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A Survey of Australian Olive Cultivars to Determine Compliance with International Standards

RIRDC Pub. No. 08/167

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A Survey of Australian Olive Cultivars to Determine Compliance with International Standards

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October 2008

RIRDC Publication No 08/167
RIRDC Project No PRJ-000497

ISBN 1 74151 756 7
ISSN 1440-6845

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Published in October 2008 by Union Offset

Foreword

Preliminary studies conducted by the Australian Oils Research Laboratories in 2005 showed that some components in Australian olive oils were outside the international standards, even though they were genuine olive oil. These findings present a major problem for olive producers, particularly those who wish to export olive oil, but also those who want to comply with international trading standards. It also presents a problem in trying to detect oils which have actually been adulterated. This project was designed to determine what parameters may exceed international standards. It was also proposed to study other methods which may be used to identify authentic oils, even though chemical components may vary due to natural growing conditions.

The results showed that many of the fatty acids in Australian olive oil will be outside International Olive Council (COI) standards on some occasions. It also identified cultivars of olives which did not comply with COI standards for individual sterol content. The importance of this report is that it provides the industry with information to assist with marketing of olive oil, either within Australia or internationally. Oil is now being constantly analysed for producers, for the components identified in this report, to ensure unhindered trading with clients. Oil which is high in linolenic acid, campesterol, or other components is blended to satisfy standards which were established to suit oils produced in traditional growing areas such as the Mediterranean.

More importantly to the Australian industry, the results are now being used to negotiate changes to the trading standards. Over the last four years, Codex Australia has mounted a progressively stronger case against the adoption of COI standards by Codex Alimentarius. Standards for linolenic acid and campesterol are being studied and ultimately should provide an equal opportunity for olive producers across the globe.

New techniques, based on changes which occur to chlorophyll with heating and refining, as well as measurement of diacylglycerols to determine the age of olive oil, have been studied to assist the Australian industry to verify the high quality of the oil. These methods show promising outcomes and will be evaluated further for routine use.

This project was funded from RIRDC Core Funds which are provided by the Australian Government from the NPP program and industry funds were provided by the Australian Olive Association

This report, an addition to RIRDC's diverse range of over 1800 research publications, forms part of our New Plant 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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Acknowledgments

This study was carried out by staff at the NSW Department of Primary Industry's "Australian Oils Research Laboratory" at Wagga Wagga, NSW. A significant amount of the chemical analysis was done by Ms Kerrie Graham, Technical Officer. Samples used in this project were supplied by independent olive growers including Brian Hinson, Tasmania, Ian Delacy, Victoria, Leandro Ravetti, Victoria; Vince Tana, Western Australia and Peter and Jenni Birch NSW. The Australian Olive Association and in particular, the president, Mr Paul Miller, has been an essential component of this research.

Abbreviations

AOA	Australian Olive Association
AORL	Australian Oils Research Laboratory
Codex	Codex Alimentarius
COI	International Olive Council
COOC	Californian Olive Oil Council
DAGS	diacylglycerol
EC	European Commission EC No1989/2003.
EVOO	extra virgin olive oil
GC	gas chromatograph
HPLC	high performance liquid chromatograph
Moro 45	AORL name for a virgin oil serving as a standard
NATA	National Association of Testing Authorities
NSW DPI	New South Wales Department of Primary Industries
NSW	New South Wales
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

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Executive Summary

What the report is about
The report describes quality characteristics of Australian olive oil and compares the findings to standards used in international trade. The report shows the effects of olive cultivars, the influence of harvest timing and the changes to quality as a result of site and seasonal growing conditions. Currently genuine olive oil can be rejected as adulterated when it is outside existing regulations. Results in this report clearly describe quality characteristics of premium quality, extra virgin olive oil grown under Australian conditions.

Who is the report targeted at?
This report is targeted at those who set international standards for olive oil. It describes quality characteristics of olive oil and the impact that natural factors can have on the final product. Although standards of the International Olive Council (COI) have been developed in traditional olive oil producing countries, the standards of Codex Alimentarius are universal standards which are by definition, the regulations which cover all member countries. The Codex Alimentarius Commission was created in 1963 by FAO and WHO and one of their main purposes is "to ensure fair trade practices in the food trade, and to promote coordination of all food standards work undertaken by international governmental and non-governmental organizations" (Codex Alimentarius, 2007). Codex has however adopted many of the COI standards without consideration of the type of data outlined in this report. Organisations including FOSFA, the European Community, USDA and others within individual countries also set regulations which can impede trade of authentic high quality products if consideration is not given to natural product variability. The authors would hope that these organisations will become aware of the discrimination against high quality products by inappropriate trade standards.

> One of the main purposes of the Codex alimentarius programme is to ensure fair trade practices in the food trade, and to promote coordination of all food standards work undertaken by international governmental and non-governmental organizations.

Background
Australia is a relatively new commercial sized producer of olive oil and has recently begun to export significant quantities of oil to regions including USA, Asia and Europe. Although there are no current official Australian government standards, the Australian Olive Association (AOA) has based its standards on those of the International Olive Council. The AOA is part of a national infrastructure developed to ensure that Australian product is genuine and at least as good as oil from other olive oil producing countries.

During the mid 1990s, research at the Australian Oils Research Laboratory, Wagga Wagga (AORL) identified fresh and genuine olive oils which exceeded international standards for one of a range of fatty acids, namely linolenic acid. Although linolenic acid is not harmful to the consumer, olive oil contains only low concentrations and it is therefore useful as an indicator of adulteration. It can be used to detect the presence of oils from the *Brassica* family such as canola, rapeseed, colza or mustard which contain high levels of linolenic acid, generally in excess of 10%. International standards require that olive oil have $\leq$ 1.0% linolenic acid whereas Australian oil may have up to 1.5% and therefore may appear to be adulterated.

Further testing at AORL in recent years has shown an additional problem where some individual sterols, particularly campesterol, also exceed international limits. In this case, it appears that the problem is more due to the cultivar rather than the environment. Other countries also experience problems with cultivars such as Barnea, Cornicarbra and Koreneiki which exceed the limits.

> …this is not an Australian problem but an issue for all olive producing nations.

Collaboration with researchers in other countries including Chile, Argentina, New Zealand, Spain, France and Italy have shown that this is not an Australian problem but an issue for all olive producing nations. As such the outcomes have far more significance than the investigation initially expected. It has now become apparent that shipments of genuine olive oil are being rejected on the basis of inappropriate regulations.

> …shipments of genuine olive oil are being rejected on the basis of inappropriate regulations.

Codex Australia have been made aware of the concerns highlighted by this research and have reacted accordingly. Delegations, including those from Codex Australia, Codex New Zealand, a Technical Expert from NSW DPI (Rod Mailer), and a representative of AOA (Paul Miller), have attended biennial Codex Alimentarius Fats and Oils meetings in London in 2003, 2005 and 2007 in an to attempt to correct the clearly unacceptable standards set for olive oil which discriminate against Australasian producers and those from other countries.

Aims/Objectives

The aim of this study has been to present a list of basic data which describe Australian olive oil. In all cases, these data have been produced from oil extracted in the laboratory, from fresh olives, using official Spanish oil extraction apparatus, within 24 hours of harvest.

The objectives of this report are to benefit oil producers, traders and particularly exporters of olive oil who are experiencing problems selling genuine unadulterated extra virgin olive oil. It is hoped that the results will be considered by regulators when developing new standards for olive oil so as not to create trade barriers which restrict genuine high quality product.

An additional aim in this study was to evaluate new methods developed in Germany to test for adulteration in olive oil. These methods measure changes in pigments and triacylglycerol structure as an alternative to traditional methods. This objective was to find new methods which are not environmentally sensitive and do not discriminate against oil grown outside of traditional growing areas.

The study also aimed to measure oleocanthal, a compound in olive oil with reputed pharmaceutical benefits. This compound may provide further value to olive oil particularly if it can be extracted from olive waste.

Methods used

With funding from RIRDC and the Australian Olive Association, and with the support of several growers within the industry, 2-3 kg of 11 of Australia's most predominant cultivars of olives were sourced from four environmentally different sites at early and late fruit maturity. The study was repeated over two years. Intact fruit was transported in calico bags to Wagga Wagga. The fruit was treated in a manner to keep decomposition to a minimum. For the study, only oil which was extracted

within the laboratory was acceptable to ensure that no adulteration of the oil had occurred prior to delivery to the AORL. The oil was extracted by official mechanical means using an Abencor extractor sourced from Spain and using the protocol provided by Abencor. The extracted oil was subjected to testing of all quality parameters which normally might be used to determine if the oil was genuine, based on COI trading standards.

Results/Key findings

The project generated over 6,000 individual data points which describe the possible ranges which occur for Australian produced extra virgin olive oil. Rather than study the data as combined statistical results, the raw data has been provided to describe influences of each of the individual variables, cultivar, site, season and harvest maturity. The outcomes show that the number of deviations from existing trading standards is in fact greater than previous predictions. As expected, all oils were shown to pass the tests designed to show the presence of refined or heated oils or the presence of solvent extracted pomace oil as none of these oils had been treated other than by acceptable mechanical extraction techniques. However, tests used to indicate if there is presence of other types of oils, showed considerable non-conformity. Almost all of the 13 fatty acids used to determine if the oil is genuine were outside the limits in some cases. The sterol profile was also shown to have numerous outliers with campesterol alone being greater than the standard of 4.0% in 20 cases.

> Almost all of the 13 fatty acids were outside the limits in some cases.

Implications for relevant stakeholders

The implication of these findings is clear. For all of the situations in which authentic extra virgin olive oil is outside the limits, these products could be rejected on the grounds of fraud. Not only does this limit the sale of authentic products, it may cost exporters large amounts of money to send oil outside the country, only to have it rejected as adulterated. Another very important implication is that Australian producers are now blending high quality oil to meet standards. As a result, oil with exceptional characteristics such as organoleptic quality and oxidative stability are being blended with inferior oil to achieve compliance with inappropriate trade standards. The maintenance of these standards may well limit the profitability of olive production in Australasia, and other regions, and see some highly successful cultivars removed due to non-compliance.

> - oils with exceptional organoleptic quality and oxidative stability are being blended to comply with inappropriate trade standards.

Consumers in particular will be implicated as oil is no longer produced to achieve the highest possible sensory product with the best stability but it is being designed to be within regulations with no relevance to oil quality.

The latter part of this project describes new methods proposed by German chemists which may identify adulterated oils where existing methods fail to do so. These methods include measuring the change in chlorophyll pigments under the influence of high temperature or determining changes in triacylglycerols to diacylglycerols over long storage times. Analysis of components such as pyropheophytins and diacylglycerols were carried out as part of this project. Both methods were proposed by the German Society for Fat Science as being useful indicators of adulteration of olive oil. Our results indicate that although EVOO can develop pyropheophytins over time, heating has a much greater effect on conversion of the pigments, particularly the high temperatures employed for bleaching. Therefore, analysing pyropheophytins will be a useful test to determine the possible presence of refined, bleached and deodorised oil in EVOO. Analysis of diacylglycerols showed that changes occurred only when the oil was heated above 160^{o}C for 60 minutes.

We have not yet completed studies to determine the effects of shelf life on diacylglycerols. Although the results shown in this report are preliminary, and not in any way exhaustive, we have shown that changes in pigments and triacylglycerols are potential detection methods for refined or old oils. This work is continuing and may partially replace existing methods.

This study measured oleocanthal, a component in olive oil and waste product described as being equivalent to ibuprofen by American researchers as an anti-inflammatory compound. The AORL will continue to assess the presence and concentration of this compound in Australian olive oils which may increase olive oil value.

Recommendations

Australia at this stage does not have Australian standards. The Australian Olive Association is currently establishing a new olive industry Code of Practice which could use these data in setting standards, to be upgraded as research progresses. The Code of Practice should be backed by relevant current Australian data such as the outcomes of this research. These standards can then be applied to all products to ensure that not only are Australian producers following the regulations, but imported product is genuine and consumers within Australia get what they pay for.

> The Australian Olive Association is currently establishing a new olive industry Code of Practice which could use these data

Australian standards need to be clearly identified for the purpose of trade and particularly export. However, international organisations and particularly Codex Alimentarius need to continue to make changes to standards which will allow free flow of high quality olive oil products and prevent any barriers to trade. This information should be disseminated to world standards organisations and logical discussion on realistic standards be pursued. This report will provide weight to discussions regarding changes to world trade regulations to assist in setting relevant standards.

> Codex Alimentarius need to make changes to standards to allow free flow of quality product and prevent any barriers to trade.

1. Introduction

1.1 Background

Olives have been grown in Australia since early European settlement. The first olives are reported to have been introduced as early as 1800 by George Suttor, a London market gardener (Spennemann, 2000). In 1805 an olive tree was planted by John Macarthur on the Elizabeth Farm in Parramatta and this tree is still in existence. Despite that, the industry has been slow to develop over time. In the early 1990s however, olives became a burgeoning industry in Australia. There has been a rapid increase from a negligible crop to an estimated production of about 8,700 tonnes of oil in 2007. By 2010 the industry is expected to reach 25,000 tonnes (Miller, 2007) as orchards reach maximum production.

Even with the rapid development of the industry, Australia continues to import a large part of the olive oil consumed in this country. Major sources of the oil are from Spain, Italy and Greece. As a net importer Australia has had little expertise in determining the quality of the product and it appears that there have been instances where the imported oil has not been good quality. Undoubtedly some of the imported oil is also older than necessary to retain the fresh characteristics that consumers of olive oil are beginning to appreciate.

In the mid 1990's the Wagga Wagga Agricultural Institute became involved in measuring quality characteristics of both imported and locally produced olive oil. The Institute already had an active oils research program and a well equipped laboratory, the Australian Oils Research Laboratory, AORL, for doing the necessary analysis. The laboratory was invited to nominate for accreditation by the International Olive Council, (COI) Madrid. In 2001 the NSW Department of Primary Industries AORL underwent evaluation and was successful in becoming accredited by COI to carry out analysis using techniques provided by them. Since then the laboratory has been evaluated each year and has been successful in maintaining the accreditation.

The International Olive Council has developed eight categories for olive oil. These include:

- Extra virgin olive oil
- Virgin olive oil
- Ordinary virgin olive oil
- Lampante virgin olive oil
- Refined olive oil
- Olive oil
- Crude olive-pomace oil
- Refined olive-pomace oil
- Olive-pomace oil

Basically, Australian growers are only interested at this time in producing extra virgin olive oil (EVOO). There are no solvent extraction plants in Australia for producing pomace oil from the waste product. In some few cases where oil does not meet the stringent COI requirements for EVOO there is some refining done but this is rare. This oil is termed refined or "Pure" olive oil. Pure olive oil is often misleading and can suggest to consumers that it is premium quality whereas it has been refined and lost many of the attributes of olive oil. Extra virgin oil is the best olive oil with the natural flavours and antioxidants for which olive oil is well known.

There are three definitions of importance:

Olive oil is the oil extracted from olive fruit (*Olea europaea L.*), free of any solvent extracted or re-esterification oils or oils of any other kind.

Virgin olive oils are oils extracted from olive fruit by mechanical or physical means which does not cause any changes to the oil. The only processes acceptable are washing, decantation, centrifugation and filtration.

Extra virgin olive oil is virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 0.8 grams per 100 grams, and other characteristics of which correspond to those fixed for this category in this standard.

One of the major issues for COI is to ensure that olive oil sold to consumers is authentic and meets the standards of the product as described by COI. Many of the number of standards shown in the detailed COI document (http://www.internationaloliveoil.org/) relate to measures which ensure authenticity of olive oil and to detect possible adulteration with other oils. Some however are directly related to the oil quality and factors which are dependent on good processing and harvest techniques. These specifically are the free fatty acid content and the peroxide level.

Free fatty acids (FFA) in extra virgin olive oil must be less than 0.8%, measured as oleic acid. Olives naturally contain lipase enzymes which are capable of breaking down oil molecules to form free fatty acids. Free fatty acids are therefore largely affected by fruit quality and time and temperature of oil extraction from fruit, prior to the oil being separated from the water and solid portions of the fruit.

Peroxide value for extra virgin olive oil must be less than 20 milliequivalents of oxygen per kilogram of oil (mEq O_2/kg). Peroxide is an intermediate product in oxidation which eventually leads to rancidity of oil and typically occurs when oil is exposed to oxygen and/or light, particularly at elevated temperatures. Oxidation, and production of peroxides, occurs during oil extraction and prior to bottling but continues even after bottling, although at a reduced rate.

Adulteration of oils may include the blending of extra virgin olive oil with cheaper seed oils or with refined olive oil. COI have designed a range of sophisticated tests to detect such adulteration. The limits for EVOO set by COI are generally adequate to detect adulteration (International Olive Council, 2003). There are instances however where oil is treated in a way which makes detection of fraud more difficult and the search for new and better methods to prevent this is ongoing. One of the limitations to these methods and the limits imposed on what is acceptable for olive oil is the fact that sometimes natural, unadulterated oils do not meet the standards. This may occur due to the olive cultivar as some cultivars have unusual chemical composition. There are also differences due to environmental influences (Mailer, 2007; Mailer 2005), particularly as a result of the temperature during the fruit maturation period.

To ensure growers are aware of issues in trading olive oil, it is important for them to know if their oils meet the international standards. It appears that some growers may blend genuine olive oil which is outside the acceptable standards with oil that is within the limits to achieve a final product which meets the standard requirements. The approval of COI for this practice seems to be questionable although it is well known to exist.

At this time around 50% of Australian olive oil is being exported and sold internationally (Miller 2007). This is despite the fact that a large quantity of oil is still imported to meet local consumer demand. The export of oil, particularly to European destinations, must be unadulterated virgin olive oil and must meet all of the COI specifications. Due to the natural variation of olive oil and the influence of environmental growing conditions, this is not always the case.

1.2. Oil components

1.2.1. Fatty acids

One of the major characteristics which makes olive oil nutritionally superior to many vegetable oils is its characteristic fatty acid profile. Many edible oils contain high levels of mono- and polyunsaturated fats. Canola oil is high in linolenic acid (up to 14%), sunflower oil is high in linoleic acid (up to 75%), as is soybean oil. Other oils with vastly different fatty acid profiles are cottonseed oil and palm oil. If these oils are added to olive oil, the fatty acid profile of the olive oil will change. For example if canola oil was added to olive oil in large enough volumes, the linolenic acid content of the oil mixture would increase.

COI has a minimum and maximum limit for each fatty acid, based on the natural variation found in most olive oil. If fatty acid profiles are found to be outside this range, it could be presumed that another type of oil has been added which has a different fatty acid profile to the olive oil.

Seed oils are vastly cheaper to produce than olive oil due to lower crop establishment, maintenance, harvesting and production costs. Olive oil has been known to be adulterated with cheaper seed oils (Firestone, 2001; Li-Chan, 1994) although this has not been seen at this time in the young Australian industry.

Fatty acids profiles in olive oil can be influenced by a number of factors. Paz Romero *et al*. (2003) suggest that seasonal conditions have a significant effect on fatty acid composition, particularly rainfall events and prevailing weather conditions. Altitude has also been shown to have a significant effect (Mousa et al, 1996). Beltran *et al* (2004) found harvest timing also significantly affected the fatty acid profile.

A number of cases have been found where olive oil fatty acids are outside the limits set by COI, occurring naturally and not due to any adulteration. Scientists in Morocco (El Antari et al 2000), Italy (Dettori and Russo, 1993), France (Ollivier et al , 2003) and Portugal (Gouveia, 1997) have found several cultivars to have fatty acids outside the COI limit. In all of these cases, linolenic acid (C18:3) has been found to be above 1% in some of the varieties analysed. Similar results have also been found in Australia (Mailer, 2005).

A recent study conducted in Argentina (Ceci and Carelli, 2007) found that a number of cultivars did not meet COI regulations. Arbequina was shown to have high palmitic acid levels (>20%), low oleic acid (<55%) and high linoleic acid content (>20%). Some Barnea samples were high in linoleic acid (>21%). Linolenic acid was also shown to exceed the COI limit in a number of cultivars including Picual, Frantoio, Manzanillo Californiana and Manzanillo criolla (Ceci and Carelli, 2007).

1.2.2 Sterols and erythrodiols

Sterols are important components in human health and nutrition. Phytosterols found in vegetables and plant oils, such as β-sitosterol and Δ-5-avenasterol have been shown to reduce cholesterol absorption in the human digestion resulting in reduced health problems caused by high cholesterol levels (Moreau *et al*.1999).

Sterols form a major portion of the unsaponifiable matter of olive oil. The sterol profile of plant species is characteristic of that species and therefore plays an important role in detecting adulteration in olive oil with other oils. Seed oils have different sterol profiles to olive oil. For example, canola oil has a significant level of brassicasterol (approx. 5-13% of total sterols) and campesterol (25-39%) and has a total sterol content of approximately 4,000-11,000 mg/kg. Sunflower oil however has high levels of campesterol (approx 7-12%) and stigmasterol (8-13%, AOCS, 1998). Olive oil in contrast has high levels of β-sitosterol and Δ-5-avenasterol, only trace amounts of brassicasterol and usually small amounts of campesterol and stigmasterol. If vegetable or seed oils are added to olive oil they will change the sterol profile of the oil. For example, if canola oil was mixed with olive oil the level of brassicasterol in the oil would increase (Aparicio and Aparicio-Ruiz, 2000). The high content of Δ 7

stigmastenol in high oleic sunflower (14-22%) and safflower oils (16-23%) can reveal their addition to olive oil (Aparicio and Aparicio-Ruiz, 2000).

COI has set a minimum and maximum limit for each type of sterol based on the natural levels found in traditional olive oil types. Sterol profiles outside this range could suggest that another oil type has been added to the olive oil.

A number of cases have found where olive oils naturally exceed the COI limit for sterols. This is particularly so in the case of campesterol which should be less than 4% of total sterols according to COI standards. Cultivars in which this has not been the case include Arbequina, Corniche, Koroneiki, Cornicabra, Arauco and Barnea (Koutsaftakis et al, 1999; Stefanoudaki et al, 2000; Sanchez Casas et al, 2004; Rivera del Alamo et al, 2004; Salvador et al, 1998; Ceci and Carelli, 2007 and Mailer, 2007).

The total sterol content of olive oil varies between 1000 mg/kg and 2000 mg/kg. Refined oils contain lower levels of total sterols because the refining process gives rise to significant loss of sterols (up to 25%). The total sterol content of solvent extracted oil however, can be up to three times that of virgin olive oils (Morchio et al, 1987).

Erythrodiol content, according to COI standards, must not exceed 4.5 % of total sterols in virgin olive oil (International Olive Council, 2003). Erythrodiol levels are high in solvent extracted or refined oils (i.e. pomace oils) and therefore high levels in virgin oils would indicate adulteration with pomace oil (Reina et al, 1997).

1.2.3 Waxes

Waxes are mostly present in the external fruit wax cuticle in olives (Ranalli et al, 2000). The waxes on the surface of the fruit protects them against water loss and insect damage. In dry hot weather plants are known to produce more waxes to control the rate of transpiration in order to reduce water loss (Hamilton, 1995).

Extra virgin olive oil is characterised by the virtual absence of waxes with 40 to 46 carbon atoms as these compounds are not extracted by mechanical processing. The waxes are found in comparatively large amounts in refined and pomace oil as they are dissolved in the solvents used in the extraction process. Therefore the presence of waxes is a good method for identifying solvent extracted oil (pomace oil) in virgin olive oil (Kiritsakis and Christie, 2000).

Some researchers have found wax content of extra virgin olive oil to exceed the limits set by COI of <250 mg/kg. Arbequina and Picual were found to exceed the COI limit in a trial conducted in Argentina (Ceci and Carelli, 2007) while some oils collected in the south of Italy were also found to exceed the limit (Poiana et al, 1997).

1.2.4 α-tocopherol

Tocopherols are well known for their inhibition of lipid oxidation in foods and biological systems. Vitamin E or α-tocopherol is only synthesized by plants and is an important dietary nutrient for human.

The tocopherol content of food increases storage life by protecting food lipids from autoxidation (Kamal-Eldin and Appelqvist, 1996). There is currently no limit set by COI for α-tocopherol content in olive oil. However it is known that different oils show tocopherol concentrations and profiles which are substantially different to each other. For example, the content of tocopherols has been used to help detect adulteration of olive oils with hazelnut oils. Olive oils have only traces of δ-tocopherol whereas hazelnut oils contain higher quantities (Aparicio and Aparicio-Ruiz, 2000).

1.2.5 Trans fatty acids

Virgin olive oils contain only cis isomers of unsaturated fatty acids. In the refining process there is a partial isomerization of unsaturated fatty acids (Angerosa et al, 2006) leading to the formation of trans fatty acid isomers. Levels of the trans isomers of oleic, linoleic and linolenic acids above the limits set by COI can indicate adulteration with hydrogenated seed oils, esterified olive oils and illegally treated virgin olive oils. (Aparicio and Aparicio-Ruiz, 2000).

1.2.6 UV absorbance

The adulteration of virgin olive oil with refined olive can be detected by the presence of conjugated dienes and trienes formed during the refining process (Angerosa et al, 2006). The maximum absorption of these dienes and trienes occur at 232 and 270nm respectively. However, autoxidation reactions are also associated with the formation of conjugated bonds, resulting in a decrease of the method sensitivity (Li-Chan, 1994). However, many researchers (Tovar et al 2002; Cinquanta et al, 2001; Salvador et al, 2001) have determined UV absorbance in fresh olive oil is well within COI limits (International Olive Council, 2003).

1.2.7 Stigmastadienes

Stigmastadienes are formed by the dehydration of β-sitosterol during refining, but not present in significant quantities in virgin olive oil (Li-Chan, 1994). According to most authors, bleaching is the main refining step that causes the formation of stigmastadienes (Cert et al, 1994; Grob et al, 1992).

The COI limit for stigmastadienes in extra virgin olive oil is <0.15 mg/kg (International Olive Council, 2003). Levels of stigmastadienes in olive oils under typical refining conditions are in the range of 2-45 mg/kg (Gordon and Firman, 2001).

1.2.8 Triacylglycerides (ΔECN 42 Values)

Triglycerides are lipid molecules containing a glycerol moiety and three fatty acids.
The ECN (equivalent chain number) is a value determined by adding the actual number of carbon atoms in the triacylglycerol molecule and subtracting twice the number of double bonds in the molecule.

Olive oil, in contrast to most seed oils, has many triacylglycerols with ECN numbers of 44, 46, 48 and 50. Triacylglycerols with ECN 40 and ECN 42 are absent or present in trace amounts respectively. Therefore, the measurement of ECN 42, which varies according to the content of glycerol trilinoleate, is an effective tool to detect oils with a higher level of unsaturation than olive oil.

The difference between the theoretical ECN 42 value (a calculation based on gas chromatographic determination of fatty acid composition) and the experimental ECN 42 value (determined by measuring triacylglycerols by HPLC) is called the ΔECN42 value. To meet the COI standard for ΔECN42, extra virgin olive oils must not exceed 0.2 (Angerosa et al, 2006).

1.3 New methodology

Despite the limitations of existing methods to adequately discriminate between authentic and adulterated oil, Australia is committed to ensure that Australian olive oil is genuine. Additional methods are therefore required to determine authenticity. Current methods are costly, time consuming and often produce false negatives due to natural variation in olive oil with environment, cultivar and seasonal conditions. The new methods include determining the level of pyropheophytin, diacylglycerols and oleocanthal. These methods can determine heated or old oil by the proportions of these components in the oil.

Pyropheophytins

Pyropheophytins are by-products of chlorophyll formed when the pigment is heated. Chlorophyll is converted to pheophytin and ultimately to pyropheophytin with application of high temperature. This

is a useful test as refining, particularly bleaching and deodorising, generally requires the application of high temperature. Pyropheophytins also appear to form with age, appearing in oil which has been in long term storage and may therefore be a useful test to discriminate fresh oil from old oil.

Diacylglycerols

Diacylglycerols are molecules with a glycerol moiety and two fatty acids. This is basically a triacylglycerol which has lost one fatty acid. With oil ageing or heat treatment, fatty acids can be cleaved from triacylglycerols to form diacylglycerols. The proportions of diacylglycerols with fatty acids on the 1,2 position or the 1,3 position reportedly can be used to detect poor quality oil.

Oleocanthal

Oleocanthal is a phenolic compound which has been found in olive oil and is considered to have the same medicinal properties as ibuprofen. The compound varies in concentration between cultivars and oil quality and may be useful in determining fresh olive oil. A method will be evaluated for measuring oleocanthal to determine its usefulness in good quality olive oil.

1.4 Industry involvement

The Australian olive industry understands the need for standards which eliminate cases of fraud in olive oil and ensure that adulteration does not occur, in Australian oil or in oil which is imported into Australia. Australia supports the International Olive Council in developing sophisticated methods which can be used to detect instances of adulteration. However the industry has become aware of the problems associated with good quality, genuine extra virgin olive oil which does not meet the rigid standards of some international standards. Ample evidence exists to show that not only Australian oil, but oils from many countries consistently fail to meet the regulations in fatty acid or phytosterol profiles.

Cultivars such as *cv* Barnea have levels of campesterol between 4 to 5% of the total sterol content. This is virtually always above the limit of 4% shown in international regulations. As such, the oil is regarded as not being extra virgin olive oil.

Similar problems have come from fatty acid analysis as fatty acids vary with environment and particularly with temperature during maturation of the fruit. Cold climates produce higher levels of oleic acid and warmer climates promote higher levels of palmitic acid (Mailer 2007) both of which may exceed the regulations. Linolenic acid also varies and may exceed 1.0%.

The Australian Olive Association has supported this study to illustrate the variation in olive oil characteristics which occurs as a result of natural variation. Although the environmental conditions in parts of Australia are quite different to that of traditional olive oil producing countries, many European countries have also published scientific data showing that they also experience problems in meeting some international trading standards. This has major implications for export and trade between countries, most of which utilise these standards.

2. Objectives

2.1 Olive Survey

Previous studies have shown that olive oil produced in Australian may sometimes not meet international trading standards. Various components on olive research studies have highlighted fatty acids and sterols in particular which fluctuate depending on cultivar, seasonal conditions and fruit maturity. This survey was designed to determine what quality parameters may become issues in trade and what are the reasons for the non-compliance of those parameters. The survey would take into account issues of cultivar, environment and fruit maturity.

No prior survey of minor components has been carried out on Australian olive cultivars. This project aims to survey the chemical profile of 10 of Australia's major cultivars. These cultivars are reported to make up more than 80% of the Australian crop.

Olives would be sampled from four environmentally different sites over two subsequent years and at early and late harvest times to investigate the changes in olive composition from the effect of site and environmental differences. The outcome would be a set of data of minor components which would identify the range in quality olive producers could expect from oil extracted from these 10 cultivars.

2.2 New methodology

New methods have recently been developed by the German Society for Fat Research to help detect instances of fraud. These methods will be studied to determine their ability to discriminate between extra virgin olive oil and adulterated oil. Although they have not been accepted by international standards organisations at this time, evidence suggests that the study of pigments and degradation products of triacylglycerols may be useful in determining the presence of refined oil and oil which has been stored for long periods. This study will determine concentration of pyropheophytin, diacylglycerols and oleocanthal in olive oil and relate them to oil quality. The objective is to determine their usefulness in identifying adulteration.

3. Methodology

3.1 Materials and methods

3.1.1 Australian Oils Research Laboratory

This study was carried out at the new NSW DPI Australian Oils Research Laboratory situated in Wagga Wagga, New South Wales. The laboratory staff has had many years of experience in oil research and development working on canola breeding programs, olive oil quality and evaluation, and numerous other oil crops. The laboratory has had AS / NZS ISO 9001:2000 systems certification for over 10 years and more recently obtained ISO 17025 certification for many of the methods of analysis carried out through the National Australian Testing Authority (NATA). Dr Rodney Mailer has been a certified approved Chemist of the American Oil Chemists' Society for over 15 years. Since 2001 the AORL has maintained accreditation from the International Olive Council (COI) for chemical testing of olive oils for adulteration and in 2007 also had the sensory laboratory accredited by COI making it one of the few laboratories in the world with these COI accreditations.

Figure 3.1 New oil testing laboratory at Wagga Wagga Agricultural Institute

3.2 Samples

Olive fruit samples were provided voluntarily by individual growers from across the diverse Australian growing areas. These growers are acknowledged in this report. Olive fruit (2 kg) of each of 10 cultivars was harvested at two maturity dates at each site. These fruit were packed into calico bags, placed in polystyrene containers together with one or more frozen "bricks" and transported overnight to the AORL. The fruit were placed into cold (12^{o}C) storage on receival and the oil was extracted within 24 hours.

3.2.1 Regions

Four regions were selected to represent the extremes of growing conditions across olive growing areas within Australia. The areas included:

- Northern NSW/Southern Qld.
- Central Victoria
- Western Australia
- Southern Victoria/Tasmania

Figure 3.2 Australian sites, shown by red dot, from which olives were sourced for this project to represent extreme environmental differences

3.2.2 Cultivars

Eleven cultivars were selected for the study which represented the majority of the Australian olive crop production (Leandro Ravetti *pers. comm.*) There are a wide range of cultivars grown in Australia, over 46 of which are represented at the Wagga Wagga historic olive grove at Charles Sturt University (Mailer and May 2002). However most of these are grown in low numbers or not used commercially. The cultivars used for this study included:

Table 3.1 Olive cultivars used for the study

Arbequina
Barnea
Coratina
Corregiola
Frantoio
Koreneiki
Manzanillo
Leccino
Nevadillo
Blanco
Pendolino
Picual

Despite efforts to obtain samples from all sites for early and late harvest timing over two years, some samples were not received, as shown in Table 3.3. Mean values have been calculated for each of the parameters but it is acknowledged that these means are based on only the samples received. It is clear that results will be affected by missing samples from some sites or harvest times, particularly for *cvv.* Nevadillo blanco and Pendolino.

Table 3.2 Olive samples received for analysis

	Northern NSW/Southern Qld				Central Victoria				Western Australia				Southern Victoria/Tasmania			
	2005		2006		2005		2006		2005		2006		2005		2006	
	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
Arbequina	x	x	x	x	x	*nr*	x	x	*nr*	*nr*	x	x	x	x	x	*nr*
Barnea	x	x	x	x	x	x	x	x	x	x	x	x	x	*nr*	x	*nr*
Coratina	x	x	x	x	x	x	x	x	x	*nr*	x	x	x	x	x	*nr*
Corregiolla	x	x	x	x	*nr*	*nr*	*nr*	*nr*	x	x	x	x	x	x	x	*nr*
Frantoio	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Koreneiki	*nr*	x	x	x	*nr*	*nr*	x	x	x	x	*nr*	x	*nr*	*nr*	*nr*	*nr*
Leccino	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Manzanillo	x	x	x	x	*nr*	x	x	x	x	x	x	x	x	nr	nr	nr
Nevadillo Blanco	*nr*	*nr*	*nr*	*nr*	*nr*	*nr*	*nr*	*nr*	x	x	x	x	*nr*	*nr*	*nr*	*nr*
Pendolino	*nr*	*nr*	x	x	*nr*	*nr*	x	x	*nr*	x	x	x	*nr*	*nr*	x	x
Picual	*nr*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	*nr*

nr = no sample received

3.3 Analysis

3.3.1 Oil extraction

A mechanical extraction unit (Abencor, Spain), was used to extract the oil. The Abencor unit imitates the process used by the industry to extract olive oil. It consists of a hammer mill, a thermo-malaxer and a centrifuge. Approximately 1kg of fruit was ground to a paste using the hammer mill. The sample was thoroughly mixed and 700 g of the pulp was weighed into a mixing jar, placed in the thermo-malaxer and allowed to stir for 20 minutes at 25°C. Boiling water (300 ml) was added, and the sample was stirred for a further 10 minutes. The sample was centrifuged for 1 minute. The oily must was collected into a measuring cylinder; the pomace was rinsed with 100 ml of boiling water, centrifuged for 1 minute, and the must again collected. After allowing some time for the sample to settle, the oil was transferred to a glass bottle and sealed under nitrogen until further analysis.

3.3.2 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 (0.1g or 5-7 drops) was dissolved in 2 mL heptane. The sample was mixed and 0.2 mL of 2 N methanolic potassium hydroxide was added. The sample was mixed for 30 seconds, covered and left until the two phases separated (20-30 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 (30m, 0.25mm, 0.25μm 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. Data was analysed using Star® Workstation Chromatography software (version 6.20). Results are expressed as a percentage of the total fatty acids

3.3.3 Sterols and erythrodiols

Sterols and diols were determined using the International Olive Council method COI/T.20/Doc. No 10 "Determination of the composition and content of sterols by capillary column gas chromatography". Oil (5 g) was weighed and internal standard (0.2% α-cholestanol w/v and 0.2% betulinol w/v) was added (2 mL). A solution of 2N ethanolic potassium hydroxide (50 mL) was added and the sample boiled for one hour to allow for saponification of the sample. The sample was then liquid-liquid extracted three times using diethyl ether, and the saponifiable matter was discarded. The unsaponifiable matter was washed with 0.5N sodium hydroxide (50 mL). The sample was then washed with distilled water (50 mL) until neutral pH. It was filtered through anhydrous sodium sulphate, and the solvent evaporated using a rotary evaporator and dried in an oven at 100 ^{o}C for 15 minutes.

The sample was weighed and made up to a 5% solution in chloroform. The sample was then injected onto a TLC plate (silica gel 60 plate previously treated with 0.2N ethanolic potassium hydroxide for 10 seconds, allowed to dry at room temperature for 2 hours, and then dried in an oven at 100 ^{o}C for 1 hour).The plate was developed in a TLC developing chamber with toluene/acetone (95:5 v/v), until the solvent front reached about 1 cm from the upper edge of the plate. The plate was sprayed with 2.7-dichlorofluoroscein and examined under ultraviolet light to identify the sterols and diol bands.

The sterols band was then scraped from the plate, transferred to a G3 porous septum, and filtered under vacuum with chloroform/diethyl ether 3:1 (v:v) (15 mL). This was repeated twice, with the filtrate collected into a 50 mL flask. The filtrate was evaporated to 4-5 mL using a rotary evaporator, and transferred to a 10 mL centrifuge tube. The sample was then evaporated until dry under a gentle flow of nitrogen. A few drops of acetone were added, and the sample was again evaporated to dryness. The sample was placed in an oven at 105 ^{o}C for 20 minutes. The sample was allowed to cool and 750 μL of silylation reagent (HMDS/TMCS/Pyridine, 3:1:9) was added, allowed to stand for 20 minutes at

room temperature and then centrifuged at 3000 rpm for 30 minutes. The sample was then transferred to GC vials.
The sterols and diols were determined by gas chromatography using a J and W scientific SE-54 capillary column (30 m, 0.25 mm, 0.25 μm). The column temperature program was 265 °C for 45 minutes and then increased at 5 °C /minute to a final temperature of 300 °C and held for 5 minutes. The injector temperature was 280 °C with a split ratio of 1:20. The detector temperature was 290 °C. Data was analysed using Star® Workstation Chromatography software (version 6.20). Results for individual sterols were expressed as a percentage of the total sterols. Total sterols were expressed as mg/kg oil. Diols were expressed as a percentage of the total sterols and diols.

