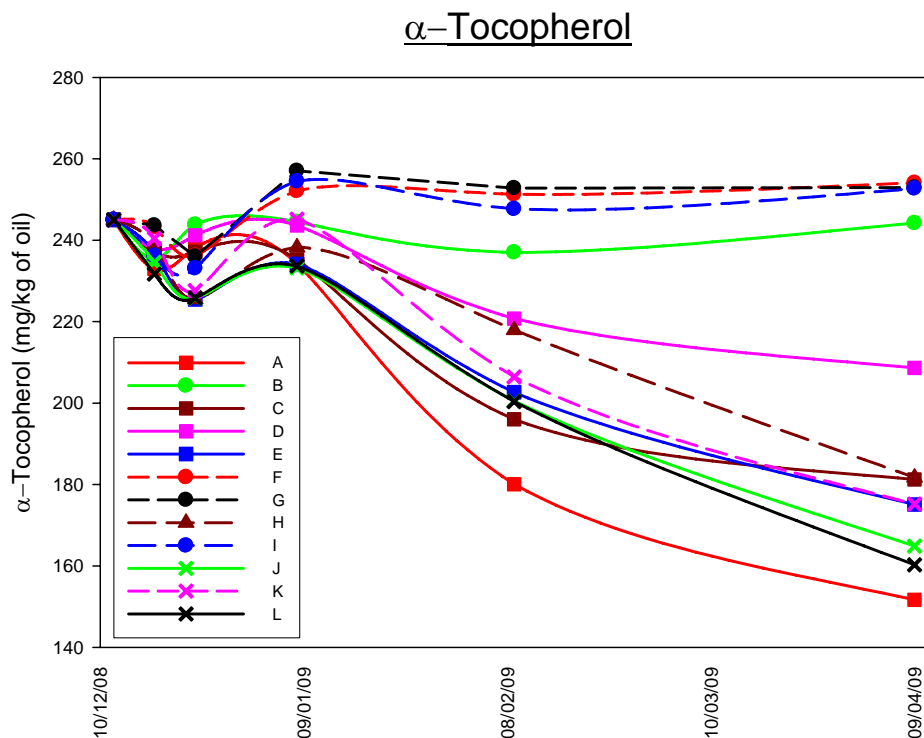
The induction time may be extrapolated to indicate the potential shelf life of the oil when stored under good conditions (not in flexible bags). Using this assumption, the original oil may be presumed to be suitable for a much longer period, when held in suitable storage conditions than sample A in which the shelf life has been reduced to less than half in only 16 weeks. Even the mid range containers have decreased to only 65% of the original shelf life.

## 4.5 $\alpha$ -Tocopherol

As for phenolic compounds, tocopherols offer stability to oil by reducing free radicals and increasing oxidative stability. The tocopherol content of this oil began at around 250 mg/kg.

Despite some minor fluctuations in the first few weeks, the three metallised bags had approximately the same tocopherol level after 16 weeks. Unlike the other tests, it is interesting to see that I, the aluminium foil bag joined the best group in retaining tocopherol levels at approximately original levels.

The plastic bottle, L has joined A in the worst performing group. Containers C, E, H, J and K were again poor performers. Sample D has been consistently mid range and indicates the LDPE/EVA material has greater protection against oxidation than simply PE.



**Figure 4.5 Effect of 12 alternative types of storage material on  $\alpha$ -Tocopherol of olive oil stored for 16 weeks.**

