**School of Public Health**

# Effect of processing techniques on yield and quality of Western Australian olive oil

**Hui Jun Chih**

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## Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: …….

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## Abstract

Many studies have shown that regular consumption of olive oil lowers the risk of cardiovascular diseases, breast, ovarian and prostate cancers. These benefits are thought to be due primarily to the high level of monounsaturated fatty acids and bioactive phenolic compounds in the olive oil. An increased awareness of these health benefits has led to a significant increase in the demand for olive oil around the world. However, the current production volume of olive oil is unable to meet the increasing demand. The techniques currently used by the industry extract less than 60 % of oil and 10 % of bioactive phenolic compounds from the olive fruits. There is therefore a need to not only increase the yield of oil extraction but also the extent of recovery of bioactive phenolic compounds.

The objective of this study was to investigate the effect of *extending the length of olive paste mixing period to 60 minutes* and *addition of processing aids to the olive paste* (citric acid, Viscozymes and Pectolyase) on the extraction and quality of olive oil. The study was conducted over a 2 year period on *Frantoio* olives harvested from Gingin, Swan Valley and Margaret River in Western Australia at various maturity levels. The effects of these processing techniques were assessed on the yield of oil extraction, oil recovery, concentration of total phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition in terms of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), level of conjugated diene (K232) and conjugated triene (K270), variation of specific extinction (ΔK), colour in terms of brightness (L*), greenness (a*) and yellowness (b*) as well as the sensory properties of the extracted olive oil samples. The oil samples extracted were also compared to the commercial olive oil samples in terms of these parameters. Significant differences between the quality of the extracted olive oil samples were detected at $\alpha=0.05$ level on the estimated marginal means value as generated by the Univariate ANOVA procedure.

The results from this study indicate that the interaction between the processing techniques and maturity levels did not significantly affect the quality of extracted oil. However, the interaction between the processing techniques and the olive growing

sites did significantly affect the quality of the extracted oil. In addition, the quality of the olive oil samples varied in relation to the different processing techniques applied.

Among the processing techniques investigated, addition of 0.15 g/mL of citric acid or Viscozymes were both effective in increasing the yield of oil extraction to around 12 % and the oil recovery to above 60 %. However, they were not effective at improving the extraction of phenolic compounds to the oil. Addition of citric acid at the higher concentration of 0.30 g/mL was the most effective technique in increasing the concentration of total phenolic compounds in the extracted oil. The concentration of total phenolic compounds was increased to 266.32 mg/kg oil when compared to the control sample (113.09 mg/kg oil). The antiradical activity of the extracted oil (47.61 % inhibition of DPPH radicals) was also higher than that of the control sample (32.49 % inhibition of DPPH radicals). Addition of 0.30 g/mL citric acid to olive paste lowered the percentage of saturated palmitic acid and increased the monounsaturated fatty acids: polyunsaturated fatty acids ratio. The addition of citric acid at 0.30 g/mL to the olive paste was beneficial in protecting the extracted olive oil against oxidation, as the peroxides value was significantly reduced. The olive oil extracted by addition of 0.30 g/mL citric acid also has comparable colour compared to the control olive oil sample. In addition, the quality of olive oil extracted by addition of 0.30 g/mL citric acid has comparable sensory profile to the commercial EVOO samples.

*Key words*: olive oil, *Frantoio*, Gingin, Swan Valley, Margaret River, extraction techniques, malaxation period, processing aids, concentration, efficiency, phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition, conjugated diene and triene, colour, sensory profile.

## Abbreviations

| | |
|---|---|
| ΔK | variation of specific extinction |
| 3,4-DHPEA | 3,4-(dihydroxyphenyl)ethyl alcohol |
| 3,4-DHPEA-EA | 3,4-(dihydroxyphenyl)ethanol-elenolic acid |
| 3,4-DHPEA-EDA | 3,4-(dihydroxyphenyl)ethanol-elenolic acid dialdehyde |
| a* | greenness |
| ABTS | 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid |
| AOAC | Association of Official Agricultural Chemists |
| AOCS | American Oil Chemists' Society |
| b* | yellowness |
| BOD | biochemical oxygen demand |
| C16:0 | palmitic acid |
| C18:0 | stearic acid |
| C18:1 | oleic acid |
| C18:2 | linoleic acid |
| CE | capillary electrophoresis |
| $CO_2$ | carbon dioxide gas |
| COD | chemical oxygen demand |
| DPPH | 2, 2-diphenyl-l-picrylhydrazyl |
| EVOO | extra virgin olive oil |
| FAME | fatty acid methyl esters |
| FRAP | ferric reducing antioxidant power |

| | |
|---|---|
| FTIR | Fourier Transform-InfraRed |
| FT-NIR | Fourier Transform-Near InfraRed |
| GC | gas chromatography |
| GPA | Generalised Procrustes Analysis |
| HPLC | high performance liquid chromatography |
| IOC | International Olive Council |
| K232 | level of conjugated diene |
| K270 | level of conjugated triene |
| S | growing sites |
| L* | brightness |
| LC | liquid chromatography |
| LOX | lipoxygenase |
| mEq | milliequivalent |
| MI | maturity index |
| MS | mass spectroscopy |
| MUFA | monounsaturated fatty acid |
| NMR | nuclear magnetic resonance |
| $O_2$ | oxygen gas |
| OO | olive oil |
| ORAC | oxygen radical absorbance capacity |
| *p*-HPEA-EDA | *p*-(hydroxyphenyl)ethanol-elenolic acid dialdehyde |
| POD | peroxidase |
| POO | pomace olive oil |

| | |
|---|---|
| PPO | polyphenoloxidase |
| PUFA | polyunsaturated fatty acids |
| Q | quality parameters |
| rpm | rotation per minute |
| Sample A30 | Olive paste was malaxed for 30 min with addition of 0.15 g/mL citric acid |
| Sample A30[H] | Olive paste was malaxed for 30 min with addition of 0.30 g/mL citric acid |
| Sample C30 | Olive paste was malaxed for 30 min with no addition of processing aids |
| Sample C60 | Olive paste was malaxed for 60 min with no addition of processing aids |
| Sample P30 | Olive paste was malaxed for 30 min with addition of 0.15 g/mL Pectolyase |
| Sample V30 | Olive paste was malaxed for 30 min with addition of 0.15 g/mL Viscozymes |
| Sample V30[H] | Olive paste was malaxed for 30 min with addition of 0.30 g/mL Viscozymes |
| T | techniques |
| Univariate ANOVA | Univariate Analysis of Variance |
| USA | United States of America |
| USDA | United States Department of Agriculture |
| VOO | virgin olive oil |
| w/w | weight by weight |
| WAOC | Western Australian Olive Council |

**Table of Contents**

**Page**

## List of Figures

**Page**

## List of Tables

**Page**

# Chapter 1 Introduction

Olive oil is a natural juice extracted from olive fruit (*Olea europaea*). It is produced by the mechanical pressing of olive fruits. The benefits of incorporating olive oil into the diet have been well recognized. Regular consumption of olive oil helps to reduce the risk of cardiovascular diseases, metabolic diseases and cancers. The protective effects of olive oil against cardiovascular diseases are due to the high monounsaturated fatty acid (MUFA) to polyunsaturated fatty acid (PUFA) ratio as well as the presence of bioactive phenolic compounds in olive oil. Indeed, these health beneficial compounds are not found in other widely consumed vegetable oils, such as soybean, peanut and coconut oil. The phenolic compounds in olive oil are strong antioxidants which are effective in scavenging free radicals. The antioxidant effect of olive oil prevents the free radicals from initiating oxidation process. As a result, it minimizes the occurrence of metabolic diseases, including atherosclerosis, arthritis, cell mutation and cancer. Therefore, dietitians encourage regular inclusion of olive oil as a source of lipid in the diet (Covas et al. 2006b; Perez-Jimenez et al. 2007).

Increased awareness of the health benefits of olive oil since a decade ago have resulted in a 10 % increase in the world consumption of olive oil to about 2.8 million tonnes as recorded in year 2005/2006. In particular, countries such as the United States of America (USA), Japan and Brazil have increased their olive oil imports since 2002/03 (IOOC 2005). The domestic demand of olive oil in the USA has increased steadily from 2003 and is currently costing the country 9.25 million Australian dollars to import olive oil (Harcourt 2007). Australia recognized the economic impact of importing olive oil and has initiated local production of olive oil. The Australian olive oil producers are striving to increase the production volume of oil to meet the increasing demand. Such approach is expected to satisfy the nation's demand of quality virgin olive oil and to save the nation approximately 200 million Australian dollars on importing olive oil (Sweeney 2006; *Western Australia Agri-food and Fibre Industry Outlook* 2005).

Presently, olive oil is extracted from the fruit by a rather conservative mechanical extraction which only confers an oil recovery of approximately 60 % (Artajo, Romero

& Motilva 2006; Ranalli et al. 2005). The continued use of such low-yield techniques is probably due to the restrictions enforced by the International Olive Council (IOC) that forbids the use of chemical solvents in the production of virgin olive oil. There is a need to look at natural alternatives to improve the yield of oil extraction in order to meet the increasing demand for olive oil. In addition, approximately 90 % of the bioactive phenolic compounds in olives are not extracted into the oil by the mechanical extraction technique; they often end up in the by-product (Artajo, Romero & Motilva 2006; Rodis, Karathanos & Mantzavinou 2002; Vlyssides, Loizides & Karlis 2004). The concentration of phenolic compounds in the olive oil is directly related to the health benefits, the stability as well as the sensory property of olive oil (Bendini et al. 2007; Gutierrez, Arnaud & Garrido 2001; Morales & Tsimidou 2000; Servili et al. 2009). Therefore, it is necessary to evaluate new extraction techniques in terms of their ability to improve both the yield of olive oil and the concentration of phenolic compounds during the extraction process. Such improvements however need to also be evaluated in terms of their effect on various measures of the quality and acceptability of the extracted olive oil. Indeed, the Australian Olive Association (2002) has pointed out in the *Research and Development Plan for the Australian Olive Industry 2003-2008* that Australia needs to "*develop best practice post-harvest technologies guidelines*" to achieve greater yield of oil extraction with high quality oil. The practices need to ensure that we are "*maximizing the health active components of olive oil through various production and/or processing variables*" in order to compete with imports (AOA 2002).

Studies conducted in the Mediterranean countries have indicated the potential of adding processing aids during the processing of olive oil. The use of enzymes is one of the main areas being investigated. Results have shown encouraging outcomes in terms of their ability to improve the yield of oil extraction (Chiacchierini et al. 2007). However, the effect of adding processing aids on increasing the concentration of bioactive phenolic compounds in olive oil has not been fully investigated. Citric acid as a chelating agent may prevent the loss of phenolic compounds during the extraction of olive oil. It is proposed that the addition of enzymes and citric acid to olives may increase the yield of oil extraction and the concentration of bioactive phenolic compounds in olive oil. In addition to the effect of extraction techniques, the quality of olive oil is also dependent on the cultivars of olives, growing sites and growing

conditions of olives as well as maturity level of the harvested olives (Garcia, Seller & Perez-Camino 1996; Leon et al. 2008; Salvador et al. 2003; Tura et al. 2007). Research activities should therefore be conducted to determine the effect of all these factors on the quality of olive oil. In particular, the yield of oil extraction, oil recovery and concentration of phenolic compounds in the extracted olive oil should be the focus of these research activities.

## 1.1 Objectives

To the best of the knowledge of the author, no literature has examined all the effect of olive growing sites, maturity level and processing techniques on the yield or quality of Western Australian olive oil. In particular, the combined effects of these variables are absent. Three main objectives were developed for this research project. They are listed as follow:

- To evaluate the interaction between processing techniques, olive growing sites and maturity level of olives on the quality of extracted olive oil over a 2 year period
- To determine the effects of processing techniques (extended length of olive paste malaxation period and addition of enzymes preparation or citric acid) on the quality (mainly the yield of oil extraction, oil recovery and concentration of phenolic compounds) of extracted Western Australian *Frantoio* olive oil
- To establish and validate optimum processing techniques for the production of Western Australian *Frantoio* olive oil with competitive yield of oil extraction and quality parameters to the IOC standards and commercial extra virgin olive oil (EVOO) samples

## 1.2 Significance of this study

The knowledge gained from this project is expected to establish optimum processing techniques that will result in the production of olive oil that has:

- A higher yield of olive oil
- Increased concentration of bioactive phenolic compounds
- Comparable quality to the standards set by the IOC for EVOO

As the effect of olive growing sites and maturity of the olive fruits on the yield and quality of the extracted oil will be evaluated and made available to the Western Australian olive oil producers, they will be in a better position to accommodate the increased demand for high quality olive oil.

# Chapter 2 Literature Review

## 2.1 Origin of olives

According to Vossen (2007), olives originated 5000 years ago along the eastern Mediterranean Coast around Turkey, Syria, Lebanon, Palestine, and Israel. They were then introduced to the west into Greece and Egypt, followed by Sicily, Sardinia, Italy, France, Spain, Portugal, Algeria, Tunisia, and Morocco. Spain and Portugal were the largest production areas of olive oil during the time. The olive oil was mainly shipped to England, Germany, France, and Italy. The spread of olive plantings and oil processing facilities all around the Mediterranean basin was facilitated by the expansion of the Roman Empire (Vossen 2007). It was a sign of peace during the time.

During the Middle Ages, olive oil production continued to increase primarily in Spain, Italy, and Greece. The greatest expansion of olive oil production came after the 1700s. However, very limited quantity was used for human consumption. Olive oil was primarily used as lamp fuel, with other uses including fragrant offerings to the Gods, pharmaceutical ointments to cure diseases, and to make the skin and hair appear healthier. It was also used for athlete's skin during competition, for soap production, and for blessing the dead. It was regarded as too luxurious to be consumed as food (Vossen 2007).

Today, Spain, Italy and Greece continue to be the main olive growing countries although some olive varieties have been introduced for cultivation to countries outside the Mediterranean regions, such as the USA, South Africa, Australia, New Zealand, Chile, and Argentina (Vossen 2007). The spread of cultivation area for olives at present is illustrated in Figure 2-1. The main olive cultivars planted in Australia are *Frantoio*, *Correggiolo* and *Leccino*. Other cultivars include *Barnea*, *Picual*, *Manzanillo*, *Paragon*, *Koroneiki*, *Nevadillo Blanco*, *Verdale*, *Bouquettier*, *Arbequina* and *Coratina*. Each of them varies in sizes and has different lipid and carbohydrate contents. *Manzanillo* and *Kalamata* are mainly used in the production of table olives and tapenades for their large fruit size and lower lipid content. Meanwhile, cultivars

such as *Frantoio*, *Barnea* and *Picual* are mainly for the production of olive oil (Gawel 2006).

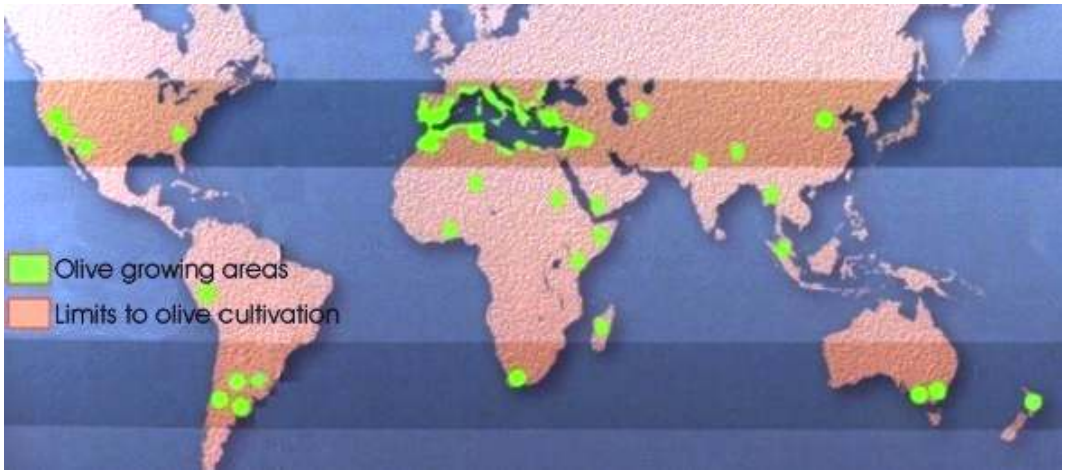



**Figure 2-1: Current major olive cultivation areas**

Source:http://www.internationaloliveoil.org/downloads/Projects/paginas/Section-a.htm

## 2.2 Structure and composition of olive fruit

Olives (*Olea europaea*) are botanically classified as drupes. An olive fruit comprises of three fundamental parts, the epicarp (skin), mesocarp (flesh) and the endocarp (stone or seed), as illustrated in Figure 2-2. The mesocarp is the largest part of an olive fruit, contributing to 70-90 % of the total fruit weight while endocarp and epicarp contributing to 9-27 % and 2-3 %, respectively, of the total fruit weight (Conde, Delrot & Geros 2008).

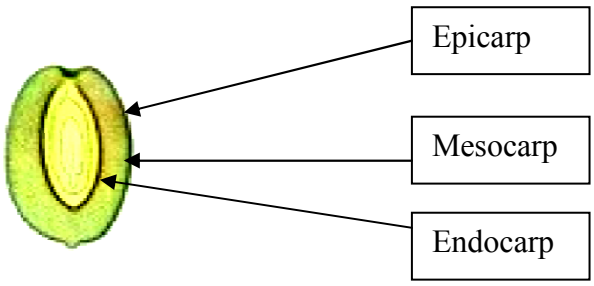



**Figure 2-2: Structure of an olive fruit**

Source: http://www.absoluteastronomy.com/topics/Olive#encyclopedia

The olive consists of mainly water, lipid, carbohydrate, fiber, ash and small amount of protein. The major nutrient composition of olive fruits is shown in Table 2-1.

**Table 2-1: Nutrient composition of olive fruit**

| Nutrients | % w/w |
|---|---|
| Water | 80 |
| Protein | 0.8 |
| Total lipid | 10.7 |
| - Palmitic (C16:0) | -1.1 |
| - Stearic (C18:0) | -0.2 |
| - Oleic (C18:1) | -7.7 |
| - Linoleic (C18:2) | -0.9 |
| - Linolenic (C18:3) | -0.1 |
| Carbohydrate | 6.4 |
| Total dietary fiber | 3.2 |
| Ash | 2.3 |

Source: Medeiros and Hampton (2007)

## 2.3 Processing of olive fruits

Olive fruits can be processed into various products found on the market shelves. The main olive products include olive oil, table olives and tapenades. The high content of lipid in olive fruits, in particular, of the cultivar *Frantoio*, *Barnea* and *Picual* is perfect for olive oil production, where the olives are crushed and the lipid is extracted from the fruit. Meanwhile, production of table olives and tapenade employs cultivars with less lipid content, such as *Manzanillo* and *Kalamata*. Table olives are produced by applying lye treatment to the olives, or by natural fermentation of the olives, while tapenade is produced from pureed table olives added with capers and olive oil (Fernández 1997). The difference between the world market share of olive oil and table olives is shown in Table 2-2. The olive industry is dominated by the production of olive oil (Table 2-2). The following section thus focuses on processing of olive oil.

**Table 2-2: World market shares of the main olive products in year 2008**

| Olive products | Production (1,000 tonnes) | Consumption (1,000 tonnes) |
|---|---|---|
| Olive oil | 2,867 | 2,876 |
| Table olives (and tapenades) | 2,032 | 2,146 |

Source: International Olive Council (IOC 2008a, b)

## 2.3.1 Extraction of olive oil

Extraction of olive oil from the fruit *Olea europaea* dated back to 5000 B.C. (Di Giovacchino 2000). The following sections cover both the ancient method and the modern method applied during the production of olive oil. There have been developments in the area to improve both the yield of olive oil extraction and the quality of the extracted oil.

### 2.3.1.1 Ancient method

In the early days, olives were ground in a mortar with stones and the paste was pressed by heavy rocks to release the oil. Separation of the oil and the vegetable water was done solely by gravity force. Millstone crusher was developed during the Roman Age to replace the use of mortar with additional presses to facilitate the separation of oil. The invention of screw press in 50 B.C. by the Greeks was regarded as a major milestone in the history of the development of olive oil production. Heavy labour forces were required to operate the wooden and iron screw presses (Di Giovacchino 2000).

### 2.3.1.2 Modern method

In the early 17$^{th}$ century, hydraulic press was firstly developed to assist the production of olive oil. Labour requirements were minimized through the development of electrically driven hydraulic pumps, cage presses, column presses and super presses with pressure up to 350-500 atmospheres. Invention of percolation system and centrifugation system came in place in mid 1900s after realizing the disadvantages of the pressing system. The machinery involved in the pressing system was complex and required large investment of labour forces. In addition, the discontinuous system reduced the working capacity (Di Giovacchino 2000). The innovative percolation and centrifugation system promoted continuous production of olive oil and thus improved the efficiency of olive oil production. In particular, the development of automated centrifugal decanter in 1960s was an major achievement in the development of olive oil production (Di Giovacchino 2000).

#### 2.3.1.2.1 Three-phase decanter

The three-phase olive oil decanter is an example of the automated centrifugal decanter. It consists of a crusher, malaxer and centrifuge. The high centrifuge speed (3500-3600 rpm) applied in a three-phase decanter greatly enhanced the separation of the liquid oil and water phase from the solid pomace phase (Di Giovacchino 2000). The centrifuged products include the olive oil, oily by-product and wastewater. Addition of lukewarm water to the three-phase decanter in the ratio of 1:1 olive paste:water (w/w) is a common practice in the industry to further improve the extraction of olive oil. However, this step reduces the concentration of phenolic compounds in the oil and the subsequent storage stability of the oil. In addition, the wastewater produced from the three-phase decanter poses a disposal problem for the industry. With every 100 kilograms of olives processed, approximately 100 liters of vegetation wastewater is produced (Alburquerque et al. 2004). The organic wastewater, consists of sugars, polyalcohols, pectins, lipids and notable amounts of aromatic compounds (tannins and polyphenols), makes it highly polluted to the environment. Indeed, its biochemical oxygen demand (BOD) is 89-100 g/L and the chemical oxygen demand (COD) is 80-200 g/L (Alfano et al. 2008). The high BOC and COD values indicated an urgency to reduce (if not totally eliminate) the wastewater, particularly as the production of olive oil was expected to increase.

#### 2.3.1.2.2 Two-phase decanter

The phototoxic effect of the large amount of wastewater produced from the three-phase decanters initiated the modification the decanter. In this regard, a two-phase decanter was invented for the production of olive oil. The two-phase decanter is very similar in structure compared to the three-phase decanter. The only difference lies on the number of outlets the decanters contain. The two-phase decanter comprises of two outlets; one for olive oil and the other for the by-products. As no water is added to the two-phase decanter, it does not produce problematic wastewater high in organic matters.

The yield of oil extraction is similar between the two types of decanter, with the two-phase decanter producing an extraction yield of 1 % greater than that extracted by the three-phase decanter (Di Giovacchino 2000). However, the concentration of phenolic

compounds in the oil extracted by the two-phase decanter is higher than that extracted by three-phase decanter. Indeed, the concentration of total phenolic compounds in Spanish *Arbequina* olive oil extracted by two-phase decanter was reported as 80.88 mg/kg oil, compared to a lower concentration of 42.07 mg/kg oil in olive oil extracted by a three-phase decanter (Gimeno et al. 2002). The significant 48 % increment in the concentration of total phenolic compounds in the extracted oil is attributable to the zero amount of water required during the olive oil extraction process when applying the two-phase decanter. In this regard, the two-phase decanter is widely used nowadays due to its greater energy- and cost-efficiency.

## 2.4 Olive oil

Olive oil, the natural juice extracted from the olive fruit (*Olea europaea*), has been consumed since 1700s as its health benefits were revealed. The production of olive oil has spread to countries outside the Mediterranean region (Figure 2-1) as the demand for olive oil around the world increases.

### 2.4.1 Worldwide production and consumption

The vegetable oil market is dominated by soybean, palm kernel, peanut, canola, sunflower and coconut (ASA 2009). According to a 2008 United States Department of Agriculture (USDA) report, olives contribute to a minor consumption proportion of 3 %. However, as we learn more about the health benefits of olive oil, it is expected that the world demand of olive oil will continue to increase (IOC 2008a). As illustrated in Table 2-3, the estimated world consumption volume is higher than the capacity the world can produce (production volume). The consumers would have to compromise with the quality and consume old olive oil if the production volume was not increased. Producers of olive oil are catching up on the production volume, with expectations being laid on those outside the Mediterranean region, such as the USA and Australia (UNCTAD 2006), where the domestic consumption is higher than the production volume (Table 2-3).