3.3.4 Waxes

Waxes were determined using the International Olive Council method COI/T.20/Doc. No 18 "Determination of wax content by capillary column gas chromatography". Silica gel 60 (15 g) (hydrated to 2%) was mixed with anhydrous hexane to form a slurry. The slurry was then transferred to a glass column and allowed to settle by gently tapping the lower part of the column. Oil (0.5 g) was weighed, and 1 mL of the internal standard (lauryl arachidate, 0.1% w/v in hexane) was added. The sample was mixed with some hexane (approx 2 mL) and transferred to the previously prepared glass column. The hexane was allowed to pass through the column until the solvent meniscus was about 2mm above the silica gel. A solution of hexane/diethyl ether (99:1 v:v) was added to fill the column. The flow rate was adjusted to 15 drops every 10 seconds. Using a measuring cylinder 140 mL of the hexane /diethyl ether solution was collected and discarded. A further 50 mL of the solution, containing the wax fraction was collected. This was then evaporated using a rotary evaporator. Heptane (2 mL) was added and transferred to a GC vial.

The waxes were determined by gas chromatography using an SGE BPX5 capillary column (12 m, 0.53 mm,0.25μm). The column temperature program was initial temperature 80 °C , increasing to 120 °C at 30 °C/minute, hold for 1 minute, then increasing to 340 °C at 5 °C/minute, hold for 17 minutes. The injector temperature was 230 °C and the detector temperature was 350 °C. Data was analysed using Star® Workstation Chromatography software (version 6.20). Results were expressed as mg/kg of oil.

3.3.5 α-tocopherol

α-tocopherol was measured using the IUPAC method 2-432 with slight modification. Oil (2g) 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 1 mL/minute. A Phenomonex Luna 5μ silica column (250 x 4.60mm) was used. The peaks were measured using a UV detector set at 292 nm. Data were 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.

3.3.6 Trans fatty acids

Samples were prepared as for the fatty acid profiles 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" (see section 3.4). The trans fatty acids were determined by gas chromatography using a Supelco 2340 capillary column (60m, 0.25mm, 0.25μm film) and a flame ionisation detector. The column temperature program was 165°C for 10 minutes and then increased at 2 °C / minute to a final temperature of 200 °C and held for 13 minutes. The injector temperature was set at 245 °C with a split ratio of 1:50. The detector temperature was 245°C. Data was analysed using Star® Workstation Chromatography software (version 6.20). The results were expressed as a percentage of total fatty acids

3.3.7 UV absorbance

UV absorption was measured using the International Olive Council method COI/T.20/Doc. No. 19 "Spectrophotometric investigation in the ultraviolet" with slight modification. Oil (0.1g) was weighed into a 10 mL volumetric flask and made to volume with trimethylpentane. The sample was then scanned using a UV spectrophotometer and the absorbances were recorded at 232 nm, 266nm, 270 nm and 274 nm. The absorbance values were then used to calculate Specific extinction at 270nm and ΔK

3.3.8 Stigmastadienes

Stigmastadienes were measured using the International Olive Council method COI/T.20/Doc. No 11 "Determination of stigmastadienes in vegetable oils". Oil (20 g) was weighed and 1 mL of internal standard (cholesta-3,5-diene, 20 mg/L) was added. To this was added 75 mL of ethanolic potassium hydroxide (10% w/v) and the sample was heated to boiling for 30 minutes. The sample was allowed to cool and 100 mL distilled water was added. The unsaponifiable matter was then liquid-liquid extracted twice with 100 mL hexane. The sample was washed with 100 mL mixture of ethanol/ water (1:1) until a neutral pH was achieved. The sample was evaporated to a few mLs with a rotary evaporator
A glass column was packed with silica gel 60 (2 % hydrated) the same preparation as for waxes (see section 3.3.4), with a slight difference being the addition of anhydrous sodium sulphate (height of approx. 0.5cm) to the top of the column. The sample was transferred to the previously prepared glass column. The hexane was allowed to pass through the column until the solvent meniscus was about 2mm above the silica gel. Hexane was added to fill the column. The flow rate was adjusted to 7 drops every 10 seconds. Using a measuring cylinder 25 mL of the hexane solution was collected and discarded. A further 40 mL of the solution containing the stigmastadiene fraction was collected. This was then evaporated to dryness using a rotary evaporator. Hexane (0.5mL) was added and transferred to a GC vial. The stigmastadienes were determined by gas chromatography using a J and W scientific SE-54 capillary column (30 m, 0.25 mm, 0.25 μm). The column temperature program was 235^{o}C for 6 minutes and then increased at 2 ^{o}C / minute to a final temperature of 285 ^{o}C and held for 10 minutes. The injector temperature was set at 300 ^{o}C with a split ratio of 1:15. The detector temperature was 320^{o}C. Data was analysed using Star® Workstation Chromatography software (version 6.20). Results were expressed as mg/kg.

3.3.9 Triacylglycerides (ΔECN)

Triacylglycerols were measured using the AOCS Official method Ce 5b-89 with slight modification. Oil (0.5g) was weighed into a 10 mL volumetric flask, and made up to volume with acetone. The samples were filtered and transferred to HPLC vials. The ECN 42 concentration was determined by HPLC, with acetone/acetonitrile (75:25) as the mobile phase and a flow rate of 1 mL/minute. A Phenomonex Luna 5μ C18 (2) column (250 x 4.60mm) was used. The peaks were measured using a Refractive Index detector. Data were analysed using Waters Empower Pro version 5.00. The difference between the actual ECN 42 value and the theoretical value, calculated from the fatty acid profile of the sample, was then calculated and this difference was reported.

4. Results

This project has studied the influence of natural variations on olive oil quality. This variation is due to influences of environment, seasonal conditions and genotype. Different components of olive oil are influenced to a varying degree by these three variables. To show the individual effects on each component, the variation in quality is illustrated for each of those variables.

4.1 The effect of cultivar on olive oil components

4.1.1 Fatty acid profiles

4.1.1.1 Oleic acid

The most important fatty acid in olive oil is oleic (from olea – olive). It is a monounsaturated fatty acid and has been associated with lowering of cholesterol, reducing risk of coronary heart disease and a range of other health benefits. COI requires the level of oleic acid must be between 55 and 83% of total fatty acids. Figure 4.1 illustrates that in this study oleic acid ranged from 52.2-84.2%. Despite the higher values exceeding the range, this is not detrimental to the quality of the oil and, in fact, more oleic acid may be considered to be beneficial.

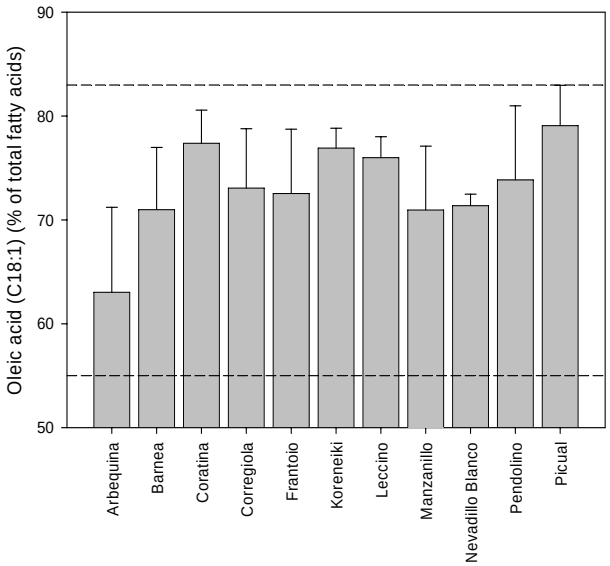


Figure 4.1 Mean oleic acid (C18:1) content for eleven cultivars. Each bar represents the mean for the cultivar ± standard deviation. The COI range is shown by the dotted lines

4.1.1.2 Linoleic acid

Linoleic acid is a polyunsaturated fatty acid commonly associated with seed oils such as sunflower and safflower oil. COI standards have set limits of 3.5% to 21.0% of total fatty acids for linoleic acid. This study found levels of 2.2 to 23.8 (Figure 4.2). Both the maximum and minimum levels found were outside the COI standard.

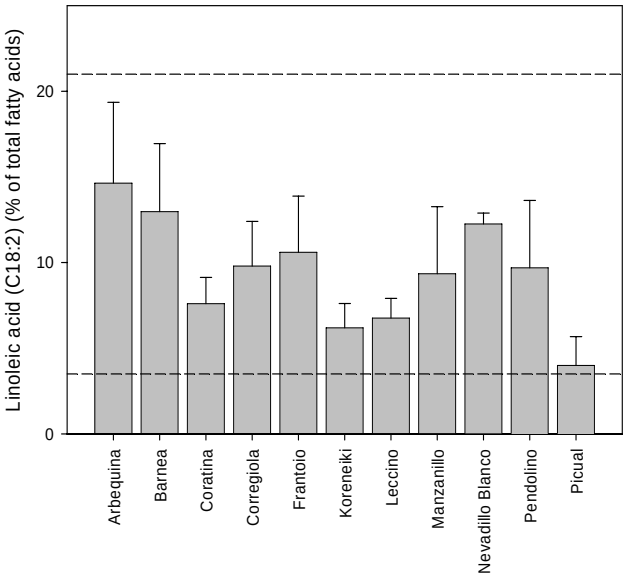


Figure 4.2 Mean linoleic acid (C18:2) content for eleven cultivars. Each bar represents the mean for the cultivar ± standard deviation. The COI range is shown by the dotted lines

4.1.1.3 Linolenic acid

Linolenic acid is also polyunsaturated and is associated with oil seed crops such as canola, colza, rapeseed and mustard. These crops contain greater than 10% linolenic acid and therefore this component is an easy way to detect the presence of these oils in olive oil. Linolenic acid also has the disadvantage of being rapidly oxidised due to three double in the fatty acid molecule. For many years the COI standard was set at 1.5% as an allowable level for linolenic acid. However, to make the standard more efficient in avoiding canola adulteration COI reduced the standard to 1.0%. This has caused problems for the Australian industry where genuine olive oil has been found to contain levels of linolenic acid above 1.5%. In this study the average level ranged from about 0.5 to 1.3%, however some individual samples were as high as 1.7%."

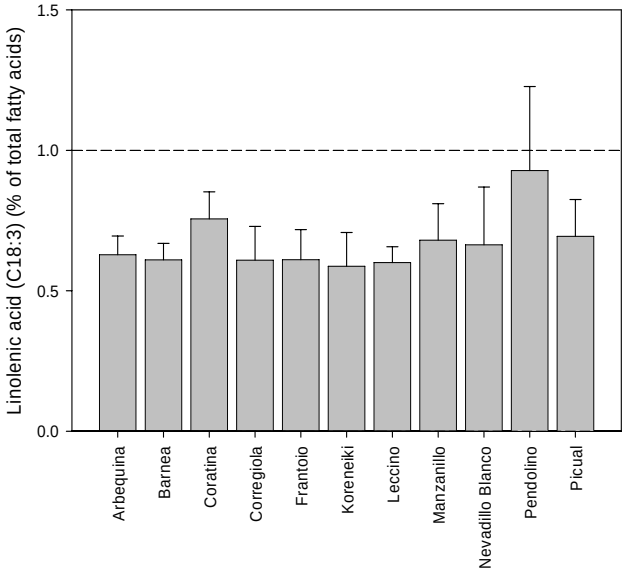


Figure 4.3 Linolenic acid (C18:3) content for eleven cultivars. Each bar represents the mean for the cultivar ± standard deviation. The COI range is shown by the dotted line

4.1.1.4 Palmitic acid

Palmitic acid is a saturated fatty acid with no double bonds in the molecule. Although this has the advantage of being very stable in high temperature applications, saturated fats are linked to various medical disorders such as hardening of the arteries and coronary heart disease. COI requires the level must be between 7.5% and 20.0% of total fatty acids, as indicated by the dotted lines in Figure 4.4. *cv* Arbequina had the greatest amount of palmitic acid, at one stage exceeding 20%. In some instances the level was lower than the official standard in *cv* Barnea although this may be considered advantageous.

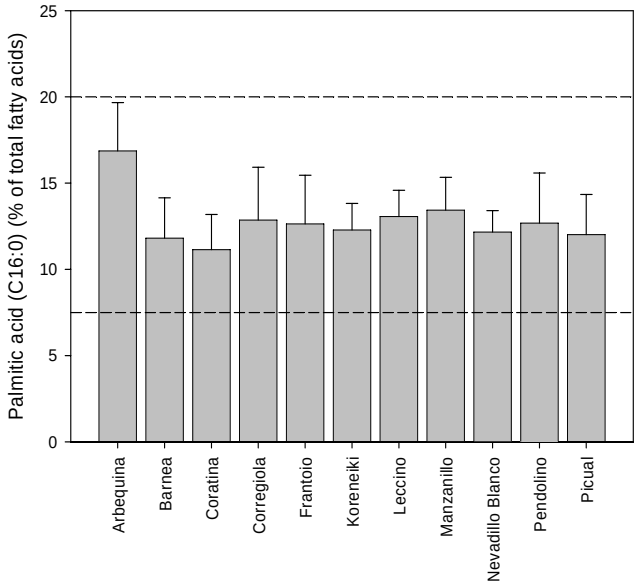


Figure 4.4 Palmitic acid (C16:0) content for eleven cultivars. **Each bar represents the mean for the cultivar ± standard deviation. The COI range is shown by the dotted lines**

4.1.1.5 Other fatty acids

The relative concentrations for minor fatty acids C14:0, C16:1, C17:0, C17:1, C18:0, C20:0, C20:1, C22:0 and C24:0 are shown in Table 4.1.

The fatty acids C14:0, C20:0, C22:0 and C24:0 are saturated fats and showed virtually no difference between cultivars. All are within COI limits.

The component C16:1 is a monounsaturated fatty acid of some interest and was significantly higher in *cv* Arbequina, low in Coratina, but similar in all other cultivars. C20:1 is also monounsaturated but shows little fluctuations between cultivars and all meet the limit of 0.4% except Coratina at 0.41%.

C17:0 and C17:1 are generally absent in most edible oils but always present in low amounts in EVOO. Nevadillo Blanco had relatively higher levels of both these components compared to other cultivars but all were less than 0.3% and within COI regulations.

C18:0 ranged from a low of 1.3% in Pendolino to a high of 3.0% in Manzanillo but all remained within the acceptable limits.

Table 4.1 Mean and standard deviation (s.d.) for minor fatty acids for eleven olive cultivars. Results are a percentage of the total fatty acids. COI limits are shown below the table

	C14:0		C16:1		C17:0		C17:1		C18:0		C20:0		C20:1		C22:0		C24:0	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Arbequina	0.01	0.00	2.42	0.96	0.09	0.02	0.21	0.03	1.39	0.20	0.32	0.04	0.25	0.06	0.09	0.02	0.05	0.02
Barnea	0.01	0.00	0.89	0.28	0.04	0.01	0.08	0.02	1.91	0.24	0.34	0.05	0.23	0.02	0.09	0.02	0.04	0.01
Coratina	0.00	0.00	0.41	0.09	0.04	0.01	0.07	0.01	1.74	0.29	0.33	0.06	**0.41**	0.11	0.09	0.02	0.05	0.01
Corregiola	0.01	0.00	1.02	0.40	0.04	0.01	0.08	0.02	1.80	0.20	0.30	0.06	0.30	0.06	0.09	0.03	0.04	0.02
Frantoio	0.00	0.00	1.01	0.50	0.04	0.01	0.08	0.02	1.72	0.31	0.31	0.05	0.30	0.05	0.09	0.03	0.04	0.01
Koreneiki	0.01	0.00	0.91	0.18	0.03	0.01	0.06	0.01	2.20	0.21	0.37	0.05	0.28	0.02	0.11	0.02	0.04	0.02
Leccino	0.01	0.00	1.08	0.27	0.04	0.01	0.08	0.02	1.75	0.34	0.27	0.05	0.25	0.03	0.07	0.02	0.03	0.01
Manzanillo	0.01	0.01	1.44	0.38	0.11	0.02	0.22	0.05	3.03	0.48	0.40	0.07	0.24	0.03	0.10	0.02	0.05	0.01
Nevadillo Blanco	0.01	0.00	0.72	0.11	0.14	0.01	0.29	0.03	1.66	0.07	0.29	0.04	0.32	0.06	0.08	0.02	0.03	0.02
Pendolino	0.00	0.00	0.75	0.29	0.03	0.01	0.09	0.02	1.27	0.14	0.24	0.03	0.33	0.03	0.08	0.01	0.04	0.02
Picual	0.00	0.00	1.15	0.60	0.04	0.01	0.09	0.01	2.25	0.59	0.30	0.06	0.24	0.04	0.08	0.02	0.04	0.02
IOC Limit	**<0.05**		**0.3-3.5**		**<0.3**		**<0.3**		**0.5-5.0**		**<0.6**		**<0.4**		**<0.2**		**<0.2**	

4.1.2 Sterols and erythrodiols

Phytosterols are an important component of plant products and particularly important in olive oil. Plant sterols have been shown to be useful in human digestion in that they block the absorption of cholesterol (Moreau *et al*.1999). The profile of individual sterols in plants is related to the plant species. As a result it can be used as a "fingerprint" and useful to determine the presence of oils of another species in olive oil. For example, brassicasterol is predominant in *brassica* plants and yet only trace amounts exist in olive oil. COI have used this phenomenon to set regulations regarding individual plant sterols.

4.1.2.1 Total sterols

The amount of phytosterol in individual plant species is also significant with some species having higher levels than others. Where unscrupulous processors might try to desterolize olive oil to remove traces of inappropriate sterol types from the oil; or to blend refined, bleached and deodorized oil with virgin oil; COI have set minimum levels of total sterols for extra virgin olive oil. This defines EVOO as having a minimum of 1000mg/kg of total sterol content. There is clearly a strong relationship between the olive cultivar and the amount of phytosterol. In this study of eleven cultivars, Pendolino and Koreneiki had the lowest levels sometimes below the limit. The lowest level found was 789 mg/kg in a Koreneiki sample.

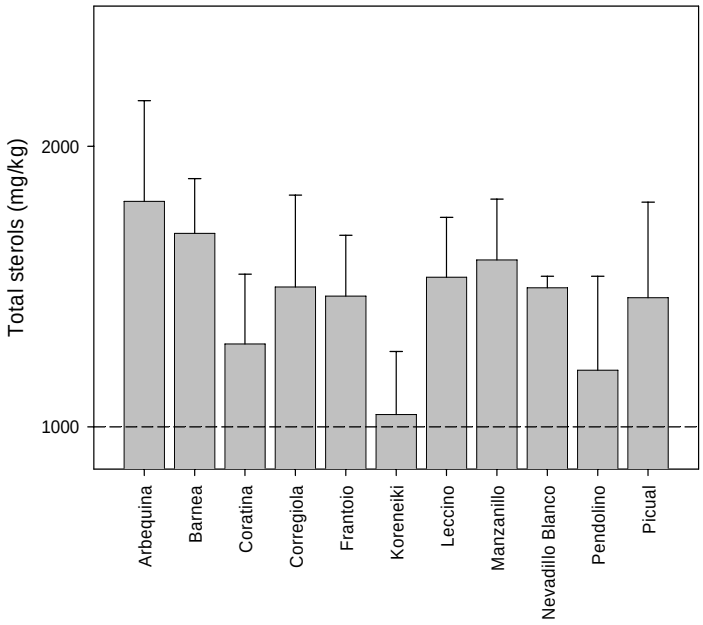


Figure 4.5 Total sterol content for eleven cultivars. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be greater than 1000 mg/kg as indicated by the dotted line

4.1.2.2 Campesterol

Perhaps the sterol component which has been of most interest to Australian producers in recent years is campesterol. This component is high in sunflower oil and is therefore a useful tool to identify adulteration. However, it is also high in some olive oils, particularly in cultivars such as Cornicarbra (Rivera del Alamo et al, 2004), Koreneiki (Koutsaftakis et al, 1999) and Barnea (Ceci and Carelli, 2007). With a maximum level of 4.0% in the COI standard, many samples of these cultivars will exceed that limit. This is illustrated in Figure 4.6 in which the mean value for Barnea is 4.5%. Koreneiki also had high levels of campesterol, with the mean value approximately 4%.

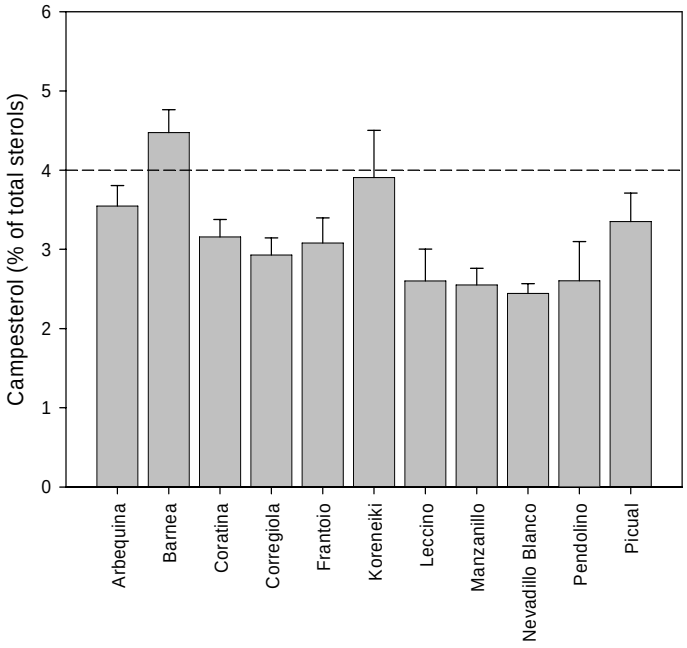


Figure 4.6 Campesterol content for eleven cultivars of olives. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be less than 4% of total sterols, as indicated by the dotted line

4.1.2.3 Stigmasterol

The acceptable level of stigmasterol is determined by the level of campesterol. COI requires that the level must be below the level of campesterol (as a % of total sterols). All of the cultivars tested met the requirements and only Manzanillo had appreciable levels of Stigmasterol (Figure 4.7).

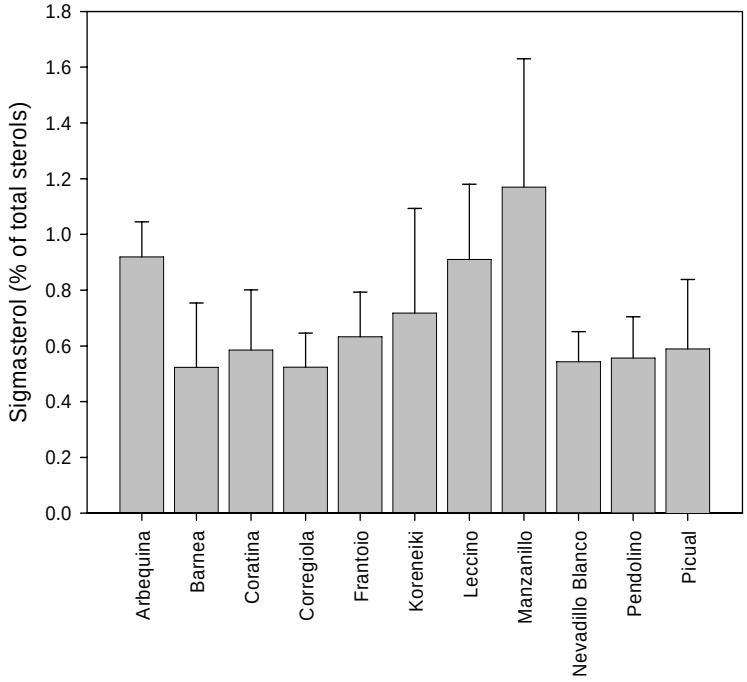


Figure 4.7 Stigmasterol content for eleven cultivars. Each bar represents the mean for the cultivar ± standard deviation

4.1.2.4 Apparenț β-sitosterol

Apparent β-Sitosterol is the sum of β-Sitosterol and a number of other less abundant sterols (COI Trade Standards, 2003). COI requires the level must be greater than 93% of total sterols. Only some Koreneiki and Barnea failed to meet this standard with a minimum level overall of 91.7%. The range of mean values is illustrated in Figure 4.8.

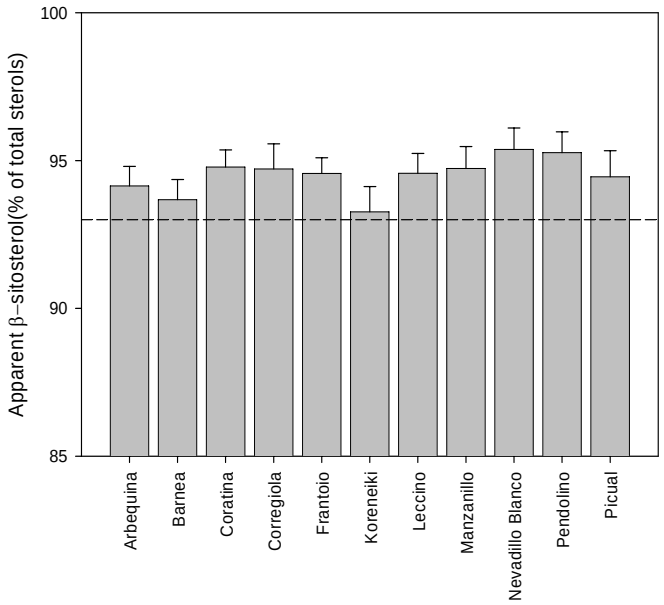


Figure 4.8 Apparent β-sitosterol content for eleven cultivars. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be greater than 93% of total sterols, as indicated by the dotted line

4.1.2.5 Cholesterol

The minor sterol products are shown in Table 4.2. Cholesterol is basically an animal product and contributes only a trace amount of the total sterols in olive oil. The COI limit is <0.5% of total sterols and none of the samples tested in this study exceeded that value (table 4.2).

4.1.2.6 Brassicasterol

Brassicasterol is of interest as it is a major component of *brassica* oils (rapeseed, colza, canola, mustard,) and as these oils are abundant and cheap they are obvious products used for adulteration of olive oil. The presence of brassicasterol is easy to detect. It is present in very low levels (<0.1%) in olive oil and is a good indicator of fraud. No oils exceeded the allowable limits (table 4.2).

4.1.2.7 Δ-7- stigmastenol

Δ-7- Stigmastenol is also of low concentration (<0.5) and of limited interest (table 4.2). Most samples were within the limits in the samples tested, although one sample of Koreneiki was very high (1.36%).

4.1.2.8 Erythrodiols

The analysis of erythrodiol and uvaol (also known as triterpene dialcohols) is commonly used as an indicator for the presence of solvent extracted oils. Absolute concentrations of erythrodiol and uvaol in pressed oils are much lower than those from solvent extracted oils (Blanch et al, 1998). Most of the samples analysed in this study had very low concentrations of erythrodiols, however the levels in the Koreneiki cultivar were higher (table 4.2), with a maximum result of 6.69%.

Table 4.2 Mean and standard deviations of some sterols for eleven cultivars. Results are a percentage of total sterols. COI limits are shown below

	Cholesterol		Brassicasterol		Δ-7- Stigmastenol		Diols	
	Mean	**s.d.**	**Mean**	**s.d.**	**Mean**	**s.d**	**Mean**	**s.d**
Arbequina	0.11	0.06	0.01	0.02	0.19	0.13	1.36	0.34
Barnea	0.12	0.07	0.00	0.00	0.16	0.09	0.99	0.44
Coratina	0.18	0.11	0.00	0.02	0.20	0.10	1.56	0.53
Corregiola	0.16	0.06	0.01	0.02	0.33	0.14	1.14	0.42
Frantoio	0.12	0.06	0.01	0.02	0.35	0.26	1.26	0.73
Koreneiki	0.22	0.10	0.01	0.02	0.39	0.42	3.55	1.49
Leccino	0.10	0.05	0.01	0.02	0.25	0.10	1.27	1.18
Manzanillo	0.15	0.08	0.00	0.00	0.22	0.11	1.80	0.67
Nevadillo Blanco	0.16	0.16	0.00	0.00	0.24	0.17	1.30	0.52
Pendolino	0.11	0.05	0.02	0.02	0.22	0.12	0.90	0.24
Picual	0.13	0.06	0.01	0.02	0.27	0.13	1.18	0.67
IOC limits	**<0.5**		**<0.1**		**<0.5**		**<4.5**	

4.1.3 Wax content

Wax content is generally very low in mechanically extracted olives as the wax is not easily removed from the outer skin of the fruit. It is a useful indicator of the presence of pomace oil as solvent extraction of oil from the pomace also removes waxes which end up in the oil. Although we found no appreciable levels of wax in this study, it was clear that some cultivars had more than others and the standard deviation between tests was large. This is largely due to the effect of growing site, which is discussed in the following section. All samples were within the COI limits at less than 250 mg/kg.

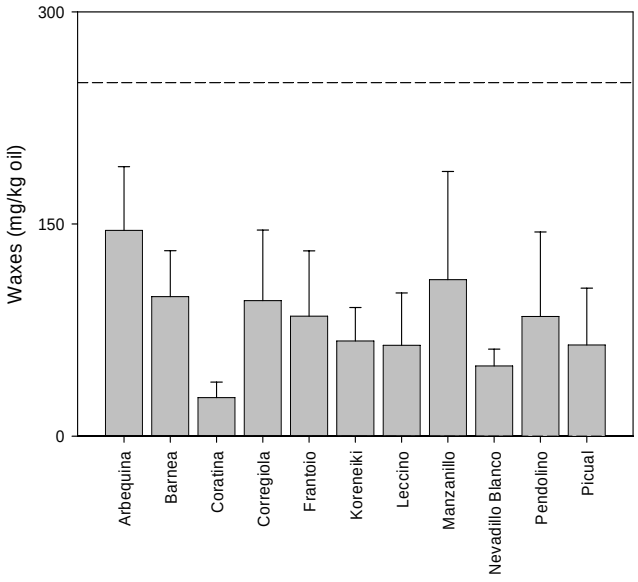


Figure 4.9 Wax content of eleven cultivars of olives. **Each bar represents the mean for the cultivar ± standard deviation. COI require the level to be less than 250 mg/kg, as indicated by the dotted line**

4.1.4 α-tocopherol

Although there is no COI limit for α-tocopherol (vitamin E), it is a very effective antioxidant in olive oil and contributes significantly to oxidative stability or shelf life. These results indicate *cv* Manzanillo contains relatively low levels of α-tocopherol whereas Leccino and Pendolino are relatively high. There was considerable variation between cultivars.

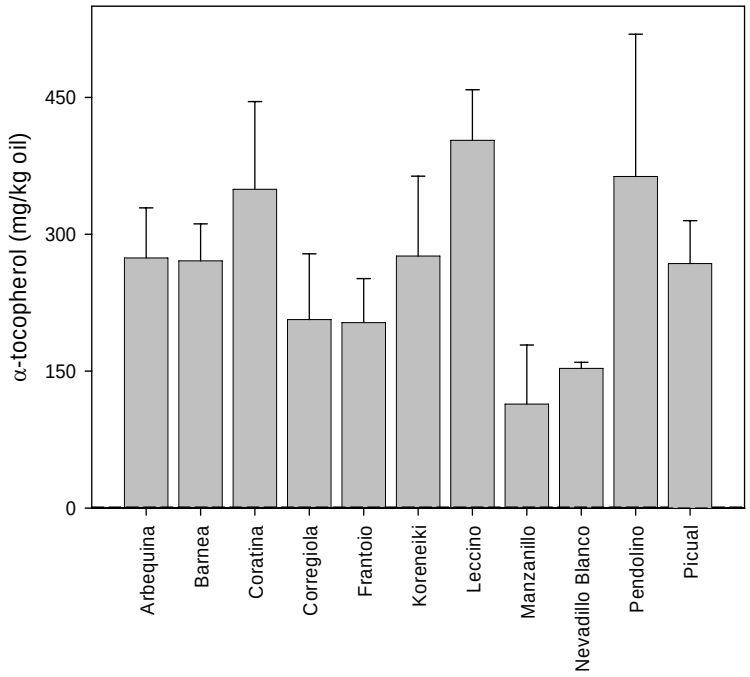


Figure 4.10. The α-tocopherol content for eleven cultivars. Each bar represents the mean for the cultivar ± standard deviation

4.1.5 Trans fatty acids

Fatty acids in the natural form are in the cis- configuration. Only trace amounts are in the trans fatty acid form. However, numerous processes, particularly hydrogenation with nickel catalysts, can result in formation of significant amounts of trans-fatty acids. Oil which has been heated or undergone long term storage may also have elevated levels. It was unlikely that oil extracted in the laboratory from fresh fruit would produce any results with high trans-fatty acids. However, to cover all of the standard requirements of EVOO, eleven cultivars were tested to see what the natural levels were. As suspected, none of the samples had more than trace amounts of any fatty acid in the trans form (Table 4.3).

Table 4.3 Means and standard deviations for trans-fatty acids for eleven cultivars. Results are a percentage of total fatty acids. COI limits are shown below

	C18:1T		C18:2T + C18:3T		Total trans	
	mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Arbequina	0.00	0.00	0.02	0.01	0.02	0.01
Barnea	0.00	0.00	0.02	0.01	0.02	0.01
Coratina	0.00	0.00	0.01	0.01	0.01	0.01
Corregiola	0.00	0.00	0.01	0.01	0.01	0.01
Frantoio	0.00	0.00	0.01	0.01	0.01	0.01
Koreneiki	0.00	0.00	0.01	0.00	0.01	0.01
Leccino	0.00	0.00	0.01	0.00	0.01	0.00
Manzanillo	0.00	0.00	0.01	0.01	0.02	0.01
Nevadillo Blanco	0.01	0.00	0.01	0.01	0.02	0.01
Pendolino	0.00	0.00	0.01	0.01	0.01	0.01
Picual	0.00	0.00	0.00	0.01	0.01	0.01
COI Limit	**<0.05**		**<0.05**			

4.1.6 UV absorbance

UV-absorbance is a method of determining the conformation of fatty acids. Changes to structure such as in conjugated fatty aids, will alter the absorbance at 270 nm wavelength. Such fatty acid changes occur when oil is heated or in contact with metal catalysts. No significant levels were expected from fresh olive oil and none were found (Table 4.4).

Table 4.4 Means and standard deviations for UV absorption for eleven cultivars. COI limits are shown below

	ΔK		Specific extinction coefficient 270nm	
	mean	**s.d.**	**mean**	**s.d.**
Arbequina	0.00	0.00	0.10	0.03
Barnea	0.00	0.00	0.09	0.03
Coratina	0.00	0.00	0.13	0.04
Corregiola	0.00	0.00	0.10	0.03
Frantoio	0.00	0.00	0.10	0.03
Koreneiki	0.00	0.00	0.13	0.04
Leccino	0.00	0.00	0.08	0.03
Manzanillo	0.00	0.00	0.09	0.05
Nevadillo Blanco	0.00	0.00	0.11	0.04
Pendolino	0.00	0.00	0.10	0.04
Picual	0.00	0.00	0.08	0.04
IOC Limit	**<0.01**		**<0.25**	

4.1.7 Stigmastadienes

Stigmastadienes are formed by the dehydration of β-sitosterol during refining, but not present in significant quantities in virgin olive oil (Li-Chan, 1994). According to most authors, bleaching is the main refining step that causes the formation of stigmastadienes (Cert et al, 1994; Grob et al, 1992). The level of stigmastadienes was expected to be low in freshly extracted olive oil and this is shown to be the case in Table 4.5.

Table 4.5. Means and standard deviations for stigmastadienes and difference between actual and theoretical ECN 42 for eleven cultivars. COI limits are shown below the table

	Stigmastadienes (mg/kg)		Difference between actual and theoretical ECN 42	
	mean	**s.d.**	**mean**	**s.d.**
Arbequina	0.05	0.04	0.11	0.04
Barnea	0.03	0.03	0.12	0.18
Coratina	0.03	0.02	0.08	0.05
Corregiola	0.03	0.03	0.10	0.08
Frantoio	0.05	0.03	0.08	0.05
Koreneiki	0.03	0.03	0.09	0.03
Leccino	0.04	0.03	0.08	0.05
Manzanillo	0.05	0.03	0.11	0.08
Nevadillo Blanco	0.02	0.01	0.08	0.06
Pendolino	0.05	0.03	0.07	0.06
Picual	0.04	0.03	0.08	0.07
IOC Limit	**<0.15**		**<0.2**	

4.1.8 Triacylcerides (ΔECN 42)

Edible oils are composed basically of mixtures of triacylglycerols, each made up of a cluster of three fatty acids attached to a glycerol moiety. The mixtures of these triacylglycerols depend on the fatty acid profile of the oil and are therefore different for oil from different species of oil crops. The main fatty acids in common edible oils are oleic, linolenic and linolenic fatty acids, each with 18 carbons in the molecule.
The ECN (equivalent carbon number) value can be measured by analysing intact triacylglycerols or it can be calculated from the fatty acid profile. Section 1.2.8 has a comprehensive explanation of ECN 42.
There were no differences between cultivars for ECN 42 as expected and all were acceptable by COI standards (Table 4.5).

4.2 The effect of site on olive oil components

The influence of cultivar on the differences between chemical components has been discussed in the previous section. However, the area, or site, at which the fruit are grown, can also have a strong influence on quality due to the different temperatures, rainfall, soil types and other variables. These 10 fruit cultivars were sourced from four extreme sites across the Australian olive production sites including the far north, central NSW, Western Australia and the far southern districts. The overall effect of site on the components in olive oil, regardless of growing season, cultivar or time of harvest is discussed in the following section

4.2.1 Fatty acid profiles

4.2.1.1 Oleic acid

High levels of oleic acid are desirable and from Figure 4.11 it was shown that the highest levels come from the southern regions of Central and Southern Victoria. The most northerly site, Northern NSW, was consistently lower in oleic acid.

4.2.1.2 Linoleic acid

The effect on polyunsaturated, linoleic fatty acid was directly the inverse to that of oleic acid. The coolest region was consistently low in linoleic acid and the northerly regions were consistently high (Figure 4.12). Northern areas in Australia are warmer climates and temperature appears to be the major influence on fatty acid profile.

4.2.1.3 Linolenic acid

Linolenic acid, as previously discussed, is of importance to Australian producers due to the higher levels than normally experienced in Europe. Although there was virtually no difference in levels from the three more southern sites, Northern NSW was higher (Figure 4.13). This might be expected considering the increasing level of polyunsaturated linoleic acid at more northerly, and warmer, sites.