## 4.6a Specific extinction coefficient

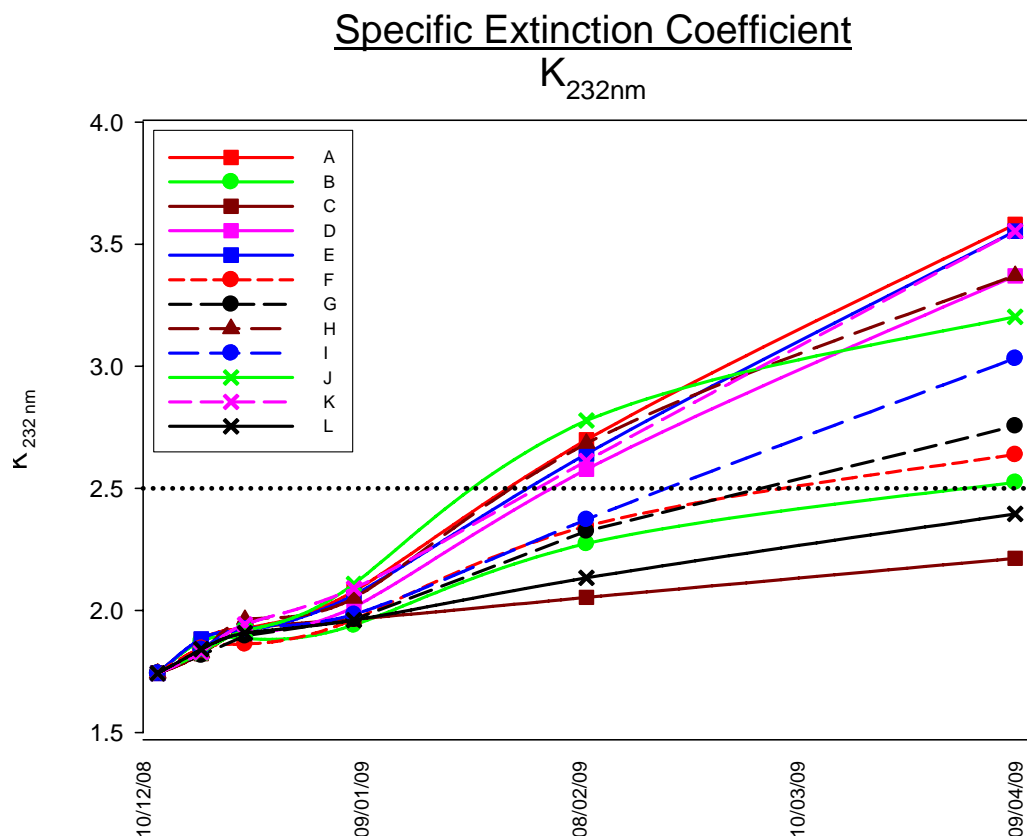
$K_{232nm}$ : UV absorption at  $K_{232}$  and  $K_{268}$  are used to determine if oil has been heated or is in some way degraded to cause changes in the configuration of the fatty acids. The maximum level for  $K_{232}$  must be less than 2.5. The original sample in this study was 1.74.

The results shown here are of high significance because they show the relationship of the K values to the oil quality. However, there is not a clear relationship with any of the chemical parameters and the  $K_{232}$  value.

A, K and E, all PE bags, have shown the greatest increase in UV absorption. H and D, also PE bags, have also increased significantly.

Even the best containers for other chemical parameters, B, F and G have changed significantly and would fail the IOC test.

The nylon (C) and the plastic bottle (L) have shown least change, remaining below the IOC limit.



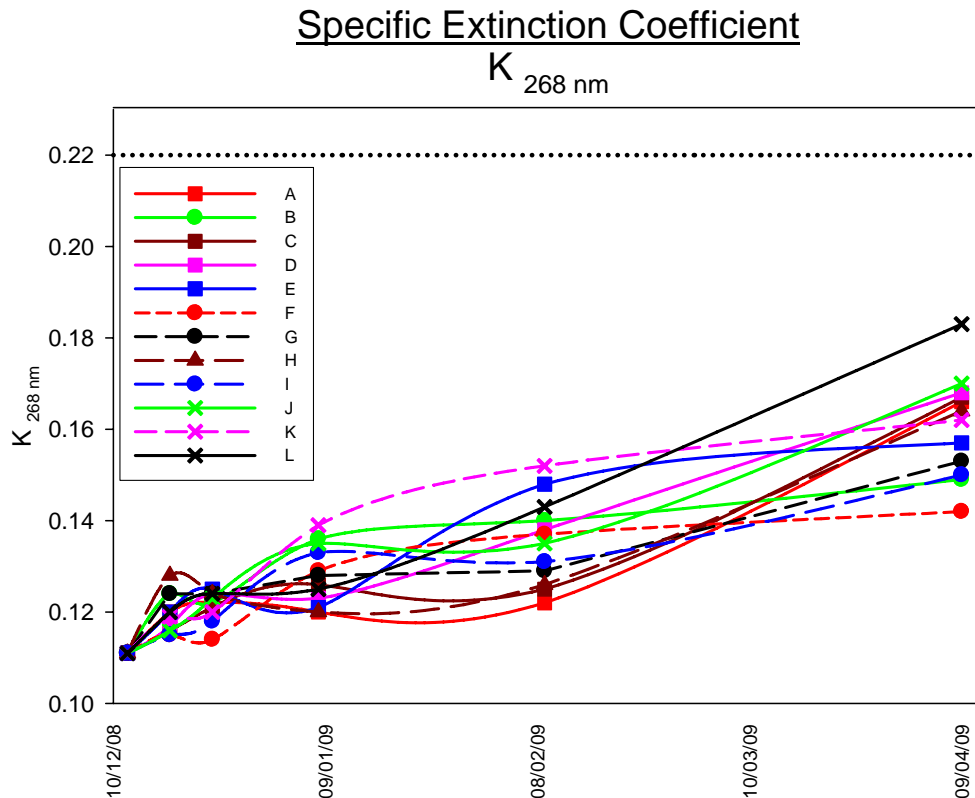
**Figure 4.6a Effect of 12 alternative types of storage material on  $K_{232nm}$  of olive oil stored for 16 weeks**

$K_{232nm}$  measures increases in primary oxidation products and therefore it can be seen that there is a direct relationship between peroxide value (primary oxidation) and  $K_{232nm}$ .