**Table 2-3: Comparison of production and consumption of olive oil in some countries as reported in year 2008**

| | Production (1,000 tonnes) | Consumption (1,000 tonnes) |
|---|---|---|
| Spain | 1,150 | 600 |
| Italy | 560 | 750 |
| Greece | 370 | 265 |
| Turkey | 159 | 90 |
| Argentina | 20 | 5 |
| Australia | 13 | 45 |
| USA | 2 | 251 |
| World | 2,866 | 2,875 |

Source: International Olive Council (IOC 2008a)

## 2.4.2 Production and consumption in Australia

In Australia, the total number of olive trees planted is estimated to be 10 million, producing a total volume of 12,000 tonnes of olive oil in 2008 (AOA 2009). The main production areas of olive oil in Australia are marked in orange colour in Figure 2-3. They are the Moore River Region, Margaret River and Great Southern Regions of Western Australia; the Fleurieu Peninsula and the East/South East of South Australia; the North, Central and Western Victoria; and Northern Slopes of New South Wales, Hunter Valley and the Murray Irrigation Area as well as South Eastern Queensland (Gawel 2006). Victoria is the major producer of olive oil in Australia, contributing to 49 % of the total production volume. Western Australia comes second (24 %) with South Australia ranking the third (15 %). New South Wales (8 %) and Queensland (4 %) are the two minor producer of olive oil in Australia (AOA 2009).

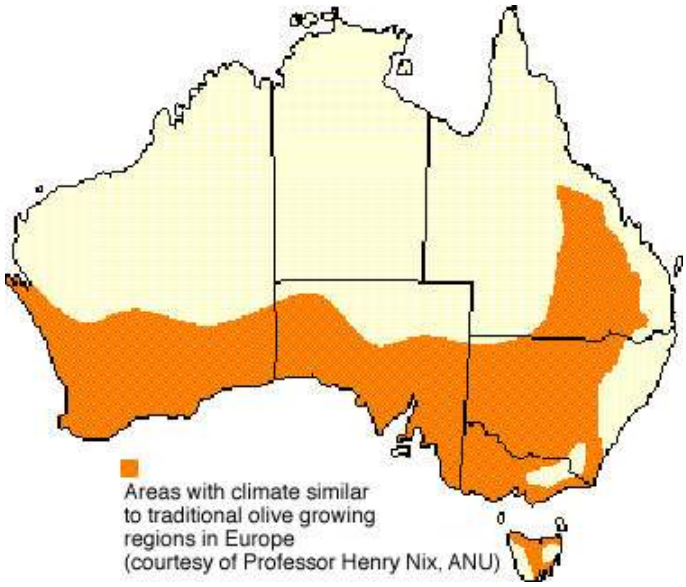



**Figure 2-3: Main olive cultivation areas in Australia**

Source: www.australianolives.com.au/page.php?19

Consumption volume of olive oil in Australia is approximately 32,000 tonnes per year; the largest consumer of olive oil per capita outside the Mediterranean region. The significant differences between the production and the consumption volume highlight the need to increase the efficiency of the olive oil extraction process in Australia (AOA 2009).

## 2.4.3 Health benefits

The increasing demand of olive oil is attributable to the increased awareness of its health benefits. Olive oil contains a good ratio of fatty acids as well as the bioactive unsaponifiable compounds. The unique composition of olive oil is protective against cardiovascular diseases, metabolic syndrome and cancer. For instance, the high ratio of monounsaturated to polyunsaturated fatty acids (MUFA:PUFA) of olive oil help protect against cardiovascular diseases and type II diabetes while its phenolic compounds have anti-cancerous effects (Medeiros & Hampton 2007; Tuck & Hayball 2002).

### 2.4.3.1 Cardiovascular diseases and metabolic syndrome

The low incidence of cardiovascular disease and cancer in people living in the Mediterranean region has raised the involvement of their diet in such reductions. It is believed that the Mediterranean diet, which consists of 50 mL of extra virgin olive oil per day, has protective effect on human health (Bogani et al. 2007). Their study recruited 12 men with a body mass index of 22. After 2 hour of ingestion, there was a significant reduction in inflammation. In addition, the plasma antioxidant status was increased. The study speculated that long term consumption of olive oil with high concentration of phenolic compounds could prevent atherosclerosis.

In addition to the bioactive phenolic compounds, the high MUFA:PUFA ratio in olive oil also contributes to the health profile of olive oil. Over a three-week trial on 200 men over 6 research centres in 5 European countries, consumption of 25 mL olive oil per day was found to increase the level of high density lipoprotein and decrease the ratio of total cholesterol: high density lipoprotein as well as the concentration of triglycerides in circulation. The mechanism by which these benefits occur is not clear, however is thought to be due to the high MUFA to PUFA ratio in olive oil (Covas et al. 2006a; Medeiros & Hampton 2007). When olive oil with a high concentration of phenolic compounds was consumed, the positive health benefits as stated above was even more apparent with a lower level of oxidized lipoproteins (Covas et al. 2006b). The study confirmed that daily consumption of 25 mL olive oil, in particular olive oil with high level of phenolic compounds (336 mg/kg), was associated with a reduced risk of heart diseases. The results were in agreement with the findings of Tuck and Hayball (2002), Grignaffini et al. (1994) and Salami et al. (1995). Based on their studies, it is suggested that phenolic compounds, particularly hydroxytyrosol, of olive oil inhibits oxidation of low density lipoprotein cholesterol and thus reduces the formation of atherosclerotic plaques and the risk of occurrence of coronary heart disease (Grignaffini et al. 1994; Salami et al. 1995; Tuck & Hayball 2002).

In addition to the cardiovascular protective effects of olive oil, consumption of olive oil reduces other aspects of the metabolic syndrome such as obesity and diabetes (Medeiros & Hampton 2007). Metabolic syndrome is reported to be directly related to the risk of cardiovascular diseases (Alessi & Juhan-Vague 2006). In a 2 year cohort study, consumption of 8 g olive oil per day was found to significantly affect several

aspects of the metabolic syndromes, such as reducing body weight and abdominal obesity, decreasing insulin resistance and improving endothelial function in 90 adults (Esposito et al. 2004). The outcome suggested that consumption of olive oil could reduce the occurrence of metabolic syndrome and therefore also the risk of cardiovascular diseases.

Results from other studies have also suggested that olive oil has the ability to prevent age-related cognitive decline and Alzheimer's disease (Berr et al. 2008; Burgener et al. 2008). A study on 8028 subjects aged 65 years old and above over a 4 year period found that consumption of olive oil improves the visual memory of these subjects.

#### 2.4.3.2 Cancer

The antioxidant activities exerted by the phenolic compounds in olive oil can reduce the occurrence of cancer (Tuck & Hayball 2002). Indeed, as summarized by Escrish et al. (2006) using the epidemiological data collected from case-control and cohort studies, there was a strong association between cancer and consumption of olive oil. The phenolic compounds in olive oil were found effective in preventing damage to the DNA and initiation of cancerous cells *in vitro*. In this regard, olive oil consumption could reduce the occurrence and progression of breast, ovarian, prostate, colon and stomach cancers (Escrish et al. 2006; Galeone et al. 2007; Medeiros & Hampton 2007; Menendez et al. 2006; Sotiroudis & Kyrtopoulos 2008).

In addition to the epidemiological data, in vitro studies were also conducted to understand the protective mechanism exerted by phenolic compounds in olive oil on cancers. Menendez et al. (2008) studied the anticarcinogenic effect of the isolated single phenols (hydroxytyrosol and tyrosol), polyphenol acid (elenolic acid), lignans ((+)-pinoresinol and 1-(+)-acetoxypinoresinol) and secoiridoids (deacetoxy oleuropein aglycone, ligstroside aglycone, and oleuropein aglycone) in olive oil on cultured human breast cell lines. Of all the tested fractions, (+)-pinoresinol, 1-(+)-acetoxypinoresinol, deacetoxy oleuropein aglycone, ligstroside aglycone, and oleuropein aglycone were found to induce strong tumoricidal effects. The breast cancer cell proliferation and survival were significantly prevented as the protein expression was inhibited (Menendez et al. 2008). The study highlighted the reversible

effect on breast cancer by the phenolic compounds in olive oil, the oleuropein aglycone.

Many clinical studies have pointed out the protective effect of olive oil on cancer. However, no information is published on the effect of consumption of the specific phenolic compounds in olive oil by human subjects and the occurrence of cancer in these subjects. As a result, the actual mechanism by which phenolic compounds act in reducing the prevalence of cancers remains unknown. However, the strong association between consumption of olive oil and the reduction in cancer prevalence derived from the epidemiological studies encourages consumption of olive oil with high concentration of phenolic compounds.

## 2.4.4 Composition

The health benefits of olive oil are attributable to the composition of olive oil. Olive oil consists of 98 % of saponifiable compounds, which include mainly triacylglycerols and free fatty acid, and 2 % of unsaponifiable compounds, such as hydrocarbons, sterols, triterpene alcohols, tocopherols, phenols, chlorophylls, flavour compounds, mono and diacylglycerols, phosphatides, waxes and esters of sterols (Kiritsakis & Christie 2000). Both the saponifiable and unsaponifiable compounds are responsible for the stability and health benefits of olive oil.

### 2.4.4.1 Saponifiable compounds

Saponifiable compounds in olive oil are mainly fatty acids. The types of fatty acids found in olive oil and their percentage of distribution are tabulated in Table 2-4. They are responsible for the unique fatty acid ratio in olive oil which is good for preventing cardiovascular diseases. The main triacylglycerols in olive oil are constructed by mainly oleic acid (O), palmitic acid (P) and linoleic acid (L) in the form of OOL, OOO and POO.

**Table 2-4: Composition of fatty acids of olive oil**

| Fatty acids | % in olive oil |
|---|---|
| C14:0 | ≤ 0.05 |
| C16:0 | 7.5-20.0 |
| C16:1 | 0.3-3.5 |
| C17:0 | ≤ 0.3 |
| C17:1 | ≤ 0.3 |
| C18:0 | 0.5-5.0 |
| C18:1 | 55.0-83.0 |
| C18:2 | 3.5-21.0 |
| C18:3 | ≤ 0.9 |
| C20:0 | ≤ 0.6 |
| C20:1 | ≤ 0.4 |
| C22:0 | ≤ 0.2 |
| C24:0 | ≤ 0.2 |

Source: Kiritsakis & Christie (2000)

### 2.4.4.2 Unsaponifiable compounds

The main constituents of the unsaponifiable compounds in olive oil are hydrocarbon, tocopherols, aliphatic alcohols, diacylglycerols, sterols, phenolic compounds and the flavour compounds. They are indicators of the quality of olive oil.

Squalene ($C_{30}H_{50}$) is the main hydrocarbon found in olive oil. Its concentration can vary between cultivars and processing techniques, covering the range of from 2500 to 9250 µg/g in olive oil (Kiritsakis & Christie 2000; Eleni Psomiadou & Tsimidou 1999). Other hydrocarbons include polycyclic aromatics such as phenanthrene, pyrene, fluoranthrene, 1,2-benzanthracene, chrysene.

Tocopherols have the structure of a complex phenol, with the hydroxyl group accountable for antioxidant activity. They are influential on the oxidative stability of olive oil. The main type of tocopherols occur in olive oil is α–tocopherols. Their concentration ranges between 12 and 200 ppm, depending on the processing techniques applied during the production of olive oil. It was found that temperatures above 33 ºC can degrade the α–tocopherols (Morales & Przybylski 2000; Oliveras-López et al. 2008).

Aliphatic alcohols in olive oil are mainly saturated straight chain with even number of atoms up to $C_{28}$. The most abundant ones are hexacosanol, octacosanol and

tetracosanol. They can be used as an indicator of solvent extracted olive oil, with a typical concentration of 224-434 mg/100g compared to pressed olive oil of 10-70 mg/100g.

When harvested olives are stored under inappropriate conditions, the lipases present in the fruit break down the triacylglycerols. Consequently, diacylglycerols are generated. Therefore, the presence of diacylglycerols indicates the oil has undergone hydrolysis and is an indication of inferior olive oil quality (Rosati et al. 2006; Shimizu et al. 2008).

Sterols in olive oil are synthesized from squalene (Kiritsakis & Christie 2000). The main sterols found in olive oil are β–sitosterol, $\Delta^5$-avenasterol and campesterol. The amount of sterols is vital for authenticity determination of olive oil (Pardo, Cuesta & Alvarruiz 2007) as well as for protection against oil oxidation. In this regard, the total concentration of sterols in olive oil should be between 180-265 mg/100g (Morales & Przybylski 2000).

Phenolic compounds in olive oil are responsible for its oxidative stability and the sensory profile. A series of simple and complex phenolic compounds are present and unique to olive oil. They are 3,4-(dihydroxyphenyl)ethyl alcohol (3,4-DHPEA), 3,4-(dihydroxyphenyl)ethanol-elenolic acid (3,4-DHPEA-EA) and 3,4-(dihydroxyphenyl)ethanol-elenolic acid dialdehyde (3,4-DHPEA-EDA). They are shown to have strong antioxidant activities (Medeiros & Hampton 2007). The strong antioxidant activity of these phenolic compounds is attributable to the number of its hydroxyl groups. More than one hydroxyl groups is essential in providing substantial antioxidant activity. Indeed, it was reported that these phenolic compounds have greater antiradical activity than those phenols containing single hydroxyl group and/or $–COOCH_3$ group such as oleuropein aglycone (Servili et al. 2009). Meanwhile, the concentration of phenolic compounds in olive oil is dependant on the agronomic practices and processing conditions of the olive oil. For example, water deficits cause stress to olive tress and lead to high concentration of phenolic compounds in the olive fruits (Gomez-Rico et al. 2006; Servili et al. 2007a) while centrifuge reduces the concentration of phenolic compounds in the oil (Di Giovacchino 2000).

Flavour compounds of olive oil are produced during biogenesis pathway induced by endogenous enzymes as well as during the production of olive oil. Examples of

volatile compounds that give rise to positive aromatic profile of olive oil are from the $C_6$ family. For example, hexanal, hexanol, hexenol and hexyl acetate are responsible for the green, fruity aroma of olive oil (Morales & Tsimidou 2000; Servili et al. 2008).

## 2.4.5 Quality parameters

The components in olive oil play an influential role in affecting the quality of olive oil. For example, peroxides value, acidity level, level of conjugated diene (K232) and conjugated triene (K270) as measured at ultraviolet wavelengths are important markers on the oxidative state of the oil. On the other hand, the percentage of fatty acids, such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) are important for authenticity determination of olive oil. In this regard, it is necessary to have standard levels for these components.

The IOC recognized the need and had developed a trade standard for different classes of olive oil (Table 2-5). There are four main classes of olive oil; the extra virgin olive oil (EVOO), virgin olive oil (VOO), olive oil (OO) and pomace olive oil (POO), each with different standards to adhere to. EVOO is exclusively produced by mechanical pressing of the olives. Such practice is expected to retain all the bioactive compounds in the olive oil. The acidity level of EVOO should be below 0.8% m/m oleic acid while the peroxides value should be below 20 mEq/kg oil. The level of conjugated diene and triene indicates the stage of oxidation in oil. The level of conjugated diene and triene of EVOO should be below 2.50 and 0.22 %, respectively. VOO is produced in an identical approach. However, as the quality of olives has a direct impact on the quality of olive oil (Di Giovacchino, Sestili & Di Vincenzo 2002), olive oil extracted by mechanical techniques with an acidity level of between 0.8 and 2.0 % m/m oleic acid is classified as VOO. On the other hand, OO is not restricted by the method of production. Chemical extraction is permitted in the production of ordinary OO. The oil primarily extracted from the pomace (solid waste product generated from the first press of olive paste) is classified as POO. Second or third presses of the olive pomace are often carried out to ensure the oil is fully extracted despite the fact that the quality of the extracted oil could be jeopardized by this approach.

**Table 2-5: Quality standards of the four main classes of olive oil**

| Quality parameters | Classes of olive oil | | | |
|---|---|---|---|---|
| | **Extra Virgin Olive Oil (EVOO)** | **Virgin Olive Oil (VOO)** | **Olive Oil (OO)** | **Pomace Olive Oil (POO)** |
| **Acidity level (% m/m oleic acid)** | ≤ 0.8 | ≤ 2.0 | ≤ 1.0 | ≤ 1.0 |
| **Peroxides value (mEq/kg oil)** | ≤ 20 | ≤ 20 | ≤ 15 | ≤ 15 |
| **K232 (%)** | ≤ 2.50 | ≤ 2.60 | N/A | N/A |
| **K270 (%)** | ≤ 0.22 | ≤ 0.25 | ≤ 0.90 | ≤ 1.70 |
| **C16:0 (%)** | 7.5-20.0 | 7.5-20.0 | 7.5-20.0 | 7.5-20.0 |
| **C18:0 (%)** | 0.5-5.0 | 0.5-5.0 | 0.5-5.0 | 0.5-5.0 |
| **C18:1 (%)** | 55.0-83.0 | 55.0-83.0 | 55.0-83.0 | 55.0-83.0 |
| **C18:2 (%)** | 3.5-21.0 | 3.5-21.0 | 3.5-21.0 | 3.5-21.0 |

Source: International Olive Council (2006)

The standards established by IOC need to be strictly adhered to and followed during the production of olive oil. Such practice ensures the quality of olive oil for international exports, which has been the main interest of the Australian olive industry in order to supply the increasing demand of olive oil around the world (Mailer & Ayton 2008). For the interest of the consumers, they should be able to identify their desirable classes of olive oil with matching quality as specified on the label of the oil bottle.

### 2.4.5.1 Measurements of quality parameters

In order to trade olive oil in the (international) market, the producer has to ensure that the quality standards set by IOC are achieved. In doing so, the quality parameters need to be analyzed accurately. A range of analytical methods have been studied to assess the key quality markers of olive oil, namely the fatty acid profile, acidity level, oxidative stability (peroxides value and absorbances of conjugated dienes and trienes at ultraviolet wavelengths), total phenolic compounds, antiradical activity, volatile compounds and sensory profile (Kiritsakis & Christie 2000). Innovative rapid methods have been investigated by researchers to replace some time-consuming and highly health and environment hazardous chemical analytical methods.

#### 2.4.5.1.1 Fatty acid composition

Olive oil has a unique fatty acid profile of high amount of oleic acid (83 %). Fatty acid composition of olive oil is therefore an important marker of the authenticity and quality of the oil. Fatty acid is commonly analyzed in the form of fatty acid methyl esters (FAME) by gas chromatography (GC). Fatty acids are derivatized into FAME as they are more volatile and apolar than fatty acids. Such molecular characteristic of FAME helps their elution in the chromatographic column, giving rise to fast and accurate determination of the fatty acid composition in olive oil (Morales & Leon-Camacho 2000).

Derivatization of the fatty acids is conducted in methanolic medium, either by alkaline or acid catalysis. Alkaline catalysis involves addition of hexane and methanolic potassium hydroxide to the olive oil in converting the fatty acids to FAME. The isomerization of unsaturated fatty acids is preventable using such technique. However, the drawback of alkaline catalysed derivatization is that it does not derivatize free acids well. As a result, it is not applied to olive oil with high acidity level. Meanwhile, acid catalysis of the fatty acids involves the addition of hexane and methanolic sulfuric acid to olive oil. During the 90 minute reflux process, most of the fatty acids are derivatized into FAME. Boron trifluoride or boron trichloride can be added in place of sulfuric acid to shorten the reaction time. Indeed, Morales and Leon-Camacho (2000) stated that this technique is more efficient as it is not necessary to saponify the fatty acids.

New developments in the determination of fatty acids include rapid vibrational spectroscopy, such as Fourier Transform-InfraRed (FTIR) and Fourier Transform-Near InfraRed (FT-NIR) instruments. They represent the new clean and rapid analyses of oil samples. The former measures the fatty acid composition in a few minutes while the latter can provide results within 30 seconds (Armenta, Garrigues & de la Guardia 2007; Christy & Egeberg 2006).

#### 2.4.5.1.2 Acidity level

Acidity level, also known as level of free fatty acids, should not be confused with the fatty acid composition of oil. The latter measures the composition of fatty acids in intact triacylglycerol compounds in the oil while acidity level reflects the level of

those fatty acids that are not intact with a triacylglycerol molecule. The value of acidity level thus gives an indication of the extent of deterioration of quality, particularly during the storage period. Indeed, value of acidity level indicates the degree of hydrolytic rancidity of triacylglycerols and predicts the quality of the oil. In the case of olive oil, value of acidity level plays an important role in classification of the extracted olive oil.

Acidity value is measured by titration against potassium hydroxide solution according to the American Oil Chemists' Society (AOCS) method. The result is expressed as grams of oleic acid per 100 gram of oil (Armenta, Garrigues & de la Guardia 2007). The rapid NIR method mentioned in Section 2.4.5.1.1 could also be applied to determine the acidity level of olive oil (Armenta, Garrigues & de la Guardia 2007). However, the set up of an NIR system can be costly. Another rapid method was introduced by an Italian company to measure the acidity level in oil samples (Kamvissis et al. 2008). It applies the photometric principle to measure the acidity value at a wavelength of 505 nm as a result of chemical reaction between the alcoholic potassium hydroxide and phenolphthalein derivative. Briefly, the cuvette containing the reagents and the oil sample is heated before inserting it to the pre-warmed OxiTester to determine the acidity level. Within a minute, the OxiTester generates the value of acidity level of the oil based on the reaction between alcoholic potassium hydroxide and phenolphthalein derivative and express it in the percentage of oleic acid. The result acquired from OxiTester was validated by Kamvissis et al. (2008). OxiTester is shown to provide results of high accuracy and sensitivity. The CDR® Food Lab OxiTester method is faster and easier than the official AOCS method.

#### 2.4.5.1.3 Oxidative stability

Oxidative stability of an oil sample can be measured in terms of the primary product from oxidized oil (such as peroxides) and secondary product (such as aldehydes, ketones and conjugated dienes).

Peroxides value, also known as peroxide index, is an important indicator of oxidative rancidity of the oil. Measurement of peroxide value is carried out by iodemetric titration that measure iodine liberated from potassium iodide by oil peroxides. It requires accurate experimental technique as it is easily affected by the time and the

reaction mixture (Armenta, Garrigues & de la Guardia 2007). In this regard, the Italian CDR® Food Lab Company invented the OxiTester to measure the peroxides value of an oil sample in a rapid approach. OxiTester measures the peroxides value as the intensity of ferric ($Fe^{3+}$) ions in the oxidized oil. Addition of reagent containing a mix of alcohols and the redox solution to the oil sample is incubated in the OxiTester to induce oxidation. After an incubation of 3 minutes, the peroxides value is measured as mEq/kg oil. The technique is rapid and less time-consuming comparing to the titration method.

Oxidation initiates the formation of peroxyl radicals and hydroperoxides in olive oil. As a result, there is an increase in the conjugated structure. The conjugated diene and conjugated triene were found to have a maximum absorption at ultraviolet wavelength of 232 nm and 270 nm, respectively (Morales & Przybylski 2000). In this regard, the IOC has developed a method to measure the extent of oxidation by measuring the level of conjugated diene and conjugated triene in the oxidized oil. Briefly, the oil is dissolved in cyclohexane and the absorption of this 1 % solution is measured at 232 and 270 nm to determine the K232 and K270 extinction coefficients, respectively, using a 1 cm quartz cuvettes. The K232 and K270 values are used for the classification of olive oil.

#### 2.4.5.1.4 Phenolic compounds

Phenolic compounds were shown to correlate with antiradical activity as well as sensory properties of olive oil (Gutierrez, Arnaud & Garrido 2001; Morales & Tsimidou 2000). Determination of this important quality marker is thus mandatory to evaluate the quality of extracted olive oil.

The determination of the concentration of total phenolic compounds in olive oil is commonly measured via the reaction of Folin-Ciocalteau reagent with the extracted phenolic compounds present in methanolic solution. Sodium bicarbonate is added to the mixture to create an alkaline condition that facilitates this reaction. Consequently, blue colour is developed as a result of the formation of tungsten and molybdenum oxides. The colour intensity is measured at a wavelength of 725 nm. Caffeic acid and gallic acid are the two common phenolic compounds employed to construct the

standard curve. Concentration of total phenolic compounds in the olive oil is then determined from the standard curve developed (Gutfinger 1981).