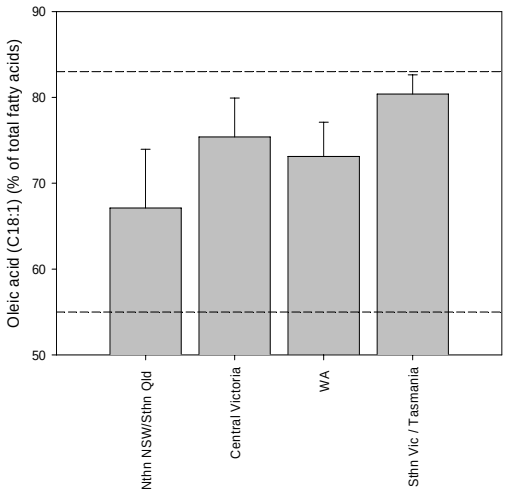


Figure 4.11 Oleic acid (C18:1) content for four sites. Each bar represents mean for site ± standard deviation. COI standard is 55% to 83% of total shown as dotted lines

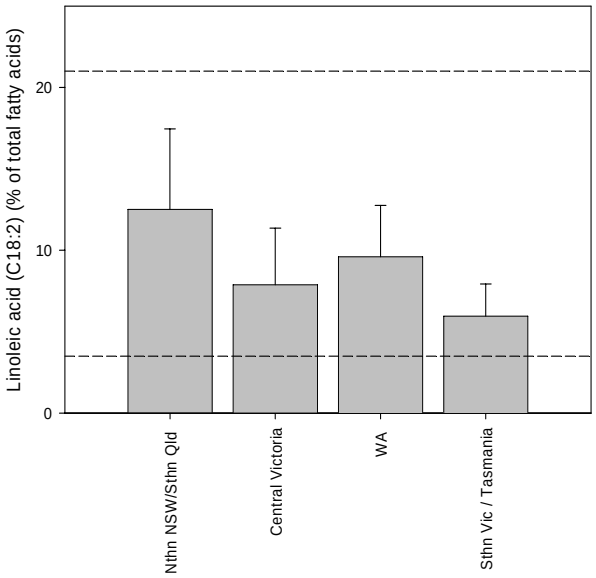


Figure 4.12 Linoleic acid (C18:2) content at four sites. Each bar is mean for cultivar ± standard deviation. COI limits are 3.5 - 21.0% of total, indicated by dotted lines

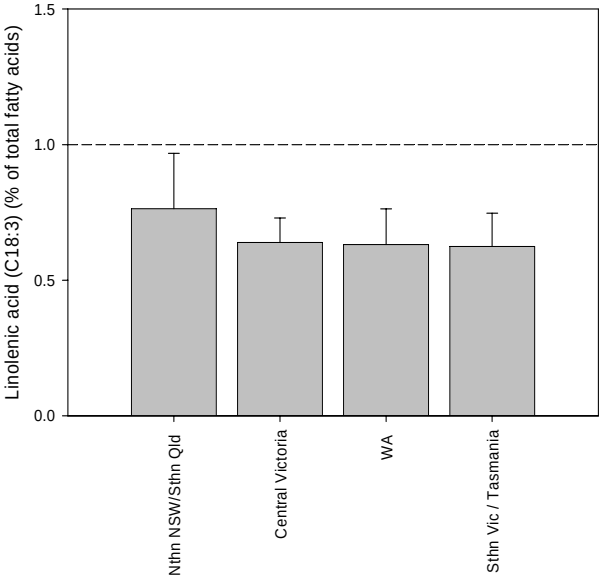


Figure 4.13 Linolenic acid (C18:3) content at four sites. Bar represents mean for cultivar ± standard deviation. COI limit is < 1.0% of total fatty acids as indicated by dotted line

4.2.1.4 Palmitic acid

Saturated palmitic acid, as for polyunsaturated linoleic acid, was seen to increase with more northerly, warmer, sites. From these results, it seems apparent that cooler climates result in increased oleic acid level with a decrease in saturated and polyunsaturated fatty acids. All samples were within the standard (Figure 4.14).

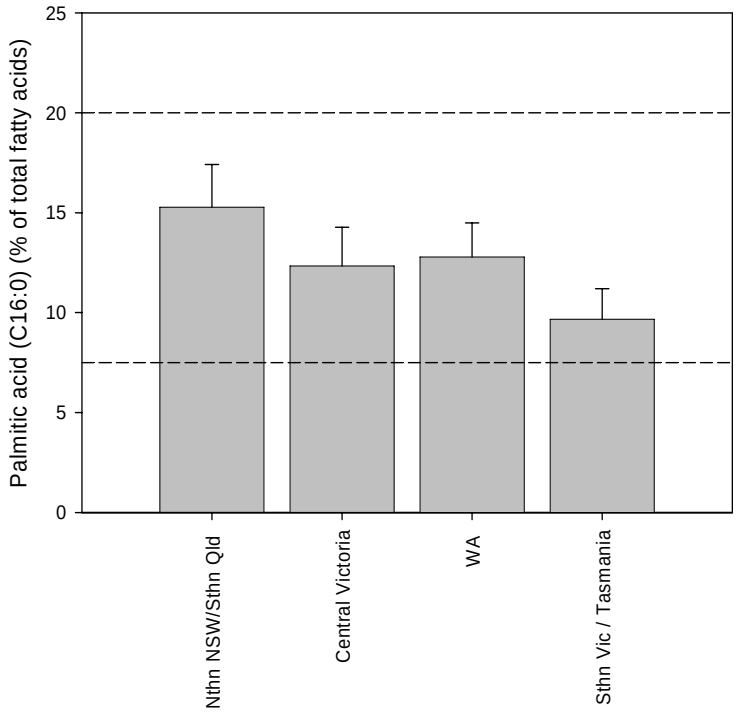


Figure 4.14 Palmitic acid (C16:0) content at four sites. Each bar represents the mean for the cultivar ± standard deviation. COI limits are 7.5 - 20.0% of fatty acids, as indicated by dotted lines

4.2.1.5 Other fatty acids

Saturated fatty acids C14:0, C17:0, C18:0, C20:0, C22:0 and C24:0 showed no site effect and all were within COI limits (Table 4.6). Monounsaturated C17:1 and C20:1 were also uninfluenced by site. However, C16:1 showed a clear increase in warmer, northerly sites with almost three times the level between the two extremes.

Table 4.6 Means and standard deviations for major fatty acids from four sites. Results are a percentage of total fatty acids. COI limits are shown below the table

	C14:0		C16:1		C17:0		C17:1		C18:0		C20:0		C20:1		C22:0		C24:0	
	mean	s.d	mean	s.d	mean	s.d	mean	s.d	mean	s.d	mean	s.d	mean	s.d	mean	s.d	mean	s.d
Northern NSW/	0.01	0.01	1.60	0.83	0.05	0.03	0.10	0.07	1.86	0.62	0.31	0.06	0.28	0.08	0.08	0.02	0.04	0.02
Central Victoria	0.01	0.00	1.01	0.46	0.05	0.03	0.11	0.06	1.82	0.54	0.32	0.06	0.29	0.08	0.09	0.02	0.04	0.01
Western Australia	0.01	0.00	0.98	0.33	0.06	0.04	0.12	0.08	1.96	0.57	0.32	0.07	0.29	0.07	0.09	0.02	0.04	0.02
Sthn Vic / Tasmania	0.00	0.00	0.56	0.24	0.05	0.01	0.10	0.02	1.93	0.39	0.31	0.06	0.28	0.06	0.10	0.02	0.03	0.01
IOC limit	**<0.05**		**0.3-3.5**		**<0.3**		**<0.3**		**0.5-5.0**		**<0.6**		**<0.4**		**<0.2**		**<0.2**	

s.d. – standard deviation

4.2.2 Sterols

Although sterols have been shown to be clearly related to the olive genotype, the site effect appears less clear. However, understanding natural effects on sterols may help produce overcome difficulties with current international standards.

4.2.2.1 Total sterols

The total sterol content of all olives tested was consistently well in excess of the minimum level set by the COI standard of 1000mg/kg. Northern NSW, the warmest climate, produced the highest level of sterols whereas the cooler regions were lower. There was not a significant difference among the three southern sites.

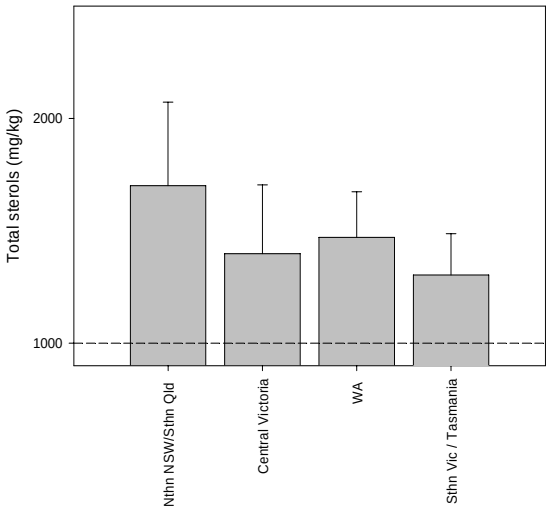


Figure 4.15 Total sterol content for four sites. Each bar represents the mean for the cultivar ± standard deviation. COI level is >1000mg/kg, as indicated by the dotted line

4.2.2.2 Campesterol

The importance of campesterol is a result of it consistently exceeding the COI standard in some cultivars. It was of interest that although the most northern site was the highest in total sterols, Central Victoria was the highest in campesterol Figure 4.16). This is a concern as the majority of cv Barnea trees are grown in Central Victoria. Unlike other sterols, campesterol is independent of the total amount of sterol.

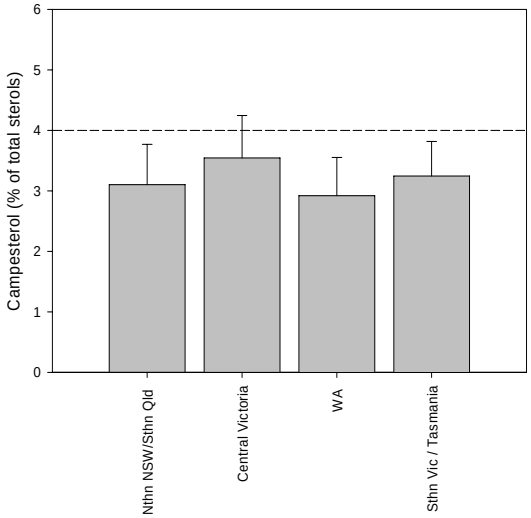


Figure 4.16 Campesterol content at four sites. Each bar represents mean for the cultivar ± standard deviation. COI limit is <4% of total sterols, as indicated by the dotted line

4.2.2.3 Stigmasterol

Stigmasterol content was in the same relative order as total sterols with the Northern site the highest and progressively lower with more southerly sites. All samples met COI requirements (Figure 4.17).

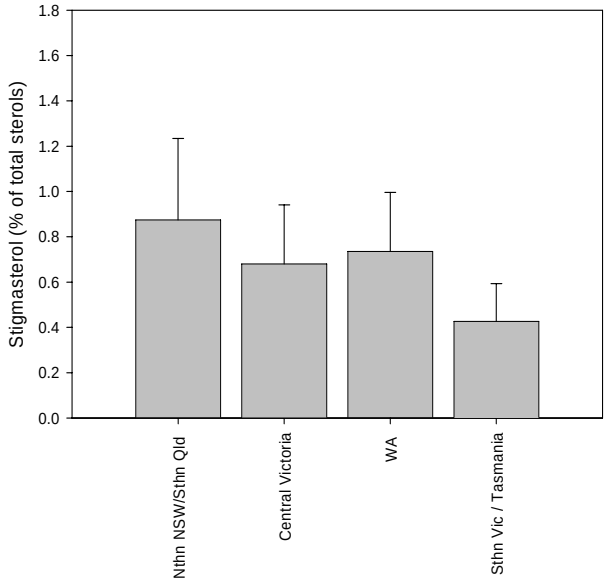


Figure 4.17 Stigmasterol content at four sites. Each bar represents the mean for the cultivar ± standard deviation. COI limit states the level must be below that of campesterol (as a % of total sterols)

4.2.2.4 Apparent β-sitosterol content

The apparent β-sitosterol content at four sites was surprisingly similar showing that β-sitosterol maintains a consistent proportion of the total sterols. All samples were above the minimum level required to meet the standard (Figure 4.18).

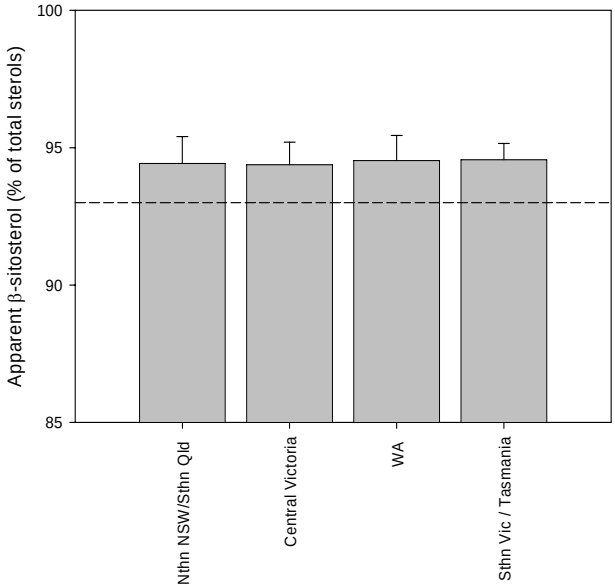


Figure 4.18 Apparent β-sitosterol content for four sites. Each bar represents the mean for the cultivar ± standard deviation. COI require the level to be greater than 93% of total sterols, as indicated by the dotted line

4.2.2.5 Cholesterol

Cholesterol is a minor component in olive oil and there were no significant differences in cholesterol content between four environmentally different sites (Table 4.7). No samples exceeded the standard.

4.2.2.6 Brassicasterol

Brassicasterol was also negligible in all samples and no site differences (Table 4.7). All samples were less than the maximum level allowed.

Table 4.7 Means and standard deviations of some sterols for four sites. Results are a percentage of total sterols. COI limits are shown below

	Cholesterol		Brassicasterol		Δ-7-stigmastenol		Diols	
	Mean	**s.d.**	**Mean**	**s.d.**	**Mean**	**s.d.**	**Mean**	**s.d.**
Nthn NSW/Sthn Qld	0.13	0.09	0.00	0.01	0.34	0.26	1.59	1.28
Central Victoria	0.13	0.07	0.00	0.01	0.18	0.11	1.37	0.75
WA	0.14	0.08	0.01	0.02	0.26	0.13	1.22	0.74
Sthn Vic / Tasmania	0.17	0.06	0.01	0.02	0.23	0.11	1.50	0.70
IOC limit	**<0.5**		**<0.1**		**<0.5**		**<4.5**	

4.2.2.7 Δ-7-stigmastenol

Δ-7-stigmastenol was slightly higher in the warmer northern region than the south but it was not the lowest in the coldest region of Tasmania (Table 4.7). The order of magnitude was virtually the opposite of the levels in campesterol for the four regions with Central Victoria being the lowest despite being located centrally between the other sites.

4.2.2.8 Erythrodiols

There was no clear trend with diols with Northern NSW being highest and WA being lowest (Table 4.7). All samples were within the permissible standard.

4.2.3 Wax content

Wax was shown to vary significantly between and within cultivars and genotype. However, it is clear from Figure 4.19 that site differences are also significant. The wax content increased the further north the site. Although all olive oils were extracted in the laboratory at the same temperature, oil from the warmer climate contained more wax. This may be because the olives actually had more wax on the flesh and therefore there was a larger amount dissolved in the oil on extraction. It may however indicate that there are waxes of different molecular size on the olives from different regions which are more soluble in mechanically extracted olive oil. Wax analysis by GC chromatography to determine individual components could determine if this was the case.

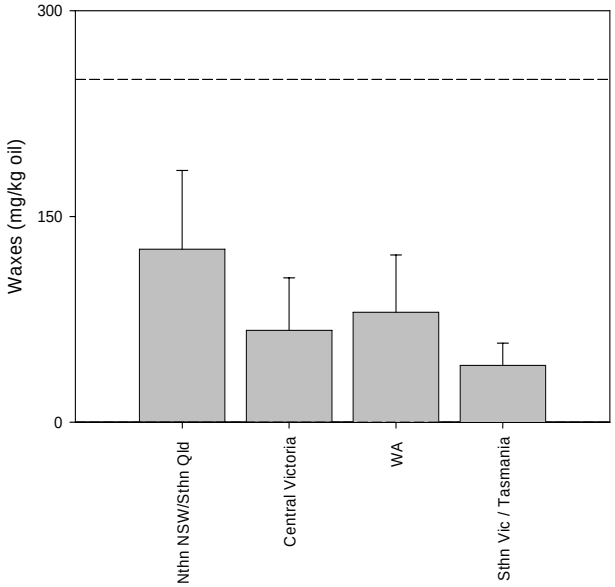


Figure 4.19 Wax content for four sites. Each bar represents the mean for the cultivar ± standard deviation. COI limit is <250 mg/kg, as indicated by the dotted line

4.2.4 α-tocopherol

The α-tocopherol content of olives from four regions was different among regions with the highest levels in Northern NSW. The other three regions did not show significantly different levels of α-tocopherol with Western Australia being the lowest (Figure 4.20).

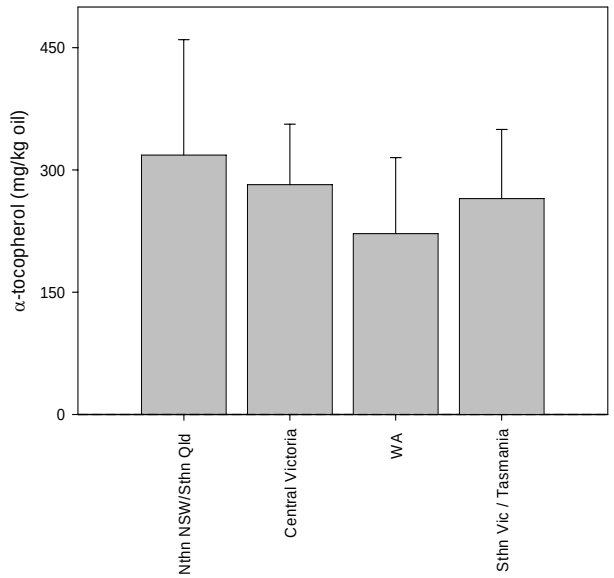


Figure 4.20 α-tocopherol content for eleven cultivars. Each bar represents the mean for the cultivar ± standard deviation. There is no COI limit

4.2.5 Trans fatty acids

There were no site effects for trans-fatty acids and levels found were negligible (Table 4.8).

Table 4.8 Mean and standard deviation for trans-fatty acids for four sites. Results are a percentage of total fatty acids. COI limits are shown below

	C18:1T		C18:2T +C18:3C		Total trans	
	mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Northern NSW	0.00	0.00	0.02	0.01	0.02	0.01
Central Victoria	0.00	0.00	0.01	0.01	0.01	0.00
WA	0.00	0.00	0.01	0.01	0.02	0.01
Southern Vic / Tasmania	0.00	0.00	0.00	0.00	0.01	0.01
IOC Limit			**<0.05**		**<0.05**	

4.2.6 UV absorbance

UV absorbance showed no difference between sites. All samples were within COI limits (table 4.9).

Table 4.9 Means and standard deviations for UV absorption for four sites. COI limits are shown below

	ΔK		Spec.ext 270nm	
	mean	**s.d.**	**mean**	**s.d.**
Northern NSW	0.00	0.00	0.10	0.03
Central Victoria	0.00	0.00	0.09	0.03
WA	0.00	0.00	0.09	0.04
Southern Vic / Tasmania	0.00	0.00	0.13	0.04
IOC limit	**<0.01**		**<0.25**	

4.2.7 Stigmastadienes

Stigmastadienes showed no relationship to site (Table 4.10). All samples were within internationally acceptable levels.

Table 4.10 Means and standard deviations for Stigmastadienes and difference between actual and theoretical ECN 42 content for four sites. COI limits are shown below

	Stigmastadienes (mg/kg)		Difference between actual and theoretical ECN 42	
	mean	**s.d.**	**mean**	**s.d.**
Northern NSW	0.04	0.04	0.15	0.13
Central Victoria	0.04	0.03	0.07	0.05
WA	0.04	0.03	0.08	0.05
Southern Vic / Tasmania	0.03	0.02	0.07	0.05
IOC limit	**<0.15**		**<0.2**	

4.2.8 Triacylglycerides (Δ ECN)

Although the level for ECN42 appeared greater in the warmer Northern region, the difference was not significant (Table 4.10).

4.3 The effect of harvest timing on olive oil components

Previous studies (Mailer et al., RIRDC Report 2005) have shown that there is a major change in oil quality as olives mature. In many cases, oil quality is more different between early and late harvested fruit than the difference between the cultivars. This phenomenon can be utilised by growers to harvest at a time suitable to them to produce the type of oils which they desire. Early harvested oil is greener, pungent, bitter and has higher levels of polyphenols. More mature olives produce mellow and less pungent oil but more fragile, with reduced oxidative stability. The comparison between early and late harvest is carried out here to determine if the variability between oil qualities is sufficient to cause the oil to be outside the international limits for EVOO.

4.3.1 Fatty acid profiles

The previous chapters have indicated that some fatty acids are cultivar dependant and some are influenced by site (temperature and environment). This chapter compares the fatty acid profiles of olive harvested at two levels of maturity to determine the influence of harvest timing.

4.3.1.1 Oleic acid

The variation due to genotype described in Section 4.1 is also obvious in Figure 4.21. There is also some difference in oleic acid between early and late harvested fruit. However, there is no consistency with some cultivars showing a reduction in oleic acid with maturity and others showing an increase. Our previous studies also showed a slight reduction overall but no real relationship between maturity and oleic acid content.

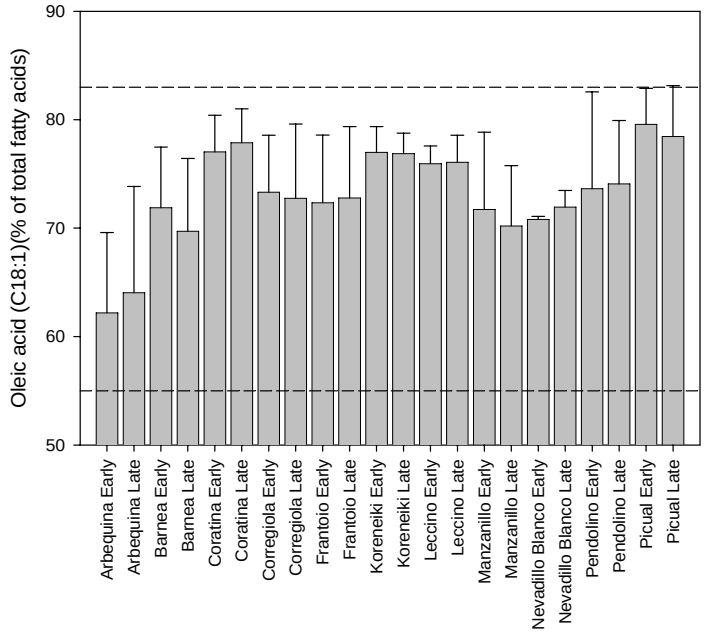


Figure 4.21 Oleic acid (C18:1) content for eleven cultivars at different harvest times. Each bar represents mean for the cultivar ± standard deviation. COI limits are 55 - 83% of total fatty acids as indicated by the dotted lines

4.3.1.2 Linoleic acid

Although *cv* Arbequina did not show an increase in linoleic acid with late harvest, all other cultivars did (Figure 4.22). This is in agreement with our previous study showing linoleic

acid increases with maturity. The result is that the oil is less stable as the polyunsaturated fatty acids increase as the rate of oxidation increases with the number of double bonds. Linoleic acid is much more reactive than oleic acid. Although some cultivars were lower than the minimum allowable levels, no oils exceeded the maximum allowable linoleic acid content.

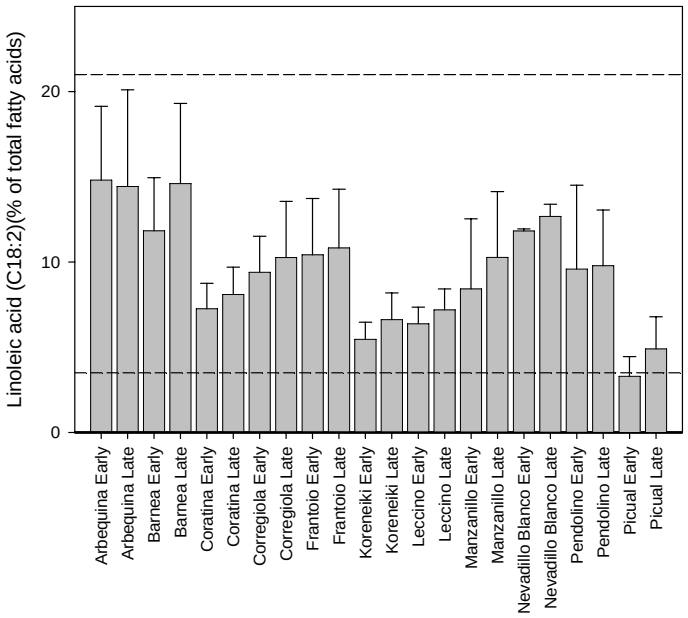


Figure 4.22 Linoleic acid (C18:2) content for eleven cultivars at different harvest times. **Each bar represents the mean for the cultivar ± standard deviation. COI limits are 3.5 - 21.0% of total fatty acids, as indicated by the dotted lines**

4.3.1.3 Linolenic acid

Linolenic acid variation between young and mature fruit was not clear with some showing a decrease and others showing an increase in concentration (Figure 4.23). Only *cvv* Coratina, Koreneiki and Pendolino showed significant differences and all three showed a decrease in linolenic acid with maturation. Pendolino early harvest exceeded the limit of 1.0% imposed by COI.

4.3.1.4 Palmitic acid

Again the variation between early and late harvest is not highly significant although mean values for all samples show a decrease in palmitic acid with delayed harvest time (Figure 4.24). Palmitic acid is a saturated fatty acid and provides additional oxidative stability to the oil. This reduction in palmitic acid results in reduced oxidative stability of late harvested olives. The benefits are that reduced palmitic acid increases nutritive value. Reduced palmitic acid also reduces the melting point and therefore reduces the solidification temperature i.e. the oil will remain liquid at lower temperatures than olive oil with high palmitic acid levels.

4.3.1.5 Other fatty acids

Table 4.11 shows the minor fatty acids for all cultivars at two harvest times. Only stearic acid, C18:0 showed consistent differences between harvest times with all but two higher in stearic acid with later harvest. Fatty acids C14:0, 16:0, 17:0, 17:1, 20:0, 20:1, 22:0 and 24:0 showed no significant difference between harvest times. These are all minor fatty acids of little importance and low concentration.

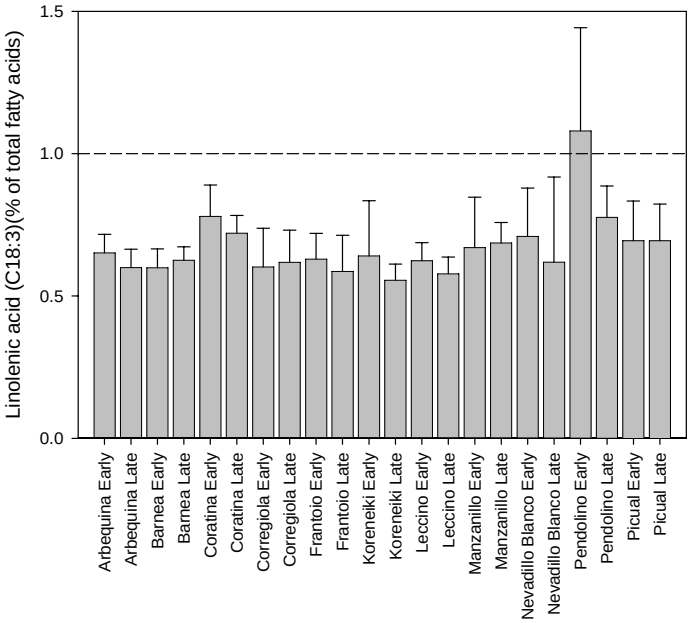


Figure 4.23 Linolenic acid (C18:3) content for eleven cultivars for different harvest times. Each bar represents the mean for the cultivar ± standard deviation. The COI limit is <1.0% of total fatty acids, as indicated by the dotted line

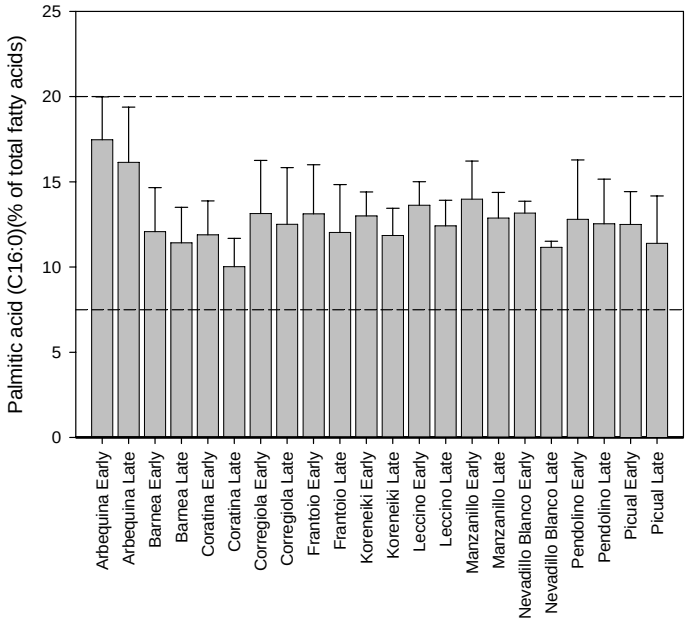


Figure 4.24 Palmitic acid (C16:0) content for eleven cultivars for different harvest times. Each bar represents the mean for the cultivar ± standard deviation. COI limits are 7.5 - 20.0% of total fatty acids, as indicated by the dotted lines.

Table 4.11 Means and standard deviations for some fatty acids for 11 cultivars at different harvest times. Results are a percentage of total fatty acids. COI limits are shown below

	C14:0		C16:1		C17:0		C17:1		C18:0		C20:0		C20:1		C22:0		C24:0	
	mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Arbequina Early	0.01	0.01	2.48	0.84	0.09	0.03	0.22	0.04	1.38	0.24	0.33	0.03	0.24	0.05	0.09	0.02	0.05	0.02
Arbequina Late	0.01	0.00	2.36	1.19	0.08	0.01	0.21	0.02	1.40	0.16	0.30	0.05	0.27	0.06	0.10	0.02	0.05	0.02
Barnea Early	0.00	0.00	0.90	0.28	0.04	0.01	0.07	0.02	1.91	0.27	0.33	0.06	0.23	0.03	0.08	0.02	0.04	0.02
Barnea Late	0.01	0.00	0.87	0.28	0.04	0.01	0.08	0.02	1.91	0.21	0.34	0.03	0.24	0.01	0.10	0.01	0.04	0.01
Coratina Early	0.00	0.00	0.42	0.10	0.04	0.01	0.07	0.02	1.66	0.21	0.32	0.07	0.38	0.14	0.09	0.02	0.05	0.01
Coratina Late	0.01	0.00	0.40	0.08	0.04	0.01	0.07	0.02	1.85	0.37	0.35	0.05	0.44	0.04	0.10	0.01	0.05	0.01
Corregiola Early	0.00	0.00	1.01	0.39	0.04	0.01	0.08	0.03	1.70	0.14	0.29	0.06	0.30	0.07	0.09	0.03	0.04	0.02
Corregiola Late	0.01	0.00	1.03	0.46	0.04	0.01	0.09	0.02	1.91	0.20	0.32	0.06	0.31	0.05	0.10	0.03	0.04	0.01
Frantoio Early	0.00	0.00	1.03	0.47	0.04	0.01	0.08	0.02	1.61	0.33	0.31	0.06	0.29	0.05	0.09	0.03	0.04	0.01
Frantoio Late	0.00	0.00	0.99	0.56	0.04	0.01	0.09	0.01	1.85	0.24	0.32	0.03	0.32	0.05	0.10	0.02	0.04	0.02
Koreneiki Early	0.01	0.01	0.92	0.16	0.03	0.01	0.06	0.02	2.10	0.13	0.35	0.07	0.28	0.04	0.10	0.02	0.05	0.02
Koreneiki Late	0.01	0.00	0.91	0.21	0.04	0.00	0.07	0.01	2.27	0.23	0.38	0.04	0.27	0.02	0.12	0.02	0.04	0.02
Leccino Early	0.01	0.00	1.11	0.28	0.04	0.01	0.08	0.02	1.60	0.35	0.25	0.06	0.26	0.04	0.06	0.02	0.03	0.01
Leccino Late	0.01	0.00	1.04	0.27	0.04	0.01	0.08	0.02	1.92	0.26	0.29	0.03	0.25	0.02	0.08	0.01	0.03	0.01
Manzanillo Early	0.01	0.01	1.45	0.50	0.11	0.02	0.19	0.04	2.72	0.42	0.36	0.08	0.24	0.04	0.09	0.02	0.05	0.02
Manzanillo Late	0.01	0.00	1.43	0.26	0.12	0.03	0.24	0.05	3.34	0.32	0.44	0.04	0.23	0.02	0.11	0.01	0.05	0.01
Nevadillo Blanco Early	0.01	0.00	0.79	0.12	0.14	0.01	0.28	0.04	1.63	0.01	0.28	0.04	0.29	0.07	0.07	0.03	0.02	0.03
Nevadillo Blanco Late	0.01	0.00	0.65	0.02	0.14	0.00	0.30	0.02	1.69	0.10	0.31	0.03	0.35	0.00	0.09	0.01	0.04	0.01
Pendolino Early	0.00	0.00	0.72	0.34	0.03	0.01	0.09	0.01	1.30	0.16	0.26	0.03	0.35	0.02	0.09	0.01	0.04	0.01
Pendolino Late	0.00	0.00	0.79	0.25	0.03	0.01	0.09	0.02	1.24	0.14	0.23	0.02	0.31	0.02	0.08	0.01	0.03	0.02
Picual Early	0.01	0.00	1.15	0.54	0.04	0.01	0.08	0.02	2.01	0.43	0.28	0.06	0.25	0.05	0.08	0.02	0.04	0.02
Picual Late	0.00	0.00	1.15	0.72	0.04	0.01	0.09	0.01	2.56	0.65	0.33	0.04	0.24	0.03	0.09	0.01	0.04	0.02
IOC limit	**<0.05**		**0.3-3.5**		**<0.3**		**<0.3**		**0.5-5.0**		**<0.6**		**<0.4**		**<0.2**		**<0.2**	

4.3.2. Sterols

Sterols, as discussed previously, are an important character used in detecting adulteration of olive oil with other oils. Variation which occurs therefore as a result of natural growing conditions may indicate that genuine olive oils are adulterated. The existing standards sometimes are too restrictive to allow for this natural variation.

4.3.2.1 Total sterols

The influence of genotype on total sterol content is apparent (Figure 4.25) with Koreneiki having a low level of total polyphenols and Arbequina being the highest. The influence of harvest date however was only apparent on *cv* Pendolino which was considerably higher in early harvested fruit. As Pendolino was the only cultivar to show this difference, and the study was only done over two years, the comparison may need to be repeated to ensure this is a genuine and repeatable difference.

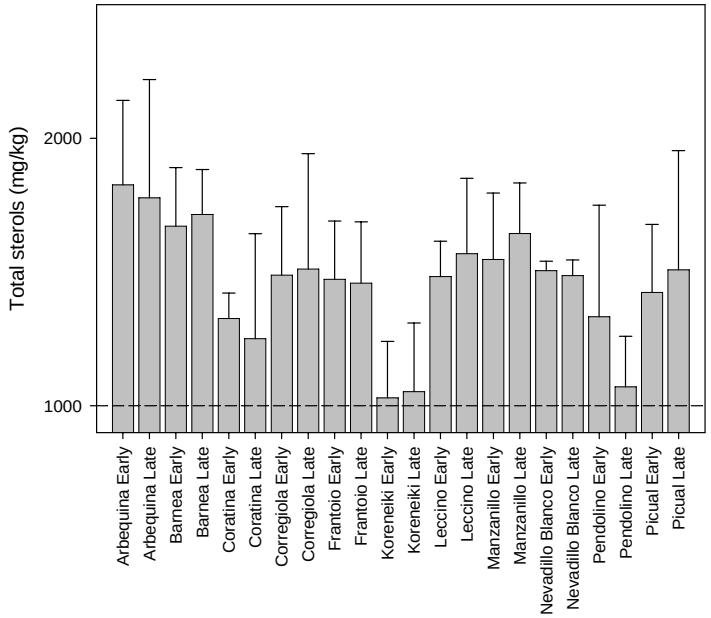


Figure 4.25 Total sterol content for eleven cultivars at different harvest times. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be greater than 1000mg/kg, as indicated by the dotted line

4.3.2.2 Campesterol

We have shown that campesterol is an issue for some cultivars, regularly exceeding the maximum level of 4.0% of total sterols allowed in the COI standards. Methods for reducing this level have become important to growers to try to meet standards for export. Harvest timing is one method used to achieve quality variation to suit the grower's aims. However, there was little variation in harvest timing for all cultivars tested with six cultivars having slightly lower levels in late harvest fruit. The cultivars Barnea and Koreneiki, where excessive campesterol is a problem, there was no difference between early and late harvested fruit (Figure 4.26).

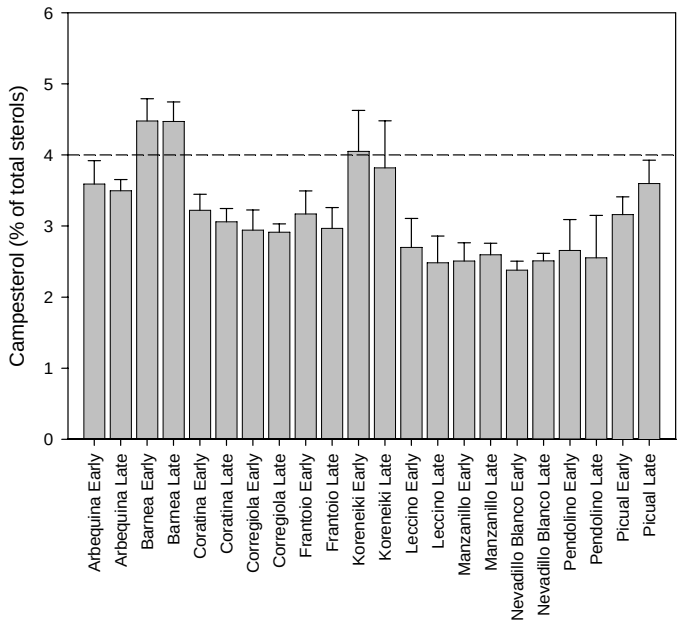


Figure 4.26 Campesterol content for eleven cultivars at different harvest times. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be less than 4% of total sterols, as indicated by the dotted line

4.3.2.3 Stigmasterol

Stigmasterol must be less than the campesterol content for each cultivar. All cultivars were well within the standard. The influence of harvest timing was mixed with six cultivars showing slightly elevated levels in late harvested fruit and the others being the same or less. Only Koreneiki showed a significant reduction with late harvesting and two years of testing is insufficient to know if this is consistent for this cultivar (Figure 4.27).