## 4.6b Specific extinction coefficient (continued)

**$K_{268\text{nm}}$ :** The  $K_{268}$  of EVOO must be less than 0.22. The original oil in this study was 0.11. All of the oils stored under different conditions remained under the IOC limit after 16 weeks. In contrast to the  $K_{232}$  result, the best performers at  $K_{232}$  were two of the worst at  $K_{268}$ .

Containers B, F and G were the best in this case, joined by I, the aluminium foiled barrier. This is a similar outcome to the results for tocopherols in which the metallic containers including the aluminium foil were the best. Aluminium foil, although generally being better than most, was not generally as good as metallic PE for other parameters.



**Figure 4.6b** Effect of 12 alternative types of storage material on  $K_{268\text{nm}}$  of olive oil stored for 16 weeks

$K_{268\text{nm}}$  is a measure of secondary oxidation products. Unlike  $K_{232\text{m}}$  there has been far less change in the test period as the oil has not developed the secondary oxidation products which will ultimately see this parameter increase (Bilancia *et al*, 2007).



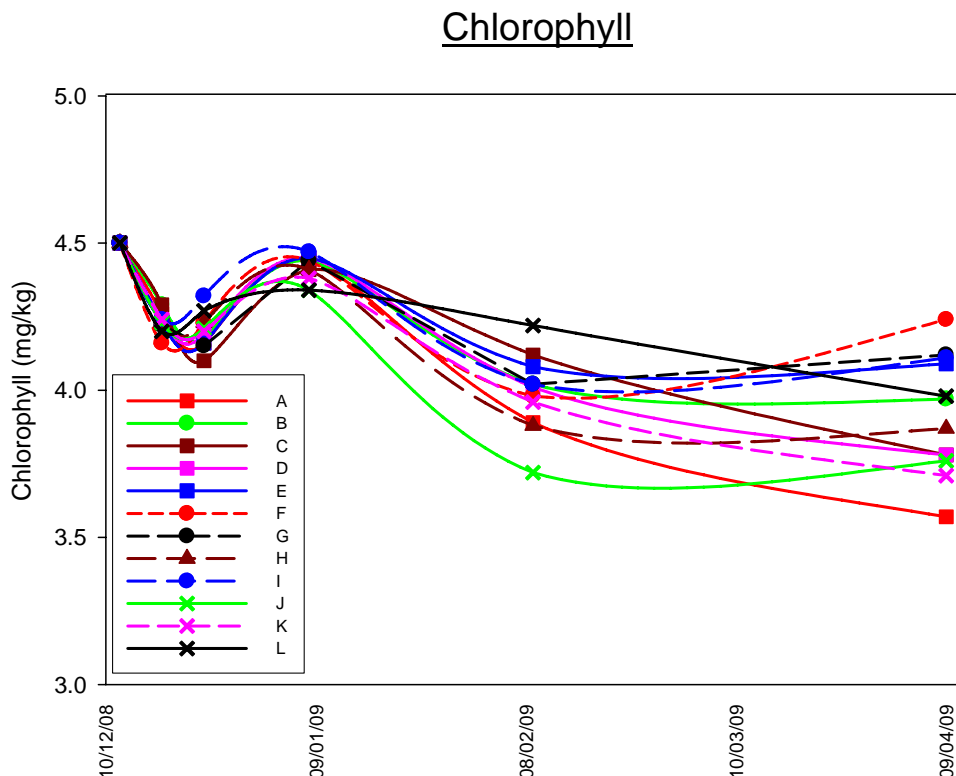
## 4.7 Chlorophyll

Chlorophyll decreases as fruit begins to ripen and continues to decrease after the oil has been extracted from the fruit. This process would be expected to be influenced by oxygen transfer rates as chlorophyll acts as an antioxidant in the dark but as a pro-oxidant in the light, capturing light energy.

Chlorophyll in the original sample was only 4.5 mg/kg. The amount of change has been shown over 16 weeks to be relatively small but this is a factor of the low initial chlorophyll content.