Siger, Nogala-Kalucka and Lampart-Szczapa (2008) commented on the performance of determination of the total concentration of phenolic compounds using Folin-Ciocalteau reagent. Folin-Ciocalteau reagent is favoured over the Folin-Denis reagent as it contains higher percentage of molybdate and sulfate ions. The molybdate acts as catalyst to increase the reaction rate while the sulfate ions solubilize the salts and remove turbity otherwise occur in the mixture. However, the analysis should be conducted cautiously to ensure there is no other highly oxidized compound in the system. The reason is that the reagent is sensitive to all compounds (not necessarily exclusive to phenolic compounds) that are capable of reducing phosphomolybdic and phosphotungstic acids to a blue complex. The presence of other easily oxidized compounds in the system will cause a higher absorbance reading at the wavelength of 725 nm, resulting in an overestimation of the total concentration of phenolic compounds in the oil (Siger, Nogala-Kalucka & Lampart-Szczapa 2008). In this regard, when the identification of individual phenolic compound is required, capillary electrophoresis (CE), gas chromatography (GC), high performance liquid chromatography (HPLC), liquid chromatography (LC), nuclear magnetic resonance (NMR) and mass spectroscopy (MS) are some promising methods to employ (Carrasco-Pancorbo et al. 2005b). In particular, HPLC provides higher resolution of the individual phenolic compounds while MS is capable of determining the molecular weight and provides structural information on the individual separated phenolic compounds (Carrasco-Pancorbo et al. 2007; Morales & Tsimidou 2000).

#### 2.4.5.1.5 Antiradical activity

Antiradical activity is important for preventing oxidation at cellular levels (Lavelli 2002). In addition to promoting good health, antiradical activity also serves as an indirect indication of the storage stability of the oil (Del Carlo et al. 2004; Gutierrez, Arnaud & Garrido 2001).

Measurement of the antiradical activity can be conducted based on either the hydrogen atom transfer or electron transfer method (Sun & Tanumihardjo 2007). The hydrogen atom transfer method measures the ability in quenching free radicals by

hydrogen donation, such as the oxygen radical absorbance capacity (ORAC) method; while the electron transfer method measures the ability to reduce radicals by transferring the electron, such as the 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and 2, 2-diphenyl-l-picrylhydrazyl (DPPH) methods. Amongst these methods, the DPPH method is most widely employed in the determination of antiradical activity in olive oil (Carrasco-Pancorbo et al. 2005a; Kiralan, Bayrak & Özkaya. 2009). The reason is that it is an efficient and simple method. Briefly, the oil is dissolved in hexane and mixed with methanol prior to exposure to the DPPH solution. As the DPPH radicals are reacted with the antioxidant present in the olive oil, the radicals are reduced by the electron transferred from the antioxidant. Consequently, the DPPH radicals are deactivated as they becomes colourless in the methanolic solution. The antiradical activity of a compound is then determined by examining the colour changes of the DPPH radicals at a visible wavelength of 515 nm using a spectrophotometer.

#### 2.4.5.1.6 Volatiles

Olive oil should have fresh, green and fruity aroma on the nose. Green apples, tropical fruits, fresh salad leaves, nutty, floral aroma are the common descriptions associated with fresh olive oil. The types of volatile compounds contribute to these pleasant aroma are the $C_5$ and $C_6$ compounds.

The $C_5$ and $C_6$ volatile compounds are generated by endogenous enzymes naturally present in the olive fruits (Morales & Tsimidou 2000). The biochemical lipoxygenase (LOX) pathway is activated when the structure of the olive cells are ruptured. Upon cell disruption, the endogenous lipoxygenases, fatty acid hydroperoxide lyase, acylhydrolase, alcohol dehydrogenase and alcohol acyltransferase are activated. They use the $C_{18}$ PUFAs present in the fruit as substrate and produce $C_5$ and $C_6$ volatile compounds (Angerosa et al. 2000; Ranalli et al. 2001; Sonia et al. 2009). The presence of these volatile compounds in the olive oil gives rise to the pleasant fresh, green and fruity aroma. On the other hand, deactivation of these endogenous enzymes, such as through heat treatments, reduces the concentration of $C_5$ and $C_6$ compounds in the oil (Luaces, Sanz & Perez 2007). The absence of $C_5$ and $C_6$ compounds coupled with the presence of aldehydes contributes to the poor sensory quality of olive oil

(Morales & Przybylski 2000), of which rancid, winey-vinegary and fusty sensations can be detected. The types of pleasant and unpleasant aromatic compounds found in olive oil and their corresponding sensory attributes are listed in Table 2-6 (Angerosa 2000).

**Table 2-6: Volatile compounds in olive oil**

| Volatile compounds | Corresponding aromatic perceptions |
|---|---|
| *Pleasant volatiles* | |
| hexanal | green, apple, sweet |
| hexanol | fruity, aromatic, soft, cut grass |
| hexyl acetate | fruity, sweet, floral |
| trans-2-hexenal | lawn, banana, almond, bitter, astringent |
| trans-2-hexenol | flowers, fruity, tomato, astringent, bitter |
| cis-2-hexenyl acetate | fruity, green leaves |
| cis-3-hexenol | banana, leaf-like, green-fruity, pungent |
| cis-3-hexenal | green leaves, grassy, green, apple-like |
| cis-3-hexenyl acetate | green-banana, fruity, green leaves, floral, ester |
| trans-3-hexenol | bitter, astringent |
| *Unpleasant volatiles* | |
| nonanal | soapy, citrus-like |
| 2-heptenal | oxidized, tallowy, pungent |
| 2-decenal | fatty |
| pentenal | cut grass |
| 2,4-heptadienal | citrus-like, soapy |
| octanal | fatty, sharp, rancid |
| 2,4-decadienal | deep-fried, fatty |
| 2-hexenal | green |
| 2-octenal | fruity, soap, fatty |

Source: Angerosa (2000)

Kiritsakis and Christie (2000) summarized a range of methods available for measuring the volatile compounds in olive oil. The most common method is by gas chromatography-mass spectroscopy (GC-MS). Other available techniques are the aroma extract dilution analysis and the sensory wheel composed of the 7 basic perceptions of oil (green, bitter-pungent, undesirable, ripe olives, ripe fruity, fruity and sweet). The compounds can then be verified by a GC-sniffing method (Kiritsakis & Christie 2000). New development in the area includes the application of high field NMR spectroscopy for direct analysis of volatiles (Kiritsakis & Christie 2000).

#### 2.4.5.1.7 Colour

Although colour of olive oil is not directly related to its quality, it has a significant effect in influencing the consumers' buying decision (Criado et al. 2008). In addition, it is speculated that the reading of greenness (a*) and yellowness (b*) of an oil are correlated to the amount of antioxidant chlorophyll and carotenoid respectively, present in the olive oil (Minguezmosquera et al. 1991; Ranalli et al. 2007). Therefore, it is worthwhile to evaluate the colour of olive oil as an indirect measurement of its quality.

Colour of an oil sample can be measured in terms of chroma, brightness, hue and integral colour index via transmittance measurements (Ranalli et al. 2005). Alternatively, the colour of an oil sample can be converted from the percentage of carotenoids and chlorophyll detected colorimetrically via separation employing thin-layer chromatography (Ranalli et al. 2005). However, a more efficient and less time-consuming approach is via the CIELAB colorimetric system. The colour can be measured directly without pre-treatment or dilution of the oil. The brightness (L*), greenness (a*) and yellowness (b*) of an oil sample are reported directly from the CIELAB absorption spectrum (Escolar, Haro & Ayuso 2007; Minguezmosquera et al. 1991).

#### 2.4.5.1.8 Sensory profile

Olive oil has its own unique flavour and palate profile distinct from other vegetable oil. It has pungent, sweet aroma with some degree of peppery sensation at the back of the throat. Assessment of the quality of an olive oil is commonly conducted on both the positive and negative characteristics of the oil sample by a group of trained panelists. Positive characteristics include fruitiness, bitterness and pungency of the olive oil, which are attributable to the concentration of phenolic compounds and volatile $C_5$ and $C_6$ compounds in the oil. Olive oil producers are encouraged to produce olive oil with positive sensory characteristics. The descriptions of these characteristics are listed in Table 2-7.

**Table 2-7: Specific descriptive vocabulary for the positive characteristics of olive oil as defined by IOOC**

| Standards | Descriptions |
|---|---|
| ***Positive characteristics*** | |
| Fruity | Set of the olfactory sensations characteristic of the oil which depends on the variety and comes from sound, fresh olives, either ripe or unripe. It is perceived directly or through the back of the nose |
| Bitter | Characteristic taste of oil obtained from green olives/olives turning colour |
| Pungent | Biting tactile sensation characteristics of oils produced at the start of the crop years, primarily from olives that are still unripe |

Source: IOOC (1996)

The negative characteristics of the olive oil are caused by improper post-harvest handling of the olive fruits. Olive oil should be produced appropriately as soon as possible after the olives are harvested from the tree. Olives that are dried out, stained with mud and/or stored in piles under humid conditions contribute to the negative quality of olive oil, such as hay-wood, earthy, fusty, musty characteristics, respectively. The extracted olive oil should also be stored properly without contact with sediment and/or metallic surfaces to prevent the development of muddy and metallic characteristics, respectively. Oxidation of the extracted olive oil can lead to formation of hexanal and $C_9$ aldehydes, which are observed as unfavourable sensory characteristic in olive oil (Morales & Przybylski 2000). The negative sensory attributes of olive oil are described in Table 2-8.

**Table 2-8: Specific descriptive vocabulary for the negative attributes of olive oil as defined by IOOC**

| Standards | Descriptions |
|---|---|
| ***Negative attributes*** | |
| Fusty | Characteristic flavour of oil obtained from olives stored in piles which have undergone an advanced stage of anaerobic fermentation |
| Musty-humid | Characteristic flavour of oil obtained from fruit in which large numbers of fungi and yeasts have developed as a result of its being stored in humid conditions for several days |
| Muddy-sediment | Characteristic flavour of oil that has been left in contact with the sediment that settles in underground tanks and vats |
| Winey-vinegary | Characteristic flavour of certain oils reminiscent of wine or vinegar. This flavour is mainly due to a process of fermentation in the olives leading to the formation of acetic acid, ethyl acetate and ethanol |
| Metallic | Flavour reminiscent of metals resulted from prolonged contact with metallic surfaces during crushing, mixing, pressing or storage |
| Rancid | Flavour of oils which have undergone a process of oxidation |
| Heated or burnt | Characteristic flavour of oils caused by excessive and/or prolonged heating during processing, particularly when the paste is thermally mixed, if this is done under unsuitable thermal conditions |
| Hay-wood | Characteristic flavour of certain oils produced from olives that are dried out |
| Rough | Thick, pasty mouthfeel sensation produced by certain oils |
| Greasy | Flavour of oil reminiscent of that of diesel oil, grease or mineral oil |
| Vegetation water | Flavour acquired by the oil as a result of prolonged contact with vegetable water |
| Brine | Flavour of oil extracted from olives which have been preserved in brine |
| Esparto | Characteristic flavour of oil obtained from olives pressed in new esparto mats. The flavour may differ depending on whether the mats are made of green esparto or dried esparto |
| Earthy | Flavour of oil obtained from olives which have been collected with earth or mud on them and not washed |
| Grubby | Flavour of oil obtained from olives which have been heavily attacked by the grubs of the olive fly (*Bactrocera oleae*) |
| Cucumber | Flavour produced when oil is hermetically packed for too long, particularly in tin containers, and which is attributed to the formation of 2-6 nonadienal |

Source: IOOC (1996)

The profile sheet developed by IOC for sensory evaluation of the olive oil (Appendix 1) is commonly adapted by many countries despite its poor reproducibility (Angerosa 2000; Guerrero, Romero & Tous 2001). In this regard, development and revision on the original document is required. For instance, Guerrero (2001) from Spain suggested

a “Generalised Procrustes Analysis (GPA)” in evaluating the sensory characteristics of olive oil. The method allowed ranking of each of the descriptor in intensity from 0 to 5. It is more informative in providing information on the intensity of each of the descriptor of the attributes and is independent of odd descriptors, when compared to the IOC profile sheet.

In Australia, the development of the evaluation sheet of olive oil is primarily led by Richard Gawel and the Australian Olive Association. The latest evaluation sheet used by the Western Australian Olive Council (WAOC) for judging of olive oil samples in the annual Royal Show Competition is illustrated in Appendix 2. Both the aroma and the taste of olive oil are judged and marks are allocated for each attributes. Olive oil sample with total marks of between 17 and 20 (highest score) is awarded a gold medal, marks between 15 and 16.5 receives a silver medal, and marks between 13 and 14.5 entitled to a bronze medal.

Recognition of the subjective nature of human sensors inspired the sensory scientists to develop electronic nose and artificial neural network to mimic human sensory receptors (Gomez et al. 2008; Lozano, Santos & Horrillo 2008; Yu et al. 2008). Employment of these devices could provide reliable sensory data of olive oil (Galmarini et al. 2008; Guerrero, Romero & Tous 2001; Huang, Kangas & Rasco 2007). Electronic nose is reported as more effective in distinguishing olive oil and physically degraded olive oil than human subjects (Messina et al. 2005).

### 2.4.6 Factors affecting the yield and quality

As previously stated in Table 2-5, the IOC has established a trade standard for each class of olive oil. Due to their better quality than the ordinary OO and POO, the selling price of EVOO is higher in the market. According to an IOC report (IOC 2009), the market price of EVOO is approximately €205/100kg compared to €175/100kg of OO and €100/100kg of POO. The significant trading price difference amongst EVOO, OO and POO is one of the reasons the olive oil producers focus on production of premium quality EVOO.

It is understood that the quality of the olive fruits and processing techniques affect the quality of olive oil (Di Giovacchino, Sestili & Di Vincenzo 2002). EVOO is

exclusively produced by the mechanical pressing of top quality olives. The IOC has forbidden the use of sub-quality olives and/or chemical solvents during the production of EVOO. Such practice is expected to retain all the bioactive compounds in the olive oil.

However, the rather conservative mechanical extraction of olive oil from the fruit results in an oil recovery of approximately 60 % (Artajo, Romero & Motilva 2006; Ranalli et al. 2005). The lower extraction yield compared to other oil crops and the loss of approximately 40 % of oil during olive oil production (Table 2-9) is due to the restrictions enforced by the IOC that forbids the use of chemical solvents in the production of extra virgin olive oil. In addition, the conservative mechanical extraction technique causes an approximately 90 % loss of bioactive phenolic compounds during the olive oil production (Artajo, Romero & Motilva 2006; Rodis, Karathanos & Mantzavinou 2002; Vlyssides, Loizides & Karlis 2004). There is a need to look at natural alternatives to improve the yield of oil extraction and concentration of bioactive phenolic compounds in the oil in order to meet the increasing demand of good quality olive oil.

**Table 2-9: Transfer of lipids and total phenolic compounds during the production of olive oil**

| Components (%) | In oil | In pomace | % lost |
|---|---|---|---|
| Lipids | 58.78 | 36.18 | 38.10 |
| Total phenolic compounds | 12.71 | 87.29 | 87.29 |

Source: Artajo, Romero and Motilva (2006)

The Australian olive industry is keen on improving the current olive oil processing techniques to improve the yield of oil extraction. In addition, the olive oil producers are interested in increasing the recovery of bioactive phenolic compounds, which contribute to the health benefits, stability as well as sensory profile of olive oil. Therefore, it is necessary to understand the contributing factors that affect the yield and quality of olive oil.

### 2.4.6.1 Growing sites

Rainfall, temperature, types of soil, nutrient composition of the soil, irrigation and drainage system throughout the development stage of olive fruits all affect the quality of the fruit and the quality of the extracted oil. Studies have stated that latitude, rainfall and temperature of the growing environment significantly affect the concentration of antioxidants and oil stability (Romero et al. 2003; Salvador et al. 2003; Tura et al. 2007). Olives are best grown in regions with a Mediterranean climate characteristic, such as a typical cool and wet winter with warm and dry summer.

Indeed, changes in the growing environment affect the yield of oil extraction and the quality of the extracted oil. For example, irrigation can increase the yield of the fruit (Ismail, Stavroulakis & Metzidakis 1999; Nuzzo et al. 1997; Vossen 2007) although such practice increases the acidity level of the oil (Dag et al. 2008; Ismail, Stavroulakis & Metzidakis 1999). The concentration of total phenolic compounds was reported lower in olive oil extracted from irrigated trees (Dag et al. 2008; Gomez-Rico et al. 2006). However, such observation is not in agreement with the work conducted by Ismail, Stavroulakis and Metzidakis (1999). The discrepancies are due to the differences in the maturity level of the fruit at harvest and the olive cultivars. The effect of maturity level and olive cultivars on the quality of extracted olive oil will be discussed in the following sections.

Olives grown in more southerly latitudes were reported to have a higher level of oleic acid in the oil (Oueslati et al. 2009; Psomiadou et al. 2003). Such observation is also valid for olives grown in southerly latitudes of Australia (Mailer & Ayton 2008). Furthermore, fatty acid composition of olive oil is affected by rainfall and prevailing weather conditions. It is reported that the level of oleic acid in oil extracted from olives exposed to cold climates is higher than those to warmer climates. In addition, the level of palmitic acid is higher in oil extracted from olives grown at a warmer climate (Mailer & Ayton 2008). As the fatty acid composition is an important factor in determination of authenticity of an olive oil sample, the effect of growing sites and the growing environment where the olives are grown should be investigated. Currently, no conclusive data is available on the effect of olive growing sites on the quality of Western Australian olive oil. The data should help the olive oil producers in

Western Australia to understand the variation in olive growing sites on the fatty acid composition and other quality parameters of the extracted olive oil.

### 2.4.6.2 Cultivar

There are different cultivars of olives. Each of them varies in sizes and has different lipid and carbohydrate contents. Cultivars with large fruit size but lower lipid contents such as *Manzanillo* and *Kalamata* are more suitable for production of table olives and tapenades. Meanwhile, cultivars such as *Frantoio*, *Barnea* and *Picual* are mainly employed for the production of olive oil.

Similar to olive growing sites, the cultivar of olive fruits leads to major differences in the composition of the fruit and the extracted oil. For example, fatty acid profiles of Spanish *Arbequina*, *Frantoio* and *Picual* olive cultivars were found to have different fatty acid profiles (Leon et al. 2008). In particular, there were significant differences on the percentage of oleic acid (C18:1) and linoleic acid (C18:2). *Arbequina* and *Frantoio* olive oil were characterized by a low percentage of oleic acid and high percentage of linoleic acid. However, *Picual* olive oil was reported to have high percentage of oleic acid and low percentage of linoleic acid. In their study, the effect of olive growing sites was adjusted and the effect of cultivars on the fatty acid composition of olive oil was found significant. In addition, the triacylglycerol and phytosterol of olive oil were found to vary depending on the cultivar of olives (Haddada et al. 2007).

The aroma profile of monovarietal olive oil varies between one another. It was reported that the total volatile compound present in 39 Spanish olive oil samples range from 9.83 to 35 g/kg (Luna, Morales & Aparicio 2006). For example, E-2-hexenal which is responsible for the green, fruity pungent aroma in olive oil, range from a concentration of 2.52 to 18.1 mg/kg amongst the oil samples. The amount of endogenous enzymes present in the olive fruits is speculated to be the cause of the variation between volatile compounds in olive oil extracted from different olive cultivars (Gómez-Rico, Fregapane & Salvador 2008). As these endogenous enzymes act on different position of the fatty acid chain (the precursors of volatiles), a different range of volatile compounds is formed. Consequently, the aroma profile of olive oil

extracted from different olive cultivars is different (Gómez-Rico, Fregapane & Salvador 2008; Morales & Tsimidou 2000).

The concentration of phenolic compounds in the oil also varies according to cultivars of olives. The variation lies between 37.1 to 615.5 mg/kg (Tura et al. 2007). The large range of variation is attributable to the effect of olive growing sites and the maturity level of the harvested olive fruits. Coupled with the effect of growing sites and maturity levels, it is difficult to identify an individual olive cultivar with high concentration of phenolic compounds based on the literatures available. Meanwhile, Tura et al. (2007) has confirmed the effect of cultivar on the oxidative stability of olive oil by examining the effect of both cultivar and olive growing sites in their study. The higher oxidative stability is attributable to the high concentration of total tocopherols, which is found cultivar-dependant (Tura et al. 2007). For example, *Leccino* olive oil with greater concentration of total tocopherols of greater than 250 mg/kg oil has higher oxidative stability than that of *Frantoio* olive oil.

The effect of cultivar on the quality of olive oil has been demonstrated. Cultivar of olives affect the amount of endogenous enzymes present in the fruits, which directly affects the phenolic compounds, volatile compounds as well as the oxidative stability and sensory profile of the extracted olive oil. The effect of olive cultivar on the quality of extracted olive oil should not be neglected. Understanding of the influences of cultivar on the quality of olive oil is vital for production of olive oil that meets the IOC standards for EVOO. Nevertheless, no investigations have been conducted on the Western Australian olive cultivars.

#### 2.4.6.3 Maturity level

The varietal nature of olives and their maturity levels affect the composition of the fruits and thus the quality of the extracted olive oil. Indeed, olive fruits change in size, composition, colour, texture and aroma as they undergo different stages of development and maturity level (Conde, Delrot & Geros 2008). The main constituents of each section of the olive fruit at the commercial maturity for oil production are reported in Table 2-10.

**Table 2-10: Distribution of the major nutrient components in the three sections of olive fruit at commercial maturity**

| Nutrients (%) | Sections of olive fruit | | |
|---|---|---|---|
| | Mesocarp | Epicarp | Endocarp |
| Water | 60 | 10 | 30 |
| Oil | 30 | 1 | 27 |
| Carbohydrates | 4 | 70 | 27 |
| Protein | 3 | 0 | 10 |
| Ash | 2 | 19 | 13 |

Source: Conde, Delrot and Geros (2008)

The quality of extracted olive oil has been documented as largely affected by the maturity level of the harvested olives (Conde, Delrot & Geros 2008; García et al. 2001; Garcia, Seller & Perez-Camino 1996; Salvador, Aranda & Fregapane 2001). A general indication deduced from these studies is that the yield of oil extraction, concentration of phenolic compounds, oxidative stability, pigments and sensory profile of the olive oil are all affected by the maturity level.

As mesocarp is developed during the first 15 weeks after flowering, there is intense increase in the percentage of oil accumulation in the fruit (Ayton et al. 2001; Conde, Delrot & Geros 2008). Indeed, the percentage of oleic acid (C18:1) increases rapidly in the first 8 weeks (Ayton et al. 2001). In addition, the yield of oil extraction increases during this period.

The synthesis of lipid continues into the 25$^{th}$ week until the fruit enters the ripening stage (García et al. 2001). The beginning of the ripening stage is signified by the change of green olive skin colour to the spotted purple stage as the chlorophyll is replaced by anthocyanins (García et al. 2001). Conde, Delrot and Geros (2008) reported that it is at this ripening stage that the olives have a balance of fatty acid composition and a high concentration of phenolic compounds.

As the ripening stage continues, the activity of cytoplasmic glycosidases, cell wall-linked glycosidases and pectinesterase increases. Consequently, the pectin, hemicelluloses and cellulose of the cell wall are degraded. As a result, the cell wall loses its rigidity and the fruit becomes soft (Jimenez et al. 2001). The softening of the fruit indicates that the olives have reached their matured stage. As the skin of the olive

fruits appear black coupled with soft fruit flesh, the oil content reaches its maximum of 58 % of the fruit weight (Conde, Delrot & Geros 2008). However, the concentration of total phenolic compounds can decrease by up to 50 % while the concentration of tocopherols could drop up to 35 % (Angerosa 2000; Aparicio 2000; Rotondi et al. 2004). In addition, the percentage of C18:1 oleic acid is reduced as the fruit ripens. There is also a drop in the pleasant sensory quality, in which the desirable green leafy aroma (contributed by hex-3-en-1-ol and hex-2-en-1-ol), bitterness and pungency (Aparicio 2000) are less intense.

On the other hand, maturity level was found to not significantly affect the oil content of the Spanish *Arbequina* and *Picual* olives (Zamora, Alaiz & Hidalgo 2001). The results indicate that olive growing sites could be the contributing factor. Indeed, olive growing site (rather than olive cultivar) is established as one of the major factors affecting oil content in olive fruits as they mature (Mailer, Ayton & Conlan 2007). However, no research activity has been published on the effect of growing sites on the oil content of Western Australian olives. It is therefore necessary to investigate the effect of maturity level on the yield of oil extraction as well as the quality of extracted Western Australian olive oil. The results are anticipated to assist the olive oil producers in improving the efficiency of olive oil extraction process and the quality of the extracted oil.

#### 2.4.6.4 Storage of harvested olive fruits

Improper storage of harvested olive fruits can increase the bulk core temperature and promote activity of endogenous enzymes that accelerate the deterioration of olive quality. Ideally, harvested olive fruits should be processed within 24 hours to avoid jeopardizing the quality of the extracted oil. However, not all olive growers have the capacity to process the harvested fruits almost immediately. Such phenomenon is particularly common to small olive growers. Often, the small olive growers have to send their harvested olives to a processing plant in order to get the oil extracted. In this regard, the harvested olives are often stored for a period longer than 24 hours before being processed into olive oil.