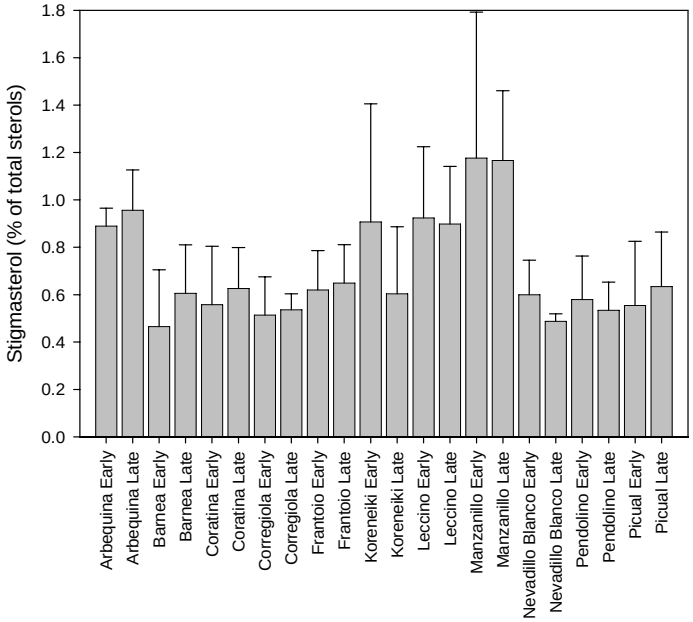


Figure 4.27 Stigmasterol content for eleven cultivars at different harvest times. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be below the level of campesterol (as a % of total sterols)

4.3.2.4 Apparent β-Sitosterol

The average concentration of β-sitosterol was above the minimum of 93% although some samples were below this. The majority of cultivars showed slightly reduced levels with late harvesting although *cv* Koreneiki was the reverse, increasing in β-sitosterol with late harvest (Figure 4.28).

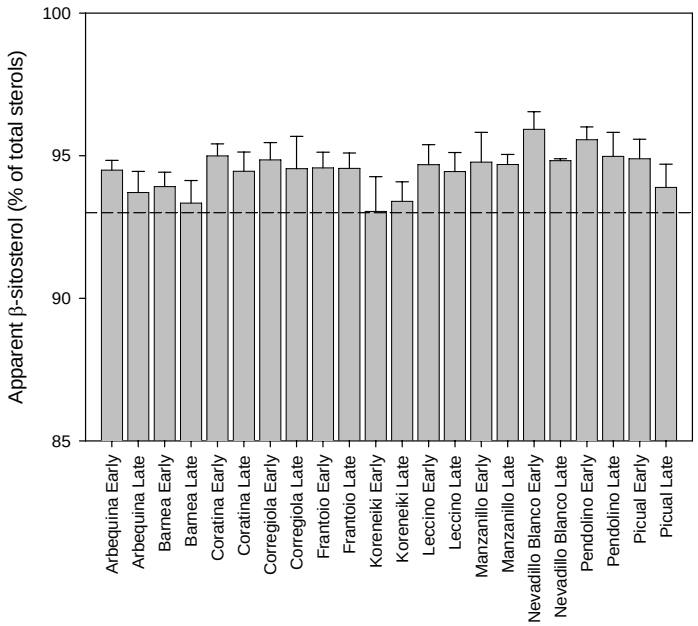


Figure 4.28 Apparent β-sitosterol content for eleven cultivars at different harvest times. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be greater than 93% of total sterols, as indicated by the dotted line

4.3.2.5 Cholesterol

All cultivars were similar or slightly higher in cholesterol with late harvested olives except for Koreneiki in which concentration increased with maturity (Table 4.12).

4.3.2.6 Brassicasterol

Brassicasterol was an insignificant component of the sterol profile and showed no relationship with fruit maturity in any cultivar (Table 4.12).

4.3.2.7 Δ-7-Stigmastenol

All cultivars except had similar levels of Δ-7-Stigmastenol for both early and late harvest (Table 4.12)

4.3.2.8 Erythrodiols

The cultivars Barnea and Koreneiki showed significant reduction in diols with late harvest. The level recorded for Koreneiki was actually above the limit of 4.5 permitted by the standards (Table 4.12). Other cultivars were the same or slightly higher with late harvest.

Table 4.12 Means and standard deviations of some sterols for eleven cultivars at different harvest times. Results are a percentage of total sterols. COI limits are shown below

Cultivar	Cholesterol		Brassicasterol		Δ-7-Stigmastenol		Diols	
	mean	s.d	mean	s.d	mean	s.d	mean	s.d
Arbequina Early	0.08	0.03	0.00	0.00	0.11	0.05	1.36	0.21
Arbequina Late	0.14	0.07	0.02	0.02	0.28	0.13	1.35	0.48
Barnea Early	0.09	0.05	0.00	0.00	0.17	0.08	1.06	0.49
Barnea Late	0.16	0.07	0.00	0.00	0.14	0.10	0.89	0.37
Coratina Early	0.18	0.15	0.00	0.00	0.18	0.10	1.50	0.52
Coratina Late	0.17	0.05	0.01	0.02	0.24	0.11	1.65	0.58
Corregiola Early	0.15	0.07	0.00	0.00	0.29	0.11	1.11	0.35
Corregiola Late	0.16	0.07	0.01	0.02	0.38	0.17	1.17	0.54
Frantoio Early	0.13	0.07	0.00	0.01	0.31	0.18	1.30	0.86
Frantoio Late	0.11	0.05	0.01	0.02	0.39	0.35	1.23	0.59
Koreneiki Early	0.15	0.09	0.00	0.00	0.52	0.73	4.86	1.78
Koreneiki Late	0.26	0.10	0.02	0.02	0.32	0.14	2.77	0.47
Leccino Early	0.13	0.06	0.01	0.02	0.21	0.08	0.86	0.29
Leccino Late	0.12	0.04	0.01	0.02	0.29	0.11	1.73	1.62
Manzanillo Early	0.13	0.06	0.00	0.00	0.20	0.11	1.72	0.63
Manzanillo Late	0.16	0.09	0.00	0.00	0.24	0.11	1.87	0.76
Nevadillo Blanco Early	0.14	0.09	0.00	0.00	0.11	0.01	1.02	0.23
Nevadillo Blanco Late	0.18	0.26	0.00	0.00	0.37	0.15	1.58	0.65
Pendolino Early	0.09	0.05	0.00	0.00	0.28	0.09	0.85	0.25
Pendolino Late	0.13	0.04	0.03	0.03	0.16	0.12	0.95	0.24
Picual Early	0.12	0.06	0.01	0.03	0.27	0.13	1.14	0.48
Picual Late	0.15	0.06	0.01	0.02	0.26	0.14	1.23	0.90
IOC Limits	**<0.5**		**<0.1**		**<0.5**		**<4.5**	

4.3.3 Wax content

There were clear differences in wax content in some cultivars depending on the harvest date. Arbequina, Leccino, Manzanillo and Pendolino were clearly lower in wax content with late harvest. Only the total wax content has been recorded and the difference in the wax profile is not shown. However, the different waxes may have varying levels of solubility in olive oil. Despite the variation with harvest time, all samples were within the required level of <250 mg/kg (Figure 4.29).

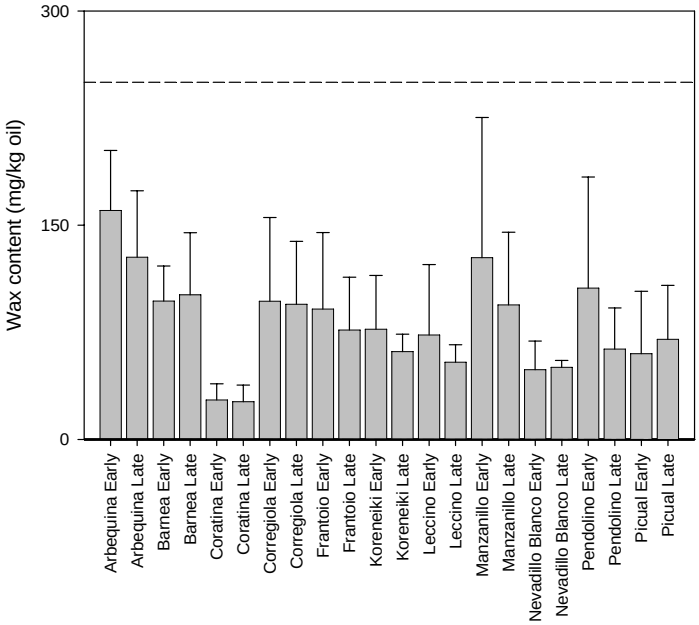


Figure 4.29 Wax content for eleven cultivars at different harvest times. Each bar represents the mean for the cultivar ± standard deviation. COI require the level must be less than 250 mg/kg, as indicated by the dotted line

4.3.4 α-tocopherol

The component α-tocopherol is a strong antioxidant and is shown here to decrease substantially as olive fruit mature. All cultivars except for *cv* Nevadillo banco showed some reduction (Figure 4.30). The reduction in oxidative stability in olive fruit appears to decrease in all aspects as the level of polyunsaturated fatty acids increase and antioxidants such as α-tocopherol and polyphenols decrease. There is no standard for α-tocopherol in olive oil.

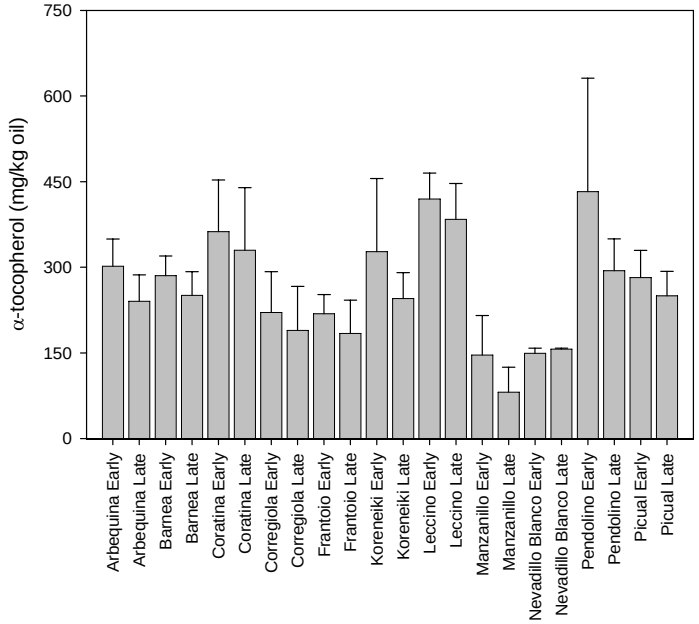


Figure 4.30 α-tocopherol content for eleven olive cultivars at different harvest times. Each bar represents the mean for the cultivar ± standard deviation. There is no COI limit

4.3.4 Trans fatty acids

Trans fatty acids were analysed for all samples but the levels were insignificant and there was no apparent influence of harvest time. The results are not shown here.

4.3.5 UV absorbance

There was only a very slight decrease in UV absorbance for most cultivars although the difference is not significant (table 4.13).

Table 4.13 Means and standard deviations for UV absorption for eleven cultivars at different harvest times. COI limits are shown below

Cultivar	ΔK		Specific extinction coefficient, 270nm	
	mean	s.d	mean	s.d
Arbequina Early	0.00	0.00	0.10	0.02
Arbequina Late	0.00	0.00	0.11	0.04
Barnea Early	0.00	0.00	0.10	0.03
Barnea Late	0.00	0.00	0.08	0.02
Coratina Early	0.00	0.00	0.14	0.04
Coratina Late	0.00	0.00	0.12	0.05
Corregiola Early	0.00	0.00	0.11	0.04
Corregiola Late	0.00	0.00	0.09	0.03
Frantoio Early	0.00	0.00	0.11	0.03
Frantoio Late	0.00	0.00	0.10	0.03
Koreneiki Early	-0.01	0.00	0.16	0.03
Koreneiki Late	0.00	0.00	0.11	0.03
Leccino Early	0.00	0.00	0.09	0.04
Leccino Late	0.00	0.00	0.07	0.03
Manzanillo Early	0.00	0.00	0.10	0.06
Manzanillo Late	0.00	0.00	0.08	0.02
Nevadillo Blanco Early	0.00	0.00	0.13	0.05
Nevadillo Blanco Late	0.00	0.00	0.10	0.05
Pendolino Early	0.00	0.00	0.13	0.04
Pendolino Late	0.00	0.00	0.07	0.02
Picual Early	0.00	0.00	0.09	0.04
Picual Late	0.00	0.00	0.07	0.02
IOC Limit	**<0.01**		**<0.25**	

4.3.6 Stigmastadienes

There was no harvest timing effect for stigmastadienes and results have not been included in this report. All samples met international standards.

4.3.7 Triacylcerides (ΔECN 42)

There was no harvest timing effect for ECN 42 and results have not been included in this report. All samples met international standards.

4.4 The effect of growing season on olive oil components

Samples were taken at early and late harvest from four sites over two years. The purpose of harvesting over two years harvest was to overcome seasonal differences and to provide an average result. However, two years provides very limited data on the wide range of environmental conditions which may occur from year to year in Australia. To have more reliable figures such a study should be carried out over three or more years to account for annual climatic differences. However, this study has provided a reasonable range of data which would be expected to reflect the olive oil quality produced across the Australian growing regions.

The two years studied have been used as duplicate analysis to help develop the standard deviation between sites, cultivars and harvest dates. Some limited data is shown in section 4.4 comparing two years to illustrate the differences which may occur from year to year. This data is a mean value for each cultivar x four sites x two harvest dates. Much of the data is not shown within the text if it is not significant.

4.4.1 Fatty acid profiles

4.4.1.1 Oleic acid

Although there is not a consistent effect for oleic acid across all sites between the two years, the difference for Arbequina is considerable. The change in oleic acid was not explained by fruit maturity and it is unknown why cv Arbequina has shown such a difference for the two years. Most cultivars showed consistent levels over the two periods (Figure 4.31).

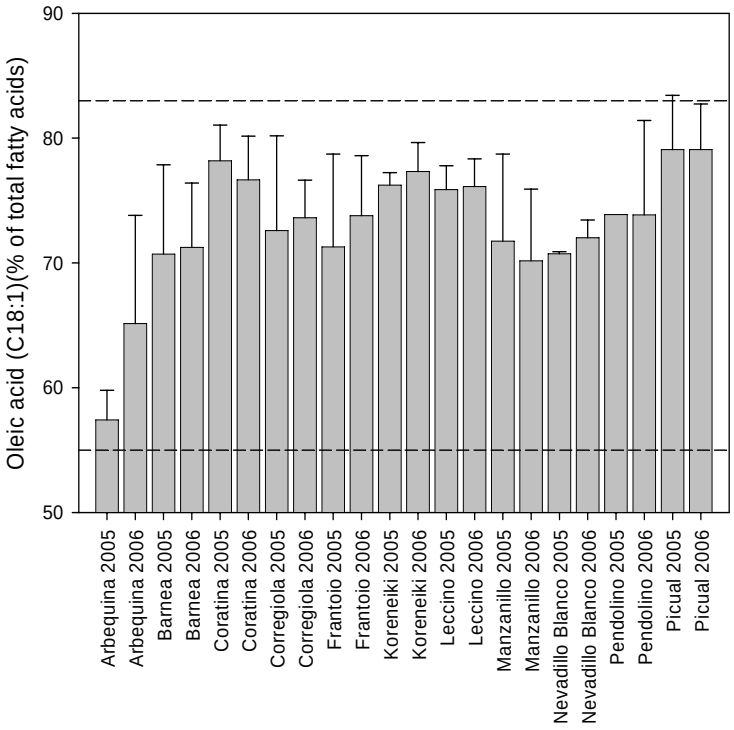


Figure 4.31 Oleic acid (C18:1) content for eleven cultivars for each of two years. Each bar represents the mean for the cultivar ± standard deviation. COI limit is 55 - 83% of total fatty acids, as indicated by the dotted lines

4.4.1.2 Linoleic acid

The linoleic acid level in most cultivars was consistent although *cvv* Arbequina, Corregiola and Frantoio had higher concentrations in 2005 than in 2006 (Figure 4.32). This is in contrast to the high level of oleic acid seen in *cv* Arbequina in 2006 (Figure 4.31).

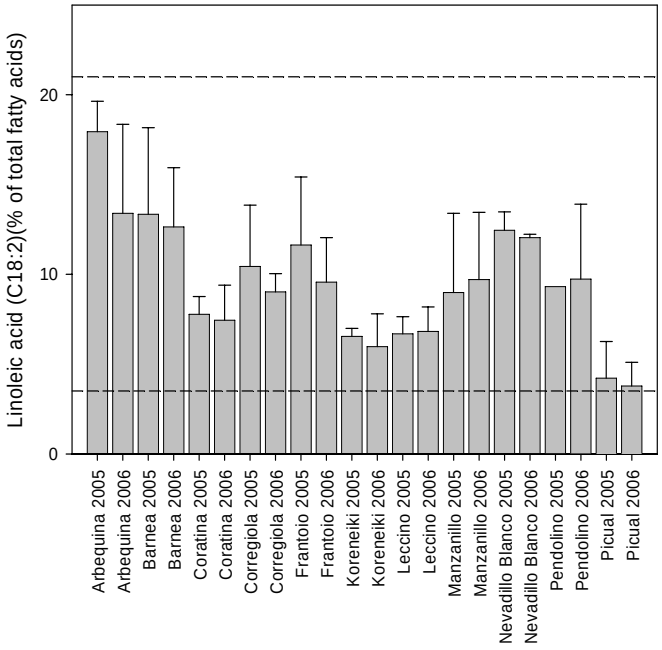


Figure 4.32 Linoleic acid (C18:2) content for eleven cultivars for each year studied. Each bar represents the mean for the cultivar ± standard deviation. COI limit is 3.5 - 21.0% of total fatty acids, as indicated by the dotted lines

4.4.1.3 Linolenic acid

The mean value of all but one cultivar was higher for linolenic acid in 2006 than in 2005 (Figure 4.33). This is in contrast to the increased levels of linoleic acid in 2005. This suggests that there may be some relationship with linoleic and linolenic acid as a result of environmental conditions.

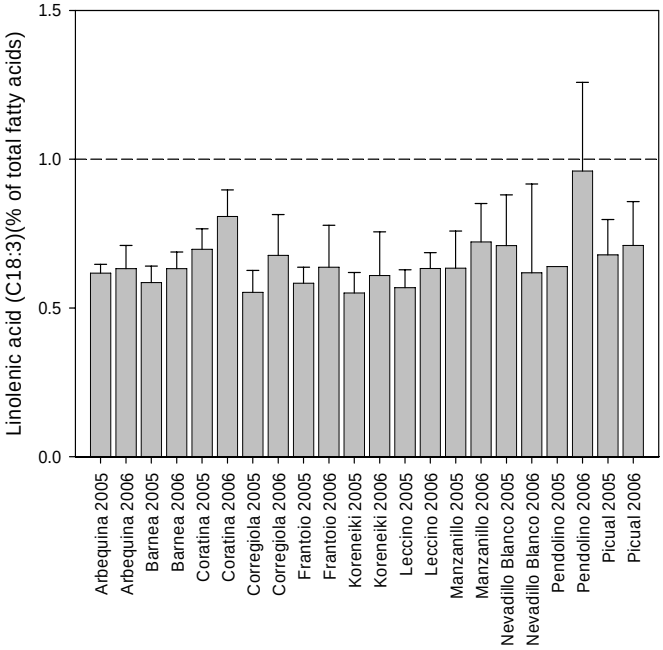


Figure 4.33 Linolenic acid (C18:3) content for eleven cultivars for each year studied. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be less than 1.0% of total fatty acids, as indicated by the dotted line

4.4.2 Sterols

4.4.2.1 Total sterols

Total sterols have been shown to vary between environmentally different sites and therefore might be expected to vary in consecutive years. This appears to be the case (Figure 4.34) in which total sterols was less in 2006 than 2005 in all but three cases. For *cv* Koreneiki the total sterol content failed to meet the standard in some instances in 2006.

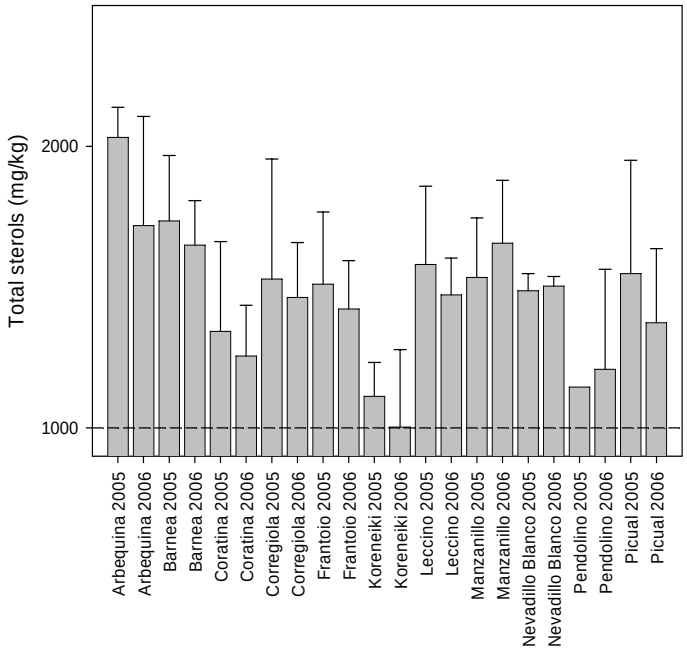


Figure 4.34 Total sterol content for eleven cultivars for each year studied. Each bar represents the mean for the cultivar ± standard deviation. The COI standard is >1000mg/kg

4.4.2.2 Campesterol

There was an increase in campesterol in *cvv* Koreneiki and Pendolino from 2005 to 2006 although there were no differences for the two seasons for campesterol in the other cultivars tested (Figure 4.35).

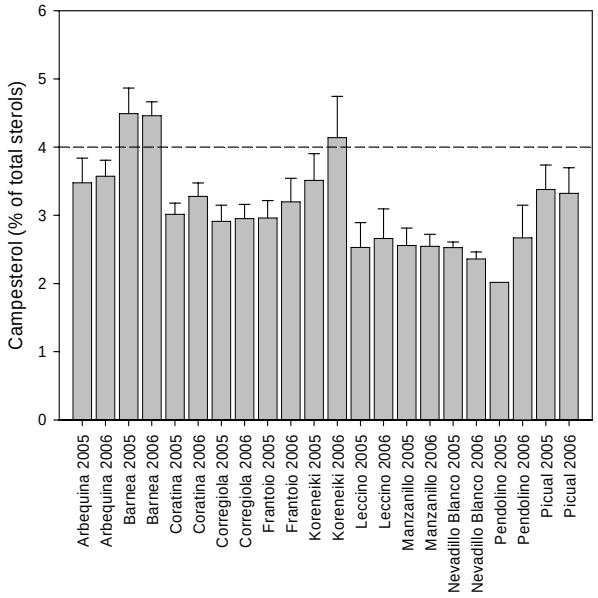


Figure 4.35 Campesterol content for eleven cultivars for each year studied. Each bar represents the mean for the cultivar ± standard deviation. COI standard is <4% of total sterols

4.4.2.3 Stigmasterol

Although some cultivars showed no difference in stigmastadienes over two years, some cultivars, notably Frantoio, Koreneiki, Leccino, Manzanillo and Nevadillo Blanco, were considerably higher in 2005 than in 2006. There is no consistency with this result and the levels of total or other individual sterols between years (Figure 4.36).

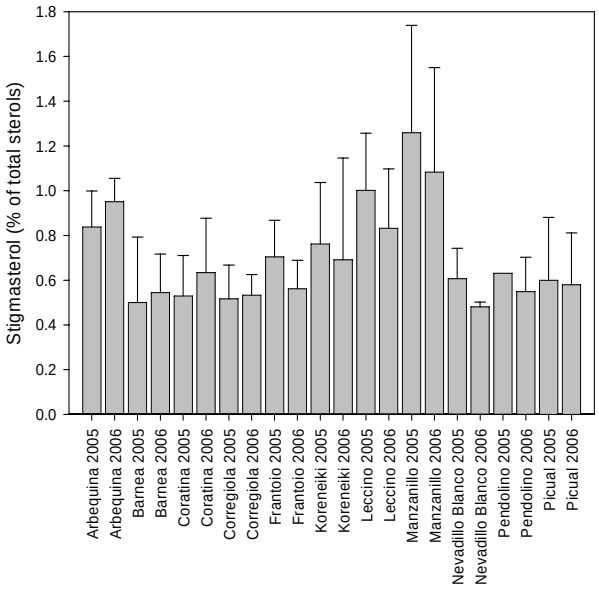


Figure 4.36 Stigmasterol content for eleven cultivars for each year studied. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be below the level of campesterol (as a % of total sterols)

4.4.3 Wax content

There was little influence in seasonal effect on wax with most cultivars being the same for each of the two years and only Arbequina, Leccino and Frantoio being higher in 2005 and Pendolino being higher in 2006 (Figure 4.37).

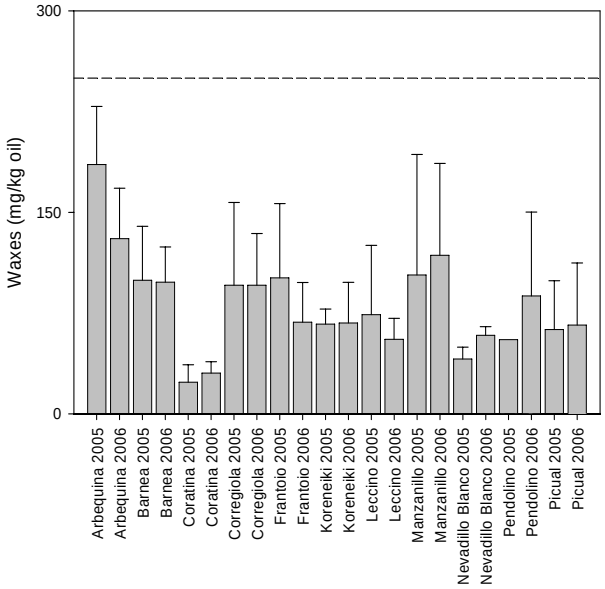


Figure 4.37 Wax content for eleven cultivars for each year studied. Each bar represents the mean for the cultivar ± standard deviation. COI requires the level must be less than 250 mg/kg, as indicated by the dotted line

4.4.4 α-tocopherol

In all but a few cases α-tocopherol was higher in 2006 than it was in 2005. It has been shown that maturity affects the α-tocopherol level although there is no explanation for the difference between years. This may be further investigated with a study of environmental conditions over the two, or more, seasons.

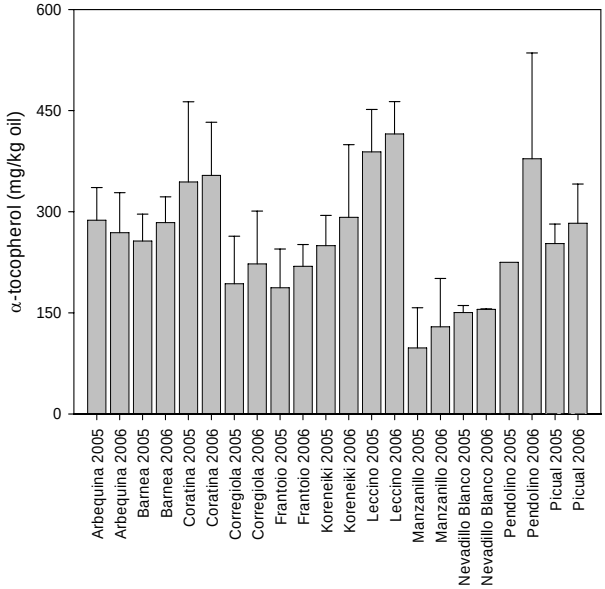


Figure 4.38 α-tocopherol content for eleven cultivars for each year studied. Each bar represents the mean for the cultivar ± standard deviation. There is no COI limit

4.4.5 Trans fatty acids

As for other fruit growing conditions, there were no effects on trans-fatty acids for the two years and data has not been shown.

4.4.6 UV absorbance
The results were similar with UV absorbance as for trans fatty acids with no seasonal difference recorded and therefore the results are not shown.

4.4.7 Stigmastadienes
There were no seasonal differences found between cultivars and therefore the results are not shown.

4.4.8 Triacylcerides (ΔECN 42)
There were no seasonal differences found between cultivars and therefore the results are not shown.

5. Supplementary Tests

5.1. Introduction

The results described in this report indicate the difficulty in identifying genuine olive oil and discriminating it from adulterated oil. COI have developed a wide range of sophisticated methods which come close to determining if the oil is not genuine. However, there are still opportunities to adulterate oil without it being detected. Additionally, and of much importance to Australian growers, as well as growers from many other countries, the limits imposed by international standards of COI, EEC, Codex Alimentarius and others may often determine oil is adulterated when in fact it is genuine extra virgin, high quality olive oil.

Various researchers have worked to develop new methods to overcome this problem. In particular, chemists from the German Fat Society have proposed some new methods which are capable of detecting refined oil and the presence of seed oils. These tests include capillary GC analysis of triacylglycerols which they indicate will differentiate seed oils from olive oil. Additionally, testing for diacylglycerols is possibly a method to detect old oil from fresh olive oil. Also, and perhaps the most useful test proposed is the measure of pyropheophytins, a by-product of chlorophyll, formed when chlorophyll is heated. Refining olive oil generally requires the application of heat and this test has the potential to identify situations in which oil has been heated. The presence of significant amounts of pyropheophytin in oil labelled EVOO would indicate that sample contained refined oil.

This chapter has looked at the measurement of pyropheophytins and diacylglycerols, firstly to evaluate the methods in terms of simplicity for laboratory application in routine testing, and secondly to evaluate the potential of the method to discriminate between fresh EVOO and aged or refined oil.

Additionally, this chapter will discuss the measurement of oleocanthal, a recently identified substance in olive oil which may have health benefits to the consumer. The purpose was to evaluate the method and to determine the level of oleocanthal in some Australian and imported olive oils, both fresh and after long term storage.

5.1.1 Pyropheophytins

5.1.1.1 Introduction.

The method for the "Determination of pyropheophytin A in olive oil" was evaluated as a quick method to distinguish fresh extra virgin olive oil from refined olive oils. Other COI methods such as the analysis of stigmastadienes can also indicate if the oil has been refined.

Chlorophyll constitutes an important component of olive oil. The level of chlorophyll is high in oil from immature fruit and gradually reduces as the fruit matures (Mailer et al. 2005, RIRDC Report). The degree of green colour of olive oil therefore depends largely on the fruit maturity at time of harvest. Chlorophyll is a fragile compound and will degrade rapidly in the presence of light to form pheophytins. With the application of heat, further breakdown of the pheophytins occur to produce pyropheophytins (Figure 5.1). The compound will change from the various forms of green chlorophyll to yellow and brown pheophytins with exposure to light. As refining generally requires the application of heat, particularly for deodorising, the presence of pyropheophytins has been suggested as a method to indicate the presence of refined olive oil in bottles labelled EVOO.

5.1.1.2 Aim

This study investigated the level of chlorophyll by-products in both fresh and aged oil as well as oil which has been heated for various times and at different temperatures. The study looked at the viability of this method for application to routine screening for refined oils.

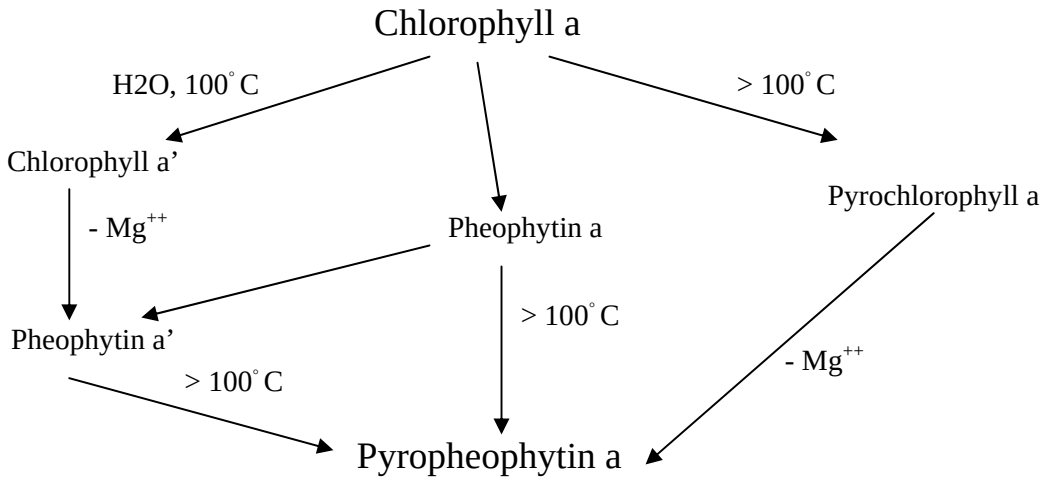


Figure 5.1 Chlorophyll degradation products in olive oil (Aizetmuller 1986)

5.1.1.3 Materials and Methods

Principle of the method: The pigments (pheophytins, pyropheophytin A, chlorophyll a and chlorophyll b) were separated using silica gel columns. The eluate was analysed by HPLC using a RP C18 column and a UV-detector at 410 nm. The concentration of pigments including pyropheophytins was calculated using peak areas.

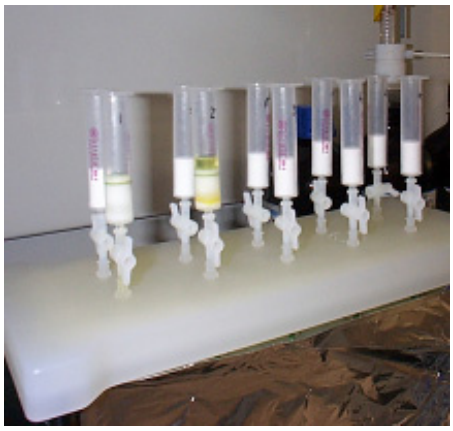
A.  B. 

Figure 5.2 A. Commercial "ready made" columns and; B. "in-house" made columns for separating pigments

Method: Olive oil (300 mg) of each sample was weighed into small beakers. The oil was rinsed twice with 1 mL portions of hexane into silica gel columns. The solvent was drained to just above the top of column packing, then eluted twice with 10 mL petroleum ether / diethyl ether (90:10 v/v). The pheophytin fraction was then eluted with 10 mL acetone and collected in a pear–shaped flask. The acetone was evaporated to approximately 2 mL on a rotary evaporator at 20°C. The solution was analysed immediately by HPLC as pheophytins are very unstable at light. The mobile phase was water/methanol/acetone (4:36:60). Column flow was isocratic (1 mL/min).

Figure 5.3 Components for "in-house" made columns for separating pigments

The use of ready made columns, though readily available, was quite expensive ($359/30 columns). In-house columns were made at a much cheaper cost (approximately $359 for 1000 columns). They were made using a 25 ml pipette tip, silica gel 60 at 5% moisture and non-absorbent cotton wool. A small wad of cottonwool was used to block the opening, a 1 g layer of silica gel was added and a final wad of cottonwool covered the gel (gently tapped down).

The amount of pyropheophytin A in area % (W) is calculated using the formula:

$$W = Apppa * 100 / (A_{ppp\,A} + A_{pp\,A}' + A_{pp\,A})$$

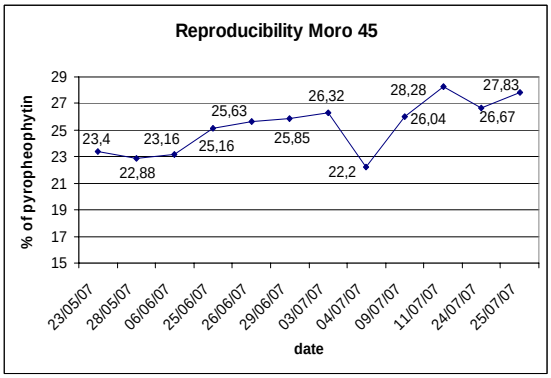
Where:

A_{pppA} is the peak area of pyropeophytin A
$A_{pp\,A}$ is the peak area of pheophytin A
$A_{ppA'}$ is the peak area of pheophytin A'

5.1.1.4 Results

Reproducibility:
The reproducibility limit (R) is equivalent to the difference between the maxima and minima value of pyropheophytin A in the sample. The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, should not be more than 5 %.
To test reproducibility of this method, a standard sample of commercial virgin olive oil was analysed (Figure 5.4(a)). This oil was used as a standard during this study.
The reproducibility was within 6.08 % (28.28%-22.20%), however, except for four values, 22.8%, 22.2%, 28.28% and 27.83%, the remaining values were relatively constant. These four values are due to the difficulty to keep chlorophyll A in good condition during analysis. Even with many precautions such as working in a dark room, covering the silica columns with aluminium foil and using the same HPLC, the results obtained for reproducibility were slightly high.

(a)



(b)

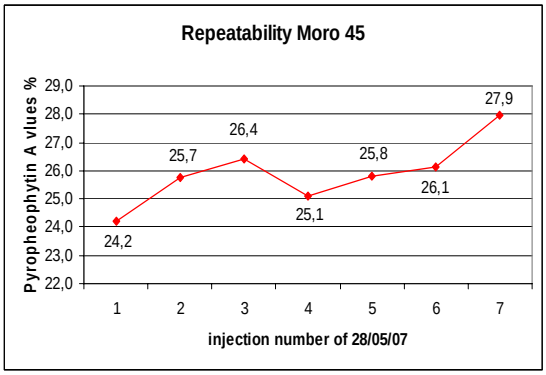


Figure 5.4 (a) reproducibility and (b) repeatability of olive oil standard

Repeatability*:*
The repeatability limit (r) is the value less than or equal to the absolute difference of two test results which can be expected with a probability of 95%, under repeatable conditions. Repeatability conditions are defined as conditions under which test results are obtained with the same method, on identical test material, in the same laboratory, by the same operator, using the same equipment and reagents, within a short interval of time. Repeatability is calculated from the difference between the maxima value and the minima value for the sample analysed on the same day with 7 injections (Figure 5.4(b)). The repeatability for this study was 3.7%. The protocol specifies that this value must not exceed 5%. Unlike the reproducibility, it was easier to obtain a good repeatability as the samples were prepared on the same day therefore chlorophyll A was less likely to be exposed to the same oxidation conditions, notably the light.