Despite the small change, the results are in the same relative order with B, F and G, together with E, I and L showing the least change. The metallised containers would be expected to better protect the chlorophyll due to the opaque nature of the material.

The PE containers again showed the greatest variation from the original sample.



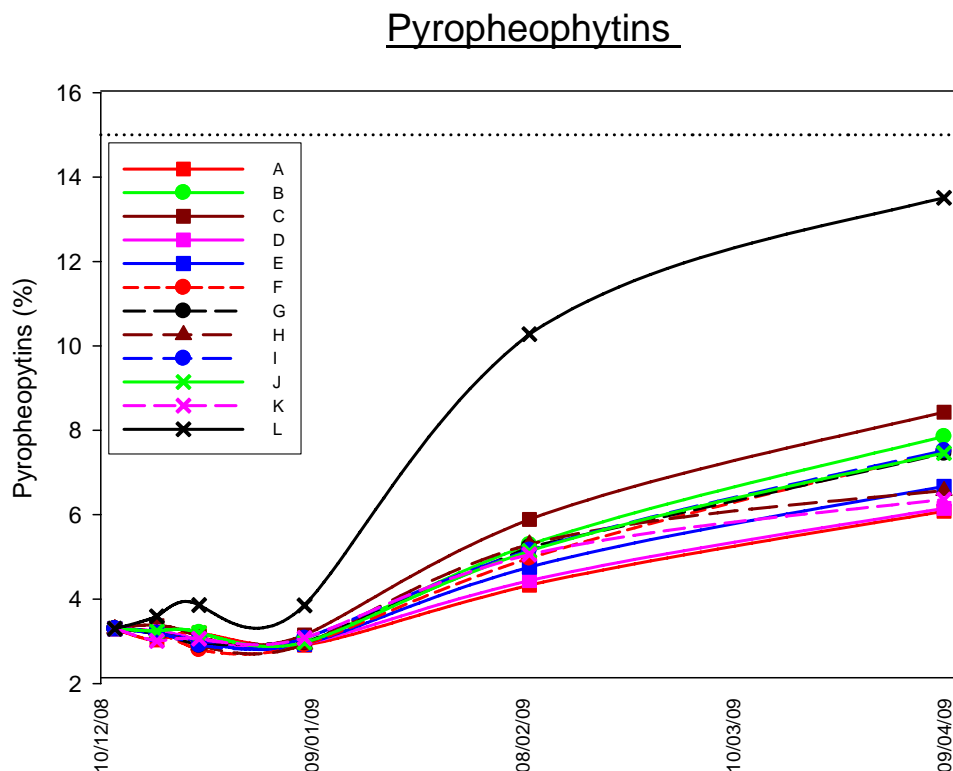
**Figure 4.7 Effect of 12 alternative types of storage material on chlorophyll content of olive oil stored for 16 weeks**

## 4.8 Pyropheophytin

Pyropheophytins (PPP) are by-products of chlorophyll pigments, changing from chlorophyll to pheophytin to pyropheophytin under the influence of time and / or heat treatment. No results have been published to the author's knowledge of the influence of storage containers on pheophytin content. The Australian Olive Association has adopted a standard of <15% for PPP in EVOO. The original olive oil sample had a PPP level of 3%.

The container material in this study has shown a highly significant effect with the plastic bottle showing a rapid increase in PPP within a few weeks. Although the level increased in sample L from 3% to 13% in 16 weeks, the oil was still within specification. This study will continue to determine how much it will increase.

It is of interest that the PE material in several of the containers, in which quality characteristics were adversely affected, showed least effect for PPP. Sample A, for example, which had the poorest storage ability based on peroxide level, has shown the least change for PPP. Containers B, F, G and I, all having good storage characteristics, were in the highest category for PPP increases although overall the changes were small.



**Figure 4.8 Effect of 12 alternative types of storage material on pyropheophytin content of olive oil stored for 16 weeks**

This is the first information which has been published on storage effects on pyropheophytins but it is a good indication of the usefulness of this method. These samples have changed within four months but it is likely that they will fail the AOA imposed limits of 15% within 12 months. The oil has already deteriorated beyond good quality olive oil within four months. The limit of 15% is therefore flexible enough to avoid rejection of good quality oil in routine screening. The near linear response of pyropheophytin to time indicates that the majority of these oils will fail within around 12 months.