In order to verify the appropriateness of storing harvested olives, studies have been conducted to investigate the effect of storage on the quality of extracted olive oil. For example, it is shown that *Frantoio* olives, when stored at 4±2 $^0$C for 2 weeks before processing by the industrial two-phase decanter, led to an increase of 13 % in the oil yield (Kalua et al. 2008). The result suggested that endogenous enzymes, such as pectic enzymes, within the fruit matrix reached their highest activity in breaking down the cell structures after two weeks of harvesting. The oil inside the cells was thus more accessible and could be extracted freely resulting in an increased oil yield.

However, extended period of storage of up to three weeks promotes the hydrolytic rancidity of triacylglycerols. The fatty acids are broken down during the hydrolytic process. Together with the water naturally present in the olive fruits, formation of emulsion is encouraged. The emulsion traps the oil droplets within the matrix and discourages the separation of oil droplets during the processing of olive oil. As a result, less amount of oil can be extracted, leading to a lower yield of oil extraction (Kalua et al. 2008). Therefore, storage of harvested olives should not be longer than 3 weeks. Indeed, studies have shown that inappropriate storage conditions of harvested olives resulted in inferior oil quality (Clodoveo et al. 2007; Kiritsakis et al. 1998; Vichi et al. 2009). When olives were stored at below 5 $^0$C over a period of 30 days, the extracted oil had an acceptable level of acidity, peroxides value and level of conjugated dienes and trienes (Clodoveo et al. 2007; Kiritsakis et al. 1998). However, olive oil extracted from olives stored at 7.5 $^0$C for 30 days was found to have an unacceptable high level of acidity, peroxides value and level of conjugated diene and triene (Kiritsakis et al. 1998). The acidity level was recorded higher as the harvested olives were stored at 8 $^0$C for 21 days (Vichi et al. 2009). The results suggest that storage temperature is a more significant factor than the storage length in producing olive oil with inferior quality. The elevated temperature encouraged the ripening of the olive fruits. It is anticipated that the increase in storage temperature also activated the activity of polyphenoloxidase (PPO). As a result, the phenolic compounds are degraded (Rotondi et al. 2004). Indeed, hydrolysis of glycosylated phenolic compounds of oleuropein and ligstroside was documented during the storage period (Kalua et al. 2008). The lower concentration of phenolic compounds initiates the onset of oxidation, leading to higher level of acidity, conjugated diene and triene as

well as a greater peroxides value in the oil. Therefore, storage of harvested olive fruits should not exceed 5 $^{0}$C over a maximum period of 1 month.

### 2.4.6.5 Removal of stone (destoning)

The stone of olives contains high level of endogenous enzymes, such as peroxidase (POD) and lipoxygenase (LOX) (Montedoro et al. 2002). POD is responsible for degrading the phenolic compounds, while LOX plays an important role in synthesizing the volatile compounds of olive oil (Schwimmer 1981). By removing the stones prior to malaxation, the presence of POD in the olive oil is reduced. Therefore, the breakdown of phenolic compounds is minimized. It is therefore likely to increase the concentration of phenolic compounds in the extracted olive oil. Indeed, it has been observed by Servili et al. (2007) that destoning of *Frantoio* and *Coratina* olives resulted in an improved concentration of specific phenolic compounds, such as 3,4-(dihydroxyphenyl)ethanol-elenolic acid dialdehyde (3,4-DHPEA-EDA), *p*-(hydroxyphenyl)ethanol-elenolic acid dialdehyde (*p*-HPEA-EDA) and 3,4-(dihydroxyphenyl)ethanol-elenolic acid (3,4-DHPEA-EA) in the olive oil. These phenolic compounds are strong antioxidant in olive oil (Ranalli et al. 2001). They have the potential to reduce the risk of cardiovascular diseases and cancer. Therefore, destoning may greatly enhanced the health benefits of the extracted olive oil.

Removal of stones also eliminates the presence of cracked pit pieces in the olive paste. As a result, the paste is more homogeneous and can be mixed more evenly during malaxation, enabling the oil droplets to 'bind' to each other and ease the separation of the oil from the paste (Mulinacci et al. 2005). The 'rough taste' in the olive oil caused by the solid cracked pit pieces can also be avoided by removal of the olive stones (Del Caro et al. 2006). Moreover, removal of stones reduces the possibility of increase in temperature during malaxation, thus preventing the degradation of bioactive components in olive oil (Del Caro et al. 2006).

Olive oil extracted from destoned olives has also been found to maintain lower acidity level, higher values of chlorophylls, carotenoids and α-tocopherol, longer oxidative stability and higher antioxidant activity than oil extracted from whole olives (Del Caro et al. 2006). As a result, the olive oil extracted from destoned olives has greater

stability and shelf life than those extracted from whole olives. Servili et al. (2007) reported that 93 % of the total phenolic concentration of *Frantoio* olives is found in the pulp. Since the concentration of phenolic compounds in olives stones is low (Servili et al. 2007b), removing the stones prior to malaxation does not lead to major loss of phenolic compounds in the extracted olive oil.

Olive stone contains hydroperoxide lyase which breaks down 13-hydroperoxides. As a result, $C_9$ aldehydes (Sanchez & Salas 2000) which produce the unpleasant cucumber odour of olive oil are synthesized. By removing the olive stones, hydroperoxide lyase is eliminated, thus preventing the formation of unpleasant cucumber odour in the extracted olive oil. The aroma of olive oil is improved when the olive stones are removed prior to malaxation. In addition, it was shown that the concentration of $C_5$ and $C_6$ volatile compounds in the olive oil extracted from destoned olives were increased (Amirante et al. 2006).

However, it should be noted that removal of the olive stones reduces the oil yield by 1.5 % (Mulinacci et al. 2005). In addition to the loss of oil present in the olive stones, the reduction in oil yield reported from destoned olives seems to be related to the absence of the draining effect exerted by stone pieces during malaxation (Mulinacci et al. 2005). The merging of released oil droplets during the malaxation period is hindered by the absence of stones. Consequently, separation of the oil from the olive paste by means of mechanical centrifuge becomes difficult. Lower yield of oil extraction is reported from destoned olives. The finding is also reported by Amirante et al. (2006).

### 2.4.6.6 Milling equipment

There are different types of milling equipment employed by the olive oil industry. The common ones are hammer mill and stone mill. Due to the different action they exert on the olive fruits and the subsequent olive paste, the quality of the extracted olive oil varies accordingly.

Caponio, Alloggio and Gomes (1999) studied the impact of crusher on the phenolic compounds of virgin olive oil. It was found that hammer mill could extract more phenolic compounds than stone mill when the olives were processed for 20 minutes.

In particular, the concentration of simple phenolic compounds (such as 3,4-dihydroxyphenyl-ethanol, *p*-hydroxyphenyl-ethanol, *p*-hydroxybenzoic acid, hydroxycaffeic acid, *p*-hydroxyphenylacetic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, cinnamic acid) was increased, and that of the hydrolysable phenolic compounds was reduced (Caponio, Alloggio & Gomes 1999). It was also found that hydrolysable phenolic compounds were the compounds responsible for the bitter pungent taste. In order to achieve a balanced sensory profile, it is recommended to employ a stone mill for production of olive oil from olives with a natural bitter characteristic. A hammer mill is more appropriate for crushing olives with a natural sweet characteristic. The selection of machinery thus has an important role to play in determining the end quality of the extracted olive oil.

The olive paste produced by the high speed fixed-hammers metallic crusher undergoes a rise in temperature of between 13-15 °C during the process. Conversely, the lower speed executed by the stone mill imparts a rise of only 4-5 °C of the olive paste during the processing (Di Giovacchino, Sestili & Di Vincenzo 2002). In this regard, types and speeds of the crushing machine used for the production of olive oil needs to be chosen appropriately to deliver the designated quality of olive oil. In particular, the complete breakdown of the olive cell structures exerted by the violent metallic crushers allows the release of more phenolic compounds from the olives compared to the stone mill. As a result of the increased concentration of total phenolic compounds in the extracted oil, the olive oil is more bitter when processed by violent metallic crushers. For olives from the southern Italian regions which typically have very bitter organoleptic characteristics (Di Giovacchino, Sestili & Di Vincenzo 2002), the producers need to be aware of this difference caused by the crushers.

Attributable to the effect of olive cultivars and olive growing sites, French olive oil is different to Italian olive oil. A study was recently conducted by Veillet et al. (2009) to compare the differences between hammer mill and stone mill for extraction of olive oil from French *Aglandau* olives. Olives crushed by a hammer mill resulted in a higher level of polyphenols and antioxidant activity in the extracted oil (Veillet et al. 2009). The hammer mill was competent in opening the fruit and cutting the cells better without destroying the inter-cellular structure of the olives. The lower yield of extraction arising from the stone mill is possibly due to its operating mechanism where the layers of destroyed inter-cellular structure are just compressed and the oil

droplets are not released completely. A more conclusive statement could be made from their conclusions if the two mills were set to operate at the same speed.

### 2.4.6.7 Length of malaxation

Malaxation, known as kneading and/or mixing of the olive paste, is an action that allows the coalescence of free, tiny oil droplets to flocculate into bigger ones which can later be extracted more easily. Malaxation also has another function as to breakdown the emulsion formed during the crushing process, which is common when a violent crushing action was applied and/or when the moisture content of the olives were high (Di Giovacchino, Sestili & Di Vincenzo 2002). Both of these actions facilitated by malaxation are beneficial in improving the yield of extraction. In addition, aromatic compounds, such as the $C_5$ and $C_6$ volatiles are produced through the LOX pathway during malaxation. The $C_5$ and $C_6$ volatiles are responsible for the cut grass," "haylike", and "floral" profile of virgin olive oil (Ranalli et al. 2001; Servili et al. 2008).

The length of malaxation period is a critical step that influences the quality of olive oil. As large amount of carbon dioxide ($CO_2$) gas is produced during malaxation (Parenti et al. 2008), there would be certain level of protection against oxidation of the oil and the phenolic compounds in the oil. However, a balance should be maintained as increased level of $CO_2$ gas may induce deleterious 'fermented' flavour in the oil. The acidity level, peroxide value, conjugated diene, conjugated triene and sensory profile of the olive oil was not affected by the prolonged 90-minute malaxation period (Di Giovacchino, Sestili & Di Vincenzo 2002). It is believed that the naturally occurred phenolic compounds in the olive oil are protective against oil deterioration over a period of 90-minute malaxation length.

### 2.4.6.8 Oxygen level during malaxation

As previously mentioned, the level of $CO_2$ gas affects the quality of olive oil. Similarly, the level of oxygen gas ($O_2$) needs to be monitored during the malaxation period to prevent the occurrence of oil oxidation. Indeed, oxygen concentration in the headspace of the malaxer is the key determinant on the concentration and the type of

phenolic compounds present in the olive paste (Servili et al. 2008). The reason is that the activity of PPO and POD enzymes are strongly affected by the oxygen concentration in the paste. In this regard, oleuropein, 3,4-DHPEA-EDA and *p*-HPEA-EDA are the most sensitive phenolic compounds to the oxygen during malaxation (Servili et al. 2008). While being effective in reducing cardiovascular diseases and preventing occurrence of cancer, these phenolic compounds also contribute to the sensory profile of the olive oil. In particular, *p*-HPEA-EDA is related to the "pungency" of the oil while the others are responsible for the "bitter" taste in virgin olive oil (Angerosa et al. 2000; Servili et al. 2008). It is therefore necessary to minimize the loss of these phenolic compounds as they have strong antioxidant ability. In this regard, oxygen levels may be altered during the production of olive oil to optimize the concentration of total phenolic compounds in olive oil (Servili et al. 2008).

#### 2.4.6.9 Malaxation temperature

The temperature at which malaxation is carried out at may influence the quality of the extracted oil. It is believed that higher temperature facilitates the breakdown of cell structure, thus releasing more of the cell components, including the oil droplets and phenolic compounds.

Studies were conducted to validate this assumption. For example, the yield of oil extraction is improved when the malaxation temperature is increased from 20 to 30 ºC. It is thought that the increase in the yield is attributable to the viscosity of the oil phase. As temperature increases, the viscosity is reduced (Ranalli et al. 2001). As a result, it facilitates the extraction of oil from the olive paste and results in a higher yield of oil extraction. The observation was verified by Inarejos-Garcia et al. (2009).

High malaxation temperature of up to 40 ºC improves the concentration of phenolic compounds and oxidative stability of Spanish *Cornicabra* olive oil (Inarejos-Garcia et al. 2009). However, Parenti et al. (2008) reported a decrease in the concentration of phenolic compounds in Italian *Frantoio* olive oil when the olive paste was malaxed for 45 minutes at temperature greater than 27 ºC. The high temperature aids the degradation of phenolic compounds and reduces their concentration in the extracted

oil. As the concentration of phenolic compounds in the extracted oil decreases, the peroxides value increases due to the loss of oxidation protective mechanism exerted by phenolic compounds (Parenti et al. 2008). Such observations are deleterious to the quality of the extracted oil. Furthermore, the undesirable biochemical pathways and fermentation process can be triggered at high temperature, producing olive oil with negative sensory attributes. Indeed, the concentration of pleasant $C_5$ and $C_6$ volatile compounds was reduced during malaxation at temperature above 35 ºC. The mechanism is attributable to deactivation of LOX pathway at high temperature (Ranalli et al. 2001).

Based on these results, it seems that although increasing the malaxation temperature improves the yield of oil extraction, the negative impacts on the quality of the extracted oil limit its worth. Rather, a compromised temperature of 27 ºC is suggested to maximize the yield of oil extracted whilst maintaining the quality of the extracted oil. As temperature rises during malaxation, it is advisable to set off the malaxation period at a lower temperature, such as at room temperature of 22 ± 2 ºC.

### 2.4.6.10 Processing aids

The potential application of processing aids is currently receiving a great deal of interest by the olive oil industry with the aim of increasing the yield of oil extraction. In some occurrences, when the initial moisture content of the olive fruits is high, or the type of crusher employed exerts violent motion to the paste, emulsion is formed. Such circumstances complicate the separation of the oil droplets from the solid paste, thus reducing the yield of oil extraction. In this regard, processing aids may help to resolve the problem. Examples of some common processing aids include talc and enzymes. Their modes of action are described as followed.

#### 2.4.6.10.1 Talc

Depending on the agronomic practices and the volume of rainfall, some olives may have high moisture content in the fruit. When these olives are crushed, both the oil and the water in the fruit are released. As a result, an emulsion is formed. With large amount of oil being trapped in the emulsion, the efficiency of oil extraction is reduced.

Therefore, there is a need to minimize the moisture content in the olive fruits, especially during the extraction process of olive oil.

Micronized mineral talc is effective in binding the water in olives with high moisture content. As the moisture content in the olives is bound by talc, there is less formation of emulsion. As a result, less oil droplets are trapped in the emulsion. Consequently, more oil can be extracted, leading to greater olive oil extraction efficiency. The concentration of talc commonly applied is about 1-2 % of the paste. The extracted oil is clearer and has similar qualities to the non-treated olive oil (Di Giovacchino 2000). Addition of talc in a concentration of 2 and 4 % can breakdown emulsion, release the oil droplets into the system and improve the extraction yield by 2 and 5 %, respectively (Di Giovacchino 2000). Application of talc is approved in Spain but not in Australia yet.

**2.4.6.10.2 Enzymes**

The cell wall of olive fruits consists of mainly pectin, cellulose and hemicellulose (Najafian et al. 2009). In order to effectively release the oil vacuoles kept in different layers in the olive cells, it is necessary to rupture each of the pectin, cellulose and hemicellulose layers. Physical cell rupture of the olive fruits is inefficient in breaking down these cell layers. Therefore, it is necessary to apply natural processing aids with specific activity to act on the olive fruit matrix and breakdown the vacuoles of the mesocarp cells which helps to release the stored oil droplets. In this case, enzymes, such as pectinase, cellulase, hemicellulase and endopolygalacturonase may be added during the extraction of olive oil. The enzymes degrade the pectin, cellulose and hemicellulose of the olive cell wall. Therefore, it is anticipated that they can promote the release of cell components (such as oil droplets and phenolic compounds) trapped in the colloidal tissues of cytoplasm (Chiacchierini et al. 2007). Meanwhile, the enzyme endopolygalacturonase is capable of breaking down the emulsions formed during olives crushing and olive paste malaxation (Di Giovacchino, Sestili & Di Vincenzo 2002). The quantity of “free oil” and concentration of total phenolic compounds are expected to increase in the olive oil extracted with enzymes (Di Giovacchino 2000).

The effect of addition of enzymes with pectolytic and cellulolytic activities to olive paste has been studied in Italy. These enzymes, particularly the ones with specific pectolytic activity effectively break down the cell structure and release the stored oil droplets. In addition, application of enzymes as a processing aid during the production of olive oil improves the yield of oil extracted without affecting the composition of fatty acids, sterols, aliphatic and triterpene alcohols, triterpene dialcohols and other fractions of the unsaponifiable matter of the oil (Chiacchierini et al. 2007; Di Giovacchino, Sestili & Di Vincenzo 2002). Another benefit of enzyme extraction is that they do not negatively affect the acidity level and oxidative stability of the extracted olive oil. In addition, the oil extracted with enzymes is reported to have higher concentration of volatile and phenolic compounds. The sensory profile is also improved (Chiacchierini et al. 2007). The higher storage stability and greater antioxidant activity of olive oil extracted by enzyme are attributable to the higher amount of phenolic compounds extracted into the oil (Ranalli & De Mattia 1997; Servili & Montedoro 2002; Vierhuis et al. 2001). It is anticipated that enzymes, when breaking down the cell structure of olive fruits, also release the phenolic compounds into the oil. However, the actual mechanism has not yet been elucidated.

In Australia, the use of enzymes in the extraction of olive oil was first conducted by Canamasas in 2006. He applied different types of enzymes, such as pectinases and cellulases, during the extraction of olive oil from *Barnea* and *Picual* olives grown in Victoria, Australia. It is revealed that the oil yield was increased due to the enzyme treatments (Canamasas 2006). However, his study did not provide information on the chemical properties (particularly the concentration of bioactive phenolic compounds) and sensory profile of the extracted oil. As we have learnt previously on the effect of olive cultivars and growing sites on the quality of olive oil, it is possible that the observations reported by Canamasas may not be applicable to all cultivars of olive grown at different sites. It is therefore necessary to evaluate the effect of enzymes on the quality of olive oil extracted from a variety of olive cultivars and olives grown at different sites.

In summary, the unique high MUFA:PUFA ratio and the bioactive phenolic compounds contribute to the health benefits of olive oil. Olive oil is reported as protective against cardiovascular diseases and cancer. Due to the increased awareness of its health benefits, the consumption of olive oil is speculated to exceed the production volume. It is necessary to determine an optimum olive oil extraction technique with high extraction of oil and phenolic compounds. However, there is a gap of knowledge in improving the yield of olive oil extraction and the oil quality, particularly on Western Australian olive oil. Yield and quality of the olive oil can be affected by various factors, such as olive growing sites, cultivar, maturity level, storage of harvested olive fruits, removal of olive stones, milling equipment, malaxation length, level of oxygen gas during malaxation, malaxation temperature and processing aids such as talc and enzymes. No conclusive outcome on the effectiveness of a particular processing technique in improving the extraction of oil and phenolic compounds has been drawn to date. Extending the length of malaxation period and addition of processing aids can be the potential solutions to this problem. Indeed, less amount of monetary investment is involved by application of these techniques comparing to modification of extraction machinery.

# Chapter 3 Research methods

## 3.1 Raw materials

### 3.1.1 Olives

Olive fruits (*Olea europaea*) of the *Frantoio* cultivars are one of the most popular olive cultivars grown in Western Australia for the production of olive oil (Gawel 2006; Obied et al. 2005). As stated in Section 2.4.2, the main production areas of olive oil in Western Australia are located in the Moore River, Margaret River and Great Southern Regions. Three olive groves that grow *Frantoio* olives were selected from these areas. They are Gingin (located at 84 km north of Perth, Western Australia, 31°22'S, 115°54'E), Swan Valley (located at 33 km east of Perth, Western Australia, 32°3'S, 115°45'E) and Margaret River (located at 277 km south of Perth, Western Australia, 33°57'S, 115°01'E). The olive fruits are developed between January to July of each calendar year. The climate data from January to July of the studied 2008 and 2009 seasons were collected and summarized in Table 3-1.

**Table 3-1: Climate data of the regions where the olive groves were located**

| Growing sites | Total Rainfall (mm) | | Average minimum temperature ($^{o}$C) | | Average maximum temperature ($^{o}$C) | |
|---|---|---|---|---|---|---|
| | **2008** | **2009** | **2008** | **2009** | **2008** | **2009** |
| **Gingin** | 213.6 | 154.8 | 11.9 | 11.4 | 26.2 | 25.9 |
| **Swan Valley** | 223.2 | 148.0 | 13.1 | 13.0 | 26.0 | 25.8 |
| **Margaret River** | 234.7 | 230.5 | 11.0 | 11.1 | 21.2 | 21.5 |

Source: Bureau of Meterology (2009)

Approximately 20 kilograms of olive fruits were harvested from each of the selected olive grove and sorted according to the maturity index (MI) established by the IOC. The MI covers the range from 0 to 7. Each specific level has a distinctive description on the physical characteristics of the olive fruits. Descriptions for each MI level are stated in Table 3-2.

**Table 3-2: Maturity index of olive fruits as stated by IOC**

| Maturity index (MI) | Descriptions |
|---|---|
| 0 | Fruit is hard with deep-green skin colour |
| 1 | Fruit starts to soften with yellow or yellow-green skin colour |
| 2 | Fruit has yellow-green skin colour with less than half of the fruit turning red (reddish spots) and violet |
| 3 | Fruit has red to purple skin colour on more than half of the fruit |
| 4 | Fruit is light purple to black skin colour with white-green flesh colour |
| 5 | Fruit has black skin colour and purple flesh colour less than half way to the pit |
| 6 | Fruit has black skin colour and purple flesh colour almost to the pit |
| 7 | Fruit has black skin colour and dark flesh colour all the way to the pit |

Source: Sibbett (2004)

In most olive oil production, the olive fruits are harvested at between MI3 and MI5 (Sibbett 2004). However, the olive fruits contain the highest level of phenolic compounds at MI1 while the oil content is the highest at MI6 (Sibbett 2004). Therefore, greenish yellow, partial purple and completely black olives with MI1, MI4 and MI6, respectively (Figure 3-1), were used in this study.

**Figure 3-1: Olives with different maturity indices**
**(From left to right: olives at MI1, olives at MI4, olives at MI6)**

## 3.1.2 Processing Aids

The effects of three different processing aids on the yield and quality of olive oil were investigated in the present study. The processing aids included chelating agent (citric acid) and enzyme preparations (Pectolyase and Viscozymes).

Chelating agent prevents degradation of phenolic compounds by deactivating PPO. Citric acid is a PPO inhibitor. It can chelate copper metal in the structure of PPO and scavenge the available oxygen radical to inhibit PPO and thus prevent degradation of phenolic compounds. Food grade anhydrous citric acid powder was purchased from PharmaAust for this study.

Enzymes facilitate the breakdown of cell walls and release oil enclosed in the vacuoles as well as the phenolic compounds in the cell. Therefore, it is anticipated that addition of enzymes will affect the yield of oil extraction and the quality of the extracted olive oil. As the enzymes employed in this study consist of a combination of different enzymes, they are referred to as enzyme preparations in the following text. Enzyme preparation Pectolyase was purchased from Sigma-Aldrich® while enzyme preparation Viscozymes was purchased from Novozyme®. Enzyme preparation Pectolyase is produced from *Aspergillus japonicus* and is presented in the form of lypholized powder. It contains two types of pectinase, the endopolygalacturonase (EC 3.2.1.15) and endo-pectin lyase (EC 4.2.2.10) as well as a maceration stimulating factor. The enzyme activity of Pectolyase is 115.0 units/mL. Enzyme preparation Viscozymes is produced from *Aspergillus niger* and is presented in the form of liquid solution. It contains a mixture of cell wall degrading enzymes, containing mainly carbohydrases such as arabanase, cellulase, β-glucanase, hemicellulase and xylanase. The enzyme activity of Viscozymes is 172.5 units/mL.

## 3.1.3 Commercial samples

Commercial samples were purchased from a supermarket to compare the effects of the processing techniques applied in this study on the quality of extracted olive oil. The group comprises of 4 oil samples labeled as “EVOO”. Two of the oil samples were produced in Italy and Spain, the two major olive oil producing countries in the world. The two brands chosen for this study are popular in the Western Australian

market. In addition, they are the only brands that store the olive oil in light-proof amber bottles. Under such storage conditions, the risk of oxidation of these oil samples is largely reduced. It is likely that the freshness of these olive oil samples is maintained. It is therefore reasonable to employ these oil samples to assess the effectiveness of the various processing techniques applied in this study. Two Australian olive oil samples were also purchased to aid the comparison as literature suggested olives growing sites cause variation in the quality of olive oil (Section 2.4.6.1). The origins and the brand names of these commercial EVOO samples are provided in Table 3-3.