Comparison of extra virgin and refined olive oil:
To illustrate the difference between the pigment content in various olive oils, a sample of fresh olive oil was compared to a sample of refined (or pure) oil. As shown in Figure 5.5(a), the extra virgin olive oil contained only pheophytin A and pheophytin A'. The refined olive oil however had considerably less pheophytin but also some pyropheophytin (Figure 5.5(b)).

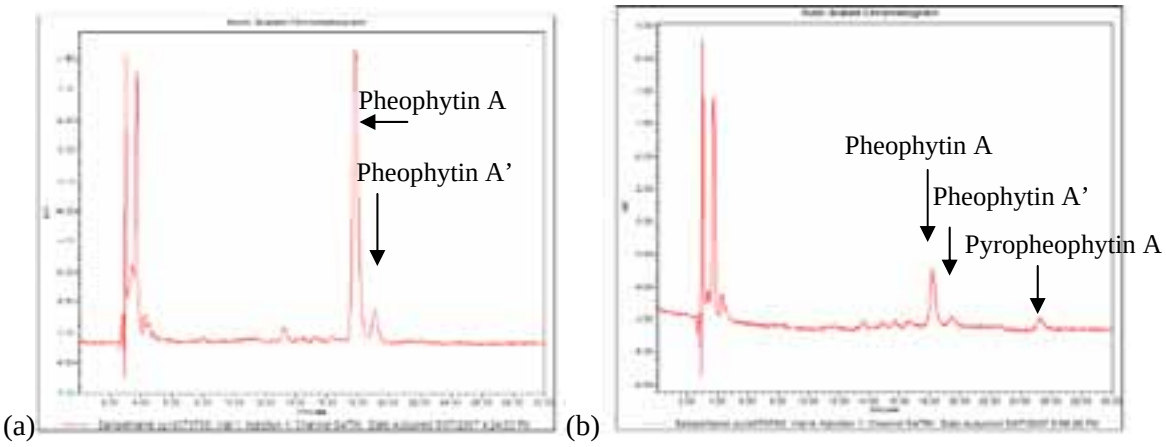


Figure 5.5. Pigment content of (a) extra virgin olive oil and (b) refined or "pure" olive oil

Comparison of industry and laboratory extraction:
To determine the influence of the extraction procedure, oil which had been extracted in an industrial press was compared to oil from a laboratory extraction (Figure 5.6(a)). In this case, Paragon oil from the industrial extraction showed no chlorophyll A peak, pheophytin B or B' peaks. Pheophytin A and pheophytin A' peak were apparent but there is no pyropheophytin peak. In the laboratory extraction, chlorophyll A was apparent at 9 minutes. Pheophytin A and A' were present although pyropheophytin peak was not present.
This may suggest that the oil from the industrial extraction had a greater exposure to light than the small sample processed under laboratory conditions.

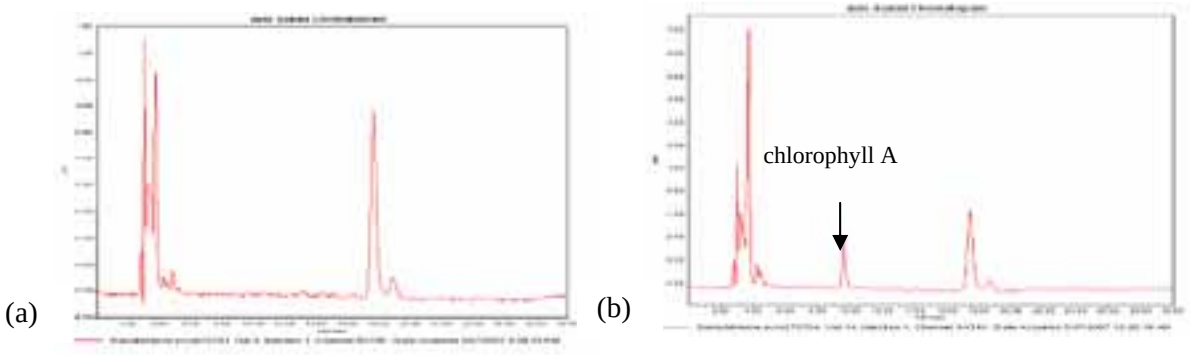


Figure 5.6. Pigment content of olive oil (cv Paragon) (a) extracted using an industrial press and (b) extracted using a laboratory press

Analysis of oils from *cv* Corregiolla showed similar results.

Old olive oil:
To determine the effect ageing has on the pigment content of olive oil, samples which had been stored in the laboratory for more than two years were analysed and compared to results with fresh olive oil. The results are shown in Figure 5.7.
Chlorophyll was not present, indicating that it had been degraded. Small peaks for pheophytin B, B', A and A' were clearly present. However, there was an abundant amount of pyropheophytin in this EVOO despite it not having been exposed to any elevated temperature.

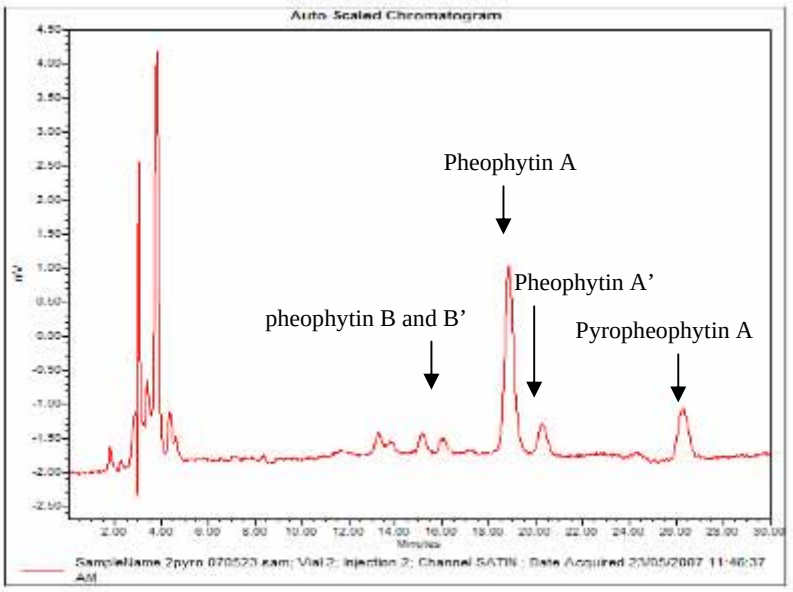


Figure 5.7 Pigment content of a sample of extra virgin olive oil stored for two years (05-203.6)

Repeated analysis of old samples, greater than two years showed the consistent presence of pyropheophytin. One sample was analysed several times and the relative amount of pyropheophytin content calculated (Fig.5.8). The results show good reproducibility in the sample with an average of 42% pyropheophytin (of total pheophytins).

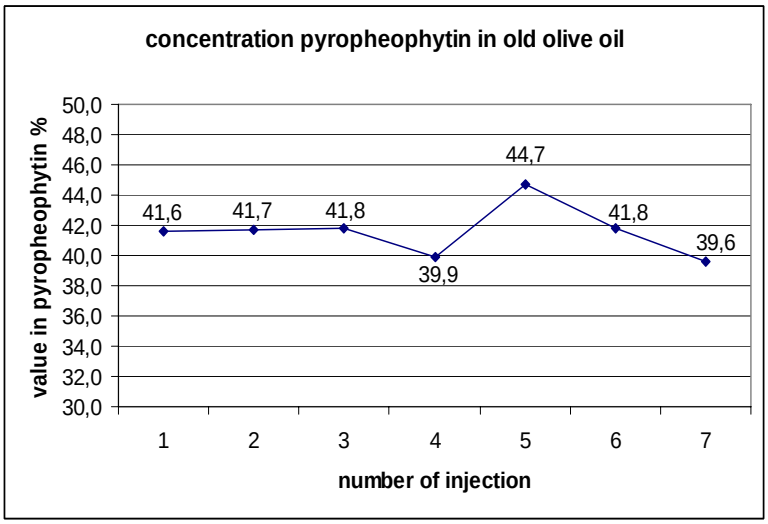


Figure 5.8 Pyropheophytin content of an old olive oil sample (05-203-14).

Supermarket samples:
To determine the pigment composition in commercial olive oil samples, 8 olive oils were purchased from local supermarkets from different origins including Spain, Australia and Italy. The samples are listed in Table 5.1.

Table 5.1 Samples of olive oil obtained from the supermarket

Sample	Source	Description
1	Italy	Olive oil 100% pure *refined*
2	Spain	Extra virgin cold pressed
3	Spain	Olive oil pure *refined*
4	Spain	Olive oil *refined*
5	Italy	Extra virgin olive oil
6	Australia	Extra virgin olive oil
7	Australia	Cold pressed extra virgin olive oil
8	Italy	Extra virgin olive oil

Supermarket sample #1, refined olive oil, had virtually no peaks with only small traces of pheophytin, pheophytin A and pyropheophytin chromatograph.

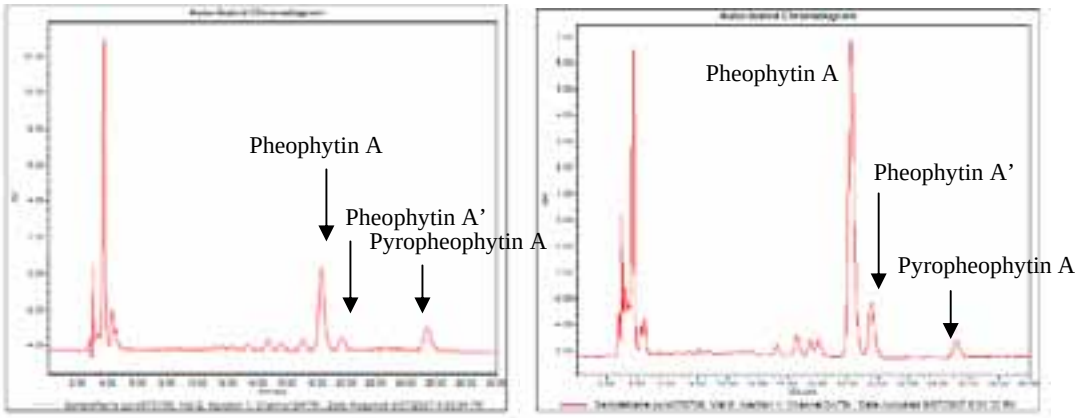


Figure 5.9 Supermarket sample #2 & #8, both extra virgin olive oils

Supermarket sample #2 had low levels of pheophytin, pheophytin A and pyropheophytin. Although #8 also had a trace of pyropheophytin, the proportion of the total pheophytin in #2 was very large and similar to refined oil. (Fig. 5.9).

Figure 5.10 shows a significant difference in pyropheophytin values between the different types of olive oil. The concentration in pyropheophytin in refined or pure olive oil samples (1, 3 and 4) was between 28.5 % and 19.8 % and this was expected. Pyropheophytin in samples 5, 6, 7 and 8, extra virgin olive oils were between 5.4 and 14.6%. However, the result of the sample number 2 was surprising as it is labelled EVOO and contained 22.8% pyropheophytin. This concentration is high and corresponds to refined olive oil. There may be several reasons for this: it may be old olive oil but there was no date on the bottle, the storage may have been poor (exposure to light or the sample too hot).

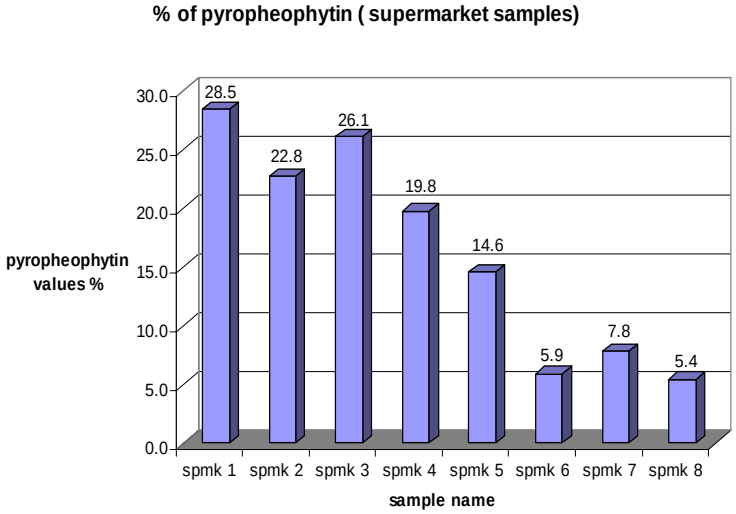
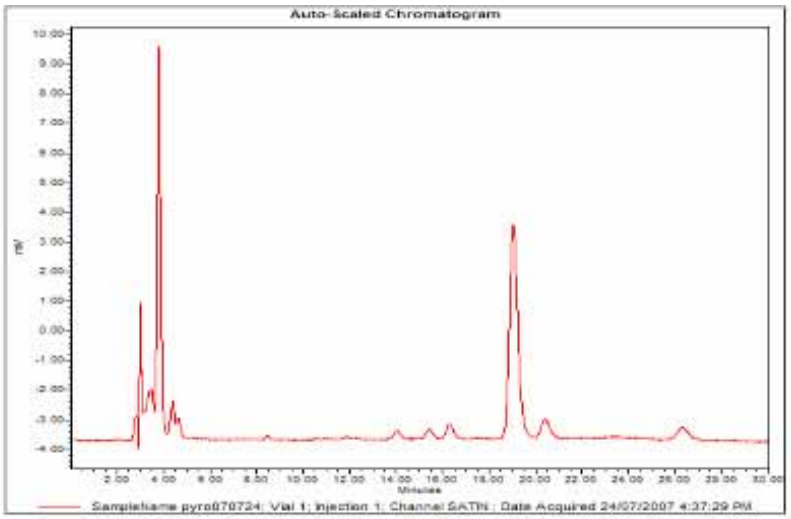


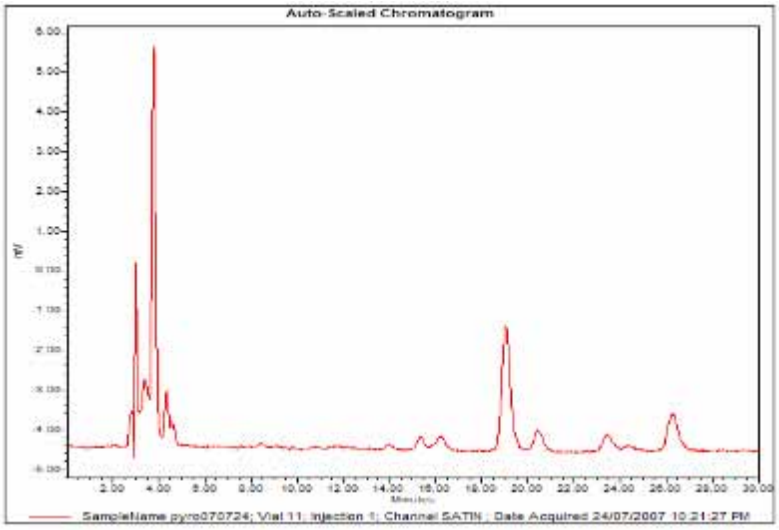
Figure 5.10 Pyropheophytin concentration in supermarket samples
1, 3 and 4 are refined oils. 2, 5-8 are labelled extra virgin.

Effect of Temperature:
The effect of heating of olive oil on pigment content was also studied. Three oil samples were heated at different temperatures and at different times to determine if pyropheophytin increased with heat. The samples were heated for 15, 30 and 60 minutes at temperatures of 80, 120, 160° C. The samples were a commercially available supermarket oil #6 (Australian) and two fresh extra virgin olive oils.

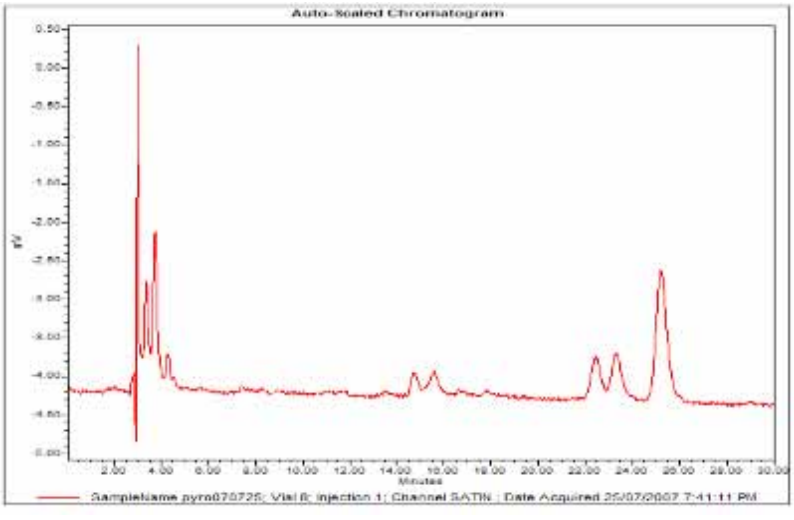
The sample heated at 80°C for 15 minutes contained mostly pheophytin and small amounts of pyropheophytin and was therefore unaffected at that temperature (Figure 5.11(a)). However pheophytin almost disappeared from the sample heated at 160◦C for 1 hour and the pyropheophytin A peak increased (Figure 5.11(c)). The oil changed colour at different stages of heating from yellow green to pale yellow (Figure 5.12 a-d). The lack of chlorophyll A in Figure 5.11 is confirmed by the yellow appearance of the oil before heating.



(a) **80◦C 15 min**



(b) **120◦C 30 min**



(c) **160°C 60min**

Figure 5.11 Supermarket sample #6 heated at different temperatures (a) 80°C for 15 minutes (b) 120°C for 30 minutes and (c) 160° C for 60 minutes

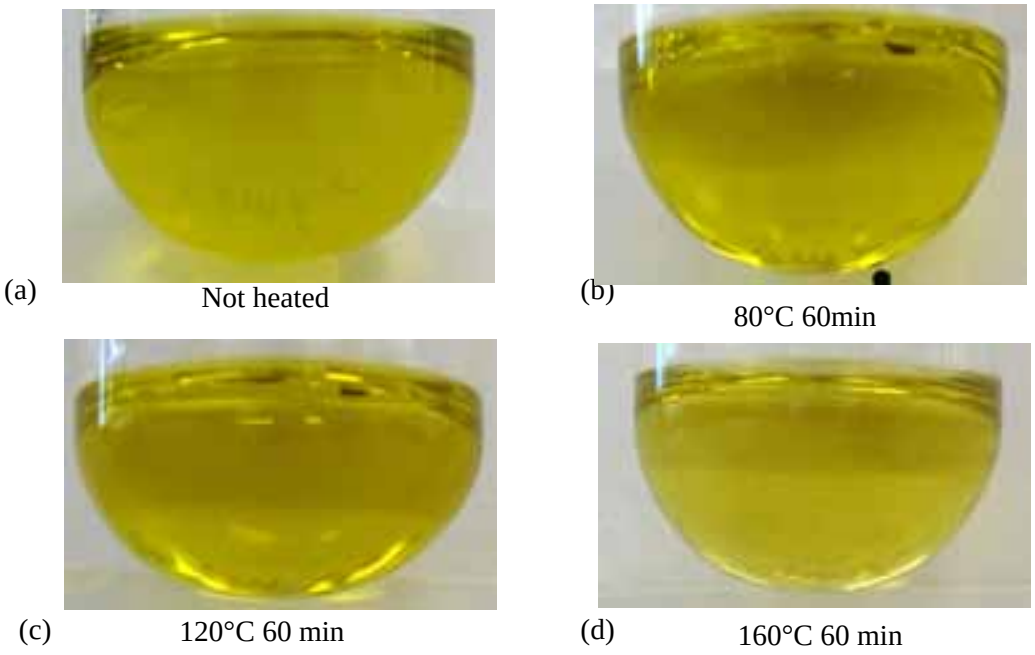


Figure 5.12. Supermarket oil #6 after heating at different temperatures

Figure 5.13 illustrates the increase in pyropheophytin concentrations when the sample was heated. The pyropheophytin concentration in the oil increased from 5.93 % to 95.5% when heated at 160° C for 60 minutes, 16 times higher than that of the original sample.

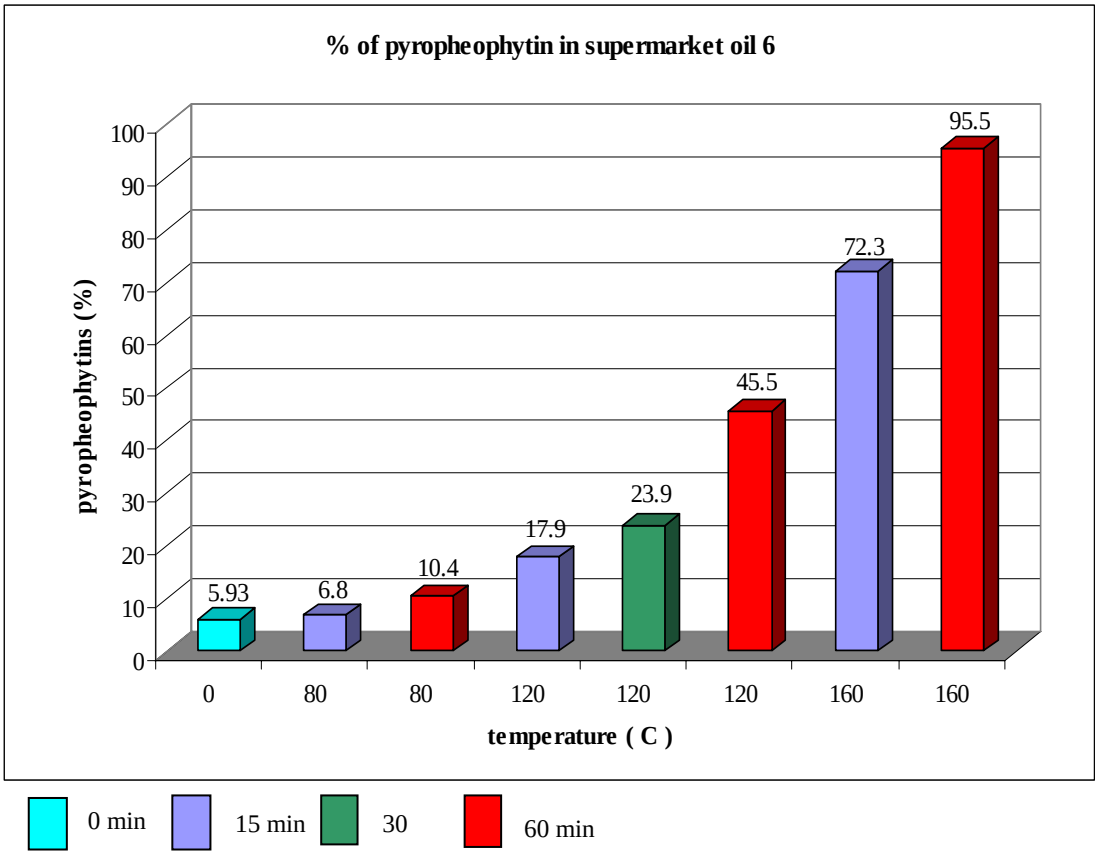
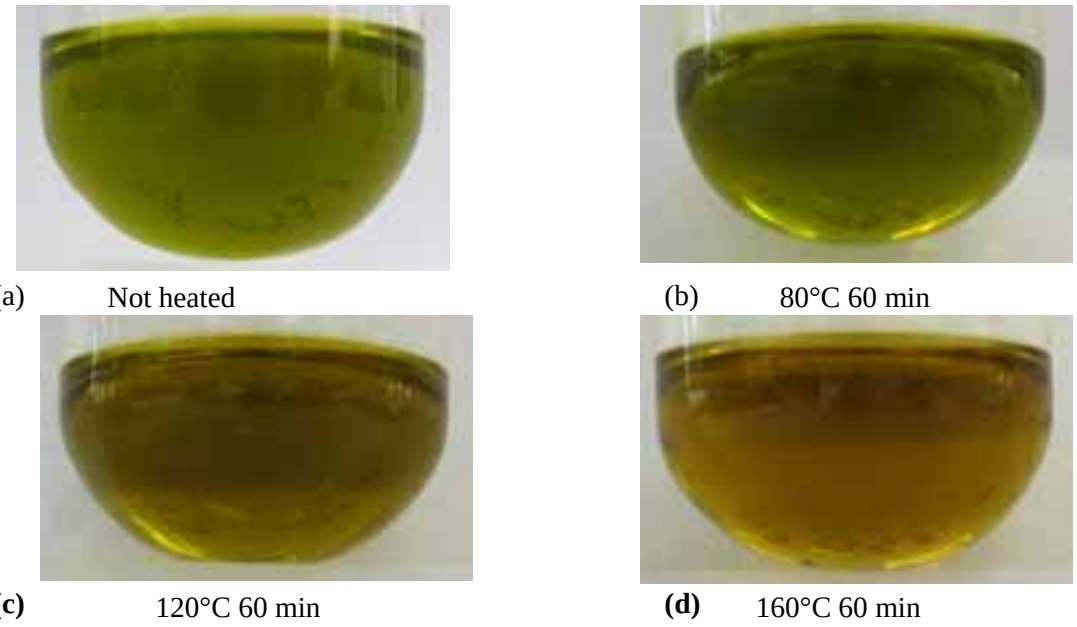


Figure 5.13 Pyropheophytin concentration in supermarket sample after heating

The process was repeated using a fresh, EVOO which contained adequate chlorophyll A to give it a rich green colour. The oil was exposed to the same heating conditions:



(a) Not heated (b) 80°C 60 min

(c) 120°C 60 min **(d)** 160°C 60 min

Figure 5.14 Fresh EVOO (07 043.2) after heating at different temperatures

Figure 5.14 shows that the colour of the oil changed dramatically from dark green to brown yellow. However, it can be seen that the olive oil retained the green colour up to 80°C. At 120°C for 15 min, the olive oil begin to change colour, becoming yellow green but only after heating at 120°C for 30 min did the change become significant, to brown green

The increase in pyropheophytins is shown in Figure 5.15. Pyropheophytin concentration increased with time and temperature although heating at 80°C the heating did not affect the pyropheophytin concentration. The pyropheophytin concentration was extremely high at 160°C regardless of heating time, 95.8% when heated for 15 minutes, 98.8% when heated for 60 minutes.

5.1.1.5 Discussion

This study compared the pigment components in fresh olive oil and refined olive oil. The changes in old oil were compared to recently extracted oil. The effect of heating for different times and temperatures to determine the degree of heat required to influence the pigment ratios was also studied.

The results of this study have shown that pyropheophytin is not present in new, fresh extra virgin olive oil. Experiments with heating oils and comparison of fresh oil with old or refined oils indicate that heating and storage can both contribute to an increase in the amount of pyropheophytin.

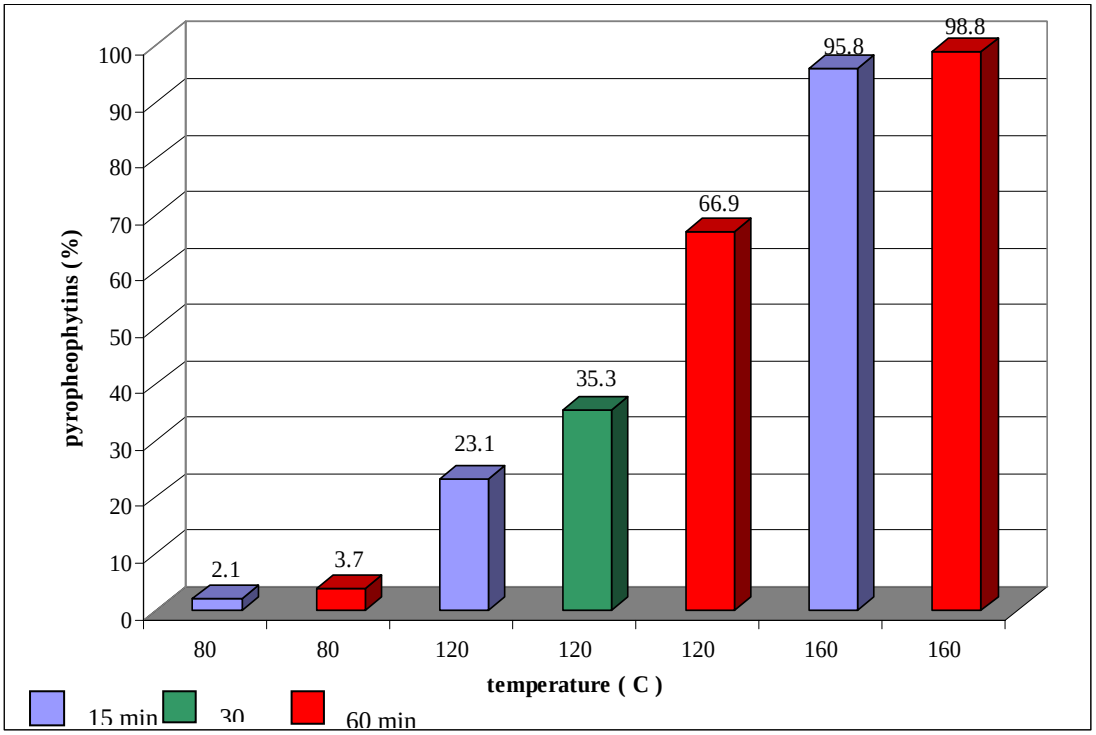


Figure 5.15 Pyropheophytin concentration in fresh olive oil sample after heating

5.1.1.6 Conclusion

This test has been shown to be useful in determining adulteration of EVOO with thermally treated virgin olive oil. Although EVOO can develop pyropheophytin over time, the effect of refining has a much greater effect on conversion of pigments, particularly the high temperatures employed for bleaching. This study found 160°C, even for short periods, converted almost all of the pigment to pyropheophytin. As such, it may be a useful test to determine the presence of refined, bleached and deodorised oil. However, this test is not capable of differentiating an extra virgin olive oil from one that has undergone a thermal treatment with a temperature less than 80°C.

In the case of one supermarket sample (sample 2), the result obtained was surprising as the pyropheophytin level was relatively high. Further testing was undertaken to determine if the oil was genuine. The sample was analysed for UV absorbance, chlorophyll, free fatty acids and fatty acid profile. The results obtained indicated that the UV absorbance was high although all other tests were acceptable.

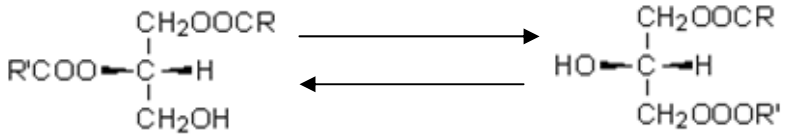
The protocol for testing is not difficult. It is important to be careful with light because chlorophyll A is very sensitive. It is necessary to ensure the room is dark when the analysis is done. The silica gel columns need to be covered with aluminium foil to protect the solution from the light. It is important also to cover the water bath during solvent evaporation.

5.1.2 Diacylglycerols

5.1.2.1 Introduction.

Diacylglycerols are products of oil formed from triacylglycerols following the cleavage of a fatty acid from the molecule. Cleavage of fatty acids occurs under various conditions, mostly commonly as a result of lipase enzyme reaction on the ester bond of the triacylglycerol.

Good manufacturing practice of virgin olive oils results in a content of more than 85% of 1,2-diacylglycerols, as a percentage of total diacylglycerols, in freshly processed virgin olive oils (DGF, 2006). Processing of rotten and fermented olives may result in a content of 40% or less of 1,2-diacyglycerols, even if freshly processed (DGF 2006). Very often, such olive oils have sensory defects. Thermal treatment of virgin olive oils is considered not to influence the content of 1,2-diglycerols. This test is therefore considered useful to determine whether olives have been processed with good manufacturing practice and retain the sensory attributes of freshly prepared virgin olive oils. A reduction of 1,2-diacylglycerols is reported to occur in storage (Gertz and Fiebig 2005) and therefore levels of less than 45% may be considered to be an indicator of low grade olive oil labelled as EVOO. 1,2-diacylglycerols are transformed to the more stable 1,3-isomers during storage or due to acidic catalysation:



1,2 diacyglycerol 1,3 diacyglycerol

Figure 5.16 Isomers of 1,2-diacylglycerols and 1,3-diacylglycerols

5.1.2.2 Aim:

The levels of diacylglycerols in a range of fresh and aged oils were studied to determine the usefulness of this method for screening EVOO for the presence of old and defective oil.
This study describes the determination of the degree of isomerisation of diacylglycerols and the application of this method to be used as quality criterion for high quality olive oil.

5.1.2.3 Materials and Methods

Samples of oil: The same samples as used for pyropheophytin analysis were utilised for this study. Also, additional samples obtained from supermarkets were analysed.
Determination of 1,2- and 1,3- Diacylglycerols in Olive Oil: Miniaturized silica gel column chromatography was used to separate the isomeric diacylglycerols from the more polar fraction of the other lipids. The ratios of 1,2- and 1,3-isomers were determined by gas-chromatography after silylation of the sample.
Preparation of the silica gel chromatography column: a small stopper of cotton wool (approx. 5 mm high) was inserted into the lower part of a 5-ml-pipette tip. Following this 1 g of silica gel 60 was added. The silica layer was covered with a 5 mm plug of cotton wool. The filling was compressed with slight pressure using a flat ended glass rod.
Separation of the fraction containing non polar lipid: The test sample was weighed (about 100.0 mg) to the nearest 0.1 mg into a 10-ml beaker and toluene (1mL) added. The sample was transferred onto the column and the remainder of the sample was washed from the beaker with 1 mL isooctane/diisopropyl ether (85:15). The column was washed with two 3.5 mL portions of the isooctane / di-isopropylether mixture. The end of the pipette tip was rinsed with eluent and the solvent

discarded. Diacylglycerols were eluted with two 3.5 ml portions of diethyl ether and collected in a 10 ml flask.
The solvent was evaporated almost to dryness (1 mL) with a rotary evaporator at 20°C and the remaining solution transferred into a reaction vial. The solvent was evaporated from the reaction vial with a stream of nitrogen.
Preparation of trimethylsilyl ethers (silylation): Silylation reagent (200 µl) was added to the reaction vial containing the diacylglycerols, sealed and the mixture allowed to react for 20 minutes at room temperature. After silylation, acetone (1 ml) was added, the sample mixed and transferred to a GC vial. The solution was injected (1-3 µL) into the gas chromatograph. The working conditions for the GC were: injector temperature 340°C, detector temperature 340°C, split ratio 1:50, carrier gas – helium. Oven programming temperature was: initial 240°C for 1 minute rising at 10°C/minute to 320°C and then held for 10min.

To calculate the area % of 1,2-diacylglycerols (W2) in reference to the sum of the areas of the individual 1,2- and 1,3-diacylglycerols (C_{32}, C_{34}, C_{36}):

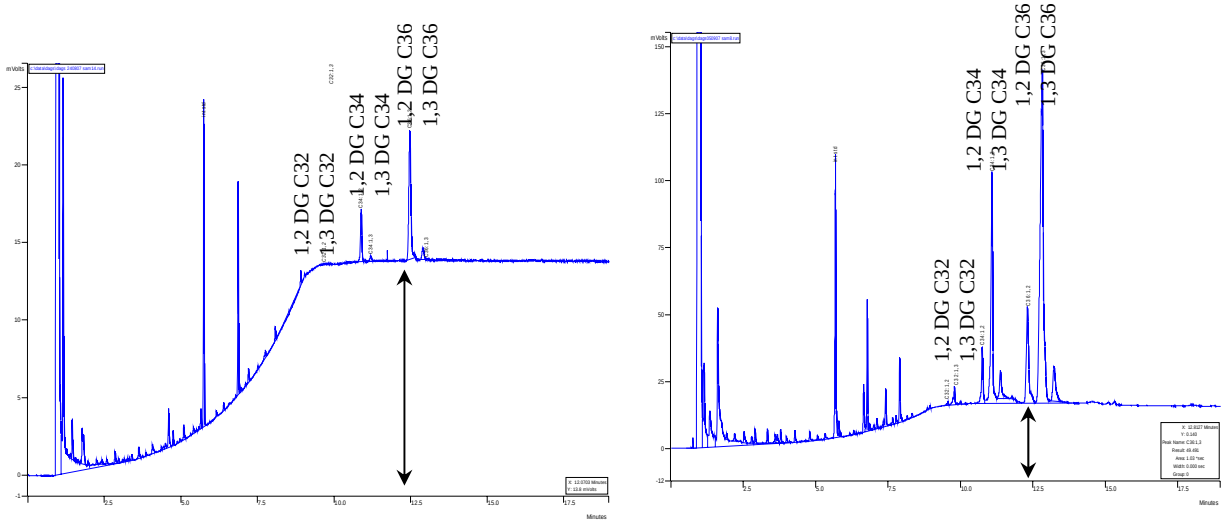
$$W2 = A_{1,2} * 100 / Ax$$

Where:

Ax is the sum of the peak areas of the individual 1,2 and 1,3-diacyglycerols (C_{34},C_{36})
$A_{1,2}$ is the peak areas of all 1,2 diacylglycerols (C_{32}, C_{34}, C_{36}) present in the test sample.

5.1.2.4 Results

To compare the difference between a good quality olive oil and an old olive oil, samples were prepared as described and analysed by gas chromatography. Figure 5.17 illustrates clearly the dramatically different proportions of 1,2 and 1,3 diacylglycerol (1,2-diacylglycerol illustrated by the arrow) in EVOO (a) and old, refined oil (b). The figures are not the same scale as can be seen by the rising baseline in the EVOO compared to that in the old, refined oil.



a. Extra virgin olive oil *b. Old or Refined olive oil*

Figure 5.17 The difference in ratio of 1,2 and 1,3 DAGs between (a) EVOO and (b)an old/refined olive oil

Reproducibility and repeatability of the method: To determine the robustness of the test, reproducibility and repeatability was determined by testing the same sample of commercial oil several times. The results (Figure 5.19) illustrate that this oil has only 32% 1,2-DAGs as a ratio of total DAGs and is therefore likely to be old oil or one of poor quality.

*Reproducibility***:** [R]The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, should not be more than 5 %.
Figure 5.19 shows the reproducibility of the DAGs method. The range of 1,2 DAGs obtained was 8.5 - 49.6%. The difference between the maxima and minima is to 41% which is exceeds the 5% limit. However, only two outliers cause this difference and with the removal of outliers the difference is only 3.2%.