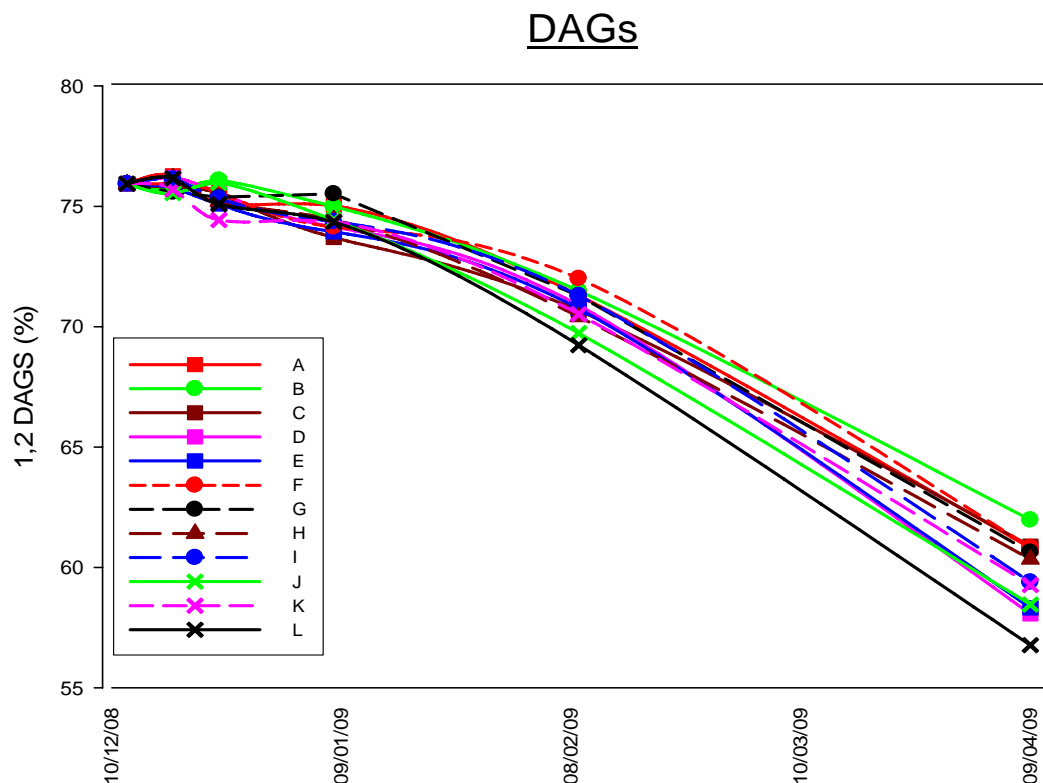
## 4.9 Diacylglycerol

Diacylglycerol (DAGs) are oil molecules derived from triacylglycerols, in which one of three fatty acids has been removed. The reason for loss of fatty acids from the molecule could be enzymatic but DAGs may form also during storage or with processing. The proportion of 1,2 DAGs to the total 1,2 and 1,3 DAGs has been proposed as a method to detect old or adulterated olive oil which may contain

refined oil. The Australian Olive Association has suggested a level of >40% as the Australian standard for EVOO. The EVOO received for this study had a 1, 2 DAG level of 76%.

This level decreased significantly and over 16 weeks had decreased by 16%. All of the oils in a range of containers still exceeded the required level of 40%. However, the near linear relationship of DAGs to time would indicate that all of these containers may fail within a further two months. Samples will be retained to measure this effect.

Unlike the results for PPP, DAG levels changed less in the metallised bags and the most in PE bags, as found for the chemical characteristics of the oil such as peroxide value and phenolic content. However, all of the containers showed significant deterioration.



**Figure 4.9 Effect of 12 alternative types of storage material on DAGS content of olive oil stored for 16 weeks**

These are the first results shown for the effect of storage on DAG content for the determination of oil quality. None of the oils have failed the AOA limit of 40% which shows that the level is sufficient to avoid rejection of good quality oil in routine screening when using this standard. The near linear response to storage on DAGs would indicate that these oils will fail within 12 months.

#### 4.10 Fatty acid profile

As expected, fatty acid profile did not vary in the different treatments. The results are shown in Appendices in Table 8.4.

## 5. Discussion of Results

This report describes the changes in oil quality which result from the oil being stored in plastic bags composed of a range of types of plastics. The oil was analysed at the time of filling the bags and then retested at regular intervals over time. Due to the cost of oil and large (1000L) sample containers, it was not possible to investigate all storage conditions. Additionally the bags were not full of oil but only partially filled and then sparged with nitrogen to remove oxygen.

Optimum storage conditions for olive oil include no contact with oxygen or light and reduced temperatures of around 16°C. This experiment was designed as a worst-case scenario and these tests were carried out in a shed, over summer, without temperature control. The containers were stored within four sided enclosures but the top was exposed to filtered light as it often is in commercial situations. As a result, the oil decomposition would be expected to be more rapid than if the investigation had been carried out at optimum storage conditions. It was not intended to investigate the range of storage conditions used by the industry. All of the samples in the range of plastic containers failed to meet the standards for extra virgin olive oil after only 16 weeks.