**Table 3-3: Origin and brand names of the commercial EVOO samples purchased from supermarket**

| Country of origin | Brand name |
|---|---|
| Italy | Colavita |
| Spain | La Espanola |
| Australia (Western Australia) | Jingili |
| Australia (Victoria) | Pro Chef |

## 3.2 Study design

In this study, the effects of olive growing sites, maturity levels of the harvested olives and processing techniques applied to the olive paste on the quality parameters of extracted Western Australian *Frantoio* olive oil were investigated. The range selected for these variables is stated in Table 3-4.

**Table 3-4: Codes of independent variables and their descriptions**

| Variables | Descriptions |
|---|---|
| *Olive growing sites* | |
| - S1 | Growing site 1: Gingin (84 km north of Perth) |
| - S2 | Growing site 2: Swan Valley (33 km east of Perth) |
| - S3 | Growing site 3: Margaret River (277 km south of Perth) |
| *Maturity levels of olive fruits* | |
| - MI1 | Maturity 1:<br>Olives are yellow or yellow-green colour and start to soften |
| - MI4 | Maturity 4:<br>Olives are light purple to black colour with white-green flesh |
| - MI6 | Maturity 6:<br>Olives are black colour with purple flesh colour almost to the pit |
| *Processing techniques applied to olive paste* | |
| - T1 | Technique 1 (*control*)<br>Olives are crushed and the olive paste is malaxed for 30 minutes with no addition of processing aids |
| - T2 | Technique 2:<br>Olives are crushed and the olive paste is malaxed for 60 minutes with no addition of processing aids |
| - T3 | Technique 3:<br>Olives are crushed and the olive paste is malaxed for 30 minutes with addition of citric acid to the olive paste |
| - T4 | Technique 4:<br>Olives are crushed and the olive paste is malaxed for 30 minutes with addition of Viscozyme to the olive paste |
| - T5 | Technique 5:<br>Olives are crushed and the olive paste is malaxed for 30 minutes with addition of Pectolyase to the olive paste |

The general factor design thus included 45 combinations of variable levels. The matrix presented in Table 3-5 only included the sample size for growing site 1 (S1), which is Gingin. In order to assess the effect of olive growing sites, identical matrices were developed for data collection at growing sites 2 and 3, which are Swan Valley and Margaret River, respectively.

**Table 3-5: General factor design and the coded level of samples**

| Samples | Coded level | | |
|---|---|---|---|
| | Growing sites | Maturity levels | Processing techniques |
| 1 | S1 | MI1 | TI |
| 2 | S1 | MI1 | T2 |
| 3 | S1 | MI1 | T3 |
| 4 | S1 | MI1 | T4 |
| 5 | S1 | MI1 | T5 |
| 6 | S1 | MI4 | TI |
| 7 | S1 | MI4 | T2 |
| 8 | S1 | MI4 | T3 |
| 9 | S1 | MI4 | T4 |
| 10 | S1 | MI4 | T5 |
| 11 | S1 | MI6 | TI |
| 12 | S1 | MI6 | T2 |
| 13 | S1 | MI6 | T3 |
| 14 | S1 | MI6 | T4 |
| 15 | S1 | MI6 | T5 |

Each condition was done in triplicate. However, in some cases, the harvested olives were of inferior quality. Hence, the number of replicates for some conditions is reduced. In particular, rotten olives that are bruised (Figure 3-2) were not used in this study. The literatures reviewed previously suggested that processing aids are more effective in enhancing the efficiency of oil extraction and the quality of the extracted oil compared to extension of olive paste malaxation period. Therefore, when there was insufficient supply of 'healthy' olives, the effect of processing aids on the efficiency of oil extraction and the quality of the extracted oil was investigated prior to the extension of olive paste malaxation period.

**Figure 3-2: Defect olives of inferior quality**

The effects of each independent variable listed in Table 3-4 were evaluated on the quality parameters of the olive oil listed in Table 3-6. Preliminary data collected in the 2008 season served as a guideline for the areas of independent variables to be focused on for the 2009 season. When the interaction between two independent variables on the quality parameters was not significant, the effect of that particular independent variable was not studied in the 2009 season. Similarly, the effect of a particular processing aid was not studied in the 2009 season if it did not show significant influences on the quality parameters in the 2008 season. On the other hand, when a particular processing aid significantly improved the quality parameters in the 2008 season, its effect was studied at a higher concentration in the 2009 season.

**Table 3-6: Codes of quality parameters of olive oil samples and their descriptions**

| Coded quality parameters | Descriptions |
|---|---|
| - Q1 | Moisture content of each of olive parts |
| - Q2 | Oil content of each of olive parts |
| - Q3 | Yield of oil extraction of olive oil |
| - Q4 | Total oil recovery of olive oil |
| - Q5 | Fatty acid composition of olive oil |
| - Q6 | Total phenolic compounds of olive oil |
| - Q7 | Antiradical activity of olive oil |
| - Q8 | Peroxides values of olive oil |
| - Q9 | Acidity of olive oil |
| - Q10 | Oxidative stability of olive oil |
| - Q11 | Colour of olive oil |
| - Q12 | Sensory evaluations on aroma and palate attributes of olive oil |

# 3.3 Sample preparation

## 3.3.1 Olives

Olives from Gingin and Swan Valley were harvested into plastic buckets and delivered to Curtin University of Technology on the same day. Harvested olives from Margaret River were kept in a cool room at 4±2 ºC and delivered on the following day due to the travel distance. Leaves, stems, as well as bruised and semi-dried olives were disposed of manually. A damp piece of cloth was used to wipe the foreign materials such as dusts, soils and dew off the olives. After cleaning, the sound olives were laid flat on trays labeled with the date of harvest, olive growing sites and

maturity indices. Olives were then wrapped with plastic film and left in the refrigerator at 4±2 ºC until further processing. Restricted by the capacity of the olive processing unit, it should be noted that the olives could be kept in the fridge to up to 2 weeks prior to processing. In order to avoid compromising the quality of the oil, old and rotten olives were discarded prior to processing and were not included in this study.

### 3.3.2 Processing aids

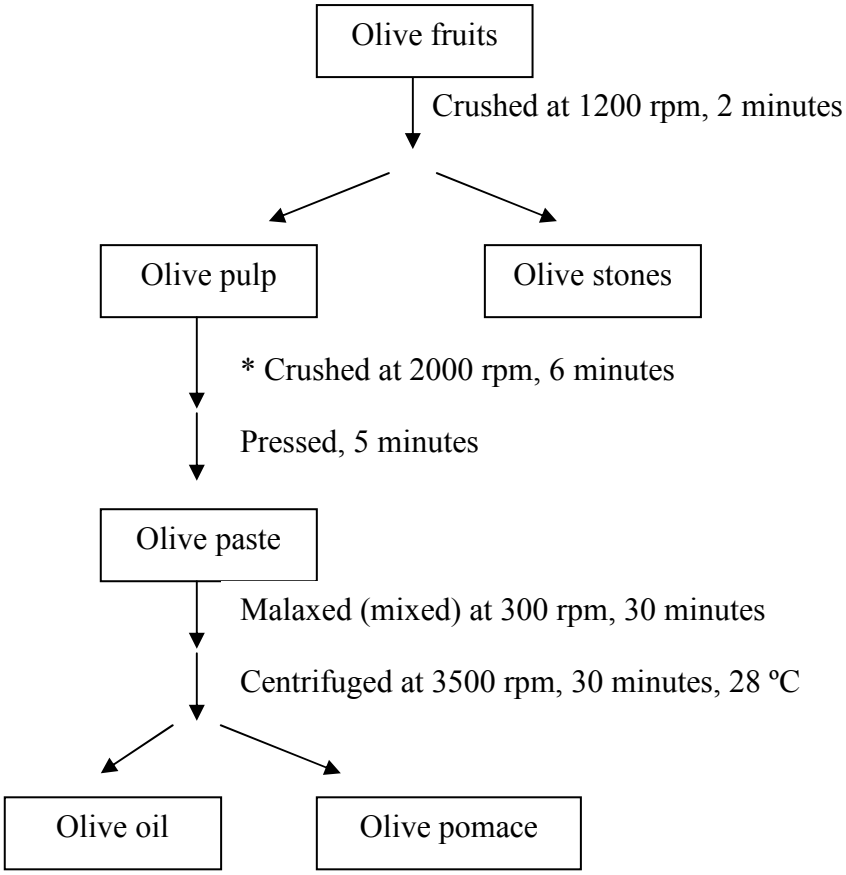
Pectolyase enzyme preparation was freshly prepared to a concentration of 0.15 g/mL in deionised water to yield 75 units/mL of enzyme activity, as recommended by Sigma-Aldrich® for maceration of cell wall. Viscozymes enzyme preparation was freshly prepared to a concentration of 0.15 g/mL in deionised water to yield 115 units/mL of enzyme activity, as recommended by Novozymes® for maceration of cell wall. Citric acid was freshly prepared to a concentration of 0.15 g/mL in deionised water according to the food safety guideline.

## 3.4 Production of olive oil

Harvested olives were prepared as stated in Section 3.3.1 and weighed to a sample size of approximately 500 grams for production of olive oil. Each sample of olives was processed by Blixer® 4.0 blender (Robot Coupe®) at 1200 rpm for 2 minutes. The olive stones were then separated manually by hand from the fruit matrix. In order to promote size reduction of the pulp, it was further processed at 2000 rpm, stopping at every 30 seconds and stirred through prior to the next processing interval of 30 seconds for a total duration of 6 minutes. Pressure was applied by using a pestle to compress the fine pulp for 5 minutes to assist the maximum release of oil from the cell structure. The two consecutive actions were followed to resemble the crushing and grinding action employed by the industrial machineries. The olive paste was malaxed at 300 rpm for 30 minutes to allow coalescence of oil droplets and assist flocculation of larger oil droplets to the top of the paste (Figure 3-3).

**Figure 3-3: Destoned olive paste being malaxed in the Blixer 4.0 Blender as oil droplets coalescene and float to the top**

An Eppendorf 5810R centrifuge (Eppendorf AG, Hamburg, Germany) was used to centrifuge the malaxed olive paste. After being centrifuged at 3500 rpm for 15 minutes in ThermoIEC polypropylene centrifuge bottles (ThermoIEC, MA) at 28 ºC, the olive oil was filtered with Whatman® Glass Microfibre filters circles (Schleicher & Schuell). The oil was then stored in amber bottles filled with nitrogen gas at room temperature for subsequent testing of the quality parameters. Production of the control sample is illustrated in Figure 3-4. The processing aids were added after the olives were crushed and the stones were separated from the crushed fruit matrix.




**Processing aids were added at this stage for treated samples*

**Figure 3-4: Flow chart of the production of olive oil**

# 3.5 Evaluation of the quality

## 3.5.1 Moisture content

Moisture contents of the olive fruit, olive stones and pomace were measured using the Association of Official Agricultural Chemists (AOAC) method 925.09. Aluminium dish was dried in Contherm Digital Series oven for 16 hours at 105 ºC. Once cooled in a desiccator to room temperature, the weight of the dish was recorded as $W_0$. Approximately 1 g of the sample was weighed into the pre-weighed dish. Total weight of the dish containing sample was recorded as $W_1$. The sample in the dish was then dried in the same oven for 16 hours at 105 ºC. Subsequently, the dish was

covered by its lid and transferred to the desiccator. The dish was cooled for 30 minutes to room temperature (20 ±2 ºC). The weight of the cooled dried dish was recorded as $W_2$. Moisture content of the samples was calculated using ***Equation 1***.

$$\textit{Moisture Content (\%)} = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \quad \text{--- Equation 1}$$

where: $W_0$= weight of empty dish (g),
$W_1$= weight of dish and sample (g),
$W_2$= weight of dried dish and sample (g)

## 3.5.2 Fat content

Fat contents of the olive fruit, olive stones and pomace were determined using the AOAC method 920.39C. A Buchi E-816 SOX Extraction Unit (Switzerland) (Figure 3-5) was employed to analyze the fat content of the samples. Accurately weighed 1 g sample ($W_s$) was placed into a thimble. Weight of the extraction cup containing two glass beads was recorded as $W_e$ and placed beneath the corresponding extraction chamber. Once sealed, the Soxhlet machine was filled with approximately 120 mL of petroleum ether to begin the fat extraction process. At the end of the 2 hour extraction period, the solvent in the extraction cup was evaporated and then dried in an oven at 105 ºC overnight. Weight of the dried extraction cup was recorded as $W_c$. The fat content of the samples was calculated based on ***Equation 2.***

$$\textit{Fat Content (\%)} = \frac{Wc - We}{Ws} \times 100 \quad \text{--- Equation 2}$$

where: $W_s$= weight of sample (g),
$W_e$= weight of empty cup (g),
$W_c$= weight of cup after extraction (g).

**Figure 3-5: Buchi E-816 SOX Extraction Unit in operation**

## 3.5.3 Yield of oil extraction

Olive oil processors used the yield of oil extraction as a guide to the appropriateness of processing techniques. The weight of the initial olive fruit samples ($W_f$) and the weight of the extracted oil ($W_o$) were used to calculate the yield of oil extraction (%) using ***Equation 3***.

$$\textit{Yield of oil extraction (\%)} = \frac{W_o}{W_f} \times 100 \quad \text{---} \quad \textit{Equation 3}$$

where: $W_o$= weight of extracted oil (g),
$W_f$= weight of olive fruits (g).

## 3.5.4 Oil recovery

Oil recovery is an important indication of the efficiency of the extraction method. The percentage of oil in the dried stones ($O_s$), the dried paste ($O_p$), and the extracted oil ($O_o$) were analyzed for each extraction process to evaluate oil recovery of each extraction process. Oil recovery (%) is calculated based on ***Equation 4***.

$$Oil\ recovery\ (\%) = \frac{O_0}{O_s + O_p} \times 100 \quad \text{--- Equation 4}$$

where: $O_o$ = Weight of oil (g) in the total amount of extracted oil (g),
$O_s$ = Weight of oil (g) in the total amount of dried stones (g),
$O_p$ = Weight of oil (g) in the total amount of dried paste (g).

## 3.5.5 Fatty acid composition

Determination of the fatty acid composition in olive oil is most appropriately measured as FAME (Section 2.4.5.1.1). Fatty acid composition of the olive oil samples prepared in this study and some commercially produced olive oil samples purchased from a local supermarket was analyzed by means of acid esterification through acid catalysis into methyl esters. Toluene (5 mL) was added to a pre-weighed amount of olive oil (0.1 g) in a test tube. Subsequently, 10 mL of boron trichloride-methanol solution was added and the mixture was flushed with nitrogen gas for 10 seconds, and later boiled for 5 minutes at 85 ºC. The mixture was then transferred to a separatory funnel and 20 mL of 20 ºC cold water was added to stop the conversion of fatty acids to FAME catalyzed by the boron trichloride-methanol solution and the intense period of reflux. The water also aided the phase separation. Subsequently 20 mL of hexane was used to extract the FAME. This step was done three times to ensure maximum extraction of FAME from the oil. The hexane extract was then collected and the pH was adjusted to neutral by washing with water, prior to the addition of anhydrous sodium sulphate. Volume of the anhydrous hexane extract was then made up to 100 mL. A volume of 1.5 mL hexane extract was pipetted into an auto-sampler vial and analyzed by the Perkin Elmer AutoSystem XL gas chromatography (Norwalk, CT, USA) equipped with an autosampler, a SGE BPX70 column (25 m x 0.32 mm; 0.25 μm), a split-splitless injector, and a flame ionization detector (FID). Helium, flowing at a rate of 1 mL/min, was employed as the carrier gas. The temperature of the injector, detector and oven were held at 200, 250 and 200 ºC, respectively. An olive oil standard (Supelco, Bellefonte, PA, USA) was analyzed in the first position of each run to ensure the correct designated peaks of the fatty acids, namely palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) were detected appropriately. Once calibrated by the standard, the peaks of fatty acids in each sample were identified and the percentage composition was calculated on a peak

area basis. The results were expressed as a relative area percent of total FAMEs by applying ***Equation 5***.

$$FA\ composition\ (\%) = \frac{As}{A_T} \times 100 \quad \text{---} \quad Equation\ 5$$

where: $A_s$= area of the specific fatty acid peak,
$A_T$ = total area of the main fatty acid peaks.

## 3.5.6 Total phenolic compounds

As stated in Section 2.4.5.1.4, determination of the concentration of total phenolic compounds in the oil is most appropriately measured by Folin-Ciocalteau reagent. Concentration of the total phenolic compounds in the olive oil samples prepared in this study and some commercially produced olive oil samples purchased from a local supermarket was determined according to the method employed by Gutfinger (1981) with slight modifications. Approximately 1 g of oil was weighed into a test tube. Hexane (5 mL) was added to dissolve the oil. The phenolic compounds were extracted three times by 3 loads of 2.5 mL 80 % methanol solution. The phenolic compounds extract with a total volume of 7.5 mL were left to stand for 5 hours to let the phenolic compounds condense. After 5 hours, 0.5 mL of Folin-Ciocalteau reagent and 1 mL of 30 % sodium carbonate solution were added to 1 mL of the phenolic compounds extract. The solution was left for an hour for blue colour development before being centrifuged at 2500 rpm for 1 minute using Eppendorf 5810R centrifuge (Eppendorf AG, Hamburg, Germany) to separate the blue solution from the white precipitate. The blue solution was transferred into a micro-cuvette and its absorbance was measured at 725 nm. The concentration of the total phenolic compounds in the olive oil was then calculated against the gallic acid standard curve and reported in mg/kg (***Equation 6***).

$$Total\ \ phenolic\ \ compounds\ (mg/kg\ oil) = \frac{Abs_S - 0.0058}{56018} \times \frac{volume\ of\ extract\ (mL)}{weight\ of\ sample\ (g)}\ x\ 10^6 \quad \text{---} \quad Equation\ 6$$

where: $Abs_s$ = Absorbance of sample,
0.0058 and 56018 are the coefficient derived from the standard curve

## 3.5.7 Antiradical activity

As stated in Section 2.4.5.1.5, the DPPH method is a rapid, straight-forward approach to accurately measure the antiradical activity of olive oil. Determination of the antiradical activity of the olive oil samples prepared in this study and some commercially produced olive oil samples purchased from a local supermarket was conducted following Kiralan, Bayrak and Ozkaya (2009)'s method. The results were expressed as the ability of the oil samples in inhibiting (scavenging) free radicals over a 30 minute period. Briefly, 1 g of oil was dissolved in 2 mL of hexane. Secondly, 2 mL of 60 % methanol was added to the mixture and agitated vigorously for 10 seconds with a vortex. After centrifuged for 10 minutes at 3500 rpm with Eppendorf 5810R centrifuge (Eppendorf AG, Hamburg, Germany), 0.5 mL of the extract was reacted with 60 % DPPH-methanolic solution for 30 minutes in the dark. The absorbance of the solution was read at 515 nm. A control sample consisted of 0.5 mL of 60 % methanol was reacted under the identical condition. Antiradical activity of the sample, reported as percentage of inhibition of the DPPH radicals (DPPH•), was calculated using ***Equation 7***.

$$\textit{\% inhibition of DPPH} \bullet = \frac{\textit{Abs}_c - \textit{Abs}_s}{\textit{Abs}_c} \times 100 \quad \text{--- Equation 7}$$

Where: $Abs_c$ = absorbance of the control,
$Abs_s$ = absorbance of sample.

## 3.5.8 Peroxides value

Oxidized lipid peroxides contribute to the rancidity of oil samples. It is necessary to measure the peroxides value over time to ensure the storage stability of oil samples. The peroxides value of the olive oil samples prepared in this study and some commercially produced olive oil samples purchased from a local supermarket was measured as the intensity of ferric ($Fe^{3+}$) ions in the oxidised oil by an OxiTester instrument (CDR®, Italy), as illustrated in Figure 3-6. The OxiTester instrument was automatically set to pre-heat mode at the beginning of the operation. Cuvettes containing a mix of alcohols were put in the heated instrument (37 ºC) for 5 minutes. After heating, the whole cuvette was agitated to ensure the reagent was well-mixed. A micro-quantity of oil (5 μL) was added to the pre-warmed cuvette, agitated vigorously,

followed by addition of 10 µL of the redox solution and subsequent vigorous agitation before incubating the cuvette for another 3 minutes. The incubated cuvette was then inserted into the designated well and the readings measured as mEq/ kg oil.

**Figure 3-6: OxiTester assessing the peroxides value of an oil sample**

## 3.5.9 Acidity

Acidity of oil serves as an indicator of the appropriateness of processing and storage condition. In the case of olive oil, acidity level aids in the classification of EVOO, VOO, OO and POO. Acidity is usually determined by the quantity of free fatty acids derived from hydrolytic rancidity of triacylglycerols. The acidity of the oil samples prepared in this study and some commercially produced olive oil samples purchased from a local supermarket was determined by an OxiTester instrument (CDR®, Italy). The method is based on the principle reaction between the alcoholic potassium hydroxide and phenolphthalein derivative. The OxiTester instrument was pre-heated to 37 ºC. Once this was completed, the blank cuvette containing alcoholic potassium hydroxide and phenolphthalein derivative was put in the heated instrument for 5 minutes. After heating, the whole cuvette was agitated in order to ensure the reagent was mixed well. A micro-quantity of oil (2.5 µL) was added to the pre-warmed cuvette and agitated vigorously before inserting it into the designated well. The readings shown on the OxiTester were expressed in the percentage of oleic acid.

### 3.5.10 Oxidative stability

Double bond configuration, as a result of the formation of peroxyl radicals and hydroperoxides, is often reflective of the oxidative stability of oil samples. The oxidative stabilities of the olive oil samples prepared in this study and some commercially produced olive oil samples purchased from a local supermarket were measured in terms of the presence of conjugated diene and triene as represented by K232 and K270 extinction coefficients, respectively. The K232 and K270 values are the absorption of 1 % oil solution in cyclohexane at 232 and 270 nm, respectively, with 1 cm of pass length in quartz cuvette as described in the IOC/T20/Doc. no. 19/Rev. 2 (2008). The absorption was measured using a Shimadzu UV-VIS (UV-1201) spectrophotometer (Shimadzu, Japan). The extinction coefficient was calculated using ***Equation 8***.

$$\textit{Extinction coefficient, } K = \frac{A}{C} \times P \quad \text{--- Equation 8}$$

Where: A = absorbance of the sample measured at the specific wavelength,
C = concentration of the sample (g/100ml),
P = thickness of the cuvette (cm).

The absorbance values were also measured at 266 nm and 274 nm in order to calculate the variation of the specific extinction at a maximum wavelength of 270 nm, delta K (ΔK). The variation was calculated based on ***Equation 9***.

$$\textit{delta } K = K270 - \frac{K266 + K274}{2} \quad \text{--- Equation 9}$$

### 3.5.11 Colour

Colour measurement can be easily conducted by CIELAB system as this technique is accurate and does not require pre-treatment or dilution of the oil sample (Section 2.4.5.1.7) The colour of the olive oil samples prepared in this study and some commercially produced olive oil samples purchased from a local supermarket was measured by Minolta CM-508i spectrophotometer (Konica Minolta Sensing Inc., Japan) as illustrated in Figure 3-7. The Minolta spectrophotometer was calibrated

against a white tile, with the illuminant set at D65 at an angle of 2 degrees. Approximately 3 mL of oil sample was poured into the Agtron cell (Part nr 311595, Agtron Inc., Nev., USA). The bottom of the Agtron cell is facilitated with an optical glass to ensure the incidence light and reflected light do not interfere with the readings. A piece of Leneta® white paper was used to cover around the Agtron cell to provide a standard background and to prevent extraneous light from entering the sample. The Agtron cell was then put on the aperture of the inverted Minolta spectrophotometer. The L*, a* and b* values of each oil sample were taken twice and an average reading was reported for each sample.

**Figure 3-7: Measurement of colour on oil sample in Agtron cell covered by Lenata paper using a Minolta spectrophotometer**

### 3.5.12 Sensory profile

Sensory analyses of the olive oil samples prepared in this study and some commercially produced olive oil samples purchased from a local supermarket (n=17) were conducted in a standardized sensory evaluation room. The description of these samples is listed in Table 3-7.