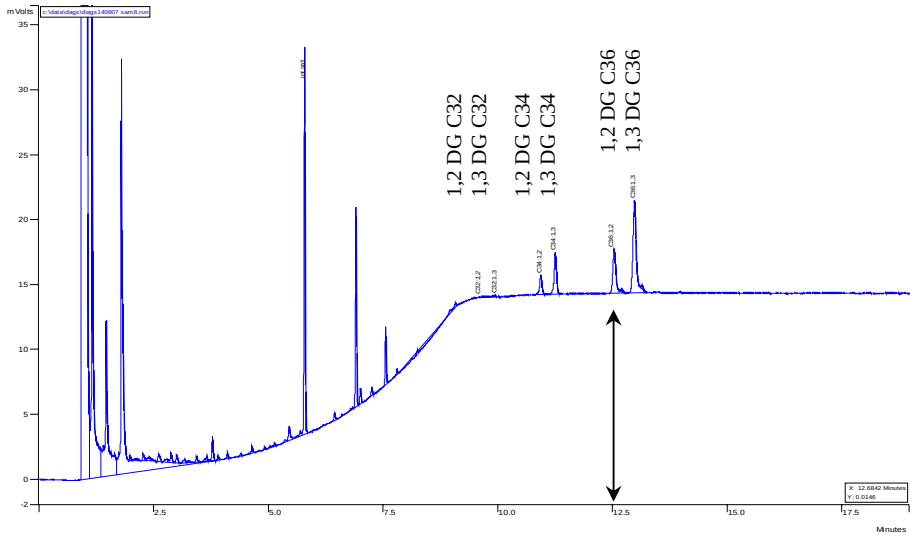


Figure 5.18 Chromatogram of commercial sample used to determine reproducibility and repeatability of the DAGS method

Repeatability: [r] The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 %.
Figure 5.19 illustrates that the difference between the maxima and minima is 1.3% and easily within the 5% limit. This is expected as it is easier to achieve better repeatability than reproducibility with the fragile nature of olive oil and sensitivity to oxidation.

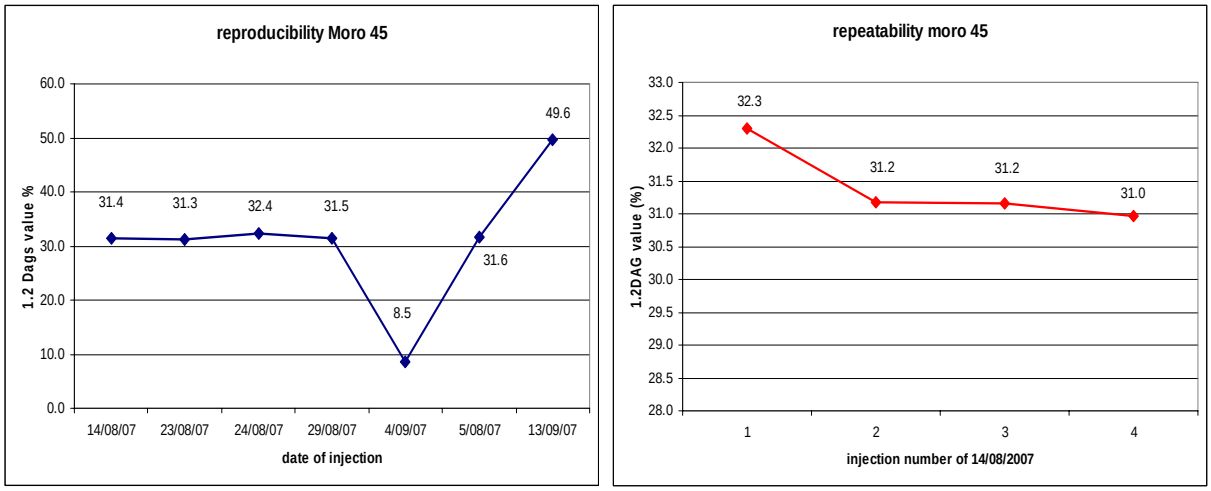


Figure 5.19 Reproducibility [R] and repeatability [r] of the DAGS method *Fresh EVOO*

To determine if the method was suitable to distinguish fresh, EVOO, several samples of recently extracted oils from various cultivars were analysed. The chromatogram of one of the oils is shown in Figure 5.20. The proportion of 1,2-diacylglycerol C36 is clearly higher than the 1,3-DAG peak indicating that the oil is good quality. Twenty one samples were analysed representing four cultivars, three replicates of each (Table 5.2a).

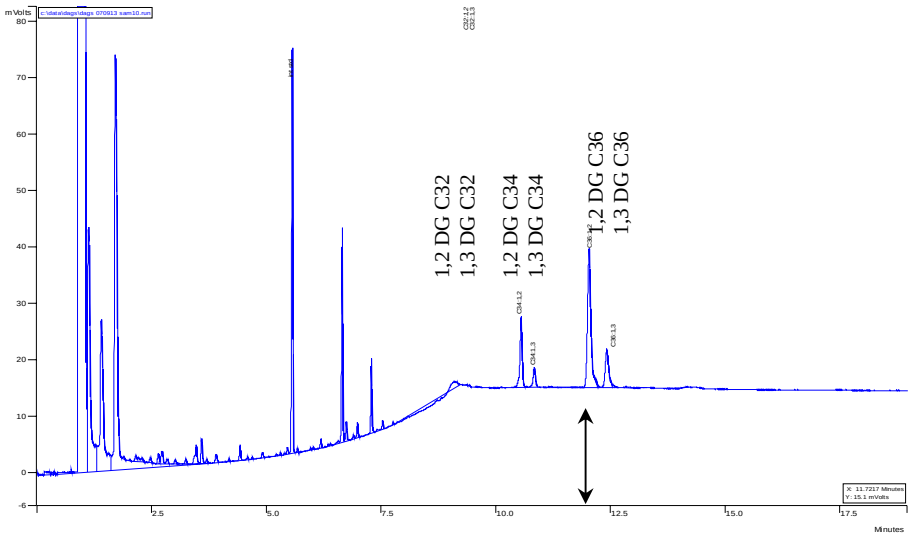


Figure 5.20 Chromatogram of freshly extracted EVOO, cv Corregiola 07-230/6, showing the 1,2 isomers are in higher concentration than the 1,3 isomers

Table 5.2a shows 1, 2-DAG concentration of the fresh samples. The literature indicates 1.2 DAGS % concentration for fresh virgin olive should exceed 60% (Gertz and Fiebig 2005). The results ranged from 59.1 – 95.6% indicating they were within or close to the recommended level for 1,2-DAG concentration.

Effect of irrigation treatment on DAG ratio in freshly harvested olive oil:
The samples analysed to represent fresh EVOO were the same samples used previously to test for the pyropheophytin method. These samples were harvested and processed at Rich Glen orchard immediately before analysis. The samples contained three irrigation treatments: deficit irrigation, partial irrigation and full irrigation.

Although there is insufficient data in this preliminary evaluation of the method, it appears, in the case of *cv* Paragon, that the deficit irrigation actually had a higher level of 1,2-DAGs than the olives under full irrigation. Despite this being apparent in the mean values of each of the three replications for each sample, the standard deviation within each set was large. This phenomenon requires further testing.

Supermarket samples:
Several supermarket samples were tested from different countries including Spain, Australia and Italy as for the pyropheophytin evaluation. Results from the initial samples were variable and inconsistent. Sample 6, an Australian oil, was 74.6% 1,2 DAG, as illustrated in Figure 5.21. Further testing of a new set of supermarket olive oils was more consistent. The results are shown in Table 5.2b. In this set of six samples selected from supermarkets in 2008, from Spain, Turkey and Italy, all six samples failed to meet the 60% level. Other IOC test utilised to determine adulteration failed to indicate any problems with three of the six oils tested. This puts the IOC tests in conflict with DGF results.

Table 5.2a Samples of freshly extracted EVOO tested for 1,2-DAGs in 2007

Name	1.2-DAGs %	Average	Cutlivar	Rep.	Irrigation
07 230 / 1	83.7		Paragon	A1	Deficit
07 230 / 2	95.6		Paragon	A2	Deficit
07 230 / 4	82.2	87.2	Paragon	A3	Deficit
07230 / 7	80.7		Paragon	C1	Deficit
07 230 / 8	81.5		Paragon	C2	Deficit
07 230 / 10	85.3	82.5	Paragon	C3	Deficit
07 230 / 12	81.4		Paragon	G1	Partial
07 230 / 15	83.1		Paragon	G2	Partial
07 230 / 17	82.9	82.5	Paragon	G3	Partial
07 230 / 9	59.1		Paragon	D1	Full
07 230 / 13	72.6		Paragon	D2	Full
07 230 / 14	73.6	68.4	Paragon	D3	Full
07 230 / 11	91.2		Nevadillo Blanco	F1	Deficit
07 230 / 16	75.7		Nevadillo Blanco	F2	Deficit
07 230 / 18	80.6	82.5	Nevadillo Blanco	F3	Deficit
07 230 / 3	76.1		Corregiola	B1	Full
07 230 / 5	86.5		Corregiola	B2	Full
07230 / 6	77.3	80.0	Corregiola	B3	Full
07 230 / 19	76.1		Manzanillo	E1	Deficit
07 230 / 20	72.9		Manzanillo	E2	Deficit
07 230 / 21	58.8	69.3	Manzanillo	E3	Deficit

Table 5.2b Samples of selected supermarket EVOO tested for 1,2-DAGs in 2008

Sample	Country of origin	1,2-DAGs%
08299/1	Spain	40.7
08299/2	Spain	38.3
08299/3	Turkey	29.6
08299/4	Italy	30.1
08299/5	Italy	30.3
08299/6	Spain	31.9

Effect of heating on olive oil 1,2 DAG ratio:

The impact of heating olive oil was evaluated by heating three olive oil samples at different temperatures and for different times (as for pyropheophytin method). This manipulation was necessary to determine if the 1, 2 DAG ratio in olive oil is influenced by thermal treatment and if it can be detected by this method. The oils were heated for 15, 30 and 60 minutes at 80, 120, 160° C. The three samples were: supermarket sample #6 (EVOO, Table 5.1) and two fresh olive samples, one extracted in the laboratory and one produced at Rich Glen (07 043/2). The results are shown in Figure 5.21.

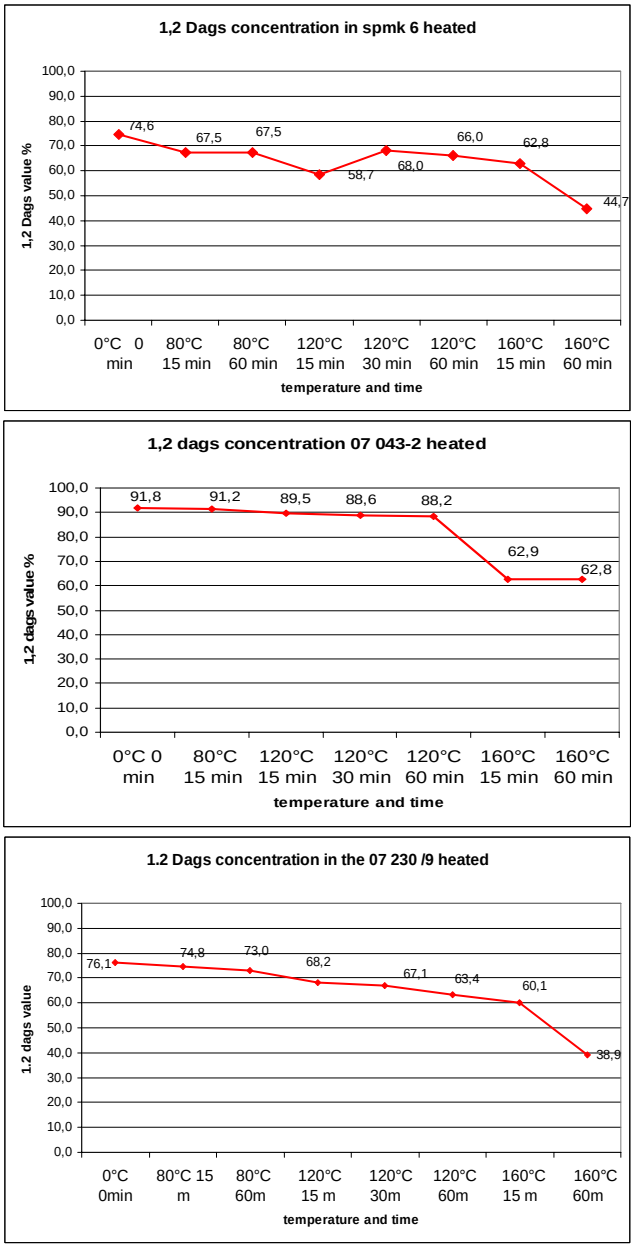


Figure 5.21 Effect of temperature in 1, 2-DAG ratio in three supermarket olive oils

From Figure 5.21 there are some interesting results.

- Temperature effect: There appears to be a clear effect of thermal treatment on 1, 2 DAGs ratio for all oils above 160°C with supermarket oil dropping from 74.6 to 44.7%. The fresh olive oil extracted in the laboratory (07 043-2) decreased from 91.8 to 62.8 %. The industry extracted fresh oil (07 230-9) dropped from 74.8 to 38.9%.
- DAGs ratios do not decrease significantly until the temperature exceeds 120°C.
- This test is probably not capable of detecting thermal treatment below 120°C.
- Heating time may be important with two samples not showing significant reductions in 1,2 DAGs at 160°C until after 60 minutes at 160°C.

5.1.2.5 Conclusion & Discussion

The method of measuring 1, 2-DAGs appears capable of discriminating between fresh olive oil and old oil. Measurement of DAGs in fresh olive oil showed in almost all cases that the level was greater than 70%. In only two samples was it less than 70% and this was due to poor reproducibility in one of the triplicate analyses done for each of the two samples. Overall, freshly extracted oils were consistently greater than 70% 1, 2-DAGs.

Changes in 1,2-DAGs were found when samples were heated above 160°C for longer than 60 minutes. This had not been shown before. However, it seems unlikely that olive oil heated below 120°C could be detected.

An advantage of this method is that only a small amount of sample is required to determine the 1, 2- and 1, 3-DAG ratios. However, reagents are very expensive. For example N-methyl-N-(trimethyl-silyl)-hepta-fluorobutyramide (MSHFBA) cost $255 for 5 mL and the Methyl imidazole cost $225 for 500 g.

For the analysis, 200 µL of silylation reagent are required (silylation reagent = add 50 µl methyl imidazole in 1 ml of MSHFBA), but only 1µL of final solution is injected in the GC. In addition, it is difficult to obtain the reagent with delivery of several weeks to months into Australia.

The silica gel with 5% moisture must be freshly prepared. Preparation of silica gel requires addition of moisture and placing the gel in the shaker overnight. It's important to not forget this step and it requires good organisation.

The solvent evaporation time at 20°C is a problem. When this study began the time to evaporate the solvent was approximately to 45 min per sample. Up to half a day was required to evaporate only 10 samples. This process has since been improved by decreasing the air pressure during drying.

5.1.3 Oleocanthal in olive oil

5.1.3.1 Introduction.

Oleocanthal is a phenolic component in olive oil which has been shown to have some health and nutritional advantages. Initial studies have described it as the component which contributes to the bitterness of olive oil, a characteristic which is considered to be an attribute to the sensory quality. Although the measurement of oleocanthal is not directly related to the theme of this study in characterising olive oils, it is an important new study and has been included here as a first stage to understanding how to measure it and what levels may be present in Australian oils. It has been difficult to access standards for oleocanthal and therefore results from our analysis have not been verified nor quantified. This report provides a summary of developments so far.

***5.1.3.2 Aim*:** This study evaluated and modified a method for the extraction and quantification of oleocanthal, a non-steroidal anti-inflammatory agent similar to ibuprofen, in olive oil to determine the levels in some Australian olive oils.

5.1.3.3 Materials and Methods

Olive oils taken from research trials being carried out at the AORL which had been assessed by the Wagga Wagga Olive Oil Sensory Panel were used for the analysis. Samples chosen had high, medium and low levels of bitterness as illustrated in Table 1.

Table 5.3 Oils used for analysis of oleocanthal

Laboratory number	Bitterness score
06304/5	**5.9**
06304/1	**3.7**
06304/22	**1.9**

Oleocanthal was extracted from the olive oil according to the procedure of Impellizzeri and Lin, (2006).

1. Olive oil (1 g) was weighed into a 15 mL centrifuge tube.
2. Hexane (2 mL) was added and vortexed twice for 15 seconds each time.
3. Acetonitrile (5 mL) was added and again vortexed twice for 15 seconds each time.
4. The tubes were centrifuged at 3500-4000 rpm for 5 minutes.
5. The solvent phase was transferred to another centrifuge tube.
6. Steps 3-5 were repeat twice more (washing the oil phase).
7. Evaporation of the combined solvent extracts was achieved using nitrogen.
8. Methanol/water (1 mL; 1:1 v:v) was added to dissolve the extract and the mixture vortexed.
9. The remaining oil was separated with hexane (3 mL) and the mixture again vortexed.
10. Tubes were centrifuged at 3500-4000 rpm for 5 minutes.
11. The methanol water phase was collected for HPLC analysis.

HPLC method:

A Phenomonex Luna C18(2) column (250mm x 4.6mm, 5µm) was used. The HPLC was fitted with a Photo-diode Array detector and measured at 278nm. The injection volume was 40 µL. The mobile phase gradient is shown in Table 2.

Table 5.4 HPLC Gradient used for oleocanthal separation

Time (min)	Acetonitrile (%)	Water (%)
0	**25**	**75**
35.00	**25**	**75**
35.01	**80**	**20**
45.00	**80**	**20**
45.01	**25**	**75**
55.00	**25**	**75**

5.1.3.4 Results

The chromatograms for the three oils are illustrated in Figures 5.22 a, b and c. As there was no oleocanthal standard available to do this analysis, it has been necessary to make some assumptions about the peaks in question. There is a reduction in all of the components prior to 30 minutes on the chromatogram and related to the bitterness score. This relationship was repeated with numerous samples of varying degrees of bitterness.

5.1.3.5 Discussion

Impellizzeri and Lin (2006) showed that the retention time for oleocanthal with the conditions described was at 20 to 21 minutes (2 peaks, cis and trans-isomers of the compound). The chromatograms shown in Figure 5.22 has a number of small peaks in that area of the chromatogram. The sample with the highest bitterness score (06304/5) shows two peaks at approximately 21 and 22 minutes, as does sample 06304/1 (bitterness 3.7). Sample 06304/22, with the lowest bitterness (1.9), does not have any significant peaks in that area of the chromatogram.

There are difficulties in proper identification and quantification of oleocanthal in the olive oils studied. Some oleocanthal, extracted or synthesised, is needed to form a calibration curve. A paper outlining the synthesis of oleocanthal was sourced (Smith et al 2005). From this paper it is clear it is beyond the capabilities of AORL to synthesise oleocanthal, due to lack of specialised equipment. The laboratories of Smith *et al*. have been contacted regarding the possible purchase of some oleocanthal (16/1/07 and 16/2/07) and as yet, no reply. It could be suggested that fractionation and collection of oleocanthal from HPLC analysis could be carried out. However, without proper identification of the peaks, this may prove difficult. Collection of a high enough concentration of standard for a calibration curve would also be problematic.

5.1.3.6 Conclusion

The extraction method and the HPLC separation of the sample seem to be successful. The purchase of pure oleocanthal standard is a priority. If this was possible, identification and quantification of oleocanthal in olive oil should be an achievable task in the near future.

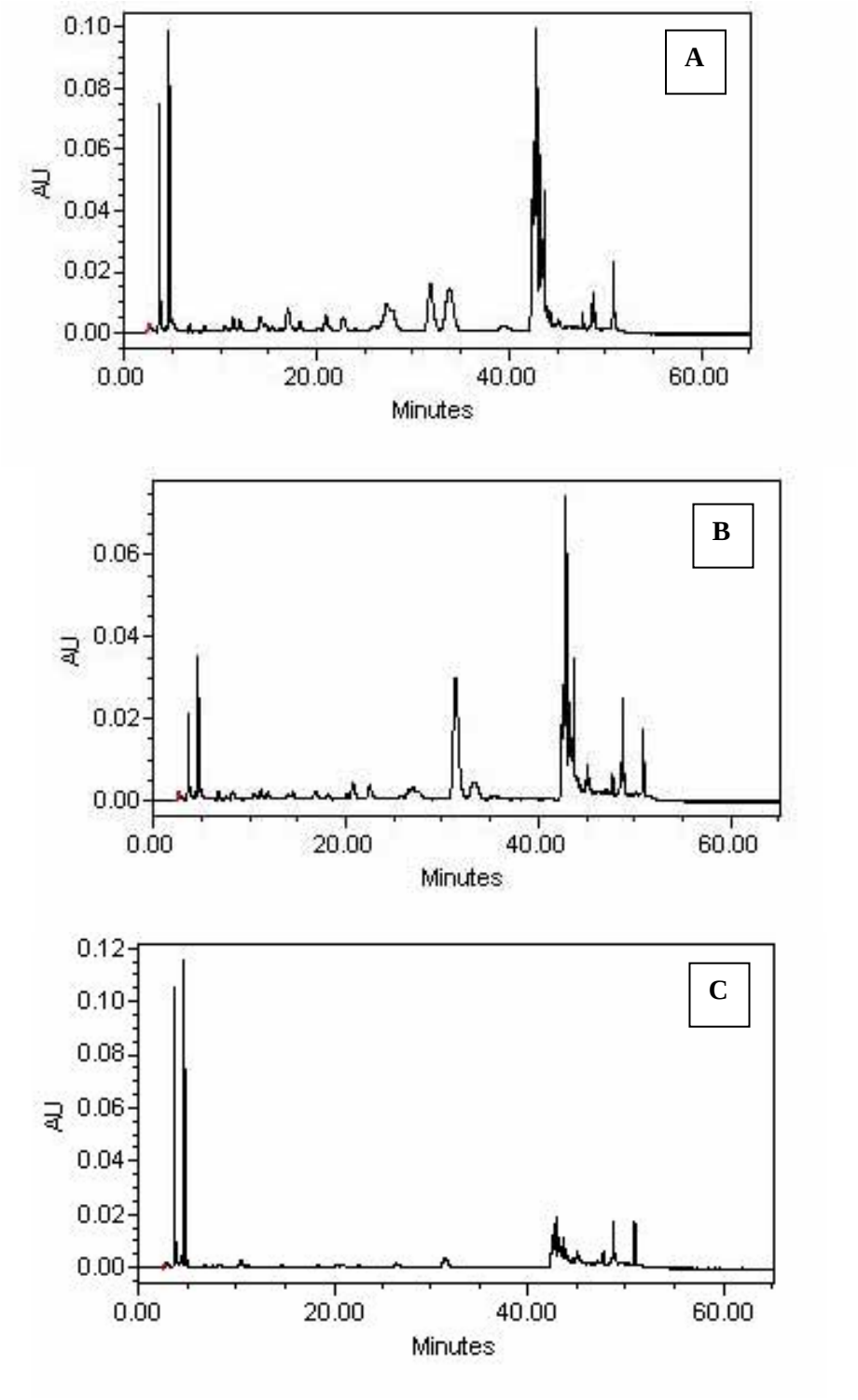


Figure 5.22 (a) 06304/5 (bitterness score 5.9); (b) Sample 06304/1 (bitterness score 3.7); (c) Sample 06304/22 (bitterness score 1.9)

6. Discussion of Results

Olive oil from the major Australian grown cultivars has been shown to vary significantly in many of the quality parameters normally used to determine if the oil is genuine or has been adulterated. This can in turn cause a buyer to reject the oil even though it is a genuine high quality extra virgin olive oil. This project has evaluated olives of all the common cultivars growing at extreme environmental sites to determine the range of quality parameters and how closely they agree with international standards for olive oil.

Ten components were tested on each of 11 cultivars. These cultivars were sourced from four environmentally different sites which represent the extremes of the Australian olive oil production areas. The olives were harvested at two times, both early and late fruit maturity. The experiment was repeated over two years. There were a total of 143 samples analysed over the two year period generating over 6,000 data points.

6.1 Fatty acid Profile

Thirteen fatty acids were measured in each of the samples. Although several of the fatty acids were outside of the standard ranges it is notable that all of the saturated fatty acids, C14:0, C17:0, C18:0, C20:0, C22:0, C24:0 were within limits.

Palmitic acid, C16:0, is the major saturated fatty acid and the COI limit is 7.5-20%. Three samples were less than 7.5% (Barnea, Corregiolla, Frantoio, all from cool climate). One sample was higher then 20% (Arbequina). Although all others were within the limit, the highest 5 results were Arbequina.

Palmitoleic acid, C16:1 is a minor component but two samples were less than the minimum of 0.3% (Coratina – Cool climate) and two samples were greater than the maximum of 3.5% (Arbequina, Northern NSW).

Heptadecenoic acid, C17:1, is common to olive oil although only at low levels and 4 samples were higher than the 0.3% standard (2 Nevadillo Blanco, 1 Manzanillo, 1 Hardys Mammoth).

Oleic acid, C18:1, is the main fatty acid in olive oil and is considered to contribute a significant amount of the nutritional value of olive oil. However, two samples were less than the minimum of 55% (Arbequina – Northern NSW). Three samples were greater than 83% (2 Picual and 1 Arbequina, all cool climate) and cool climates seemed to generally promote higher levels of oleic acid.

Linoleic acid, C18:2, is the major polyunsaturated fatty acid and nine samples were less than the allowable minimum of 3.5% (6 Picual, 2 Arbequina, 1 Manzanillo) all generally from the cooler climates. One sample exceeded the maximum of 21% (Barnea, Northern NSW). All of the highest linoleic acid contents were from Northern NSW.

Linolenic acid, C18:3, is a minor polyunsaturated fatty acid but very significant as it is used to indicate the presence of seed oils used in adulteration of olive oil. Four samples were greater than the COI standard of 1.0% (2 Pendolino, 1 Hardys Mammoth, 1 Picual). This is an important issue as these oils, although genuine olive oil which had been extracted in the laboratory, would be considered not to be olive oil be international standards.

Arachidic acid, C20:1 is also a minor component but 10 samples had greater than the maximum of 0.4% of this compound. All of these were *cv* Coratina.

6.2 Sterols

Total sterols. According to the existing standards must contain more than 1000 mg/kg of total sterols. In this study, seven samples had less than 1000 mg/kg (4 Koreneiki, 2 Pendolino, 1 Coratina). The minimum value was 789.23 mg/kg.
Cholesterol is usually present in only trace amounts. A maximum level of 0.48% was found in this study. **Brassicasterol** has a maximum of 0.08% and **Stigmasterol** was lower than the campesterol level as required by COI standards.
Campesterol is of considerable importance as some of the Australian cultivars are known to contain excessive quantities. Twenty samples were found to be higher than the COI limit of 4.0%. Sixteen of those were *cv* Barnea and four were *cv* Koreneiki. The maximum value found for campesterol was 4.8%.
Δ**7 Stigmastenol**. Eight samples were higher than the COI limit of 0.5%. Of these, three were Frantoio, two were Koreneiki, two Corregiolla and one one Picual. The maximum value found was 1.36%.
Apparent β-sitosterol, as described previously, is the sum of several individual sterols. Eight samples were lower than the COI limit of 93%. Three were Barnea, two Koreneiki, one Corregiolla, one Picual and one Arbequina. The minimum value was 91.7%.
Diols must be less than 4.5%. Three were above that level, two Koreneiki and one Leccino. The maximum value was 6.6%.
It was significance that *cv* Koreneiki was outside the limit for all of the above components in some instances.

6.3 Other components

Trans Fatty acids. Trans fatty acids are an indication of heating or refining and all samples analysed, as expected, were less than the COI limit.
Tocopherols. There is no COI standard for tocopherols. The range for α-tocopherol in these samples was from 59-766 mg/kg. It was found that tocopherols were generally lowest in *cv* Manzanillo and generally highest in *cv* Leccino.
UV-absorption. The UV-absorption was within COI limits for all samples for ΔK and specific extinction coefficient at 270nm.
Waxes. All samples were within COI limits. Most of the higher results were from Northern NSW.
Stigmastadienes. Stigmastadienes were all within limits.
Difference between actual and theoretical ECN 42. There were five samples outside the limits for ECN 42 and all of these were from northern NSW.

6.4 Summary

In total there were 87 samples which did not fit within the COI standards for these tests which are designed to determine if the oil is genuine extra virgin olive oil. As the oils were extracted from fresh olives, under controlled conditions in the laboratory, there is no reason to suggest that the oil is not extra virgin olive oil.

7. Implications

Analysis at AORL in recent years has shown that individual sterols, and particularly campesterol, exceed international limits. It appears that the problem is more due to the cultivar rather than the environment with other countries also experiencing problems with cultivars such as Barnea, Cornicarbra and Koreneiki.

Collaboration with researchers in many countries including Chile, Argentina, New Zealand, Spain, France and Italy have shown that this is not an Australian problem but an issue for many countries. As such the outcomes have far more reaching significance that the investigation initially expected and genuine olive oils are being rejected on the basis of inappropriate regulations.

As expected, all oils were shown to meet all of the tests designed to identify refining or heating processes or the presence of solvent extracted, pomace oil as none of these oils have been treated other than by accepted mechanical extraction techniques. However, tests which describe the oil composition generally used to indicate if there is a presence of other types of oils showed considerable non-conformity. Almost all of the range of 13 fatty acids, used to determine if the oil is genuine, were outside the limits at least in some cases. The sterol profile was also shown to have numerous outliers with campesterol alone being above the standard of 4.0% in 20 cases.

This report is important for oil producers, traders and particularly exporters of olive oil from Australia but hopefully also from other countries which are known to be experiencing similar problems with selling genuine unadulterated extra virgin olive oil. It is expected that the results will be considered by oil producing countries when developing standards for trade in olive oil so as not to create trade barriers which restrict trade of genuine high quality product.

The implications of these findings are clear. All of these situations where authentic extra virgin olive oil has been shown to be outside the limits, these products “could” be rejected on the grounds of fraud. Not only does this limit the sale of authentic products, it may cost exporters large amounts of money to send oil outside the country, only to have it rejected as adulterated.

Additionally, Australian producers are now blending high quality oil to meet established standards. As a result, oil with exceptional characteristics such as organoleptic quality and oxidative stability are being blended with inferior oil to achieve compliance with inappropriate trade standards. The maintenance of these standards may well limit the profitability of olive production in Australasia, and other countries, and see some cultivars which can be profitable removed due to non-compliance.

Consumers in particular will be implicated as oil is no longer produced to achieve the highest possible sensory product with the best stability but it is being designed to be within rules with no relevance to oil quality.

Australia, through the AOA, is in the process of developing a code of conduct including a set of standards for Australia. These standards can then be applied to imported product to ensure that not only are Australian producers follow the regulations but imported product is genuine and consumers within Australia get what they pay for.

As members of Codex Alimentarius, Australia is obliged to acknowledge the levels described by that organisation. Policy makers including the Australian Government, Codex Australia and Codex Alimentarius and the Australian Olive association need to describe Australian standards and they need to recognise natural variability of the product. Exporters can then trade on Australian regulations.

8. Recommendations

Australian standards need to be clearly identified for the purpose of trade and particularly export. However, international organisations and particularly Codex Alimentarius need to continue to make changes to standards which will allow free flow of high quality olive oil products and prevent any barriers to trade. This information should be disseminated to world standards organisations and logical discussion on realistic standards be pursued.

The recommendations in this report are targeted at those policy makers who have been identified above and who design trading standards. Additionally it is to provide weight to discussions regarding changes to world trade regulations to assist in setting relevant standards.

The authors would hope that the organisations which set standards for trade, such as the International Olive Council, EEC, USDA and Codex Alimentarius will become aware of the discrimination against producers of high quality products when trade standards do not correctly describe the product.

The Australian Olive Association should make use of the findings in this study to help develop the standards for the new AOA “code of conduct”.

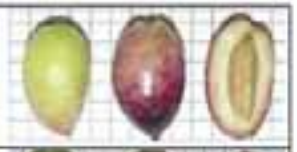
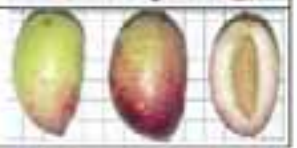
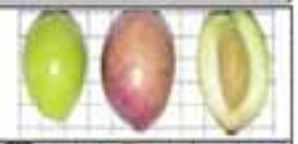
9. Appendices

Appendix 1. Photographs of olive fruit

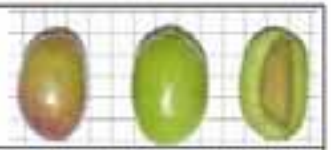
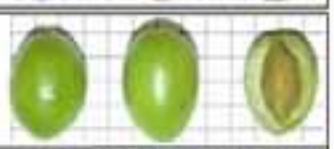
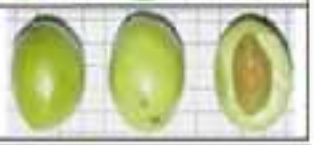
Arbequina

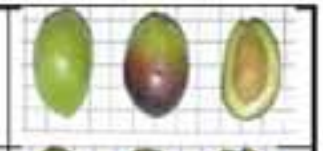
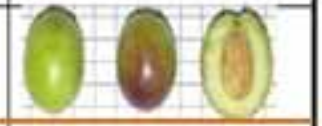
Site: Northern NSW			
Early Harvest	Harvest Date: April 06	Maturity Index: 2.19	
Late Harvest	Harvest Date: May06	Maturity Index: 5.69	
Site: Central Victoria			
Early Harvest	Harvest Date: May 06	Maturity Index: 1.05	
Late Harvest	Harvest Date: June 06	Maturity Index: 2.02	
Site: Western Australia			
Early Harvest	Harvest Date: May 06	Maturity Index: 1.95	
Late Harvest	Harvest Date: July 06	Maturity Index: 4.01	
Site: Cool Climate			
Early Harvest	Harvest Date: June 06	Maturity Index: 2.82	
Late Harvest	Not Available		

Barnea

Site: Northern NSW			
Early Harvest	Harvest Date: April 06	Maturity Index: 2.19	
Late Harvest	Harvest Date: May06	Maturity Index: 2.21	
Site: Central Victoria			
Early Harvest	Harvest Date: May 06	Maturity Index: 2.67	
Late Harvest	Harvest Date: June 06	Maturity Index: 3.95	
Site: Western Australia			
Early Harvest	Harvest Date: May 06	Maturity Index: 3.6	
Late Harvest	Harvest Date: July 06	Maturity Index: 4.43	
Site: Cool Climate			
Early Harvest	Harvest Date: June 06	Maturity Index: 4.29	
Late Harvest	Not Available		

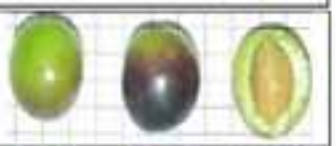
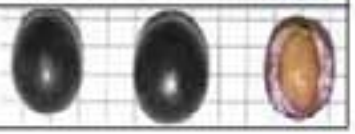
Coratina

Site: Northern NSW			
Early Harvest	Harvest Date: April 06	Maturity Index: 1.36	
Late Harvest	Harvest Date: May06	Maturity Index: 1.44	
Site: Central Victoria			
Early Harvest	Harvest Date: May 06	Maturity Index: 2.00	
Late Harvest	Harvest Date: June 06	Maturity Index: 3.76	
Site: Western Australia			
Early Harvest	Harvest Date: May 06	Maturity Index: 0.80	
Late Harvest	Harvest Date: July 06	Maturity Index: 1.04	
Site: Cool Climate			
Early Harvest	Harvest Date: June 06	Maturity Index: 1.03	
Late Harvest	Not Available		

Northern NSW		
Early Harvest - April 06	Maturity Index: 1.70	
Late Harvest - May 06	Maturity Index: 1.81	
Central Victoria		
Early Harvest - May 06	Maturity Index: 2.23	
Late Harvest - June 06	Maturity Index: 3.53	
Western Australia		
Early Harvest - May 06	Maturity Index: 1.63	
Late Harvest - July 06	Maturity Index: 2.42	
Southern - Cool Climate		
Early Harvest - June 06	Maturity Index: 1.25	
Late Harvest - June 06	Maturity Index: 2.50	

FRANTOIO

Leccino

Site: Northern NSW			
Early Harvest	Harvest Date: April 06	Maturity Index: 3.18	
Late Harvest	Harvest Date: May 06	Maturity Index: 3.98	
Site: Central Victoria			
Early Harvest	Harvest Date: May 06	Maturity Index: 2.93	
Late Harvest	Harvest Date: June 06	Maturity Index: 3.59	
Site: Western Australia			
Early Harvest	Harvest Date: May 06	Maturity Index: 3.40	
Late Harvest	Harvest Date: July 06	Maturity Index: 4.00	
Site: Cool Climate			
Early Harvest	Harvest Date: June 06	Maturity Index: 4.10	
Site: Cool Climate			
Late Harvest	Harvest Date: June 06	Maturity Index: 5.41	

Manzanillo

Site: Northern NSW			
Early Harvest	Harvest Date: April 06	Maturity Index: 2.53	
Late Harvest	Harvest Date: May 06	Maturity Index: 3.68	
Site: Central Victoria			
Early Harvest	Harvest Date: May 06	Maturity Index: 2.51	
Late Harvest	Harvest Date: June 06	Maturity Index: 3.56	
Site: Western Australia			
Early Harvest	Harvest Date: May 06	Maturity Index: 3.19	
Late Harvest	Harvest Date: July 06	Maturity Index: 3.82	
Site: Cool Climate			
Early Harvest	Not Available		
Late Harvest	**Site: Cool Climate** Not Available		

Hardy's Mammoth

Site: Northern NSW

Early Harvest	Harvest Date: April 06	Maturity Index: 3.59
Late Harvest	Not Available	

Nevadillo Blanco

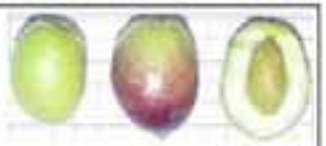
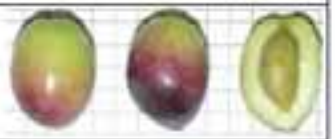
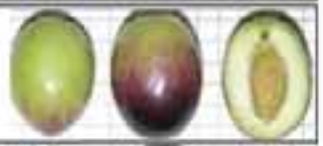
Site: Western Australia

Early Harvest	Harvest Date: May 06	Maturity Index: 2.04
Late Harvest	Harvest Date: July 06	Maturity Index: 4.85