### 5.1 Peroxide value

The first test of oil deterioration in storage is to measure the level of increase in peroxide value. The acceptable level for peroxide is less than 20 mEq/kg oxygen. In this study, after eight weeks, eight of the 12 containers had exceeded the maximum allowable for extra virgin olive oil. Low density polyethylene was the least resistant to oxidation whereas the four metallic bags showed best resistance. After four months, even the best liners had reached 16 mEq oxygen and would fail soon after. From Table 5.1 it can be seen that the four metallic bags had the least change in peroxide value. Additionally, it is clear that the bags with the greatest oxygen transfer rates performed the worst.

### 5.2 Free fatty acids

Free fatty acids are also used to test oil quality but the formation of free fatty acids relies mostly on enzymatic reactions during processing. These lipase enzymes are not present in processed olive oil and, as expected, there was virtually no change in free fatty acids in any of the samples.

### 5.3 Total polyphenols

Polyphenols are antioxidants and these protect the oil from oxidation. Contact with oxygen reduces the level of antioxidants and the resistance to oxidation. As with peroxide value, the metallic bags maintained high levels of polyphenols due to the low oxygen transfer rates through the metallic barrier. In contrast, oil stored in low density polyethylene bags showed the greatest reduction in polyphenols. Induction time is a measure of the resistance to oxidation and is therefore related directly to the level of antioxidants. As expected, the reduction for polyphenols mirrored the reduced induction time.

### 5.4 $\alpha$ -tocopherol

$\alpha$ -Tocopherol (Vitamin E) is a strong antioxidant in olive oil. Similar results were found for  $\alpha$ -tocopherol as was seen in the polyphenol results. Low density polyethylene was the first to lose  $\alpha$ -tocopherol and the metallic bags were the most resistant to change.

**Table 5.1 Container description showing gas transmission rates and peroxide value after four months storage – sorted from lowest to highest peroxide value**

<b>Sample</b>	<b>Product description</b>	<b>Product Layers</b>	<b>Peroxide value after 4 months storage (mEq O<sub>2</sub>/kg oil)</b>	<b>Gas transmission rate (cc of O<sub>2</sub> per atm for 1 mm thickness)</b>
G	Barrier Top Fill/Bottom Empty MPP Maxicon Liner	Metallised Polyester Laminate Hi Flex Polyethylene Linear Polyester	16	-2.0
B	High Barrier Bag Bulk	Metallised Polyester Laminate Low Density Polyethylene	17	-2.0
F	Multipak AMF Liquid Liner	Metallised Tri-Laminate EVOH Co-Extrusion Hi-Flex Polyethylene Linear Polyethylene	18	-2.0
I	Top of the Line Aluminium Foil Barrier	Metallised N/A	22	-2.0
C	Clear Durable Structure Bag Bulk	Nylon Laminate Low Density Polyethylene	24	1.6
L	Plastic Storage Container	N/A	33	N/A
D	Liner Bag F 1000C N 2P 2/2 CM TTF (Form Fit)	Linear Low Density Polyethylene Ethylene Vinyl Acetate	35	215
H	Standard Unit for Olive Oil	Clear plastic N/A	41	N/A
J	Ecobulk MX-EV with additional EVOH Barrier	High Density Polyethylene EVOH with 2 Adhesive Layer	44	58
K	Ecobulk MX INT – Standard Unit	High Density Polyethylene	46	58
E	Non Barrier Top Fill/Bottom Empty PPP Liner	Linear Polyethylene Hi Flex Polyethylene	47	215
A	Polyethylene Bag Bulk	Low Density Polyethylene	48	215

## 5.5 UV absorption

The specific extinction coefficient, or the UV Absorption readings at 232 and 268 nanometres (nm) measures changes in the conformation of the fatty acids. The initial changes due to oxidation, or primary oxidation products, are detected by absorbance at 232 nm and further breakdown products, or secondary oxidation products, at 268 nm. The initial changes in the oil were shown by an increase in absorbance at 232 nm with all of the containers, but most obviously in the low density polyethylene. Less obvious was the change in absorbance at 268 nm but this would continue to increase over time as the secondary products develop. The relative order was the same for all of the containers.