**Table 3-7: Oil samples evaluated on their sensory profiles**

| Oil samples | Country of origin | Descriptions on processing techniques |
|---|---|---|
| 1 | Australia (Gingin) | Olive paste was malaxed for 30 minutes with no addition of processing aids *(Sample C30)* |
| 2 | Australia (Gingin) | Olive paste was malaxed for 60 minutes with no addition of processing aids *(Sample C60)* |
| 3 | Australia (Gingin) | Olive paste was malaxed for 30 minutes with addition of 0.15 g/mL citric acid *(Sample A30)* |
| 4 | Australia (Gingin) | Olive paste was malaxed for 30 minutes with addition of 0.15 g/mL Viscozyme *(Sample V30)* |
| 5 | Australia (Gingin) | Olive paste was malaxed for 30 minutes with addition of 0.30 g/mL citric acid *(Sample A30[H])* |
| 6 | Australia (Gingin) | Olive paste was malaxed for 30 minutes with addition of 0.30 g/mL Viscozyme *(Sample V30[H])* |
| 7 | Australia (Swan Valley) | Olive paste was malaxed for 30 minutes with no addition of processing aids *(Sample C30)* |
| 8 | Australia (Swan Valley) | Olive paste was malaxed for 60 minutes with no addition of processing aids *(Sample C60)* |
| 9 | Australia (Swan Valley) | Olive paste was malaxed for 30 minutes with addition of 0.15 g/mL citric acid *(Sample A30)* |
| 10 | Australia (Swan Valley) | Olive paste was malaxed for 30 minutes with addition of 0.15 g/mL Viscozyme *(Sample V30)* |
| 11 | Australia (Swan Valley) | Olive paste was malaxed for 30 minutes with addition of 0.30 g/mL citric acid *(Sample A30[H])* |
| 12 | Australia (Swan Valley) | Olive paste was malaxed for 30 minutes with addition of 0.30 g/mL Viscozyme *(Sample V30[H])* |
| 13 | Australia (Margaret River) | Olive paste was malaxed for 30 minutes with no addition of processing aids *(Sample C30)* |
| 14 | Italy | Information on processing techniques is not available. (*Commercial EVOO sample*) |
| 15 | Spain | Information on processing techniques is not available. (*Commercial EVOO sample*) |
| 16 | Australia (Western Australia) | Information on processing techniques is not available. (*Commercial EVOO sample*) |
| 17 | Australia (Victoria) | Information on processing techniques is not available. (*Commercial EVOO sample*) |

Five experienced olive oil tasters (2 males, 3 females) as recommended by the WAOC were recruited to participate in the sensory evaluation sessions after completing the screening form (Appendix 3). An information sheet (Appendix 4) outlining the purpose and procedures of the study was presented to each panelist. Consent forms (Appendix 5) were also signed by the panelists prior to the sensory evaluation session.

Samples were served at room temperature at 25 ºC. Oil samples of approximately 10 mL were poured into individual cups labeled with randomly selected three-digit codes to prevent direct identification of the samples by the panelists (Figure 3-8). In evaluating the aroma profile of the oil, the panelists were asked to place the cup in the middle of one of their palms, and cover the cup with the other palm while swirling the cup slowly for 15 seconds. The panelists then sniffed the oil sample and recorded the aromatic profile of the oil sample on the scoring sheet (Appendix 2). Tasting of the oil sample was evaluated by taking a small sip of oil and spreading that slowly and equally in the whole mouth cavity before swallowing the sample. Apple slices and drinking water were provided for eating and drinking between sampling to rinse the panelists' palate.

**Figure 3-8: Some oil samples with randomized 3-digit codes on a standardized white plate for sensory evaluation**

The Judge's Scoring Sheet developed by the WAOC comprises of two sections; the aroma attributes and the taste (palate) attributes of the oil samples. The panelists judged the intensity of fruit aroma, complexity of the aroma as well as the faulty aromatic attributes (fusty, musty, winey/vinegary/acid/sour, muddy sediment, metallic, rancid), flavour intensity, complexity of taste on the fruitiness, bitterness, pungency and fruitiness as well as the balance of these three attributes on the palate. The judges also evaluated the fault of the oil samples based on the palate attributes. The given score of each attributes is added up for each tasted oil sample. The mean values of the tasted sample collected from the 5 panelists were reported as the score.

## 3.6 Statistical analysis

The independent variables investigated in this study are olive growing sites, maturity levels of harvested olives and the types of processing techniques applied to the olive paste. The effects of these independent variables on the quality parameters of the extracted olive oil, namely yield of oil extraction, oil recovery, concentration of total phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition in terms of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), level of conjugated diene (K232) and conjugated triene (K270), variation of specific extinction (ΔK) and colour in terms of brightness (L*), greenness (a*) and yellowness (b*) of the oil samples, as well as all the interactions between these independent variables on the quality of extracted olive oil were assessed by the Univariate Analysis of Variance (Univariate ANOVA) procedure using SPSS version 17.0 for windows software. Significant differences between the control sample and other treated groups were detected at $\alpha$=0.05 level on the estimated marginal means value as generated by the Univariate ANOVA procedure. Sensory profiles of the oil samples were analyzed separately by Kruskal-Wallis test. The differences of sensory attributes between the oil samples extracted by various processing techniques and the control oil sample were analyzed by Mann-Whitney tests at $\alpha$=0.05 level. Exact tests were used for comparison where the sample size was low.

# Chapter 4 Results and Discussions

## 4.1 Preliminary findings from the 2008 season

The olives from three growing sites in Western Australia, namely Gingin, Swan Valley and Margaret River were harvested at three different maturity levels (MI1, MI4 and MI6). The harvested olives were subjected to different processing techniques. The processing techniques applied included malaxing the olive paste for 30 minutes with no addition of processing aids, malaxing the olive paste for an extended period of 60 minutes with no addition of processing aids, malaxing the olive paste for 30 minutes with addition of either citric acid at 0.15 g/mL, Viscozymes at 0.15 g/mL or Pectolyase at 0.15 g/mL. The effects of these independent variables as well as their interactions were investigated on the quality of olive oil extracted.

In the 2008 season, some of the olives collected were bruised and/or dried. They did not meet the quality requirements of 'healthy' olives. Due to the inferior quality of the harvested olive fruits, not all of the effect of the processing techniques was evaluated. The small sample size collected in the 2008 season necessitates the collection of more olive oil samples in the 2009 season. Nevertheless, the olive oil samples extracted in the 2008 season served as a guideline on the areas of independent variables to be focused on for 2009 season.

The interactions between the 3 independent variables of 2008 season were studied using the limited information collected from the 2008 season (Table 4-1). Despite the relatively small sample size collected, the P-values of the interaction between processing techniques and maturity on the key quality variables, namely yield of oil extraction, concentration of total phenolic compounds and antiradical activity, were reported as 0.230, 0.758 and 0.608, respectively. Due to the insignificant effect ($P>0.05$) of maturity level, the effect of maturity level is not studied in the 2009 season. The olives were harvested at the commercial maturity level (MI4) during the 2009 season. On the other hand, despite the small sample size, the P-value of the interaction between processing techniques and olive growing sites on the yield of oil extraction was significant ($P=0.023$), as presented in Table 4-1. Therefore, the effect

of olive growing sites on the quality of the extracted olive oil was investigated again in the 2009 season.

**Table 4-1: Interactive effects between processing techniques, growing sites and maturity on the major dependant variables in the 2008 season**

| Dependant variables | P value of f-test | |
|---|---|---|
| | Processing techniques* Growing sites | Processing techniques * Maturity |
| Yield of oil extraction | 0.023 | 0.230 |
| Total phenolic compounds | 0.550 | 0.758 |
| Antiradical activity | 0.320 | 0.604 |

In addition to the significant interactive effect between processing techniques and olive growing sites, the qualities of the extracted olive oil samples varied when different processing techniques were applied. In particular, malaxing the olive paste for 30 minutes with addition of 0.15 g/mL Pectolyase (S*ample P30*), when compared to the control sample (*Sample C30*), had no significant effect on the key quality variables of the olive oil extracted in the 2008 season (Table 4-2). The P-values for yield of oil extraction, concentration of total phenolic compounds and antiradical activity of the olive oil extracted with Pectolyase were reported as 0.875, 0.121 and 0.295, respectively in Table 4-2. It is deduced that the activity of Pectolyase at this concentration was substandard. Hence, the effect of adding 0.15 g/mL Pectolyase to olive paste is not investigated in the 2009 season.

**Table 4-2: The effect of addition of Pectolyase at 0.15 g/mL (*Sample P30*) on the key dependant variables in the 2008 season**

| | t-test for Equality of Means Sig. (2-tailed) |
|---|---|
| **Yield of oil extraction** | 0.875 |
| **Total phenolic compounds** | 0.121 |
| **Antiradical activity** | 0.295 |

Despite the insignificant effect of Pectolyase on the key quality variables of the olive oil, addition of citric acid and Viscozymes significantly affect the quality of extracted olive oil. Therefore, the effects of adding higher concentration (0.30 g/mL) of either

citric acid or Viscozymes to the olive paste, followed by a 30 minute malaxation period were studied on the quality of the extracted oil in the 2009 season.

The total number of olive oil samples extracted throughout the 2 year study period is presented in Table 4-3. As illustrated in Table 4-3, not all of the samples were collected from all three olive growing sites at three different maturity levels and treated to all of the above stated processing techniques throughout the two year period. As elucidated previously, the reason is that there was insufficient supply of healthy olives. In particular, the quality of olives received from the Margaret River olive grove in the 2009 season was inferior. The fruits were bruised with noticeable fermented off-flavour and did not meet the quality requirements for 'healthy' olives. As a result, the olives harvested from Margaret River in the 2009 season were not treated to extended olive paste malaxation period or addition of processing aids. The olives were just processed according to the control processing conditions in the 2009 season.

**Table 4-3: The number of olive oil samples collected over the 2 year period**

| Year | Processing techniques | Growing sites | Maturity levels | | |
|---|---|---|---|---|---|
| | | | 1 | 4 | 6 |
| 2008 | *Sample C30* (control) (olive paste was malaxed for 30 min with no addition of processing aids) | Gingin | 2 | 3 | 2 |
| | | Swan Valley | 0 | 0 | 3 |
| | | Margaret River | 0 | 2 | 2 |
| | *Sample C60* (olive paste was malaxed for 60 min with no addition of processing aids) | Gingin | 3 | 3 | 3 |
| | | Swan Valley | 1 | 3 | 3 |
| | | Margaret River | 3 | 3 | 3 |
| | *Sample A30* (olive paste was malaxed for 30 min with addition of 0.15 g/mL citric acid) | Gingin | 2 | 3 | 2 |
| | | Margaret River | 3 | 2 | 2 |
| | | Swan Valley | 2 | 0 | 3 |
| | *Sample V30* (olive paste was malaxed for 30 min with addition of 0.15 g/mL Viscozymes) | Gingin | 2 | 3 | 2 |
| | | Swan Valley | 0 | 0 | 3 |
| | | Margaret River | 0 | 2 | 2 |
| | *Sample P30* (olive paste was malaxed for 30 min with addition of 0.15 g/mL Pectolyase) | Gingin | 1 | 3 | 2 |
| | | Margaret River | 0 | 2 | 0 |
| | | Swan Valley | 0 | 3 | 2 |
| 2009 | *Sample C30* (control) (olive paste was malaxed for 30 min with no addition of processing aids) | Gingin | | 3 | |
| | | Swan Valley | | 3 | |
| | | Margaret River | | 3 | |
| | *Sample C60* (olive paste was malaxed for 60 min with no addition of processing aids) | Gingin | | 3 | |
| | | Swan Valley | | 3 | |
| | *Sample A30* (olive paste was malaxed for 30 min with addition of 0.15 g/mL citric acid) | Gingin | | 3 | |
| | | Swan Valley | | 3 | |
| | *Sample V30* (olive paste was malaxed for 30 min with addition of 0.15 g/mL Viscozymes) | Gingin | | 3 | |
| | | Swan Valley | | 3 | |
| | *Sample A30[H]* (olive paste was malaxed for 30 min with addition of 0.30 g/mL citric acid) | Gingin | | 3 | |
| | | Swan Valley | | 3 | |
| | *Sample V30[H]* (olive paste was malaxed for 30 min with addition of 0.30 g/mL Viscozymes) | Gingin | | 3 | |
| | | Swan Valley | | 3 | |

The following sections of this chapter describe the effect of processing techniques on the quality parameters of extracted olive oil samples. The significant effect of olive growing sites is also presented. The quality parameters of the extracted olive oil samples are also compared with the commercial EVOO samples purchased from the supermarket. The last section of this chapter identifies the optimum processing techniques in achieving a designated level of quality parameters.

## 4.2 Effect of processing techniques on the quality parameters of olive oil

It has been the interest of olive oil producers to improve the yield of oil extraction and the concentration of phenolic compounds in olive oil as the consumers demand a significantly greater volume of healthy olive oil. The review of literature suggests that it is essential to study the effect of processing techniques, such as extended olive paste malaxation period and addition of processing aids to olive paste, on the yield of olive oil extraction, oil recovery and concentration of total phenolic compounds of the extracted oil. In this study, the quality parameters evaluated on the extracted olive oil included yield of oil extraction, oil recovery, concentration of total phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition in terms of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), level of conjugated diene (K232) and conjugated triene (K270), variation of specific extinction (ΔK) and colour in terms of brightness (L*), greenness (a*) and yellowness (b*). The quality of the oil was evaluated against each processing technique, namely: extending the length of olive paste malaxation period to 60 minutes with no addition of processing aids (*Sample C60*), addition of 0.15 g/mL citric acid followed by a 30 minute malaxation period (*Sample A30*), addition of 0.15 g/mL Viscozymes followed by a 30 minute malaxation period (*Sample V30*), addition of 0.15 g/mL Pectolyase followed by a 30 minute malaxation period (*Sample P30*), addition of 0.30 g/mL citric acid followed by a 30 minute malaxation period (*Sample A30[H]*) and addition of 0.30 g/mL Viscozymes followed by a 30 minute malaxation period (*Sample V30[H]*). The quality of these oil samples were compared with that of the control sample, of which the olive paste was subjected to a 30 minute malaxation period with no addition of processing aids (*Sample C30*).

### 4.2.1 Thirty minute olive paste malaxation period with no addition of processing aids

There is speculation that consumption of olive oil is increasing around the world (Baccouri et al. 2008). In addition, the shortfall of the estimated production volume was forecasted (AOA 2009; UNCTAD 2006). Therefore, the yield of oil extraction and oil recovery during the production of olive oil is of the utmost concern to olive oil producers.

In the industry, olive oil is commonly extracted by malaxing the olive paste without addition of processing aids to the paste. In this study, the control sample (*Sample C30*) of this study was extracted by malaxing the olive paste for 30 minutes with no addition of processing aids during the extraction process. As shown in Table 4-4, the yield of oil extraction of *Sample C30* was reported as 11.24 %. The result is similar to the 11.3 % reported from Italian olives (Chiacchierini et al. 2007). However, no information on the cultivar of these Italian olives is available. Meanwhile, the yield of oil extraction is greater than the 5.3 % reported by the non-irrigated Italian *Frantoio* olives processed in year 2003 (Tognetti et al. 2006). Conversely, the yield of *Sample C30* was lower than the average yield of 26 % reported on Australian *Frantoio* olives harvested from New South Wales in year 2005 (Kalua et al. 2008). However, details of their extraction technique were not provided in their report, making it difficult to compare to the finding of the present study. Indeed, there is a lack of recent published information on the yield of Australian *Frantoio* olive oil. In this regard, further comparison and discussion on the data collected from this study is not possible. It is anticipated that the variation in the yield of oil extraction could be attributable to the cultivar, irrigation and growing sites of the olives.

**Table 4-4: Mean values (± S.D.) of the quality of Western Australian *Frantoio* olive oil processed by malaxing the olive paste for 30 minutes with no addition of processing aids (*Sample C30*)**

| Quality parameters of olive oil | *Sample C30* (Mean ± S.D.) |
|---|---|
| Yield of oil extraction (%) | 11.24 ± 5.49 |
| Oil recovery (%) | 54.08 ± 13.94 |
| Total phenolic compounds (mg/kg oil) | 113.09 ± 86.02 |
| Antiradical activity (% inhibition of DPPH•) | 32.49 ± 5.92 |
| Peroxides value (mEq/kg) | 14.24 ± 9.17 |
| Acidity level (% m/m oleic acid) | 0.24 ± 0.13 |
| C16:0 (%) | 14.48 ± 3.56 |
| C18:0 (%) | 1.93 ± 0.97 |
| C18:1 (%) | 80.40 ± 11.83 |
| C18:2 (%) | 15.30 ± 6.64 |
| K232 (%) | 1.84 ± 0.34 |
| K270 (%) | 0.12 ± 0.10 |
| ΔK | 0.00 ± 0.02 |
| L* | 38.23 ± 0.56 |
| a* | -1.99 ± 0.81 |
| b* | 8.42 ± 3.57 |

The low yield of oil extraction indicates that it is necessary to improve the extraction technique for production of Western Australian *Frantoio* olive oil. An improved processing technique is anticipated to be capable of increasing the production volume of Western Australian olive oil and increase the market share of Western Australian olive oil in the world market.

Oil recovery of *Sample C30* was found to be 54 % (Table 4-4). In other words, approximately 40 % of olive oil is lost during the production. The high percentage of oil loss signifies the urgency to improve the processing technique in order to minimize the amount of oil present in the by-product. Potential techniques as suggested by the findings of the literature included extending the length of olive paste malaxation period and addition of processing aids to the olive paste.

Phenolic compounds in olive oil plays a major role in its health benefits and sensory profile (Bendini et al. 2007; Servili et al. 2009). Loss of these bioactive phenolic compounds reduces the quality of olive oil. In addition, it poses disposal problems and is hazardous to our environment (Alburquerque et al. 2004; Alfano et al. 2008). With

more than 90 % of the total phenolic compounds lost during common olive oil production, the concentration of total phenolic compounds retained in olive oil has been the concern of many olive oil producers. The concentration of total phenolic compounds in the olive oil extracted by malaxing the olive paste for 30 minutes with no addition of processing aids (*Sample C30*) was reported as 113 mg/kg oil. This level of total phenolic compounds is considered low, as good quality olive oil should have a minimum level of 200 mg/kg oil (Mailer 2005). As shown in Appendix 6, the concentration of total phenolic compounds in the oil samples extracted in this study was not significantly higher than that in the commercial EVOO samples. Indeed, the olive oil extracted by the control technique (*Sample C30*) was not effective in producing olive oil with high concentration of total phenolic compounds. The results emphasized the need to revise the current processing technique applied in the olive oil industry.

Due to the high correlation between phenolic compounds and antiradical activity (Appendix 7), it is believed that the low antiradical activity of *Sample C30* as stated in Table 4-4 is contributed by the low concentration of phenolic compounds present in *Sample C30*. Indeed, the antiradical activity of *Sample C30* is significantly lower than the commercial EVOO samples (Appendix 6). Both the total phenolic compounds and antiradical activity of olive oil are responsible for the health benefits and storage stability of olive oil (Del Carlo et al. 2004; Gutierrez, Arnaud & Garrido 2001; Lavelli 2002). Therefore, it is necessary to improve the current processing technique to retain the phenolic compounds during the production of olive oil.

Despite the need to improve the yield of oil extraction, oil recovery, concentration of total phenolic compounds and antiradical activity of the olive oil of *Sample C30*, the peroxides value, acidity level, fatty acid composition in terms of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), level of conjugated diene (K232) and conjugated triene (K270), variation of specific extinction (ΔK) all met the IOC standards set for EVOO. Peroxides value of *Sample C30* was found to be significantly higher than the commercial EVOO samples (Appendix 6). Such a finding indicates that *Sample C30* has undergone some degree of oxidation as hydroxyperoxide, a primary oxidation product, was detected in these oil samples. In fact, the result correlates with the significantly higher level of conjugated triene (K270) found in *Sample C30*. Conjugated triene are produced as oxidation induces structural

changes to the fatty acids. It is therefore deduced that malaxation period of 30 minutes exerted adverse effect on the oxidative stability and storage ability of the extracted oil.

In terms of colour, the brightness (L*), greenness (a*) and yellowness (b*) of *Sample C30* were found to be 38.23, -1.99 and 8.42, respectively. Colour plays an important role in affecting the purchasing decision of consumers (Criado et al. 2008; Romero et al. 2003). It is therefore necessary to determine the differences between the colour of *Sample C30* and that of the commercial EVOO samples. The brightness (L*) and greenness (a*) of *Sample C30* were comparable to the commercial EVOO samples (Appendix 6). However, the yellowness (b*) of *Sample C30* was lower than that of the commercial EVOO samples. The result suggests that 30 minute malaxation period was not effective in breaking down the hypoderm tissue of olive fruits where the natural colourings are stored (Ranalli et al. 2005). As a result, the yellow pigments in the oil, mainly contributed by carotenoids, are reported lower than the commercial EVOO samples. Another possibility of the lower reading is attributable to the antioxidant activity of these carotenoids, as supported by the low antiradical activity of *Sample C30*. As *Sample C30* was exposed to minor degree of oxidation during the extraction process, the carotenoids, which are known to have antioxidant ability, could have been oxidized during the process. Consequently, less amount of carotenoids were left in the oil, leading to lower reading of the b* value in the extracted olive oil.

The colour readings of *Sample C30* are lower than those reported by Romero et al. (2003). Spanish *Arbequina* olive oil samples collected over a 4 year period by Romero et al. (2003) were reported to have an average brightness (L*), greenness (a*), yellowness (b*) value of 82.2, -1.55 and 104.7, respectively (Romero et al. 2003). The differences could be due to the effect of growing sites and the agronomic practices of olives, as previously reviewed in Section 2.4.6. In addition, the effect of olive cultivars should not be neglected. Lack of published information on the colour parameters of Australian *Frantoio* olive oil complicated the explanation of the results collected in this study.

### 4.2.2 Thirty minute olive paste malaxation period with no addition of processing aids using olives harvested from different olive growing sites

The effect of olive growing sites on the quality of the olive oil has been reported (Dabbou et al. 2009; Mailer & Ayton 2008; Rotondi, Fabbri & Ganino 2008; Salvador et al. 2003; Temime et al. 2006; Tura et al. 2007). In agreement with these findings, olive growing sites were identified as a significant factor influencing the yield of oil extraction, oil recovery, concentration of total phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition in terms of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), level of conjugated triene (K270), and colour in terms of greenness (a*) and yellowness (b*) of the olive oil extracted in this study (Table 4-5). It is therefore necessary to investigate the variation of these quality parameters between the olive oil samples extracted from olives grown at the various sites (Gingin, Swan Valley and Margaret River).

**Table 4-5: Effect of olive growing sites on the quality parameters of olive oil**

| Quality parameters of olive oil | Growing sites<br>Significance level ($\alpha = 0.05$) |
|---|---|
| Yield of oil extraction (%) | P<0.0005 * |
| Oil recovery (%) | P<0.0005 * |
| Total phenolic compounds (mg/kg oil) | P<0.0005 * |
| Antiradical activity (% inhibition of DPPH•) | P<0.0005 * |
| Peroxides value (mEq/kg) | P<0.0005 * |
| Acidity level (% m/m oleic acid) | P<0.0005 * |
| C16:0 (%) | P<0.0005 * |
| C18:0 (%) | 0.001 * |
| C18:1 (%) | 0.014 * |
| C18:2 (%) | P<0.0005 * |
| K232 (%) | 0.241 |
| K270 (%) | P<0.0005 * |
| ΔK | 0.456 |
| L* | P<0.0005 * |
| a* | P<0.0005 * |
| b* | P<0.0005 * |

* indicates the effect of growing sites was significant at $\alpha = 0.05$ level (as assessed by Univariate ANOVA test) in affecting the quality of extracted olive oil

The olives harvested from Gingin, Swan Valley and Margaret River were subjected to a 30 minute malaxation period with no addition of processing aids under identical laboratory condition. The quality of the extracted oil samples (*Sample C30*) was tabulated in Table 4-6.