Pendolino

Site: Northern NSW			
Early Harvest	Not Available		
Late Harvest	Harvest Date: May 06	Maturity Index: 4.14	
Site: Central Victoria			
Early Harvest	Harvest Date: May 06	Maturity Index: 2.83	
Late Harvest	Harvest Date: June 06	Maturity Index: 3.98	
Site: Western Australia			
Early Harvest	Harvest Date: May 06	Maturity Index: 3.49	
Late Harvest	Not Available		
Site: Cool Climate			
Early Harvest	Harvest Date: June 06	Maturity Index: 4.24	
Site: Cool Climate			
Late Harvest	Harvest Date: June 06	Maturity Index: 5.27	

Picual

Site: Northern NSW			
Early Harvest	Harvest Date: April 06	Maturity Index: 1.89	
Late Harvest	Harvest Date: May 06	Maturity Index: 2.15	
Site: Central Victoria			
Early Harvest	Harvest Date: May 06	Maturity Index: 1.78	
Late Harvest	Harvest Date: June 06	Maturity Index: 2.96	
Site: Western Australia			
Early Harvest	Harvest Date: May 06	Maturity Index: 2.91	
Late Harvest	Harvest Date: July 06	Maturity Index: 5.27	
Site: Cool Climate			
Early Harvest	Harvest Date: June 06	Maturity Index: 2.88	
Site: Cool Climate			
Late Harvest	Not Available		

Appendix 2. Summary of raw data from chemical analysis

Sterols and Diols

Year	Region	Harvest	Variety	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7- Stigmastenol	Apparent b sitosterol	Diols	Total Sterols(mg/kg)
2005	Nthn NSW/Sthn Qld	Early	Arbequina	0.13	0.00	3.24	0.83	0.07	94.28	1.22	1929.95
2005	Nthn NSW/Sthn Qld	Early	Barnea	0.14	0.00	4.48	0.47	0.19	93.26	1.46	1539.86
2005	Nthn NSW/Sthn Qld	Early	Coratina	0.23	0.00	2.95	0.41	0.26	94.47	1.67	1383.67
2005	Nthn NSW/Sthn Qld	Early	Corregiola	0.15	0.00	3.21	0.42	0.35	93.96	1.17	1785.80
2005	Nthn NSW/Sthn Qld	Early	Frantoio	0.09	0.00	2.97	0.63	0.45	94.19	1.18	1566.84
2005	Nthn NSW/Sthn Qld	Early	Leccino	0.17	0.06	2.90	1.24	0.31	93.66	1.13	1661.32
2005	Nthn NSW/Sthn Qld	Early	Manzanillo	0.16	0.00	2.92	1.76	0.16	93.52	1.39	1602.87
2005	Nthn NSW/Sthn Qld	Late	Arbequina	0.16	0.00	3.30	0.68	0.13	94.41	1.33	2142.65
2005	Nthn NSW/Sthn Qld	Late	Barnea	0.16	0.00	4.46	0.90	0.10	92.56	1.34	1873.54
2005	Nthn NSW/Sthn Qld	Late	Coratina	0.13	0.00	2.72	0.41	0.31	95.30	2.13	2015.62
2005	Nthn NSW/Sthn Qld	Late	Corregiola	0.15	0.00	3.07	0.43	0.29	94.96	1.77	2157.68
2005	Nthn NSW/Sthn Qld	Late	Frantoio	0.11	0.00	2.53	0.63	0.52	95.12	0.57	1782.58
2005	Nthn NSW/Sthn Qld	Late	Koreneiki	0.40	0.00	3.93	1.07	0.11	93.01	2.64	973.84
2005	Nthn NSW/Sthn Qld	Late	Leccino	0.10	0.00	2.33	0.99	0.41	93.69	0.53	2101.36
2005	Nthn NSW/Sthn Qld	Late	Manzanillo	0.12	0.00	2.81	1.38	0.17	94.39	2.54	1878.01
2005	Nthn NSW/Sthn Qld	Late	Picual	0.12	0.00	2.93	0.89	0.16	94.76	0.58	2484.50
2005	Central Victoria	Early	Arbequina	0.08	0.00	3.89	1.00	0.14	94.09	1.42	2022.26
2005	Central Victoria	Early	Barnea	0.10	0.00	4.66	0.42	0.14	93.78	0.78	1759.52
2005	Central Victoria	Early	Barnea	0.10	0.00	4.52	0.44	0.10	94.06	1.06	2163.53
2005	Central Victoria	Early	Coratina	0.16	0.00	3.07	0.64	0.23	95.03	1.71	1256.72
2005	Central Victoria	Early	Frantoio	0.27	0.00	3.15	0.52	0.33	94.06	1.44	1527.73
2005	Central Victoria	Early	Frantoio	0.12	0.00	3.20	0.62	0.15	94.93	1.08	1809.49
2005	Central Victoria	Early	Leccino	0.11	0.00	2.76	0.91	0.18	95.05	0.81	1681.31
2005	Central Victoria	Early	Picual	0.18	0.00	3.25	0.38	0.50	93.59	1.71	1290.29

Year	Region	Harvest	Variety	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7- Stigmastenol	Apparent b sitosterol	Diols	Total Sterols(mg/kg)
2005	Central Victoria	Early	Picual	0.15	0.00	3.29	0.65	0.31	94.70	1.06	1707.16
2005	Central Victoria	Late	Barnea	0.25	0.00	4.89	0.33	0.00	93.21	1.34	1459.76
2005	Central Victoria	Late	Coratina	0.21	0.00	3.03	0.78	0.13	94.30	1.53	1090.20
2005	Central Victoria	Late	Frantoio	0.16	0.00	3.03	0.85	0.14	94.57	1.54	1329.92
2005	Central Victoria	Late	Leccino	0.14	0.00	2.59	0.95	0.16	95.00	1.25	1233.10
2005	Central Victoria	Late	Manzanillo	0.11	0.00	2.44	1.48	0.17	94.92	1.37	1638.93
2005	Central Victoria	Late	Picual	0.23	0.00	3.96	0.36	0.09	94.18	1.03	1278.91
2005	WA	Early	Barnea	0.19	0.00	3.89	0.89	0.31	92.86	0.61	1517.68
2005	WA	Early	Coratina	0.25	0.00	2.97	0.69	0.08	95.12	1.24	1337.80
2005	WA	Early	Corregiola	0.18	0.00	2.54	0.79	0.37	94.47	0.80	1552.89
2005	WA	Early	Frantoio	0.17	0.00	2.72	0.99	0.46	94.08	0.77	1593.81
2005	WA	Early	Koreneiki	0.26	0.00	3.45	0.67	0.06	94.13	3.12	1185.35
2005	WA	Early	Leccino	0.23	0.00	2.29	1.38	0.32	93.68	0.49	1411.71
2005	WA	Early	Manzanillo	0.23	0.00	2.26	1.39	0.32	94.04	2.48	1374.38
2005	WA	Early	Nevadillo Blanco	0.21	0.00	2.47	0.70	0.12	95.49	0.85	1530.39
2005	WA	Early	Picual	0.17	0.00	2.98	0.96	0.35	94.08	1.43	1321.82
2005	WA	Late	Barnea	0.13	0.00	4.03	0.54	0.16	94.41	0.47	1851.67
2005	WA	Late	Corregiola	0.14	0.00	2.81	0.54	0.16	95.40	0.68	1560.03
2005	WA	Late	Frantoio	0.14	0.00	2.70	0.90	0.27	94.80	1.17	1700.67
2005	WA	Late	Koreneiki	0.33	0.00	3.16	0.54	0.35	94.15	3.22	1178.27
2005	WA	Late	Leccino	0.17	0.00	1.88	1.19	0.47	93.96	1.33	1765.16
2005	WA	Late	Manzanillo	0.21	0.00	2.39	1.20	0.37	94.59	3.12	1356.02
2005	WA	Late	Nevadillo Blanco	0.36	0.00	2.58	0.51	0.27	94.87	2.05	1444.50
2005	WA	Late	Pendolino	0.19	0.00	2.02	0.63	0.17	95.62	1.16	1144.99
2005	WA	Late	Picual	0.10	0.00	3.64	0.80	0.15	94.45	0.82	1453.75
2005	Sthn Vic / Tasmania	Early	Arbequina	0.16	0.00	3.37	0.22	0.12	94.95	1.29	1417.13
2005	Sthn Vic / Tasmania	Early	Barnea	0.13	0.00	4.98	0.00	0.11	93.86	1.76	1713.53

Year	Region	Harvest	Variety	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7- Stigmastenol	Apparent b sitosterol	Diols	Total Sterols(mg/kg)
2005	Sthn Vic / Tasmania	Early	Coratina	0.29	0.00	3.23	0.27	0.12	94.72	1.30	1247.40
2005	Sthn Vic / Tasmania	Early	Corregiola	0.27	0.00	3.00	0.38	0.27	94.81	1.77	1083.22
2005	Sthn Vic / Tasmania	Early	Frantoio	0.14	0.00	3.06	0.56	0.23	94.54	3.47	1215.55
2005	Sthn Vic / Tasmania	Early	Leccino	0.14	0.00	2.99	0.71	0.13	94.23	1.14	1400.43
2005	Sthn Vic / Tasmania	Early	Manzanillo	0.15	0.00	2.52	0.36	0.22	95.06	2.55	1355.70
2005	Sthn Vic / Tasmania	Early	Picual	0.17	0.08	3.26	0.17	0.13	95.23	1.14	1447.24
2005	Sthn Vic / Tasmania	Late	Arbequina	0.24	0.00	2.74	0.61	0.18	94.51	2.00	1416.41
2005	Sthn Vic / Tasmania	Late	Coratina	0.22	0.00	3.14	0.50	0.23	94.23	2.22	1068.58
2005	Sthn Vic / Tasmania	Late	Corregiola	0.28	0.00	2.84	0.55	0.34	94.44	1.72	1032.55
2005	Sthn Vic / Tasmania	Late	Frantoio	0.15	0.00	3.28	0.64	0.34	94.08	1.92	1066.44
2005	Sthn Vic / Tasmania	Late	Leccino	0.17	0.00	2.48	0.64	0.19	95.07	0.92	1389.97
2005	Sthn Vic / Tasmania	Late	Picual	0.22	0.00	3.71	0.59	0.39	93.04	1.34	1399.37
2006	Nthn NSW/Sthn Qld	Early	Arbequina	0.05	0.00	3.16	0.83	0.20	94.97	1.26	2136.86
2006	Nthn NSW/Sthn Qld	Early	Barnea	0.04	0.00	**4.14**	0.72	0.32	94.26	1.05	1844.16
2006	Nthn NSW/Sthn Qld	Early	Coratina	0.48	0.00	3.08	1.10	0.27	94.43	1.87	1489.15
2006	Nthn NSW/Sthn Qld	Early	Corregiola	0.08	0.00	3.23	0.57	0.41	95.02	1.06	1715.60
2006	Nthn NSW/Sthn Qld	Early	Frantoio	0.06	0.00	3.03	0.72	0.39	95.15	0.87	1731.80
2006	Nthn NSW/Sthn Qld	Early	Hardys Mammoth	0.03	0.00	2.61	0.89	0.30	95.46	1.18	1509.80
2006	Nthn NSW/Sthn Qld	Early	Koreneiki	0.12	0.00	**4.10**	1.48	1.36	91.72	6.69	1114.01
2006	Nthn NSW/Sthn Qld	Early	Leccino	0.10	0.00	2.09	1.08	0.29	95.33	0.64	1440.77
2006	Nthn NSW/Sthn Qld	Early	Manzanillo	0.13	0.00	2.50	1.92	0.34	94.12	1.51	1934.59
2006	Nthn NSW/Sthn Qld	Early	Pendolino	0.04	0.00	2.24	0.89	0.43	95.63	0.90	2048.24
2006	Nthn NSW/Sthn Qld	Early	Picual	0.05	0.00	2.56	0.82	0.39	95.71	0.22	1817.24
2006	Nthn NSW/Sthn Qld	Late	Arbequina	0.05	0.01	3.39	0.98	0.48	94.59	0.83	2348.95
2006	Nthn NSW/Sthn Qld	Late	Barnea	0.06	0.00	**4.32**	0.81	0.09	94.21	1.00	1790.01
2006	Nthn NSW/Sthn Qld	Late	Coratina	0.21	0.00	3.05	0.70	0.39	95.16	1.77	917.82
2006	Nthn NSW/Sthn Qld	Late	Corregiola	0.11	0.00	2.84	0.55	0.58	95.29	0.67	1590.75

Year	Region	Harvest	Variety	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7- Stigmastenol	Apparent b sitosterol	Diols	Total Sterols(mg/kg)
2006	Nthn NSW/Sthn Qld	Late	Frantoio	0.11	0.01	2.89	0.66	1.19	94.47	0.68	1446.35
2006	Nthn NSW/Sthn Qld	Late	Koreneiki	0.17	0.04	**4.32**	0.63	0.31	93.63	2.69	869.22
2006	Nthn NSW/Sthn Qld	Late	Leccino	0.08	0.01	2.20	1.11	0.30	95.40	5.53	1657.07
2006	Nthn NSW/Sthn Qld	Late	Manzanillo	0.05	0.00	2.61	1.31	0.17	95.16	1.52	1794.88
2006	Nthn NSW/Sthn Qld	Late	Pendolino	0.11	0.00	2.11	0.63	0.12	96.07	1.00	1340.22
2006	Nthn NSW/Sthn Qld	Late	Picual	0.18	0.00	3.62	0.83	0.31	92.51	3.18	1509.46
2006	Central Victoria	Early	Arbequina	0.05	0.00	3.95	0.96	0.10	94.24	1.51	1626.58
2006	Central Victoria	Early	Arbequina	0.06	0.00	3.68	0.87	0.09	94.68	1.67	1282.10
2006	Central Victoria	Early	Barnea	0.05	0.00	**4.55**	0.49	0.11	94.17	0.58	1464.01
2006	Central Victoria	Early	Barnea	0.04	0.00	**4.63**	0.41	0.20	94.12	1.91	1570.08
2006	Central Victoria	Early	Coratina	0.04	0.00	3.55	0.61	0.11	94.87	0.87	1200.28
2006	Central Victoria	Early	Coratina	0.03	0.00	3.37	0.48	0.08	95.53	2.07	1338.44
2006	Central Victoria	Early	Frantoio	0.07	0.00	3.17	0.54	0.12	95.21	0.70	1364.57
2006	Central Victoria	Early	Koreneiki	0.08	0.00	**4.60**	0.57	0.14	93.28	4.76	789.23
2006	Central Victoria	Early	Leccino	0.06	0.00	3.38	0.82	0.19	94.68	1.08	1275.01
2006	Central Victoria	Early	Manzanillo	0.03	0.00	2.63	0.69	0.08	95.94	1.03	1299.76
2006	Central Victoria	Early	Pendolino	0.09	0.00	3.23	0.50	0.22	95.30	1.08	1006.10
2006	Central Victoria	Early	Picual	0.05	0.00	3.41	0.66	0.13	95.12	0.85	1056.25
2006	Central Victoria	Early	Picual	0.06	0.00	3.26	0.44	0.31	95.28	1.28	1113.14
2006	Central Victoria	Late	Arbequina	0.23	0.00	3.57	1.14	0.29	93.38	1.60	1580.14
2006	Central Victoria	Late	Arbequina	0.17	0.03	3.71	1.04	0.26	93.20	2.01	1336.44
2006	Central Victoria	Late	Barnea	0.23	0.00	**4.57**	0.63	0.08	93.52	0.56	1850.23
2006	Central Victoria	Late	Barnea	0.09	0.00	**4.67**	0.40	0.28	92.24	0.97	1535.54
2006	Central Victoria	Late	Coratina	0.14	0.00	3.27	0.84	0.11	94.23	0.61	1288.93
2006	Central Victoria	Late	Frantoio	0.13	0.00	2.77	0.43	0.10	95.32	0.93	1561.68
2006	Central Victoria	Late	Koreneiki	0.23	0.03	**4.56**	0.42	0.51	92.41	3.20	806.78
2006	Central Victoria	Late	Leccino	0.15	0.02	2.87	1.07	0.18	94.56	1.49	1293.21
2006	Central Victoria	Late	Manzanillo	0.30	0.00	2.60	0.94	0.19	94.85	1.42	1511.51

Year	Region	Harvest	Variety	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7- Stigmastenol	Apparent b sitosterol	Diols	Total Sterols(mg/kg)
2006	Central Victoria	Late	Pendolino	0.08	0.04	3.06	0.56	0.00	94.78	0.99	882.71
2006	Central Victoria	Late	Picual	0.15	0.04	3.49	0.65	0.23	94.22	1.04	1240.24
2006	WA	Early	Arbequina	0.10	0.00	3.62	0.84	0.06	94.71	1.10	1958.33
2006	WA	Early	Barnea	0.06	0.00	**4.22**	0.56	0.08	94.54	0.85	1707.27
2006	WA	Early	Coratina	0.06	0.00	3.21	0.49	0.09	95.64	2.10	1428.59
2006	WA	Early	Corregiola	0.09	0.00	2.68	0.56	0.26	95.74	0.89	1432.72
2006	WA	Early	Frantoio	0.09	0.00	3.22	0.72	0.14	95.00	0.79	1503.03
2006	WA	Early	Leccino	0.05	0.00	2.27	1.10	0.18	95.51	0.74	1530.64
2006	WA	Early	Manzanillo	0.10	0.00	2.22	0.94	0.10	95.97	1.38	1713.01
2006	WA	Early	Nevadillo Blanco	0.08	0.00	2.29	0.50	0.10	96.36	1.18	1480.44
2006	WA	Early	Pendolino	0.06	0.00	2.22	0.59	0.26	96.20	0.70	1319.66
2006	WA	Early	Picual	0.05	0.00	3.17	0.70	0.16	95.44	0.83	1535.63
2006	WA	Late	Arbequina	0.09	0.05	3.50	0.94	0.24	92.96	0.97	1480.11
2006	WA	Late	Barnea	0.20	0.00	**4.35**	0.63	0.24	93.19	0.56	1646.37
2006	WA	Late	Coratina	0.11	0.06	3.14	0.52	0.28	93.48	1.66	1121.48
2006	WA	Late	Corregiola	0.13	0.06	3.01	0.61	0.52	92.63	0.99	1215.88
2006	WA	Late	Frantoio	0.00	0.00	3.16	0.61	0.30	94.43	0.82	1473.99
2006	WA	Late	Koreneiki	0.19	0.03	3.12	0.36	0.30	93.77	2.08	1436.50
2006	WA	Late	Leccino	0.11	0.05	2.45	0.72	0.36	93.66	0.64	1607.13
2006	WA	Late	Manzanillo	0.19	0.00	2.73	0.70	0.38	94.23	1.28	1683.61
2006	WA	Late	Nevadillo Blanco	0.00	0.00	2.43	0.46	0.48	94.77	1.12	1528.02
2006	WA	Late	Pendolino	0.12	0.06	2.26	0.50	0.31	94.17	0.53	1085.20
2006	WA	Late	Picual	0.07	0.00	3.82	0.31	0.47	94.07	0.64	1190.20
2006	Sthn Vic / Tasmania	Early	Arbequina	0.26	0.04	3.66	0.37	0.24	94.12	2.09	1309.97
2006	Sthn Vic / Tasmania	Early	Barnea	0.09	0.00	**4.68**	0.26	0.18	94.21	0.56	1437.95
2006	Sthn Vic / Tasmania	Early	Coratina	0.10	0.00	3.55	0.33	0.35	95.14	0.65	1255.06
2006	Sthn Vic / Tasmania	Early	Corregiola	0.17	0.00	3.00	0.37	0.10	95.13	0.98	1361.11

Year	Region	Harvest	Variety	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	D-7- Stigmastenol	Apparent b sitosterol	Diols	Total Sterols(mg/kg)
2006	Sthn Vic / Tasmania	Early	Frantoio	0.11	0.00	3.19	0.37	0.66	94.93	0.73	1232.05
2006	Sthn Vic / Tasmania	Early	Frantoio	0.21	0.04	3.98	0.53	0.20	93.62	1.93	1181.69
2006	Sthn Vic / Tasmania	Early	Leccino	0.12	0.01	2.83	0.53	0.20	95.32	0.51	1407.29
2006	Sthn Vic / Tasmania	Early	Leccino	0.19	0.00	2.77	0.54	0.10	94.71	1.21	1538.92
2006	Sthn Vic / Tasmania	Early	Pendolino	0.10	0.01	2.90	0.42	0.30	95.67	1.08	1207.11
2006	Sthn Vic / Tasmania	Early	Pendolino	0.17	0.00	2.69	0.49	0.19	94.98	0.50	1082.58
2006	Sthn Vic / Tasmania	Early	Picual	0.20	0.00	3.25	0.21	0.18	94.89	1.79	1526.57
2006	Sthn Vic / Tasmania	Late	Frantoio	0.07	0.07	3.36	0.47	0.23	93.65	2.18	1305.98
2006	Sthn Vic / Tasmania	Late	Leccino	0.06	0.03	3.07	0.51	0.25	94.20	2.14	1501.17
2006	Sthn Vic / Tasmania	Late	Pendolino	0.14	0.04	3.32	0.35	0.23	94.25	1.08	900.45
	IOOC LIMITS			**<0.5**	**<0.1**	**<4.0**	**<Campesterol**	**<0.5**	**>93.0**	**<4.0**	**>1000**

Fatty Acid Profiles

				% of total fatty acids												
Year	**Region**	**Harvest**	**Variety**	**C14:0**	**C16:0**	**C16:1**	**C17:0**	**C17:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:0**	**C20:1**	**C22:0**	**C24:0**
2005	Nthn NSW/Sthn Qld	Early	Arbequina	0.02	19.54	3.21	0.07	0.19	1.11	56.41	18.18	0.64	0.29	0.24	0.07	0.03
2005	Nthn NSW/Sthn Qld	Early	Barnea	0.01	14.51	1.06	0.04	0.05	1.83	67.25	14.08	0.57	0.31	0.21	0.07	0.02
2005	Nthn NSW/Sthn Qld	Early	Coratina	0.01	12.07	0.40	0.03	0.04	1.85	76.03	8.37	0.74	0.28	0.05	0.08	0.04
2005	Nthn NSW/Sthn Qld	Early	Corregiola	0.01	15.71	1.17	0.02	0.05	1.71	69.61	10.58	0.56	0.22	0.28	0.05	0.03
2005	Nthn NSW/Sthn Qld	Early	Frantoio	0.01	15.59	1.65	0.02	0.06	1.43	67.44	12.58	0.58	0.26	0.28	0.07	0.04
2005	Nthn NSW/Sthn Qld	Early	Leccino	0.01	15.27	1.26	0.02	0.06	1.46	74.38	6.30	0.63	0.24	0.28	0.06	0.04
2005	Nthn NSW/Sthn Qld	Early	Manzanillo	0.02	15.69	1.83	0.10	0.16	2.84	65.73	12.11	0.76	0.36	0.26	0.08	0.05
2005	Nthn NSW/Sthn Qld	Late	Arbequina	0.02	19.18	3.13	0.07	0.18	1.13	55.71	19.51	0.58	0.21	0.19	0.06	0.02
2005	Nthn NSW/Sthn Qld	Late	Barnea	0.01	14.21	1.34	0.03	0.05	1.66	57.58	**23.79**	0.65	0.33	0.23	0.08	0.04
2005	Nthn NSW/Sthn Qld	Late	Coratina	0.01	9.85	0.38	0.03	0.04	2.46	76.01	9.39	0.81	0.38	**0.49**	0.09	0.04
2005	Nthn NSW/Sthn Qld	Late	Corregiola	0.01	16.32	1.53	0.02	0.05	2.03	63.44	15.40	0.65	0.24	0.22	0.05	0.03
2005	Nthn NSW/Sthn Qld	Late	Frantoio	0.01	16.19	2.03	0.02	0.06	2.10	60.35	17.96	0.64	0.28	0.25	0.06	0.03
2005	Nthn NSW/Sthn Qld	Late	Koreneiki	0.01	13.98	1.11	0.03	0.06	1.96	75.13	6.35	0.61	0.35	0.28	0.11	0.02
2005	Nthn NSW/Sthn Qld	Late	Leccino	0.01	14.86	1.21	0.02	0.04	1.70	72.89	8.12	0.55	0.25	0.25	0.07	0.03
2005	Nthn NSW/Sthn Qld	Late	Manzanillo	0.01	14.93	1.74	0.10	0.17	3.63	62.33	15.63	0.73	0.41	0.20	0.08	0.04
2005	Nthn NSW/Sthn Qld	Late	Picual	0.01	16.03	2.58	0.03	0.08	1.78	69.35	8.64	0.88	0.27	0.25	0.08	0.02
2005	Central Victoria	Early	Arbequina	0.02	18.43	2.73	0.05	0.14	1.10	60.13	16.15	0.62	0.29	0.23	0.07	0.02
2005	Central Victoria	Early	Barnea	0.01	11.61	0.72	0.03	0.06	1.68	74.23	10.60	0.55	0.26	0.19	0.05	0.01
2005	Central Victoria	Early	Barnea	0.01	14.28	1.12	0.03	0.05	1.48	66.42	15.21	0.59	0.48	0.23	0.06	0.03
2005	Central Victoria	Early	Coratina	0.00	11.73	0.38	0.03	0.05	1.47	77.72	7.28	0.65	0.25	0.33	0.06	0.03
2005	Central Victoria	Early	Frantoio	0.01	12.86	0.91	0.02	0.07	1.31	73.76	9.95	0.54	0.23	0.25	0.06	0.03
2005	Central Victoria	Early	Frantoio	0.01	15.68	1.28	0.03	0.07	1.26	66.27	14.09	0.59	0.38	0.25	0.06	0.03
2005	Central Victoria	Early	Leccino	0.01	14.99	1.36	0.03	0.07	1.18	75.38	5.81	0.66	0.19	0.24	0.05	0.03
2005	Central Victoria	Early	Picual	0.01	13.35	1.14	0.03	0.07	1.59	79.80	**2.84**	0.64	0.21	0.21	0.06	0.03
2005	Central Victoria	Early	Picual	0.01	13.51	1.11	0.03	0.07	1.59	79.01	3.59	0.60	0.21	0.20	0.06	0.02
2005	Central Victoria	Late	Barnea	0.01	8.98	0.56	0.05	0.10	2.05	76.47	10.47	0.56	0.35	0.24	0.10	0.04
2005	Central Victoria	Late	Coratina	0.01	8.70	0.35	0.04	0.06	1.69	79.56	7.94	0.69	0.34	0.44	0.11	0.05
2005	Central Victoria	Late	Frantoio	0.01	10.64	0.74	0.04	0.11	1.68	75.68	9.48	0.68	0.35	0.39	0.12	0.05
2005	Central Victoria	Late	Leccino	0.00	11.37	0.99	0.05	0.10	2.04	77.76	6.35	0.61	0.31	0.29	0.09	0.03

				% of total fatty acids												
Year	**Region**	**Harvest**	**Variety**	**C14:0**	**C16:0**	**C16:1**	**C17:0**	**C17:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:0**	**C20:1**	**C22:0**	**C24:0**
2005	Central Victoria	Late	Manzanillo	0.01	11.39	1.61	0.09	0.20	3.01	73.43	8.67	0.72	0.43	0.25	0.11	0.05
2005	Central Victoria	Late	Picual	0.00	9.00	0.70	0.05	0.08	3.25	80.88	4.61	0.64	0.38	0.25	0.11	0.05
2005	WA	Early	Barnea	0.01	13.15	0.83	0.03	0.05	1.91	71.07	11.94	0.48	0.27	0.18	0.06	0.03
2005	WA	Early	Coratina	0.01	13.68	0.43	0.03	0.06	1.53	74.58	8.12	0.73	0.29	0.43	0.07	0.04
2005	WA	Early	Corregiola	0.01	16.01	1.37	0.02	0.05	1.55	67.54	12.50	0.46	0.21	0.20	0.06	0.01
2005	WA	Early	Frantoio	0.01	16.35	1.36	0.02	0.06	1.61	64.72	14.84	0.53	0.19	0.22	0.05	0.02
2005	WA	Early	Koreneiki	0.01	13.29	0.83	0.02	0.04	2.01	76.48	6.22	0.48	0.27	0.24	0.08	0.03
2005	WA	Early	Leccino	0.01	14.31	1.01	0.02	0.05	1.31	75.16	7.17	0.51	0.18	0.21	0.04	0.01
2005	WA	Early	Manzanillo	0.01	14.89	1.32	0.11	0.18	2.42	73.73	6.33	0.47	0.27	0.17	0.06	0.03
2005	WA	Early	Nevadillo Blanco	0.01	13.66	0.88	0.13	0.25	1.62	70.60	11.73	0.59	0.24	0.24	0.05	0.00
2005	WA	Early	Picual	0.01	13.06	1.07	0.03	0.06	1.92	79.21	3.53	0.58	0.23	0.21	0.06	0.03
2005	WA	Late	Barnea	0.01	10.99	0.66	0.05	0.07	2.25	71.43	13.04	0.63	0.40	0.26	0.12	0.06
2005	WA	Late	Corregiola	0.01	12.91	0.91	0.04	0.08	2.11	71.47	10.92	0.63	0.40	0.32	0.13	0.05
2005	WA	Late	Frantoio	0.00	12.86	0.97	0.04	0.09	1.93	70.18	12.46	0.62	0.36	0.30	0.11	0.06
2005	WA	Late	Koreneiki	0.01	10.97	0.72	0.03	0.06	2.52	77.09	7.05	0.56	0.45	0.30	0.15	0.07
2005	WA	Late	Leccino	0.01	12.49	1.11	0.03	0.07	1.89	75.28	7.94	0.49	0.31	0.23	0.08	0.04
2005	WA	Late	Manzanillo	0.01	12.45	1.13	0.16	0.30	3.28	72.93	8.19	0.61	0.48	0.23	0.13	0.07
2005	WA	Late	Nevadillo Blanco	0.01	11.41	0.67	0.14	0.29	1.76	70.85	13.18	0.83	0.33	0.35	0.10	0.04
2005	WA	Late	Pendolino	0.01	13.24	0.88	0.03	0.08	1.23	73.88	9.32	0.64	0.25	0.29	0.09	0.05
2005	WA	Late	Picual	0.01	11.87	0.90	0.04	0.09	1.95	78.11	5.34	0.85	0.34	0.30	0.11	0.07
2005	Sthn Vic / Tasmania	Early	Arbequina	0.00	11.39	0.81	0.03	0.08	1.64	82.67	**2.23**	0.62	0.21	0.21	0.06	0.02
2005	Sthn Vic / Tasmania	Early	Barnea	0.00	6.75	0.39	0.06	0.09	2.46	81.20	7.65	0.63	0.35	0.24	0.11	0.05
2005	Sthn Vic / Tasmania	Early	Coratina	0.00	8.92	0.30	0.04	0.06	1.37	81.30	6.78	0.62	0.21	0.32	0.06	0.02
2005	Sthn Vic / Tasmania	Early	Corregiola	0.00	8.41	0.40	0.05	0.09	1.81	81.21	6.73	0.51	0.33	0.26	0.12	0.04
2005	Sthn Vic / Tasmania	Early	Frantoio	0.00	8.21	0.38	0.05	0.09	1.93	81.67	6.34	0.52	0.35	0.27	0.13	0.05
2005	Sthn Vic / Tasmania	Early	Leccino	0.01	13.40	1.25	0.03	0.07	1.24	77.48	5.58	0.53	0.16	0.20	0.04	0.02
2005	Sthn Vic / Tasmania	Early	Manzanillo	0.00	10.45	0.71	0.08	0.14	2.22	82.28	**2.98**	0.51	0.27	0.25	0.08	0.03
2005	Sthn Vic / Tasmania	Early	Picual	0.01	11.28	0.77	0.04	0.07	1.82	82.56	**2.28**	0.63	0.23	0.21	0.07	0.03
2005	Sthn Vic / Tasmania	Late	Arbequina	0.01	11.08	1.11	0.08	0.18	1.57	76.05	8.54	0.53	0.36	0.27	0.13	0.06
2005	Sthn Vic / Tasmania	Late	Coratina	0.01	7.78	0.29	0.05	0.08	1.72	82.06	6.53	0.64	0.33	0.37	0.11	0.04

				% of total fatty acids												
Year	**Region**	**Harvest**	**Variety**	**C14:0**	**C16:0**	**C16:1**	**C17:0**	**C17:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:0**	**C20:1**	**C22:0**	**C24:0**
2005	Sthn Vic / Tasmania	Late	Corregiola	0.00	7.43	0.34	0.04	0.09	1.99	82.30	6.49	0.51	0.33	0.31	0.12	0.04
2005	Sthn Vic / Tasmania	Late	Frantoio	0.00	7.48	0.31	0.05	0.10	2.12	81.54	7.04	0.54	0.35	0.32	0.11	0.03
2005	Sthn Vic / Tasmania	Late	Leccino	0.01	11.22	0.87	0.05	0.11	1.73	78.61	6.22	0.55	0.28	0.24	0.08	0.04
2005	Sthn Vic / Tasmania	Late	Picual	0.00	8.81	0.54	0.05	0.09	2.61	**83.69**	**2.89**	0.59	0.36	0.21	0.11	0.04
2006	Nthn NSW/Sthn Qld	Early	Arbequina	0.01	20.26	3.56	0.08	0.23	1.36	53.87	19.12	0.75	0.34	0.26	0.09	0.06
2006	Nthn NSW/Sthn Qld	Early	Barnea	0.00	15.02	1.30	0.04	0.07	1.87	63.25	17.00	0.74	0.34	0.25	0.08	0.05
2006	Nthn NSW/Sthn Qld	Early	Coratina	0.00	14.81	0.65	0.04	0.07	1.74	71.19	9.61	0.93	0.39	0.45	0.09	0.03
2006	Nthn NSW/Sthn Qld	Early	Corregiola	0.00	14.85	1.28	0.03	0.08	1.61	71.31	9.09	0.85	0.35	0.39	0.09	0.07
2006	Nthn NSW/Sthn Qld	Early	Frantoio	0.00	15.79	1.66	0.03	0.08	1.47	65.96	13.49	0.79	0.30	0.30	0.07	0.05
2006	Nthn NSW/Sthn Qld	Early	Hardys Mammoth	0.01	12.84	1.02	0.11	0.30	1.47	65.18	17.36	1.00	0.27	0.36	0.06	0.03
2006	Nthn NSW/Sthn Qld	Early	Koreneiki	0.00	14.24	1.11	0.04	0.07	2.05	74.90	5.84	0.85	0.40	0.32	0.11	0.07
2006	Nthn NSW/Sthn Qld	Early	Leccino	0.00	14.33	1.57	0.03	0.08	1.63	72.96	8.14	0.65	0.25	0.28	0.05	0.04
2006	Nthn NSW/Sthn Qld	Early	Manzanillo	0.00	16.60	2.16	0.11	0.21	3.19	62.23	13.66	0.95	0.46	0.25	0.10	0.07
2006	Nthn NSW/Sthn Qld	Early	Pendolino	0.00	18.24	1.28	0.03	0.08	1.32	58.46	18.05	1.71	0.30	0.37	0.09	0.07
2006	Nthn NSW/Sthn Qld	Early	Picual	0.00	15.74	2.38	0.04	0.12	1.62	72.74	5.53	1.04	0.31	0.34	0.09	0.07
2006	Nthn NSW/Sthn Qld	Late	Arbequina	0.02	19.94	4.08	0.08	0.21	1.39	52.19	20.73	0.71	0.32	0.21	0.09	0.04
2006	Nthn NSW/Sthn Qld	Late	Barnea	0.01	14.31	1.15	0.04	0.09	1.78	63.77	17.45	0.70	0.32	0.23	0.10	0.05
2006	Nthn NSW/Sthn Qld	Late	Coratina	0.01	12.49	0.53	0.04	0.06	1.77	72.88	10.57	0.75	0.35	0.41	0.09	0.05
2006	Nthn NSW/Sthn Qld	Late	Corregiola	0.01	14.24	1.33	0.03	0.10	1.59	71.29	9.80	0.79	0.32	0.35	0.10	0.06
2006	Nthn NSW/Sthn Qld	Late	Frantoio	0.01	14.92	1.53	0.03	0.08	1.49	69.15	11.31	0.72	0.31	0.30	0.10	0.06
2006	Nthn NSW/Sthn Qld	Late	Koreneiki	0.01	13.15	1.16	0.04	0.08	2.11	76.06	6.02	0.61	0.38	0.27	0.11	0.02
2006	Nthn NSW/Sthn Qld	Late	Leccino	0.01	14.19	1.51	0.03	0.09	1.76	72.26	8.85	0.69	0.25	0.27	0.06	0.02
2006	Nthn NSW/Sthn Qld	Late	Manzanillo	0.01	14.51	1.60	0.14	0.25	3.82	64.29	13.74	0.77	0.47	0.22	0.11	0.07
2006	Nthn NSW/Sthn Qld	Late	Pendolino	0.01	16.59	1.08	0.03	0.06	1.18	64.03	15.47	0.94	0.22	0.28	0.07	0.04
2006	Nthn NSW/Sthn Qld	Late	Picual	0.01	14.03	1.63	0.04	0.09	2.06	75.81	4.92	0.72	0.31	0.24	0.09	0.05
2006	Central Victoria	Early	Arbequina	0.01	15.82	2.02	0.09	0.23	1.52	68.12	10.89	0.65	0.35	0.15	0.10	0.06
2006	Central Victoria	Early	Arbequina	0.01	13.47	1.31	0.13	0.27	1.67	73.63	8.14	0.56	0.35	0.29	0.11	0.06
2006	Central Victoria	Early	Barnea	0.00	12.91	1.14	0.05	0.09	1.78	72.08	10.57	0.65	0.31	0.28	0.09	0.06
2006	Central Victoria	Early	Barnea	0.00	10.85	0.81	0.05	0.10	1.97	76.10	8.85	0.57	0.32	0.25	0.09	0.04
2006	Central Victoria	Early	Coratina	0.00	12.50	0.47	0.04	0.08	1.45	75.63	8.15	0.75	0.31	0.47	0.08	0.05