## 5.6 Pyropheophytin a

Chlorophyll is the green pigment in fresh olive oil. The green colour changes as the oil ages and all of these samples reacted in a similar manner. The chlorophyll is converted to alternative yellow and brown pigments pheophytins (PP) and pyropheophytins (PPP). The German Fat Society (DGF) has indicated that the proportion of PPP to the total pheophytins is a good indicator of aged oil. In this study 11 of the oils performed in a similar manner with an increase in % PPP over time. Only one, the plastic bottle, showed a marked increase. This change could not be explained but the change in all of the other oils would indicate that they will reach the limit of 15% in around 12 months. From this point it is good to indicate the oils age but it did not differentiate the types of containers which had been shown to have a wide range of oil quality.

## 5.7 1,2-Diacylglycerols

Diacylglycerols are formed as fatty acids are lost from oil molecules (triacylglycerols). Although the fatty acids are lost from the 1-position to form 2, 3-diacylglycerol, in time the molecule finds equilibrium as some molecules move to the 1, 3-diacylglycerol position. The proportion of 1,3-diacylglycerol is an indicator of the age of the oil and may also indicate some refining. In this study, all oils showed deterioration with no clear relationship to the plastic container.

Overall, the results have shown clearly that some plastic materials, such as polyethylene, are unsatisfactory for storing extra virgin olive oil. The small reduction in cost in using a cheaper bag is totally lost by the huge loss in the quality of the oil. In most cases, the metallic bags have been shown to preserve oil quality at the highest level. However, even oil stored in these bags, over time, is seen to lose quality.

It would seem clear that metallic bags should be the only type used for transporting olive oil. These results would indicate that the oil should not be stored for long periods of time in any bags. Additionally experience would indicate that the bags should be kept in a dark and cool environment although this has not been investigated in this study.

The project has been a good opportunity to evaluate the relatively new DGF methods for pyropheophytin and DAG content. In both cases the levels indicated that the oils were deteriorating with age, as they are designed to do. The AOA has set limits for pyropheophytin a at  $\leq 15\%$  (of total pheophytins), and for 1,2-diacylglycerols  $\geq 40\%$  (of total 1,2 and 1,3-diacylglycerols). It is shown that the levels set by the AOA are adequate to avoid rejection of good quality oil.

## 6. Implications

The industry needs to be made aware of these outcomes as a matter of urgency. Discussions with numerous clients of the AORL, in which oils are stored in plastic containers, are clearly not aware of the implications of this practice. The high cost of olive oil may result in significant losses to merchants. The reputation of the suppliers can also be damaged by supplying good quality oil in containers where it will lose that quality quickly and reflect on the supplier. The reputation of the Australian industry could be damaged if oil is exported in unsuitable containers to overseas countries.

Australia produces olive oil which is as good as any in the world. However, that oil is dynamic and changes with time, temperature and exposure to oxygen and light. Poor storage and transport conditions will have an adverse effect for all of the people involved in producing transporting and selling the product.

## 7. Recommendations

In this study, after eight weeks, eight of the 12 containers had exceeded the maximum peroxide value allowable for extra virgin olive oil. From Table 5.1 on page 23, it can be seen that the four metallic bags had the least change in peroxide value. Low density polyethylene was the least resistant to oxidation whereas the four metallic bags showed best resistance. After four months, even the best metallic liners had reached 16 mEq oxygen and would fail soon after. It is clear from Table 5.1 that the bags with the greatest oxygen transfer rates performed the worst.

Based on these findings, olive oil should be stored only in the recommended flexible metallic containers and only for the absolute minimum time necessary to transport the oil between the supplier and the consumer. Use of clear, porous polyethylene containers is not recommended under any conditions and not even for short periods of time. This will ensure that the quality of the oil does not deteriorate to unacceptable levels.

RIRDC and AOA need to disseminate the details of this study to the industry with some urgency. The AOA has access to a large portion of the industry through its members and the well utilised AOA web page and this data can quickly be made available to those who may be using the wrong material for oil storage.

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# 10. Appendices

**Table 10.1 Comparison of peroxide value, polyphenol content, induction time and  $\alpha$  tocopherols of oil stored in 12 containers for 4 months.**

Sample	PV		Polyphenols		Induction Time		$\alpha$ Tocopherol	
	mEq oxygen/kg oil		mg/kg of caffeic acid		Hours		mg/kg	
	0 months	4 months	0 months	4 months	0 months	4 months	0 months	4 months
A	8	48	271	148	22.94	9.56	245	152
B	8	17	271	206	22.94	16.96	245	244
C	8	24	271	198	22.94	15.90	245	181
D	8	34	271	174	22.94	12.29	245	209
E	8	46	271	173	22.94	10.59	245	175
F	8	18	271	219	22.94	17.85	245	254
G	8	16	271	217	22.94	16.57	245	252
H	8	41	271	175	22.94	11.59	245	182
I	8	21	271	199	22.94	15.63	245	253
J	8	43	271	175	22.94	11.19	245	165
K	8	46	271	177	22.94	10.34	245	175
L	8	33	271	194	22.94	14.82	245	160