**Table 4-6: Comparison between the quality parameters of control samples (*Sample C30*) extracted from olive fruits harvested from Gingin, Swan Valley and Margaret River**

| Quality parameters of olive oil | Mean ± S.D. | | |
|---|---|---|---|
| | **Gingin** | **Swan Valley** | **Margaret River** |
| Yield of oil extraction (%) | 7.05 ± 3.27 $^{\alpha}$ | 9.56 ± 4.71 $^{\beta}$ | 16.84 ± 2.38 $^{\gamma}$ |
| Oil recovery (%) | 50.59 ± 14.16 $^{\alpha}$ | 46.83 ± 13.98 $^{\alpha}$ | 63.61 ± 7.90 $^{\beta}$ |
| Total phenolic compounds (mg/kg oil) | 125.92 ± 92.45 $^{\alpha}$ | 88.74 ± 50.59 $^{\beta}$ | 118.52 ± 102.07 $^{\alpha}$ |
| Antiradical activity (% inhibition of DPPH•) | 31.99 ± 5.37 $^{\alpha}$ | 29.91 ± 6.90 $^{\beta}$ | 34.93 ± 5.12 $^{\gamma}$ |
| Peroxides value (mEq/kg) | 16.62 ± 9.05 $^{\alpha}$ | 20.06 ± 8.02 $^{\beta}$ | 7.02 ± 4.91 $^{\beta}$ |
| Acidity level (% m/m oleic acid) | 0.15 ± 0.06 $^{\alpha}$ | 0.31 ± 0.11 $^{\beta}$ | 0.25 ± 0.14 $^{\beta}$ |
| C16:0 (%) | 14.40 ± 3.77 $^{\alpha}$ | 16.47 ± 3.69 $^{\beta}$ | 12.90 ± 2.49 $^{\alpha}$ |
| C18:0 (%) | 2.20 ± 1.25 $^{\alpha}$ | 1.72 ± 0.78 $^{\beta\gamma}$ | 1.85 ± 0.79 $^{\alpha\gamma}$ |
| C18:1 (%) | 78.02 ±12.68 $^{\alpha}$ | 75.04 ± 11.83 $^{\alpha}$ | 87.25 ± 7.81 $^{\beta}$ |
| C18:2 (%) | 13.85 ± 4.20 $^{\alpha}$ | 20.26 ± 8.37 $^{\beta}$ | 12.61 ± 4.95 $^{\alpha}$ |
| K270 (%) | 0.09 ± 0.08 $^{\alpha}$ | 0.20 ± 0.12 $^{\beta}$ | 0.08 ± 0.08 $^{\alpha}$ |
| ΔK | 0.00 ±0.01 $^{\alpha}$ | 0.00 ±0.02 $^{\alpha}$ | 0.01 ± 0.02 $^{\alpha}$ |
| L* | 38.04 ±0.58 $^{\alpha}$ | 38.37 ± 0.72 $^{\alpha}$ | 38.30 ± 0.36 $^{\alpha}$ |
| a* | -2.36 ± 0.56 $^{\alpha}$ | -1.70 ± 0.69 $^{\beta}$ | -1.86 ± 1.01 $^{\beta}$ |
| b* | 9.88 ±2.78 $^{\alpha}$ | 7.80 ± 3.84 $^{\alpha\beta}$ | 7.48 ± 3.84 $^{\beta}$ |

Different Greek letters across the row indicate significant differences ($P<0.05$) between the *Sample C30*s extracted from olives harvested from different growing sites

The quality parameters of the oil samples extracted from Gingin, Swan Valley and Margaret River by the control technique (*Sample C30*) were different from each other. In particular, the yield of oil extraction and oil recovery of the *Sample C30*s were different (Figure 4-1).

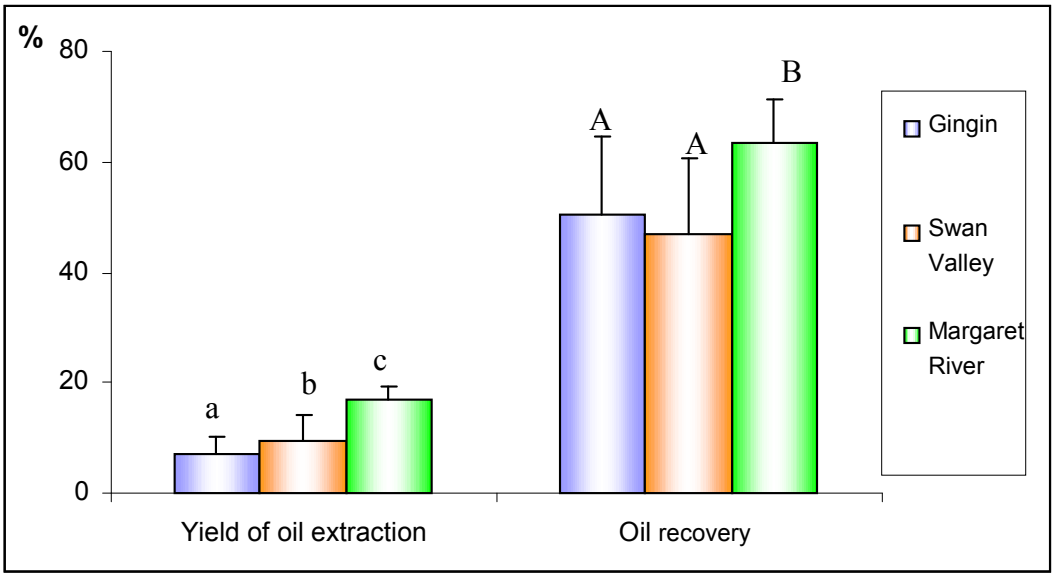



**Figure 4-1: Yield of oil extraction and oil recovery of the *Sample C30*s extracted from Gingin, Swan Valley and Margaret River olives**

**The yield of oil extraction and oil recovery are expressed as mean values with standard deviation error bars. Different letters indicate significant differences (P<0.05) on the quality parameter amongst the oil samples.**

The yield of oil extraction of all *Sample C30*s extracted using Gingin, Swan Valley and Margaret River olives was found to be different from each other (Table 4-6). The results indicated that the yield of oil extraction was the highest in Margaret River (16.84 %), followed by Swan Valley (9.56 %) with Gingin reported to have the lowest yield of oil extraction (7.05 %).

The oil recovery data provides 'mass balance' information on the processing techniques. As reported by Artajo et al. (2007), the oil recovery of industrial olive oil extraction is approximately 60 %. A low reading indicates the inefficiency of the processing technique as most of the oil was not extracted but ended up in the by product. With the industry aiming to minimize the amount of oil loss in the by product, a high reading of oil recovery is preferred. Similar to the yield of oil extraction, the oil recovery of the *Sample C30* extracted from Margaret River olives was significantly higher than those of Gingin and Swan Valley. The oil recovery of these *Sample C30*s was reported as 63.61, 50.59 and 46.83 %, respectively (Table 4-6). The oil recovery of Gingin and Swan Valley was alike to each other. The resemblance is attributable to the similar rainfall and temperature data between Gingin and Swan Valley (Table 3-1).

The slightly warmer climate in Gingin and Swan Valley compared to Margaret River necessitates irrigations of the olive trees during the olive fruit development period. Consequently, the olive fruits from Gingin and Swan Valley have higher moisture contents (data not available) compared to the non-irrigated Margaret River olives. The high moisture level in the Gingin and Swan Valley olive fruits obscures the extraction of oil as emulsion forms during the oil extraction process. As a result, the oil recovery of Gingin and Swan Valley olive oil samples is lower than that of Margaret River olive oil. Indeed, the lower moisture content in Margaret River olives minimizes the formation of emulsion. As a result, the oil vacuoles in olives of Margaret River are more easily extracted than those of Gingin and Swan Valley. As a result, the oil droplets were more easily released, resulting in a significantly greater yield of oil extraction and oil recovery of Margaret River's *Sample C30*.

Other evaluated quality parameters of the oil extracted from olives harvested from different growing sites were also found to be different. For example, the concentration of total phenolic compounds in the olive oil extracted from Swan Valley olives was reported as significantly lower than those from Gingin and Margaret River. The results shown in Table 4-6 indicate that the concentration of total phenolic compounds in *Sample C30*s extracted from Gingin and Margaret River is similar. However, the concentration of total phenolic compounds in Swan Valley *Sample C30* is significantly lower than those of Gingin and Margaret River's. As the climate data between Gingin and Swan Valley is similar (Table 3-1), the differences in the concentration of total phenolic compounds between these two olive oil samples suggest the initial composition of the olive fruits are different. The differences could be due to the effect of agronomic practices applied at these growing sites. Indeed, water status of the trees directly affects the synthesis of phenolic compounds in the olive fruits. Stressed water level has been reported to increase the presence of phenolic compounds in the olive fruits (Artajo et al. 2007; Dag et al. 2008). A good olive oil should contain a concentration of total phenolic compounds greater than 200 mg/kg oil (Mailer 2005). The significant lower concentration of total phenolic compounds of Swan Valley olive oil indicates this oil has less health benefits to offer. In addition, the storage ability and sensory profile of this oil is predicted to be inferior compared to the olive oil samples extracted from Gingin and Margaret River olives.

Antiradical activity was found to be highly correlated to the concentration of total phenolic compounds (Appendix 7). Indeed, the antiradical activity of Swan Valley *Sample C30* was significantly lower (29.91 %) than those of the Gingin and Margaret River *Sample C30*s (Table 4-6), as evidently shown by its lower concentration of total phenolic compounds present in the extracted oil. Meanwhile, the results suggest that the antiradical activity of an oil sample is also affected by the type of phenolic compounds present in the oil. The lower antiradical activity in Swan Valley olive oil is attributable to the lower concentration of strong antioxidants such as phenolic compounds 3,4-DHPEA, 3,4-DHPEA-EDA and 3,4-DHPEA-EA in the olive oil. They were reported to have greater antiradical activity than those phenols containing single hydroxyl group and/or $–COOCH_3$ group such as oleuropein aglycone (Servili et al. 2009).

As oxidized lipid peroxides contribute to the rancidity of olive oil samples, it is necessary to measure the peroxides value of the extracted oil. The results indicate that the peroxide value of the control sample (*Sample C30*) of Swan Valley reached the limit set by IOC for EVOO. The results suggest that Swan Valley olives are more susceptible to oxidation. In fact, as shown in Table 4-6, the naturally low concentration of phenolic compounds recorded in olive oil extracted from Swan Valley olives when compared to Gingin and Margaret River olives are unable to exert protective effect against oxidation of the olive oil.

The acidity level of olive oil refers to the amount of free fatty acids present in the oil. It serves as an indication of the degree of breakdown of triacylglycerols in the oil. The naturally occurring lipase enzymes in olive fruits are capable of breaking down the oil molecules. They are easily activated when the olive fruits are crushed. As a result, free fatty acids are formed. Therefore, fruit quality affects the presence of free fatty acids in the extracted oil (Mailer & Ayton 2008). Taking this factor into consideration, the IOC has established standards for the maximum acidity level in EVOO to be 0.8 % m/m oleic acid. The results shown in Table 4-6 indicate that, when the olives harvested from Gingin, Swan Valley and Margaret River were subjected to a 30 minute malaxation period with no addition of processing aids (*Sample C30*), the acidity levels of the extracted oil samples were below the IOC standards set for EVOO. Therefore, it is deduced that malaxing the olive paste for 30 minutes employing the olives harvested from Gingin, Swan Valley and Margaret River is an

appropriate technique for production of EVOO. On the other hand, the results indicate that the acidity levels of the *Sample C30*s processed using olives harvested from these three growing sites are different from each other. In particular, the acidity levels are higher when the oil samples were extracted using Swan Valley and Margaret River olives. The results indicate that alternate processing techniques should be applied to Swan Valley and Margaret River olives during the production of olive oil in order to lower the acidity level in the oil.

The control sample (*Sample C30*) extracted from Gingin olives recorded the lowest level of acidity. The result implied that the fatty acids were not broken down by the endogenous enzymes present in the olives. As a consequence, this oil may be more stable. Indeed, its higher reading of greenness (a*) found in the oil suggests that the level of the antioxidant chlorophyll is higher in Swan Valley's *Sample C30* compared to the other two *Sample C30*s from Gingin and Margaret River. This finding is possibly due to the different quality of the olive fruits harvested from these growing sites, thus resulting in different quality of the extracted olive oil.

Fatty acid composition of olive oil is reported to be affected by seasonal conditions, such as rainfall and prevailing weather conditions, as well as altitude (Mailer & Ayton 2008). In particular, growing sites with cold climates have been reported to produce olive oil with higher levels of oleic acid compared to those with warmer climates. In addition, the levels of palmitic acid are higher in oil extracted from olives grown at warmer climatic growing sites. Indeed, when the individual olives harvested from Gingin, Swan Valley and Margaret River were subjected to malaxation for 30 minutes with no addition of processing aids (*Sample C30*) under identical laboratory condition, the percentages of palmitic acid in the *Sample C30*s varied according to different growing sites (Table 4-6). In particular, the percentage of palmitic acid in the olive oil extracted from olives grown at warmer region (Swan Valley) is significantly higher than that extracted from olives grown in the cooler region (Margaret River). However, olive oil extracted from Gingin olives (also from a warmer region) is not significantly different to that extracted from olives grown at a cooler region (Margaret River). Based on the results acquired from this study, it is suggested that the latitude affects the percentage of palmitic acid in the oil. Olives grown in the eastern region (Swan Valley) tend to produce olive oil with higher percentage of palmitic acid (C16:0) when compared to the more western regions (Gingin and Margaret River).

Stearic acid (C18:0) is another saturated fatty acid present in the olive oil. Although its concentration is lower than the palmitic acid (C16:0) in olive oil, it affects the stability and health profile of the oil (Mailer & Ayton 2008). The IOC has thus established a standard for stearic acid in EVOO to not exceed 5 %. Attributable to the effect of olive growing sites, the percentage of stearic acid varied according to the three growing sites included in this study (Table 4-6). The percentage of stearic acid of the Swan Valley *Sample C30* was found to be lower than that of Gingin. The results suggested that olives grown in the more eastern latitude (Swan Valley) have lower percentage of stearic acid than those grown in the relatively western latitude (Gingin).

Oleic acid is the main fatty acid present in olive oil. The IOC has set the standard of oleic acid to be between 55-83 % in EVOO. It is therefore necessary to ensure the standard of oleic acid is kept well within this range. The olive oil extracted by malaxing the olive paste for 30 minutes with no addition of processing aids (*Sample C30*) to Gingin, Swan Valley and Margaret River olives led to 78.08, 75.04 and 87.25 % oleic acid, respectively in the extracted oil (Table 4-6). The results indicate that the percentage of oleic acid in Margaret River's *Sample C30* is significantly higher than those reported in Gingin and Swan Valley. Such finding is in agreement with those reported by Mailer and Ayton (2008). Based on the olive oil samples collected from Australia over a 2 year study period, Mailer and Ayton (2008) concluded that olives grown at a cooler region gave rise to a higher percentage of oleic acid (C18:1) in the extracted olive oil compared to those collected from warmer climate (Mailer & Ayton 2008). Margaret River is a typical example of the cooler climate regions while Gingin and Swan Valley have a warmer climate. In addition, Mailer and Ayton (2008) highlighted that the percentage of oleic acid in olive oil produced from olives harvested from cooler climate often exceeds the IOC standards set for EVOO. Such finding is also observed in this study (Table 4-6).

Linoleic acid is the main polyunsaturated fatty acid present in olive oil (Mailer & Ayton 2008). As adulteration of olive oil with sunflower oil usually contains a high level of linoleic acid (El-Abassy, Donfack & Materny 2009), the IOC has set the percentage of linoleic acid in EVOO to be between 3.5 and 21 %. The percentages of linoleic acid present in olive oil extracted from Gingin, Swan Valley and Margaret River olives are 13.85, 20.26 and 12.61 %, respectively (Table 4-6). The percentage

of oleic acid present in Swan Valley olive oil was significantly higher than those in Gingin and Margaret River olive oil samples. The result indicated that a more eastern region produces olives with higher percentage of linoleic acid. Consequently, the extracted olive oil has higher percentage of linoleic acid.

Conjugated triene is the primary product formed at the initial stage of oxidation. The level of conjugated triene of Australian olive oil as reported by Mailer and Ayton (2008) was 0.09 %. The results are in agreement with those reported in olive oil extracted from Gingin and Margaret River olives (Table 4-6). However, the level of conjugated triene reported in Swan Valley olive oil (*Sample C30*) was 0.20 %, which is significantly higher than those of Gingin and Margaret River. The results from this study indicate that olive growing site is a significant factor influencing the level of conjugated triene present in an extracted oil sample. Conversely, Mailer and Ayton (2008) stated that olive growing sites is not a significant factor affecting the level of conjugated triene in the extracted olive oil.

As shown in Table 4-6, the readings of yellowness of the control samples (*Sample C30*s) extracted from Gingin, Swan Valley and Margaret River olives were different. The yellowness of Gingin's *Sample C30* was significantly higher than that of Margaret River. It is possible that the carotenoid was more easily released from Gingin olives than that of Margaret River. The results also suggest that olive growing sites were significant in affecting the yellowness of the extracted olive oil.

In general, despite the significant effect of olive growing sites on the quality of extracted olive oil, the quality of *Sample C30*s extracted in this study met the standards established by IOC for EVOO. In addition, the quality of *Sample C30* is comparable to the commercial EVOO samples. It indicates that the quality of the harvested *Frantoio* olives as well as the control processing technique is appropriate in producing EVOO. However, there is room for improvements for all of the quality parameters of *Sample C30* extracted in this study. Other processing techniques can be evaluated for their effects in improving yield of oil extraction, concentration of total phenolic compounds and general quality of Western Australian *Frantoio* olive oil.

## 4.2.3 Extended length of malaxation period

As previously stated, the quality of *Sample C30* can be improved by various techniques. In this study, the olive paste was subjected to an extended length of olive paste malaxation period of 60 minutes (*Sample C60*). The purpose of this technique is to improve the extraction of oil and phenolic compounds to the oil. The effect of extending the olive paste malaxation period on the quality parameters of olive oil was evaluated against the control sample (*Sample C30*). The mean values and standard deviations (S.D.) of the quality parameters of the olive oil samples malaxed for 30 minutes (*Sample C30*) and 60 minutes (*Sample C60*) are tabulated in Table 4-7.

**Table 4-7: Comparison between the quality parameters (Mean ± S.D.) of olive oil subjected to an extended length of malaxation period of 60 minutes with no addition of processing aids (*Sample C60*) and the control sample (*Sample C30*)**

| Quality parameters of olive oil | [#] Samples | |
|---|---|---|
| | ***Sample C30*** | ***Sample C60*** |
| Yield of oil extraction (%) | 11.24 ± 5.49 | 10.01 ± 4.46 |
| Oil recovery (%) | 54.08 ± 13.94 | 57.15 ± 11.16 * |
| Total phenolic compounds (mg/kg oil) | 113.09 ± 86.02 | 97.36 ± 71.12 |
| Antiradical activity (% inhibition of DPPH•) | 32.49 ± 5.92 | 29.02 ± 4.80 * |
| Peroxides value (mEq/kg) | 14.24 ± 9.17 | 18.26 ± 8.10 |
| Acidity level (% m/m oleic acid) | 0.24 ± 0.13 | 0.24 ± 0.14 |
| C16:0 (%) | 14.48 ± 3.56 | 15.43 ± 3.98 |
| C18:0 (%) | 1.93 ± 0.97 | 2.18 ± 1.21 * |
| C18:1 (%) | 80.40 ± 11.83 | 81.31 ± 11.21 |
| C18:2 (%) | 15.30 ± 6.64 | 17.78 ± 7.08 |
| K232 (%) | 1.84 ± 0.34 | 1.77 ± 0.33 |
| K270 (%) | 0.12 ± 0.10 | 0.13 ± 0.09 |
| ΔK | 0.00 ± 0.02 | 0.00 ± 0.03 |
| L* | 38.23 ± 0.56 | 38.31 ± 0.59 |
| a* | -1.99 ± 0.81 | -2.01 ± 0.68 |
| b* | 8.42 ± 3.57 | 8.42 ± 3.06 |

[#] Descriptions of each sample are available in the abbreviation list
* indicates significant differences ($P<0.05$) on the quality parameter between the oil samples

As shown in Table 4-7, extending the length of olive paste malaxation period from 30 minutes (*Sample C30*) to 60 minutes (*Sample C60*) did not improve the yield of oil extraction. However, the oil recovery was increased from 54 to 57 % when the malaxation period was extended from 30 to 60 minutes. The slow malaxing action

exerted on the olive paste is not expected to significantly disrupt the cell structure. Instead, malaxation only allows coalescence of oil droplets released from the fruit matrix (Di Giovacchino, Sestili & Di Vincenzo 2002). Therefore, the 5.55 % increase in the oil recovery was not due to the cell destructive mechanism, but is attributable to the coalescence of oil droplet during malaxation. Extending the malaxation period to 60 minutes facilitated more of the released oil droplets to merge into larger oil mass, which could be easily separated from the solid phase during the centrifuge step (Di Giovacchino 2000). Therefore, extending the olive paste malaxation period to 60 minutes can be a potential solution to improve the efficiency of olive oil extraction process as well as to increase the production volume of olive oil.

Despite increasing the oil recovery, extending the olive paste malaxation period to 60 minutes reduces the antiradical activity of the extracted olive oil (*Sample C60*). The antiradical activity of the oil sample was significantly reduced from 32 to 29 % when the malaxation period was increased from 30 to 60 minutes (*Sample C60*). The significant 9 % reduction of antiradical activity, as illustrated in Figure 4-2, could be due to the oxidation of phenolic compounds in the oil during the extended period of malaxation. The statement is supported by the finding of Kalua et al. (2006), where it was observed that increasing the length of malaxation period led to a reduction in the concentration of 3,4-DHPEA. Phenolic compounds 3,4-DHPEA, 3,4-DHPEA-EDA and 3,4-DHPEA-EA were reported to have greater antiradical activity than other phenolic compounds containing only a single hydroxyl group and/or $–COOCH_3$ group such as oleuropein aglycone (Servili et al. 2009). In particular, 3,4-DHPEA is a significant phenolic compound contributing to the antiradical activity of olive oil. Consequently, it is possible that its reduction led to a significant reduction in the antiradical activity of *Sample C60*. Furthermore, it is anticipated that the health benefits and shelf life of *Sample C60* will be negatively affected due to the reduction of the antiradical activity (Del Carlo et al. 2004; Gutierrez, Arnaud & Garrido 2001; Lavelli 2002). Indeed, it should be noted that concentration of stearic acid in *Sample C60* was significantly increased from 1.9 to 2.2 %, when compared to the control sample (*Sample C30*). It is important to realize that, although higher concentration of saturated fatty acids contribute to the oxidative stability of the oil, saturated fatty acids may exert negative impacts on human health.

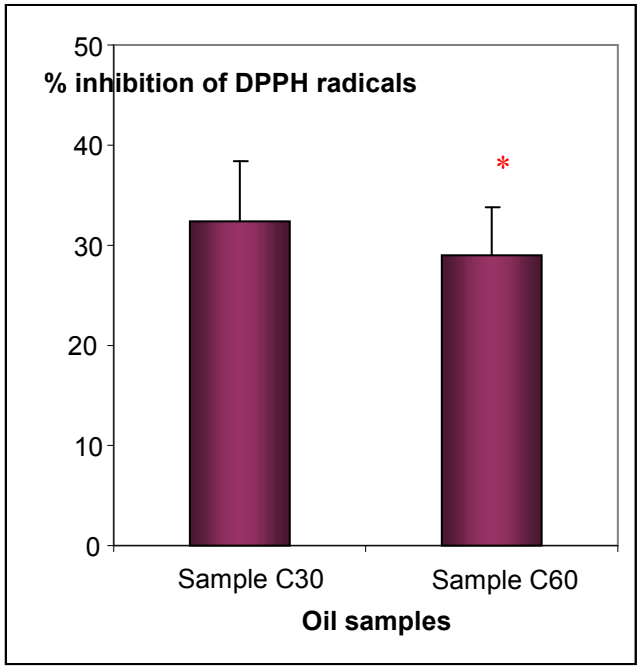



**Figure 4-2: Antiradical activity of oil samples as affected by malaxation period**

**Mean values of the antiradical activity (% inhibition of DPPH radicals) are shown with standard deviation error bars. *Sample C30* is the control oil sample extracted by malaxation for 30 minutes with no addition of processing aids. *Sample C60* is the oil sample extracted by malaxation for 60 minutes with no addition of processing aids. * indicates significant differences (P<0.05) between the antiradical activities of the oil samples.**

As shown in Table 4-7, oil recovery, antiradical activity and percentage of stearic acid (C18:0) of the oil extracted by 60 minute malaxation period (*Sample C60*) were significantly different from the control sample (*Sample C30*). In addition to the effect of extending the length of malaxation period, the changes in these quality parameters could be due to the effect of olive growing sites. For example, as shown in Appendix 8, oil recovery of Gingin's *Sample C60* was significantly increased from 50.59 to 60.15 % when compared to the control sample (*Sample C30*). However, oil recovery of Swan Valley's *Sample C60* was not significantly different from its control sample. The percentage of stearic acid (C18:0) in both Gingin's and Margaret River's *Sample C60*s was significantly increased by extending the olive paste malaxation period to 60 minutes. However, such finding is not observed in the Swan Valley's *Sample C60*.