				% of total fatty acids												
Year	**Region**	**Harvest**	**Variety**	**C14:0**	**C16:0**	**C16:1**	**C17:0**	**C17:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:0**	**C20:1**	**C22:0**	**C24:0**
2006	Central Victoria	Early	Coratina	0.00	11.24	0.40	0.05	0.08	1.86	79.15	5.37	0.82	0.40	0.45	0.11	0.06
2006	Central Victoria	Early	Frantoio	0.00	13.02	1.04	0.03	0.10	1.40	72.07	10.91	0.64	0.30	0.36	0.09	0.05
2006	Central Victoria	Early	Koreneiki	0.00	11.46	0.82	0.04	0.07	2.25	79.59	4.33	0.59	0.40	0.28	0.11	0.04
2006	Central Victoria	Early	Leccino	0.00	13.71	1.01	0.05	0.10	1.75	77.12	4.79	0.70	0.31	0.33	0.08	0.05
2006	Central Victoria	Early	Manzanillo	0.01	13.62	1.29	0.10	0.21	2.44	75.28	5.60	0.64	0.38	0.28	0.10	0.06
2006	Central Victoria	Early	Pendolino	0.00	12.59	0.67	0.03	0.09	1.19	76.85	6.83	1.00	0.25	0.35	0.09	0.04
2006	Central Victoria	Early	Picual	0.00	12.71	1.18	0.04	0.10	1.90	80.12	2.49	0.71	0.31	0.29	0.08	0.06
2006	Central Victoria	Early	Picual	0.00	11.31	0.80	0.04	0.08	2.42	81.81	2.21	0.61	0.33	0.25	0.09	0.05
2006	Central Victoria	Late	Arbequina	0.01	13.51	1.54	0.08	0.22	1.50	72.44	9.35	0.55	0.32	0.32	0.11	0.06
2006	Central Victoria	Late	Arbequina	0.01	12.90	1.39	0.10	0.23	1.53	74.08	8.31	0.59	0.33	0.34	0.12	0.07
2006	Central Victoria	Late	Barnea	0.01	11.43	0.93	0.04	0.08	1.68	71.50	13.01	0.62	0.32	0.25	0.09	0.04
2006	Central Victoria	Late	Barnea	0.00	9.99	0.73	0.05	0.10	1.94	75.56	10.38	0.58	0.32	0.24	0.09	0.04
2006	Central Victoria	Late	Coratina	0.00	10.83	0.45	0.02	0.09	1.39	78.08	7.60	0.69	0.28	0.46	0.08	0.04
2006	Central Victoria	Late	Frantoio	0.00	12.29	1.01	0.04	0.09	1.72	72.34	11.26	0.57	0.28	0.30	0.08	0.04
2006	Central Victoria	Late	Koreneiki	0.01	10.68	0.75	0.04	0.07	2.42	80.01	4.73	0.50	0.38	0.27	0.11	0.04
2006	Central Victoria	Late	Leccino	0.01	12.77	1.03	0.03	0.11	1.67	78.06	5.08	0.59	0.28	0.26	0.08	0.02
2006	Central Victoria	Late	Manzanillo	0.00	12.45	1.30	0.12	0.24	3.11	76.34	5.12	0.59	0.38	0.23	0.10	0.03
2006	Central Victoria	Late	Pendolino	0.00	11.82	0.74	0.03	0.11	1.25	77.28	7.30	0.80	0.23	0.33	0.07	0.03
2006	Central Victoria	Late	Picual	0.00	10.87	0.97	0.04	0.09	2.86	80.73	3.14	0.62	0.32	0.23	0.08	0.04
2006	WA	Early	Arbequina	0.01	17.29	2.05	0.10	0.23	1.54	60.96	16.35	0.69	0.35	0.27	0.11	0.05
2006	WA	Early	Barnea	0.01	12.54	1.06	0.04	0.08	1.88	69.21	13.89	0.59	0.33	0.23	0.09	0.04
2006	WA	Early	Coratina	0.00	13.14	0.44	0.05	0.08	1.73	75.80	6.79	0.93	0.39	0.49	0.11	0.06
2006	WA	Early	Corregiola	0.01	13.60	1.18	0.04	0.09	1.60	72.09	10.04	0.61	0.30	0.30	0.09	0.05
2006	WA	Early	Frantoio	0.01	13.73	1.07	0.04	0.09	1.66	72.55	9.35	0.69	0.33	0.33	0.09	0.06
2006	WA	Early	Leccino	0.01	13.56	1.08	0.04	0.07	1.75	75.72	6.46	0.64	0.28	0.26	0.07	0.04
2006	WA	Early	Manzanillo	0.01	12.66	1.37	0.13	0.26	3.21	71.02	9.84	0.69	0.42	0.24	0.10	0.06
2006	WA	Early	Nevadillo Blanco	0.01	12.67	0.71	0.15	0.31	1.64	71.00	11.91	0.83	0.31	0.34	0.09	0.05
2006	WA	Early	Pendolino	0.00	13.63	0.77	0.03	0.09	1.10	73.04	9.63	0.98	0.23	0.37	0.08	0.05
2006	WA	Early	Picual	0.01	12.82	1.43	0.04	0.10	2.54	76.92	4.66	0.75	0.34	0.28	0.08	0.04
2006	WA	Late	Arbequina	0.01	15.23	1.65	0.08	0.18	1.45	65.80	14.27	0.57	0.32	0.28	0.11	0.05

				% of total fatty acids												
Year	**Region**	**Harvest**	**Variety**	**C14:0**	**C16:0**	**C16:1**	**C17:0**	**C17:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:0**	**C20:1**	**C22:0**	**C24:0**
2006	WA	Late	Barnea	0.01	10.04	0.73	0.05	0.08	2.01	71.62	14.11	0.63	0.35	0.25	0.10	0.01
2006	WA	Late	Coratina	0.01	10.50	0.38	0.05	0.07	2.05	78.62	6.56	0.75	0.41	0.45	0.12	0.05
2006	WA	Late	Corregiola	0.01	11.66	1.05	0.04	0.11	1.84	75.27	8.71	0.52	0.32	0.32	0.10	0.05
2006	WA	Late	Frantoio	0.01	12.29	0.89	0.04	0.10	1.68	73.68	9.81	0.62	0.32	0.39	0.11	0.05
2006	WA	Late	Koreneiki	0.01	10.47	0.79	0.04	0.07	2.33	76.06	8.98	0.49	0.36	0.25	0.11	0.04
2006	WA	Late	Leccino	0.01	11.96	1.02	0.04	0.07	2.36	75.52	7.81	0.57	0.31	0.24	0.08	0.03
2006	WA	Late	Manzanillo	0.01	11.51	1.18	0.13	0.26	3.19	71.84	10.32	0.69	0.44	0.27	0.11	0.06
2006	WA	Late	Nevadillo Blanco	0.01	10.91	0.64	0.14	0.31	1.63	73.03	12.18	0.41	0.28	0.35	0.09	0.03
2006	WA	Late	Pendolino	0.00	11.53	0.85	0.03	0.11	1.09	76.73	8.31	0.74	0.21	0.32	0.07	0.01
2006	WA	Late	Picual	0.00	9.15	0.77	0.05	0.10	3.43	80.53	4.78	0.55	0.34	0.21	0.08	0.02
2006	Sthn Vic / Tasmania	Early	Arbequina	0.00	8.87	0.55	0.05	0.09	2.35	84.15	2.51	0.75	0.32	0.24	0.10	0.02
2006	Sthn Vic / Tasmania	Early	Barnea	0.00	9.18	0.59	0.06	0.10	2.23	78.04	8.49	0.61	0.35	0.22	0.10	0.04
2006	Sthn Vic / Tasmania	Early	Coratina	0.00	8.89	0.31	0.06	0.09	1.94	81.92	4.94	0.85	0.37	0.45	0.12	0.06
2006	Sthn Vic / Tasmania	Early	Corregiola	0.00	10.22	0.64	0.05	0.12	1.91	78.13	7.49	0.61	0.33	0.37	0.11	0.02
2006	Sthn Vic / Tasmania	Early	Frantoio	0.00	10.34	0.57	0.05	0.11	1.64	79.17	6.58	0.68	0.33	0.35	0.12	0.04
2006	Sthn Vic / Tasmania	Early	Frantoio	0.00	9.60	0.40	0.06	0.07	2.37	79.78	6.10	0.73	0.40	0.33	0.13	0.04
2006	Sthn Vic / Tasmania	Early	Leccino	0.01	12.23	0.88	0.05	0.11	1.81	77.48	6.11	0.68	0.28	0.25	0.08	0.03
2006	Sthn Vic / Tasmania	Early	Leccino	0.01	10.87	0.61	0.05	0.08	2.30	77.76	7.04	0.61	0.32	0.26	0.08	0.01
2006	Sthn Vic / Tasmania	Early	Pendolino	0.00	9.92	0.46	0.04	0.09	1.44	80.48	5.93	0.93	0.25	0.33	0.10	0.03
2006	Sthn Vic / Tasmania	Early	Pendolino	0.00	9.64	0.42	0.03	0.10	1.46	79.32	7.55	0.78	0.25	0.33	0.08	0.03
2006	Sthn Vic / Tasmania	Early	Picual	0.00	8.73	0.47	0.05	0.09	2.70	84.00	2.55	0.68	0.36	0.25	0.10	0.02
2006	Sthn Vic / Tasmania	Late	Frantoio	0.00	9.63	0.45	0.05	0.10	2.08	79.33	7.32	0.30	0.34	0.27	0.12	0.02
2006	Sthn Vic / Tasmania	Late	Leccino	0.01	10.47	0.55	0.05	0.09	2.20	78.24	7.17	0.56	0.32	0.23	0.09	0.02
2006	Sthn Vic / Tasmania	Late	Pendolino	0.00	9.54	0.40	0.05	0.10	1.46	78.45	8.55	0.76	0.24	0.33	0.08	0.03
	IOOC LIMITS			**<0.05**	**7.5-20.0**	**0.3-3.5**	**<0.3**	**<0.3**	**0.5-5.0**	**55.0-83.0**	**3.5-21.0**	**<1.0**	**<0.6**	**<0.4**	**<0.2**	**<0.2**

Trans Fatty Acids

Year	Region	Harvest	Variety	% C18:1T	% C18:2T + C18:3T	% Total trans
2005	Nthn NSW/Sthn Qld	Early	Arbequina	0.000	0.016	0.016
2005	Nthn NSW/Sthn Qld	Early	Barnea	0.000	0.017	0.017
2005	Nthn NSW/Sthn Qld	Early	Coratina	0.000	0.006	0.006
2005	Nthn NSW/Sthn Qld	Early	Corregiola	0.000	0.010	0.010
2005	Nthn NSW/Sthn Qld	Early	Frantoio	0.000	0.013	0.013
2005	Nthn NSW/Sthn Qld	Early	Leccino	0.005	0.006	0.010
2005	Nthn NSW/Sthn Qld	Early	Manzanillo	0.005	0.012	0.017
2005	Nthn NSW/Sthn Qld	Late	Arbequina	0.000	0.014	0.014
2005	Nthn NSW/Sthn Qld	Late	Barnea	0.004	0.031	0.035
2005	Nthn NSW/Sthn Qld	Late	Coratina	0.000	0.012	0.012
2005	Nthn NSW/Sthn Qld	Late	Corregiola	0.000	0.019	0.019
2005	Nthn NSW/Sthn Qld	Late	Frantoio	0.000	0.023	0.023
2005	Nthn NSW/Sthn Qld	Late	Koreneiki	0.004	0.007	0.011
2005	Nthn NSW/Sthn Qld	Late	Leccino	0.000	0.011	0.011
2005	Nthn NSW/Sthn Qld	Late	Manzanillo	0.004	0.017	0.021
2005	Nthn NSW/Sthn Qld	Late	Picual	0.005	0.014	0.019
2005	Central Victoria	Early	Arbequina	0.000	0.006	0.006
2005	Central Victoria	Early	Barnea	0.000	0.007	0.007
2005	Central Victoria	Early	Barnea	0.000	0.012	0.012
2005	Central Victoria	Early	Coratina	0.000	0.008	0.008
2005	Central Victoria	Early	Frantoio	0.000	0.006	0.006
2005	Central Victoria	Early	Frantoio	0.000	0.012	0.012
2005	Central Victoria	Early	Leccino	0.000	0.000	0.000
2005	Central Victoria	Early	Picual	0.000	0.000	0.000
2005	Central Victoria	Early	Picual	0.000	0.000	0.000
2005	Central Victoria	Late	Barnea	0.000	0.012	0.012
2005	Central Victoria	Late	Coratina	0.000	0.005	0.005
2005	Central Victoria	Late	Frantoio	0.000	0.014	0.014
2005	Central Victoria	Late	Leccino	0.000	0.010	0.010
2005	Central Victoria	Late	Manzanillo	0.005	0.012	0.017
2005	Central Victoria	Late	Picual	0.000	0.014	0.014
2005	WA	Early	Barnea	0.000	0.010	0.010
2005	WA	Early	Coratina	0.000	0.008	0.008
2005	WA	Early	Corregiola	0.000	0.006	0.006
2005	WA	Early	Frantoio	0.000	0.015	0.015
2005	WA	Early	Koreneiki	0.000	0.000	0.000
2005	WA	Early	Leccino	0.000	0.006	0.006
2005	WA	Early	Manzanillo	0.000	0.000	0.000
2005	WA	Early	Nevadillo Blanco	0.000	0.006	0.006
2005	WA	Early	Picual	0.000	0.000	0.000
2005	WA	Late	Barnea	0.007	0.019	0.026
2005	WA	Late	Corregiola	0.000	0.016	0.016
2005	WA	Late	Frantoio	0.009	0.013	0.022
2005	WA	Late	Koreneiki	0.000	0.004	0.004
2005	WA	Late	Leccino	0.000	0.011	0.011
2005	WA	Late	Manzanillo	0.012	0.018	0.029
2005	WA	Late	Nevadillo Blanco	0.008	0.025	0.032
2005	WA	Late	Pendolino	0.000	0.011	0.011
2005	WA	Late	Picual	0.006	0.006	0.012
2005	Sthn Vic / Tasmania	Early	Arbequina	0.000	0.000	0.000

Year	Region	Harvest	Variety	% C18:1T	% C18:2T + C18:3T	% Total trans
2005	Sthn Vic / Tasmania	Early	Barnea	0.000	0.008	0.008
2005	Sthn Vic / Tasmania	Early	Coratina	0.000	0.000	0.000
2005	Sthn Vic / Tasmania	Early	Corregiola	0.000	0.008	0.008
2005	Sthn Vic / Tasmania	Early	Frantoio	0.000	0.007	0.007
2005	Sthn Vic / Tasmania	Early	Leccino	0.000	0.004	0.004
2005	Sthn Vic / Tasmania	Early	Manzanillo	0.000	0.000	0.000
2005	Sthn Vic / Tasmania	Early	Picual	0.000	0.000	0.000
2005	Sthn Vic / Tasmania	Late	Arbequina	0.009	0.006	0.015
2005	Sthn Vic / Tasmania	Late	Coratina	0.000	0.000	0.000
2005	Sthn Vic / Tasmania	Late	Corregiola	0.000	0.007	0.007
2005	Sthn Vic / Tasmania	Late	Frantoio	0.000	0.003	0.003
2005	Sthn Vic / Tasmania	Late	Leccino	0.000	0.007	0.007
2005	Sthn Vic / Tasmania	Late	Picual	0.000	0.000	0.000
2006	Nthn NSW/Sthn Qld	Early	Arbequina	0.005	0.037	0.042
2006	Nthn NSW/Sthn Qld	Early	Barnea	0.004	0.027	0.031
2006	Nthn NSW/Sthn Qld	Early	Coratina	0.004	0.025	0.029
2006	Nthn NSW/Sthn Qld	Early	Corregiola	0.000	0.020	0.020
2006	Nthn NSW/Sthn Qld	Early	Frantoio	0.000	0.021	0.021
2006	Nthn NSW/Sthn Qld	Early	Hardys Mammoth	0.004	0.027	0.031
2006	Nthn NSW/Sthn Qld	Early	Koreneiki	0.005	0.006	0.011
2006	Nthn NSW/Sthn Qld	Early	Leccino	0.000	0.015	0.015
2006	Nthn NSW/Sthn Qld	Early	Manzanillo	0.006	0.019	0.025
2006	Nthn NSW/Sthn Qld	Early	Pendolino	0.005	0.031	0.036
2006	Nthn NSW/Sthn Qld	Early	Picual	0.005	0.010	0.015
2006	Nthn NSW/Sthn Qld	Late	Arbequina	0.007	0.031	0.037
2006	Nthn NSW/Sthn Qld	Late	Barnea	0.005	0.028	0.033
2006	Nthn NSW/Sthn Qld	Late	Coratina	0.005	0.017	0.022
2006	Nthn NSW/Sthn Qld	Late	Corregiola	0.004	0.019	0.023
2006	Nthn NSW/Sthn Qld	Late	Frantoio	0.008	0.020	0.028
2006	Nthn NSW/Sthn Qld	Late	Koreneiki	0.004	0.011	0.015
2006	Nthn NSW/Sthn Qld	Late	Leccino	0.006	0.013	0.019
2006	Nthn NSW/Sthn Qld	Late	Manzanillo	0.004	0.024	0.028
2006	Nthn NSW/Sthn Qld	Late	Pendolino	0.006	0.019	0.025
2006	Nthn NSW/Sthn Qld	Late	Picual	0.006	0.008	0.013
2006	Central Victoria	Early	Coratina	0.000	0.012	0.012
2006	Central Victoria	Early	Picual	0.004	0.000	0.004
2006	Central Victoria	Early	Barnea	0.004	0.013	0.017
2006	Central Victoria	Early	Arbequina	0.005	0.015	0.020
2006	Central Victoria	Early	Koreneiki	0.004	0.006	0.010
2006	Central Victoria	Early	Leccino	0.005	0.007	0.011
2006	Central Victoria	Early	Frantoio	0.000	0.015	0.015
2006	Central Victoria	Early	Picual	0.000	0.000	0.000
2006	Central Victoria	Early	Arbequina	0.004	0.009	0.013
2006	Central Victoria	Early	Barnea	0.004	0.012	0.016
2006	Central Victoria	Early	Coratina	0.000	0.010	0.010
2006	Central Victoria	Early	Pendolino	0.000	0.008	0.008
2006	Central Victoria	Early	Manzanillo	0.005	0.008	0.013
2006	Central Victoria	Late	Arbequina	0.007	0.009	0.016
2006	Central Victoria	Late	Arbequina	0.008	0.008	0.016
2006	Central Victoria	Late	Barnea	0.008	0.015	0.023
2006	Central Victoria	Late	Barnea	0.008	0.013	0.021
2006	Central Victoria	Late	Coratina	0.000	0.000	0.000
2006	Central Victoria	Late	Frantoio	0.007	0.014	0.022

Year	Region	Harvest	Variety	% C18:1T	% C18:2T + C18:3T	% Total trans
2006	Central Victoria	Late	Koreneiki	0.010	0.007	0.017
2006	Central Victoria	Late	Leccino	0.008	0.000	0.008
2006	Central Victoria	Late	Manzanillo	0.008	0.000	0.008
2006	Central Victoria	Late	Pendolino	0.005	0.014	0.019
2006	Central Victoria	Late	Picual	0.010	0.007	0.017
2006	WA	Early	Arbequina	0.006	0.026	0.032
2006	WA	Early	Barnea	0.004	0.020	0.024
2006	WA	Early	Coratina	0.000	0.013	0.013
2006	WA	Early	Corregiola	0.007	0.015	0.022
2006	WA	Early	Frantoio	0.008	0.017	0.025
2006	WA	Early	Leccino	0.004	0.012	0.016
2006	WA	Early	Manzanillo	0.005	0.010	0.015
2006	WA	Early	Nevadillo Blanco	0.006	0.015	0.021
2006	WA	Early	Pendolino	0.000	0.011	0.011
2006	WA	Early	Picual	0.005	0.004	0.008
2006	WA	Late	Arbequina	0.006	0.016	0.022
2006	WA	Late	Barnea	0.007	0.016	0.023
2006	WA	Late	Coratina	0.010	0.007	0.017
2006	WA	Late	Corregiola	0.006	0.011	0.016
2006	WA	Late	Frantoio	0.000	0.014	0.014
2006	WA	Late	Koreneiki	0.009	0.012	0.021
2006	WA	Late	Leccino	0.006	0.010	0.015
2006	WA	Late	Manzanillo	0.000	0.014	0.014
2006	WA	Late	Nevadillo Blanco	0.007	0.014	0.021
2006	WA	Late	Pendolino	0.000	0.010	0.010
2006	WA	Late	Picual	0.008	0.006	0.014
2006	Sthn Vic / Tasmania	Early	Arbequina	0.008	0.000	0.008
2006	Sthn Vic / Tasmania	Early	Barnea	0.003	0.007	0.010
2006	Sthn Vic / Tasmania	Early	Coratina	0.004	0.004	0.008
2006	Sthn Vic / Tasmania	Early	Corregiola	0.000	0.010	0.010
2006	Sthn Vic / Tasmania	Early	Frantoio	0.000	0.007	0.007
2006	Sthn Vic / Tasmania	Early	Frantoio	0.000	0.000	0.000
2006	Sthn Vic / Tasmania	Early	Leccino	0.004	0.008	0.012
2006	Sthn Vic / Tasmania	Early	Leccino	0.008	0.000	0.008
2006	Sthn Vic / Tasmania	Early	Pendolino	0.000	0.000	0.000
2006	Sthn Vic / Tasmania	Early	Pendolino	0.000	0.008	0.008
2006	Sthn Vic / Tasmania	Early	Picual	0.007	0.000	0.007
2006	Sthn Vic / Tasmania	Late	Frantoio	0.007	0.007	0.014
2006	Sthn Vic / Tasmania	Late	Leccino	0.000	0.007	0.007
2006	Sthn Vic / Tasmania	Late	Pendolino	0.000	0.007	0.007
	IOOC LIMITS				**<0.05**	**<0.05**

Waxes, stigmastadienes, ECN 42, tocopherols and UV absorption

Year	Region	Harvest	Variety	Wax Content (mg/kg oil)	Stigmastadiene Content (mg/kg oil)	difference between theoretical and acual ECN42 triglyceride content	α tocopherol (mg/kg oil)	Delta K	Spec. Ext. 270nm
2005	Nthn NSW/Sthn Qld	Early	Arbequina	221	0.000	0.18	274	0.000	0.132
2005	Nthn NSW/Sthn Qld	Early	Barnea	119	0.000	0.19	285	-0.001	0.109
2005	Nthn NSW/Sthn Qld	Early	Coratina	36	0.000	0.03	494	-0.004	0.156
2005	Nthn NSW/Sthn Qld	Early	Corregiola	204	0.000	0.14	270	0.000	0.115
2005	Nthn NSW/Sthn Qld	Early	Frantoio	127	0.080	0.12	221	0.001	0.065
2005	Nthn NSW/Sthn Qld	Early	Leccino	90	0.000	0.12	501	0.001	0.077
2005	Nthn NSW/Sthn Qld	Early	Manzanillo	245	0.000	0.19	111	0.001	0.078
2005	Nthn NSW/Sthn Qld	Late	Arbequina	199	n/a	0.15	247	-0.001	0.127
2005	Nthn NSW/Sthn Qld	Late	Barnea	174	0.000	0.78	194	0.003	0.104
2005	Nthn NSW/Sthn Qld	Late	Coratina	39	0.000	0.13	506	-0.006	0.179
2005	Nthn NSW/Sthn Qld	Late	Corregiola	123	0.000	0.25	289	0.002	0.085
2005	Nthn NSW/Sthn Qld	Late	Frantoio	148	0.022	0.17	256	0.002	0.131
2005	Nthn NSW/Sthn Qld	Late	Koreneiki	68	0.000	0.15	301	-0.003	0.099
2005	Nthn NSW/Sthn Qld	Late	Leccino	77	0.038	0.15	372	0.000	0.084
2005	Nthn NSW/Sthn Qld	Late	Manzanillo	184	0.018	0.24	62	0.001	0.088
2005	Nthn NSW/Sthn Qld	Late	Picual	142	n/a	0.27	317	0.000	0.104
2005	Central Victoria	Early	Arbequina	137	n/a	0.12	341	0.001	0.097
2005	Central Victoria	Early	Barnea	69	n/a	0.07	291	-0.001	0.079
2005	Central Victoria	Early	Barnea	99	0.089	0.18	308	0.002	0.142
2005	Central Victoria	Early	Coratina	13	0.043	0.02	336	-0.003	0.114
2005	Central Victoria	Early	Frantoio	70	0.096	0.03	205	0.000	0.080
2005	Central Victoria	Early	Frantoio	124	0.115	0.02	264	0.001	0.113
2005	Central Victoria	Early	Leccino	193	n/a	0.13	449	-0.001	0.066
2005	Central Victoria	Early	Picual	50	0.044	0.01	241	0.000	0.141
2005	Central Victoria	Early	Picual	52	n/a	0.10	248	-0.001	0.046
2005	Central Victoria	Late	Barnea	47	0.000	0.01	205	0.000	0.066
2005	Central Victoria	Late	Coratina	8	0.020	0.05	251	-0.002	0.074
2005	Central Victoria	Late	Frantoio	55	0.000	0.03	138	0.000	0.106
2005	Central Victoria	Late	Leccino	57	0.000	0.03	331	-0.001	0.037
2005	Central Victoria	Late	Manzanillo	44	0.029	0.07	61	0.001	0.086
2005	Central Victoria	Late	Picual	40	0.000	0.01	224	**-0.001**	**0.037**
2005	WA	Early	Barnea	128	0.000	0.08	256	-0.001	0.083
2005	WA	Early	Coratina	17	0.000	0.10	364	-0.003	0.128
2005	WA	Early	Corregiola	85	0.000	0.15	170	0.000	0.092
2005	WA	Early	Frantoio	210	0.026	0.07	180	0.002	0.100
2005	WA	Early	Koreneiki	77	0.000	0.08	229	-0.004	0.151
2005	WA	Early	Leccino	41	0.000	0.08	429	0.000	0.045
2005	WA	Early	Manzanillo	57	n/a	0.06	70	-0.002	0.051
2005	WA	Early	Nevadillo	35	0.011	0.17	143	-0.001	0.088

Year	Region	Harvest	Variety	Wax Content (mg/kg oil)	Stigmastadiene Content (mg/kg oil)	difference between theoretical and acual ECN42 triglyceride content	α tocopherol (mg/kg oil)	Delta K	Spec. Ext. 270nm
			Blanco						
2005	WA	Early	Picual	41	0.034	0.10	235	-0.003	0.057
2005	WA	Late	Barnea	88	0.000	0.04	255	-0.001	0.070
2005	WA	Late	Corregiola	81	0.042	0.01	167	0.000	0.149
2005	WA	Late	Frantoio	81	0.097	0.12	173	0.001	0.082
2005	WA	Late	Koreneiki	55	n/a	0.07	219	-0.002	0.094
2005	WA	Late	Leccino	49	0.032	0.03	344	-0.001	0.044
2005	WA	Late	Manzanillo	72	0.062	0.02	72	-0.002	0.056
2005	WA	Late	Nevadillo Blanco	47	n/a	0.03	158	-0.001	0.139
2005	WA	Late	Pendolino	55	n/a	0.06	225	0.000	0.068
2005	WA	Late	Picual	91	0.000	0.10	252	-0.002	0.055
2005	Sthn Vic / Tasmania	Early	Arbequina	30	0.000	0.04	291	-0.004	0.134
2005	Sthn Vic / Tasmania	Early	Barnea	72	0.000	0.06	258	-0.006	0.123
2005	Sthn Vic / Tasmania	Early	Coratina	16	0.036	0.04	249	-0.002	0.139
2005	Sthn Vic / Tasmania	Early	Corregiola	51	0.000	0.02	155	-0.003	0.172
2005	Sthn Vic / Tasmania	Early	Frantoio	53	0.038	0.07	167	-0.002	0.134
2005	Sthn Vic / Tasmania	Early	Leccino	49	0.000	0.03	340	-0.001	0.115
2005	Sthn Vic / Tasmania	Early	Manzanillo	20	0.093	0.09	214	-0.007	0.217
2005	Sthn Vic / Tasmania	Early	Picual	38	0.000	0.06	268	-0.004	0.097
2005	Sthn Vic / Tasmania	Late	Arbequina	96	0.061	0.05	229	0.000	0.099
2005	Sthn Vic / Tasmania	Late	Coratina	36	0.000	0.03	209	-0.002	0.110
2005	Sthn Vic / Tasmania	Late	Corregiola	30	0.000	0.11	108	-0.002	0.098
2005	Sthn Vic / Tasmania	Late	Frantoio	43	0.015	0.09	81	-0.002	0.078
2005	Sthn Vic / Tasmania	Late	Leccino	34	0.059	0.03	346	-0.001	0.070
2005	Sthn Vic / Tasmania	Late	Picual	48	0.000	0.03	237	-0.004	0.068
2006	Nthn NSW/Sthn Qld	Early	Arbequina	145	0.142	0.076	282	-0.001	0.117
2006	Nthn NSW/Sthn Qld	Early	Barnea	121	0.072	0.168	279	-0.002	0.106
2006	Nthn NSW/Sthn Qld	Early	Coratina	49	0.029	0.072	512	-0.005	0.135
2006	Nthn NSW/Sthn Qld	Early	Corregiola	112	0.071	0.081	340	-0.002	0.067
2006	Nthn NSW/Sthn Qld	Early	Frantoio	113	0.076	0.121	275	-0.001	0.099
2006	Nthn NSW/Sthn Qld	Early	Hardys Mammoth	103	0.026	0.357	234	-0.001	0.081
2006	Nthn NSW/Sthn Qld	Early	Koreneiki	115	0.068	0.131	472	-0.006	0.144
2006	Nthn NSW/Sthn Qld	Early	Leccino	82	0.083	0.168	447	-0.001	0.048
2006	Nthn NSW/Sthn Qld	Early	Manzanillo	243	n/a	0.059	182	0.000	0.125
2006	Nthn NSW/Sthn Qld	Early	Pendolino	223	n/a	0.173	776	-0.003	0.137
2006	Nthn NSW/Sthn Qld	Early	Picual	169	n/a	0.112	387	-0.001	0.084
2006	Nthn NSW/Sthn Qld	Late	Arbequina	151	0.031	0.058	210	0.004	0.176
2006	Nthn NSW/Sthn Qld	Late	Barnea	137	0.071	0.057	258	0.004	0.108
2006	Nthn NSW/Sthn Qld	Late	Coratina	31	0.040	0.097	410	0.001	0.118
2006	Nthn NSW/Sthn Qld	Late	Corregiola	145	0.074	0.178	250	0.003	0.073

Year	Region	Harvest	Variety	Wax Content (mg/kg oil)	Stigmastadiene Content (mg/kg oil)	difference between theoretical and acual ECN42 triglyceride content	α tocopherol (mg/kg oil)	Delta K	Spec. Ext. 270nm
2006	Nthn NSW/Sthn Qld	Late	Frantoio	102	0.073	0.013	251	0.003	0.085
2006	Nthn NSW/Sthn Qld	Late	Koreneiki	70	0.065	0.062	286	0.001	0.099
2006	Nthn NSW/Sthn Qld	Late	Leccino	60	0.074	0.068	529	0.003	0.069
2006	Nthn NSW/Sthn Qld	Late	Manzanillo	111	0.071	0.123	63	0.004	0.096
2006	Nthn NSW/Sthn Qld	Late	Pendolino	110	0.070	0.019	360	0.003	0.097
2006	Nthn NSW/Sthn Qld	Late	Picual	79	0.080	0.030	270	0.004	0.067
2006	Central Victoria	Early	Arbequina	146	0.074	0.075	376	-0.001	0.072
2006	Central Victoria	Early	Arbequina	111	0.040	0.111	291	-0.003	0.087
2006	Central Victoria	Early	Barnea	83	0.048	0.038	335	-0.003	0.085
2006	Central Victoria	Early	Barnea	89	0.059	0.041	291	-0.002	0.079
2006	Central Victoria	Early	Coratina	31	0.051	0.115	312	0.000	0.085
2006	Central Victoria	Early	Coratina	30	0.082	0.036	333	-0.005	0.152
2006	Central Victoria	Early	Frantoio	69	0.034	0.129	227	0.000	0.074
2006	Central Victoria	Early	Koreneiki	39	0.033	0.077	281	-0.009	0.197
2006	Central Victoria	Early	Leccino	63	0.065	0.058	407	-0.002	0.092
2006	Central Victoria	Early	Manzanillo	62	0.056	0.006	225	-0.002	0.066
2006	Central Victoria	Early	Pendolino	93	0.125	0.117	386	-0.002	0.089
2006	Central Victoria	Early	Picual	34	0.083	0.108	317	-0.003	0.074
2006	Central Victoria	Early	Picual	34	0.018	0.070	280	-0.006	0.129
2006	Central Victoria	Late	Arbequina	90	0.028	0.151	274	-0.002	0.075
2006	Central Victoria	Late	Arbequina	96	0.031	0.082	292	-0.004	0.090
2006	Central Victoria	Late	Barnea	99	0.026	0.096	285	-0.001	0.069
2006	Central Victoria	Late	Barnea	60	0.030	0.006	312	-0.004	0.067
2006	Central Victoria	Late	Coratina	23	0.019	0.004	300	0.000	0.050
2006	Central Victoria	Late	Frantoio	65	0.040	0.026	213	-0.002	0.074
2006	Central Victoria	Late	Koreneiki	43	0.033	0.050	221	-0.006	0.153
2006	Central Victoria	Late	Leccino	49	0.046	0.043	371	-0.002	0.058
2006	Central Victoria	Late	Manzanillo	55	0.025	0.027	170	-0.003	0.087
2006	Central Victoria	Late	Pendolino	53	0.026	0.004	337	-0.002	0.062
2006	Central Victoria	Late	Picual	36	0.042	0.176	270	-0.003	0.081
2006	WA	Early	Arbequina	202	0.044	0.132	248	0.000	0.106
2006	WA	Early	Barnea	124	0.044	0.083	220	-0.001	0.073
2006	WA	Early	Coratina	28	0.024	0.136	389	-0.004	0.140
2006	WA	Early	Corregiola	88	0.057	0.047	176	0.003	0.081
2006	WA	Early	Frantoio	75	0.099	0.015	200	-0.001	0.097
2006	WA	Early	Leccino	69	0.055	0.041	405	-0.001	0.086
2006	WA	Early	Manzanillo	138	0.072	0.187	77	0.004	0.085
2006	WA	Early	Nevadillo Blanco	63	0.027	0.045	156	-0.001	0.163
2006	WA	Early	Pendolino	137	0.067	0.026	305	0.000	0.148
2006	WA	Early	Picual	85	0.060	0.087	266	-0.001	0.043

Year	Region	Harvest	Variety	Wax Content (mg/kg oil)	Stigmastadiene Content (mg/kg oil)	difference between theoretical and acual ECN42 triglyceride content	α tocopherol (mg/kg oil)	Delta K	Spec. Ext. 270nm
2006	WA	Late	Arbequina	103	0.054	0.062	179	0.001	0.071
2006	WA	Late	Barnea	103	0.033	0.130	245	0.000	0.097
2006	WA	Late	Coratina	22	0.033	0.162	303	-0.005	0.172
2006	WA	Late	Corregiola	93	0.056	0.037	135	0.001	0.063
2006	WA	Late	Frantoio	88	0.051	0.041	166	0.000	0.067
2006	WA	Late	Koreneiki	72	0.040	0.097	199	-0.001	0.083
2006	WA	Late	Leccino	57	0.038	0.018	397	-0.001	0.060
2006	WA	Late	Manzanillo	99	0.028	0.191	59	0.001	0.047
2006	WA	Late	Nevadillo Blanco	54	0.029	0.064	155	-0.001	0.068
2006	WA	Late	Pendolino	66	0.038	0.044	255	0.000	0.058
2006	WA	Late	Picual	54	n/a	0.058	179	0.000	0.053
2006	Sthn Vic / Tasmania	Early	Arbequina	40	0.038	0.077	339	-0.011	0.209
2006	Sthn Vic / Tasmania	Early	Barnea	66	0.036	0.034	329	-0.007	0.145
2006	Sthn Vic / Tasmania	Early	Coratina	28	0.035	0.158	272	-0.006	0.230
2006	Sthn Vic / Tasmania	Early	Corregiola	40	0.048	0.029	215	-0.003	0.118
2006	Sthn Vic / Tasmania	Early	Frantoio	31	0.030	0.149	228	-0.004	0.129
2006	Sthn Vic / Tasmania	Early	Frantoio	41	0.036	0.140	218	-0.008	0.167
2006	Sthn Vic / Tasmania	Early	Leccino	37	0.025	0.147	415	-0.003	0.145
2006	Sthn Vic / Tasmania	Early	Leccino	33	0.043	0.121	384	-0.003	0.134
2006	Sthn Vic / Tasmania	Early	Pendolino	46	0.039	0.146	407	-0.008	0.185
2006	Sthn Vic / Tasmania	Early	Pendolino	30	0.037	0.036	289	-0.002	0.095
2006	Sthn Vic / Tasmania	Early	Picual	38	0.067	0.033	295	-0.008	0.152
2006	Sthn Vic / Tasmania	Late	Frantoio	32	0.050	0.102	193	-0.005	0.166
2006	Sthn Vic / Tasmania	Late	Leccino	49	0.034	0.124	384	-0.003	0.141
2006	Sthn Vic / Tasmania	Late	Pendolino	32	0.033	0.052	292	-0.004	0.088
	IOOC LIMITS			**< 250 mg/kg**	**<0.15**	**< 0.2**		**< 0.01**	**< 0.25**

10. Glossary

International Olive Council (COI), Spain www.internationaloliveoil.org

Olive oil is the oil extracted from olive fruit (*Olea europaea L.*), free of any solvent extracted or re-esterification oils or oils of any other kind.

Virgin olive oils are oils extracted from olive fruit by mechanical or physical means which does not cause any changes to the oil. The only processes acceptable are washing, decantation, centrifugation and filtration.

Extra virgin olive oil is virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 0.8 grams per 100 grams, and other characteristics of which correspond to those fixed for this category in this standard.

Free fatty acids (FFA) in extra virgin olive oil must be less than 0.8%, measured as oleic acid.

Peroxide value for extra virgin olive oil must be less than 20 milliequivalents of oxygen per kilogram of oil (mEq O_2/kg).

Oxidation and production of peroxides, occurs during oil extraction and prior to bottling but continues even after bottling, at a reduced rate.

Oxidative stability of oils is the resistance to oxidation during processing and storage.

Cold-pressed olive oil is oil which has been extracted under specific temperature limits, as described by the EC. The definition of cold-pressed or cold-extraction is given in the EC document (Appendix 10) as virgin or extra virgin oil which has been extracted at a temperature below 27^{o}C by percolation or centrifugation of the olive paste.

11. Report Author References

Book

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A Survey of Australian Olive Cultivars to Determine Compliance with International Standards

by Dr Rodney J. Mailer & Jamie Ayton
RIRDC Pub. No. 08/167

The report describes quality characteristics of Australian olive oil and compares the findings to standards used in international trade. The report shows the effects of olive cultivars, the influence of harvest timing and the changes to quality as a result of site and seasonal growing conditions.

Currently genuine olive oil can be rejected as adulterated when it is outside existing regulations. Results in this report clearly describe quality characteristics of premium quality, extra virgin olive oil grown under Australian conditions.

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