**Table 10.2 Comparison of oil specific extinction coefficient  $K_{232nm}$  and  $K_{268nm}$ , when stored in various containers for between 0 months and 4 months**

Sample	Specific Extinction Coefficient		Specific Extinction Coefficient	
	K 232 nm ( $K_{1cm}^{1\%}$ )		K 268 nm ( $K_{1cm}^{1\%}$ )	
	0 months	4 months	0 months	4 months
A	1.74	3.58	0.11	0.17
B	1.74	2.52	0.11	0.15
C	1.74	2.21	0.11	0.17
D	1.74	3.37	0.11	0.17
E	1.74	3.55	0.11	0.16
F	1.74	2.64	0.11	0.14
G	1.74	2.76	0.11	0.15
H	1.74	3.37	0.11	0.16
I	1.74	3.03	0.11	0.15
J	1.74	3.20	0.11	0.17
K	1.74	3.55	0.11	0.16
L	1.74	2.40	0.11	0.18

**Table 10.3 Comparison of containers between 0 months and 4 months for free fatty acids, chlorophyll, pyropheophytins and DAGS**

Sample	Free Fatty Acids		Chlorophyll		Pyropheophytins		1,2 DAGS	
	% (as Oleic Acid)		mg/kg		%		%	
	0 months	4 months	0 months	4 months	0 months	4 months	0 months	4 months
A	0.21	0.21	4.5	3.6	3.3	6.1	75.9	60.8
B	0.21	0.22	4.5	4.0	3.3	7.8	75.9	62.0
C	0.21	0.21	4.5	3.8	3.3	8.4	75.9	60.9
D	0.21	0.21	4.5	3.8	3.3	6.2	75.9	58.1
E	0.21	0.22	4.5	4.1	3.3	6.7	75.9	58.3
F	0.21	0.22	4.5	4.3	3.3	7.5	75.9	60.8
G	0.21	0.21	4.5	4.1	3.3	7.5	75.9	60.6
H	0.21	0.22	4.5	3.9	3.3	6.6	75.9	60.3
I	0.21	0.22	4.5	4.1	3.3	7.5	75.9	59.4
J	0.21	0.22	4.5	3.8	3.3	7.5	75.9	58.5
K	0.21	0.21	4.5	3.7	3.3	6.4	75.9	59.3
L	0.21	0.22	4.5	4.0	3.3	13.5	75.9	56.8

**Table 10.4 Comparison of fatty acid profiles at 0 months and 4 months**

		----- Fatty acid profile (%total fatty acids) -----											
	Sample	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
0 months	initial	<b>0.0 ± 0.0</b>	<b>11.2 ± 0.0</b>	<b>0.8 ± 0.0</b>	<b>0.1 ± 0.0</b>	<b>0.1 ± 0.0</b>	<b>2.2 ± 0.0</b>	<b>72.1 ± 0.0</b>	<b>12.1 ± 0.0</b>	<b>0.7 ± 0.0</b>	<b>0.4 ± 0.0</b>	<b>0.2 ± 0.0</b>	<b>0.1 ± 0.0</b>
4 months	A	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	B	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	C	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	D	0.0 ± 0.0	11.2 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.1 ± 0.1	12.1 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	E	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	F	0.0 ± 0.0	11.2 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.1 ± 0.1	12.1 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	G	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	H	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	I	0.0 ± 0.0	11.2 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.1 ± 0.1	12.1 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	J	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.1	0.7 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	K	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
4 months	L	0.0 ± 0.0	11.3 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.2 ± 0.0	72.0 ± 0.1	12.1 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0

# Effect of Storage Containers on Olive Oil Quality

RIRDC Publication No. 09/160

By Dr Rodney J. Mailer and Kerrie Graham

This report assesses the impact of storage in a range of flexible storage containers on extra virgin olive oil quality. The research was conducted by the Australian Oils Research Laboratory (AORL). It was supported by RIRDC and the Australian Olive Association.

The outcome of this study reinforces that the best storage conditions for olive oil is in opaque, impervious and inert containers, stored at cool temperatures. Stainless steel or glass would appear to be the best options for long term storage. Metallised flexible bags used for short term transport may provide reasonable protection. Storage in clear plastic, particularly in the light and at elevated temperatures, is clearly unacceptable and will result in total loss of extra-virgin olive

oil quality within weeks and perhaps days. Re-use of these containers would appear to be highly undesirable and would be expected to cause more rapid degradation.

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