On the other hand, the interactive effect between olive growing sites and extending the length of olive paste malaxation period to 60 minutes was not significant in

affecting the other quality of the extracted olive oil. The results shown in Table 4-7 indicate that extending the length of olive paste malaxation period to 60 minutes did not affect the yield of oil extraction, concentration of total phenolic compounds, peroxides value, acidity level, fatty acid composition in terms of palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2), level of conjugated diene (K232) and conjugated triene (K270), variation of specific extinction (ΔK) and colour in terms of brightness (L*), greenness (a*) and yellowness (b*) ($P>0.05$). The results are in agreement with those reported by Di Giovacchino, Sestili and Di Vincenzo (2002). As stated in their work, extended length of olive paste malaxation period up to 90 minutes did not affect the peroxides value, acidity level, levels of conjugated diene and conjugated diene of the extracted oil. It was highlighted that these quality parameters are more susceptible to the effect of cultivar than to processing techniques (Di Giovacchino, Sestili & Di Vincenzo 2002).

One of the main objectives of the present study is to improve the concentration of total phenolic compounds in the extracted olive oil. However, extending the length of olive paste malaxation period to 60 minutes could not achieve this objective. The lack of significant effect of olive paste malaxation period on the concentration of total phenolic compounds in *Sample C30* and *Sample C60* is also reported by Aliakbarian, Dehghani and Perego (2009). In their study, destoned Italian *Coratina* olives were crushed and malaxed for periods ranging from 30 to 90 minutes. It is reported that the concentration of phenolic compounds was at its maximum when the olive paste was subjected to a malaxation period of 30 minutes. The higher concentration of total phenolic compounds in the extracted olive oil malaxed for 30 minutes compared to a 60 minute malaxation period could be attributed to the activity of PPO. If the activity of PPO is not deactivated over the 60 minute malaxation period, it is possible that the phenolic compounds were continuously degraded by the PPO. Consequently, there is no increase in the concentration of total phenolic compounds in the extracted olive oil when the olive paste was malaxed for an extended malaxation period of 60 minutes.

Similar to the other quality parameters, acidity level of the olive oil extracted by extending the length of olive paste malaxation period to 60 minutes (*Sample C60*) was not significantly different to that of the control sample (*Sample C30*). The resemblance between the acidity levels of *Sample C30* and *Sample C60* is also reported by the study conducted by Kalua et al. (2006). It is stated that the acidity

level of the oil sample subjected to a 30 minute malaxation period was not significantly different to those subjected to 60, 90 and 120 minute olive paste malaxation period (Kalua et al. 2006). The acidity level of the extracted olive oil is likely to be dependent on the quality of the olive fruits. Endogenous enzymes present in the olive fruits are not triggered if the olives were not previously subjected to destructions by olive fruit fly (Pereira et al. 2004). Indeed, extending the olive paste malaxation period to 60 minutes did not significantly breakdown the olive cell structure (Di Giovacchino, Sestili & Di Vincenzo 2002). As most of the cell structure remains intact, the endogenous enzymes present in the olive cell were unlikely to be activated. Consequently, the breakdown of fatty acids is not initiated, resulting in a low acidity level in the extracted olive oil. In addition, the acidity level of *Sample C60* was reported as similar to that of Iranian *Koroneiki* olive oil (Najafian et al. 2009). Another finding generated from these results is that the acidity level of the extracted olive oil is independent of the olive cultivars.

Due to the lack of published information on the quality of Western Australian *Frantoio* olive oil, the findings of the present study can only be compared with those reported in international studies. The peroxides value of *Frantoio* olive oil processed by malaxing the olive paste for 60 minutes without addition of processing aids (*Sample C60*) was higher than that of the Iranian *Koroneiki* olive oil (Najafian et al. 2009). The effect of olive growing site on the peroxides value of the extracted olive oil is again verified by this study. The result also indicates the possibility that *Frantoio* olives are more susceptible to oxidation than *Koroneiki* olives. It is suggested that the production of Western Australian *Frantoio* olive oil to be handled properly to minimize the possible occurrence of oxidation. Inappropriate techniques can lead to substandard olive oil that may not meet the IOC standards set for EVOO.

Comparison of the oil quality with commercial samples is essential. The commercial samples can be used to validate the efficiency and effectiveness of the processing techniques applied in this study. In particular, the concentration of phenolic compounds and the antiradical activity are the focuses of this study. Assessing the quality of *Sample C60* against commercial EVOO samples indicated that *Sample C60* had lower concentration of phenolic compounds and lower antiradical activity (Appendix 6). As extending the olive paste malaxation period to 60 minutes did not

improve the total phenolic compounds in the extracted oil, it is necessary to explore alternate processing techniques to improve its concentration.

In summary, extending olive paste malaxation period to 60 minutes with no addition of processing aids positively increased the oil recovery. Such increment is encouraging. However, malaxing the olive paste for 60 minutes reduced the antiradical activity while increasing the percentage of saturated stearic acid (C18:0) in the oil. The changes in these quality parameters are attributable to the interactive effect between the processing techniques and the olive growing sites. It is necessary to explore alternate processing techniques that do not compromise the quality of olive oil for high oil recovery. Alternate processing techniques were explored to improve the yield of oil extraction, concentration of total phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition, oxidative stability and colour of the olive oil in this study. The effect of adding processing aids to the olive paste followed by a 30 minute malaxation period on the quality parameters of the extracted olive oil is presented in the following section.

### 4.2.4 Addition of processing aids

As previously suggested in the literature, addition of processing aids is another potential solution to improve the extraction of oil and total phenolic compounds in the oil. Another part of this study involves investigation of the effect of adding processing aids to the olive paste followed by a 30 minute malaxation period on the quality of extracted oil. The processing aids, namely citric acid, Viscozymes or Pectolyase were added at 0.15 g/mL to the olive paste. The extracted olive oil samples were named *Sample A30*, *Sample V30* and *Sample P30*, respectively. The effect of adding processing aids was evaluated on the yield of oil extraction, oil recovery, concentration of total phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition, level of conjugated diene (K232) and conjugated triene (K270), variation of specific extinction (ΔK) and colour in terms of brightness (L*), greenness (a*) and yellowness (b*) of the extracted oil. The quality parameters of these olive oil samples were compared with those of the control sample (*Sample C30*). Mean values and standard deviations (S.D.) of each quality parameter are tabulated in Table 4-8.

**Table 4-8: Effect of addition of processing aids on the quality of olive oil**

| Quality parameters of olive oil | [#] Samples (Mean ± S.D.) | | | | | |
|---|---|---|---|---|---|---|
| | ***Sample C30*** | ***Sample A30*** | ***Sample V30*** | ***Sample P30*** | ***Sample A30[H]*** | ***Sample V30[H]*** |
| Yield of oil extraction (%) | 11.24 ± 5.49 $^{a}$ | 12.06 ± 5.01 $^{b}$ | 12.61 ± 5.13 $^{b}$ | 10.54 ± 5.47 $^{ab}$ | 14.31 ± 2.12 $^{a}$ | 14.94 ± 2.40 $^{a}$ |
| Oil recovery (%) | 54.08 ± 13.94 $^{a}$ | 62.16 ± 6.11 $^{cd}$ | 64.79 ± 7.09 $^{c}$ | 54.47 ± 10.21 $^{ab}$ | 69.10 ± 2.35 $^{abd}$ | 73.32 ± 2.83 $^{bc}$ |
| Total phenolic compounds (mg/kg oil) | 113.09 ± 86.02 $^{a}$ | 96.44 ± 91.62 $^{b}$ | 105.65 ± 95.71 $^{ab}$ | 59.38 ± 17.88 $^{a}$ | 266.32 ± 70.33 $^{c}$ | 235.42 ± 73.59 $^{abc}$ |
| Antiradical activity (% inhibition of DPPH•) | 32.49 ± 5.92 $^{a}$ | 26.47 ± 8.69 $^{b}$ | 30.28 ± 8.94 $^{ad}$ | 27.56 ± 8.26 $^{ad}$ | 47.61 ± 9.25 $^{c}$ | 40.54 ± 5.61 $^{ad}$ |
| Peroxides value (mEq/kg) | 14.24 ± 9.17 $^{a}$ | 18.38 ± 8.40 $^{b}$ | 17.66 ± 8.15 $^{b}$ | 19.98 ± 5.84 $^{ab}$ | 6.92 ± 2.10 $^{b}$ | 6.61 ± 1.64 $^{b}$ |
| Acidity level (% m/m oleic acid) | 0.24 ± 0.13 $^{a}$ | 0.40 ± 0.33 $^{c}$ | 0.41 ± 0.34 $^{c}$ | 0.42 ± 0.26 $^{b}$ | 0.16 ± 0.03 $^{ab}$ | 0.18 ± 0.05 $^{ab}$ |
| C16:0 (%) | 14.48 ± 3.56 $^{a}$ | 17.15 ± 4.82 $^{b}$ | 16.48 ± 4.46 $^{bc}$ | 17.00 ± 2.08 $^{ac}$ | 11.98 ± 1.31 $^{bc}$ | 11.11 ± 2.20 $^{ab}$ |
| C18:0 (%) | 1.93 ± 0.97 $^{a}$ | 2.36 ± 1.32 $^{b}$ | 2.19 ± 1.04 $^{ab}$ | 2.97 ± 1.13 $^{b}$ | 0.79 ± 0.18 $^{ab}$ | 0.74 ± 0.30 $^{ab}$ |
| C18:1 (%) | 80.40 ± 11.83 $^{a}$ | 86.71 ± 15.87 $^{b}$ | 83.73 ± 10.07 $^{ab}$ | 84.85 ± 7.69 $^{ab}$ | 76.55 ± 3.53 $^{ab}$ | 72.06 ± 12.79 $^{ab}$ |
| C18:2 (%) | 15.30 ± 6.64 $^{a}$ | 18.23 ± 5.96 $^{b}$ | 17.76 ± 5.88 $^{ab}$ | 18.01 ± 5.10 $^{ab}$ | 12.29 ± 2.64 $^{b}$ | 11.08 ± 2.38 $^{ab}$ |
| K232 (%) | 1.84 ± 0.34 $^{a}$ | 2.14 ± 0.24 $^{b}$ | 2.11 ± 0.22 $^{b}$ | 1.93 ± 0.33 $^{a}$ | 1.82 ± 0.09 $^{ab}$ | 1.80 ± 0.08 $^{ab}$ |
| K270 (%) | 0.12 ± 0.10 $^{a}$ | 0.16 ± 0.12 $^{b}$ | 0.14 ± 0.11 $^{ab}$ | 0.14 ± 0.07 $^{a}$ | 0.04 ± 0.12 $^{ab}$ | 0.03 ± 0.13 $^{ab}$ |
| ΔK | 0.00 ± 0.02 $^{a}$ | 0.00 ± 0.02 $^{a}$ | 0.00 ± 0.02 $^{a}$ | 0.00 ± 0.03 $^{a}$ | 0.01 ± 0.01 $^{a}$ | 0.01 ± 0.02 $^{a}$ |
| L* | 38.23 ± 0.56 $^{a}$ | 38.52 ± 0.47 $^{b}$ | 38.50 ± 0.37 $^{ab}$ | 38.25 ± 0.47 $^{b}$ | 39.03 ± 0.37 $^{ab}$ | 38.94 ± 0.44 $^{ab}$ |
| a* | -1.99 ± 0.81 $^{a}$ | -1.80 ± 0.78 $^{b}$ | -1.80 ± 0.80 $^{ab}$ | -1.67 ± 0.78 $^{b}$ | -2.25 ± 0.36 $^{ab}$ | -2.25 ± 0.46 $^{ab}$ |
| b* | 8.42 ± 3.57 $^{a}$ | 7.05 ± 2.96 $^{b}$ | 7.03 ± 2.94 $^{b}$ | 7.09 ± 3.67 $^{b}$ | 9.11 ± 1.57 $^{ab}$ | 9.08 ± 2.06 $^{ab}$ |

[#] Descriptions of each sample are available in the abbreviation list

Different letters across the row indicate significant differences ($P<0.05$) of the quality parameter amongst the oil samples

The IOC has established standards for all of the key quality parameters for different classes of olive oil. It is therefore important to ensure the production of olive oil met the IOC standards in order to legally trade the olive oil according to the various classes. The results shown in Table 4-8 indicate that all of the oil samples extracted with addition of either citric acid, Viscozymes or Pectolyase at 0.15 g/mL met the IOC standards set for EVOO. The peroxides value, acidity level, fatty acid composition in terms of palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2), level of conjugated diene (K232) and conjugated triene (K270) of all *Sample A30*, *Sample V30* and *Sample P30* lied within the acceptable range set for EVOO. However, it should be noted that the level of oleic acid of all *Sample A30*, *Sample V30* and *Sample P30* reached the upper limit set for EVOO. High level of oleic acid is in fact a characteristic of Australian olive oil as documented in the RIRDC report (2008). The relatively high percentage of oleic acid in Australian olive oil is attributable to the cool climates (Mailer & Ayton 2008). As reported by Mailer and Ayton (2008), the Australian Olive Association is currently liaising with the IOC to review the standards set for oleic acid in EVOO.

The three different processing aids were added with different purposes. Briefly, citric acid was added to improve the concentration of phenolic compounds. It can deactivate the enzyme PPO which breaks down the phenolic compounds. It is anticipated that the concentration of phenolic compounds in the oil can be increased with less activated PPO. On the other hand, the enzyme preparations, namely Viscozymes and Pectolyase, were added to break down the cell structure in order to increase the release of the oil vacuoles entrapped within the fruit matrix. Incidentally, addition of the three different processing aids significantly increased the acidity levels and reduced the yellowness of the extracted oil samples. Due to the lack of information on the effect of processing aids on the acidity level of Australian olive oil, validation of these findings is impossible. It is possible that these processing aids applied break down the cell structure of the olive fruit and release the endogenous enzymes present in the cell (Mailer & Ayton 2008). The endogenous enzymes then induced oxidation through the LOX pathway, which led to formation of free fatty acids and an increase in the acidity level of the extracted oil (Luaces, Sanz & Perez 2007; Mailer & Ayton 2008). Despite the significant increment in the acidity level of *Sample A30*, *Sample V30* and *Sample P30*, they were all within the IOC acceptable range set for EVOO. Oxidation process

induced by the endogenous enzymes could have reduced the concentration of antioxidant carotenoid in the olive oil. Carotenoid is the main pigment that contributes to the yellow colour of oil (Alfano et al. 2008).

### 4.2.4.1 Addition of 0.15 g/mL citric acid

As shown in Table 4-8, the effect of the processing aids on the extracted oil varied. When compared to the control sample (*Sample C30*), addition of 0.15 g/mL citric acid to the olive paste followed by a 30 minute malaxation period (*Sample A30*) produced olive oil with improved yield of oil extraction and oil recovery from 11.24 to 12.06 % and 54.08 to 62.16 %, respectively. The significant improvement of oil recovery by 14.9 %, as illustrated in Figure 4-3, could be due to the effective rupturing effect exerted by the citric acid particle at the microscopic condition. As the degree of breakdown of the cell structure was increased, more entrapped oil droplets were released into the liquid phase, thus improving the amount of oil extracted. The significant increment in oil extraction highlighted the potential of applying citric acid during olive oil production. With less loss of oil during the extraction process, citric acid indicates a new approach to efficient and cost-effective production of olive oil.

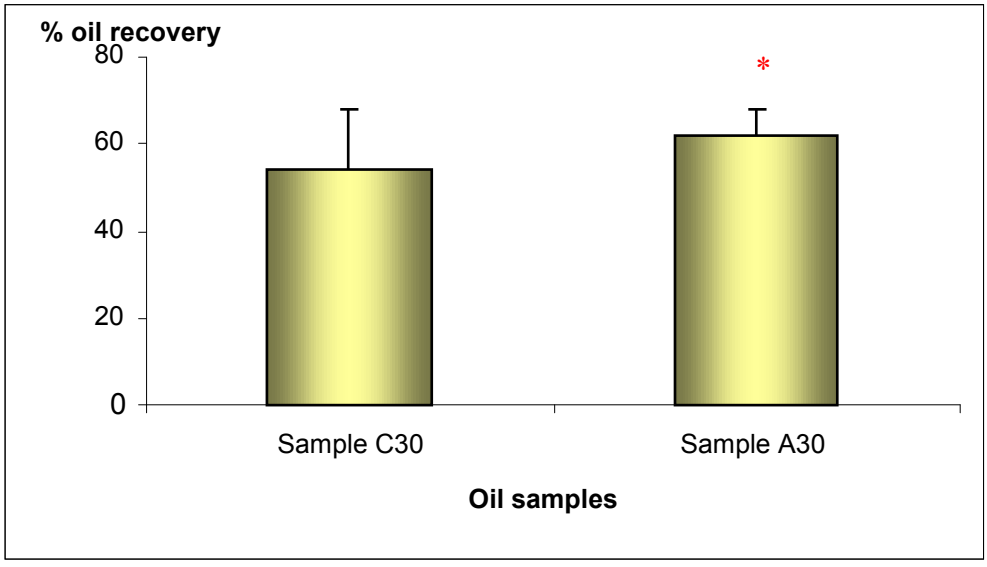



**Figure 4-3: Oil recovery as affected by addition of 0.15 g/mL citric acid**

**The mean values of the oil recovery (%) are shown with standard deviation error bars. *Sample C30* is the control oil sample extracted by malaxation for 30 minutes with no addition of processing aids. *Sample A30* is the oil sample extracted with addition of 0.15 g/mL citric acid. * indicates significant differences (P<0.05) between the percentages of oil recovery of the oil samples.**

Evaluation on the quality of *Sample A30* is necessary before such processing technique can be applied during the production of olive oil. Indeed, it is necessary to ensure the quality of olive oil extracted with citric acid meet the IOC standards. The fatty acid composition, peroxides value, acidity level, level of conjugated diene (K232) and conjugated triene (K270) of *Sample A30* are within the IOC standard set for EVOO. However, the level of conjugated diene (K232) and triene (K270) were significantly higher in *Sample A30* than those of the control sample (*Sample C30*). Such observation indicates that *Sample A30* could have been exposed to oxidation. Indeed, the concentration of total phenolic compounds in *Sample A30* is lower than that in *Sample C30*. It is thought that the phenolic compounds in *Sample A30* could have acted as an antioxidant to protect the oil from oxidation. Consequently, the concentration of total phenolic compounds in the oil is reduced. As expected, the antiradical activity of *Sample A30* was lower than that of *Sample C30* due to the high correlation ($r^2$=0.778) between phenolic compounds and antiradical activity (Appendix 7).

Citric acid has been reported as an effective chelating agent and is widely used in the food industry (Cerretani et al. 2008). In this study, citric acid was added as a chelating agent to the production of olive oil. The intention of adding citric acid was to chelate PPO, inhibit its activity in degrading phenolic compounds and reduce the oxidation of phenolic compounds during the production. With lesser amount of total phenolic compounds being oxidized, it is anticipated that the concentration of total phenolic compounds in the extracted olive oil can be improved by addition of citric acid. As antiradical activity is positively correlated to the concentration of total phenolic compounds (Appendix 7), increment of total phenolic compounds is expected to also improve the antiradical activity of the oil sample. However, such observation is not witnessed in this study. It is possible that the concentration of citric acid applied in this study was too low to be effective. It is therefore necessary to investigate the effect of adding a higher concentration of citric acid to the olive paste.

The interaction between addition of citric acid and olive growing sites on the quality of olive oil was significant. In particular, the antiradical activity of Margaret River's olive oil extracted with 0.15 g/mL citric acid (*Sample A30*) was significantly reduced. It is possible that the addition of 0.15 g/mL citric acid is not sufficient to completely inactivate the enzyme PPO present in Margaret River olives. Consequently, the

concentration of total phenolic compounds is not increased in Margaret River's *Sample A30* (Appendix 9). Such an observation has again highlighted the correlation between antiradical activity and total phenolic compounds.

As colour affects the consumers buying behaviour (Criado et al. 2008; Romero et al. 2003), it is mandatory to study the changes of brightness (L*), greenness (a*) and yellowness (b*) in the extracted oil sample. There was an increase in the brightness (L*) of *Sample A30* despite the reduction in the readings of greenness (a*) and yellowness (b*). Such phenomenon can be elucidated by the inverse correlations between the brightness of the oil and the chroma parameters, as illustrated by Ranalli et al. (2005).

It is also necessary to compare the quality of the oil sample extracted by addition of 0.15 g/mL citric acid (*Sample A30*) to that of commercial EVOO samples. The concentration of total phenolic compounds and the antiradical activity of *Sample A30* were significantly lower than those of commercial EVOO samples (Appendix 10). Meanwhile, the significantly higher readings of peroxides value and acidity level of *Sample A30* suggested that the oil has undergone oxidation. The results indicated the possible protective effect of high concentration of total phenolic compounds and antiradical activity against oxidation. Nevertheless, comparison between *Sample A30* and commercial EVOO samples suggested that higher concentration of citric acid may be necessary to produce oil samples with comparable quality to that of commercial EVOO samples.

#### 4.2.4.2 Addition of 0.15 g/mL Viscozymes

Enzyme preparation Viscozymes comprises of a mixture of cell wall degrading enzymes, containing mainly carbohydrases such as arabanase, cellulase, β-glucanase, hemicellulase and xylanase. It was added to break down the cell structure within the olive fruit matrix to release the entrapped oil vacuoles. Indeed, addition of Viscozymes at 0.15 g/mL to the olive paste followed by a 30 minute malaxation period (*Sample V30*) significantly increased the yield of oil extraction and oil recovery from 11.24 to 12.61 % and from 54.08 to 64.79 %, respectively (Table 4-8). In particular, the oil recovery of *Sample V30* was significantly improved by 19.80 %

when compared to the control sample (Figure 4-4). The improvement is due to the arabanase, cellulase, β-glucanase, hemicellulase and xylanase activity of Viscozymes. The enzyme complex is likely to be effective in breaking down the three-dimensional matrix of the cell structure at each cell layer. Consequently, the entrapped oil droplets are released from the cell by the disruptive action of Viscozymes into the liquid phase, contributing to an increase in the amount of oil extracted (Najafian et al. 2009). In this regard, addition of Viscozymes at 0.15 g/mL can be a potential solution to improve the efficiency of oil extraction. Indeed, peroxides value, level of oleic acid and oxidative stability of *Sample V30* were comparable to those of the commercial EVOO samples (Appendix 10).

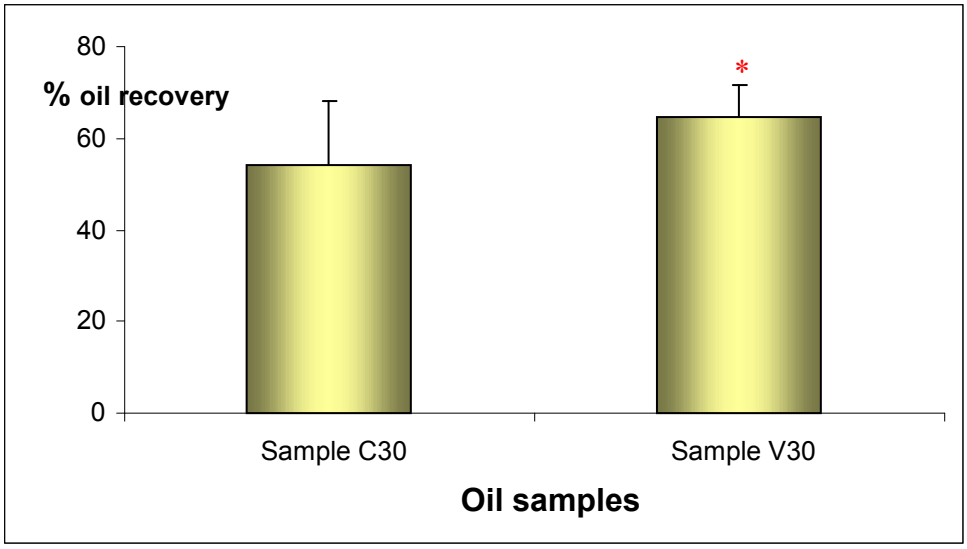



**Figure 4-4: Oil recovery as affected by addition of 0.15 g/mL Viscozymes**

**The mean values of the oil recovery (%) are shown with standard deviation error bars. *Sample C30* is the control oil sample extracted by malaxation for 30 minutes with no addition of processing aids. *Sample V30* is the oil sample extracted with addition of 0.15 g/mL Viscozymes. * indicates significant differences (P<0.05) between the percentages of oil recovery of the oil samples.**

Despite the significant effect of Viscozymes on improving the efficiency of olive oil extraction, it is necessary to assess if the oil extracted by Viscozymes met the IOC standard for EVOO. The results indicate that the readings of fatty acid composition, peroxides value, acidity level, levels of conjugated diene (K232) and conjugated triene (K270) of *Sample V30* lied within the IOC standard set for EVOO (Table 4-8). It is therefore verified that addition of 0.15 g/mL Viscozymes to the olive paste

followed by a 30 minute malaxation period is an appropriate technique for production of EVOO.

Colour is one of the significant factors affecting the consumers' buying behaviour (Criado et al. 2008). While assessing the effect of adding 0.15 g/mL Viscozymes on the colour of the extracted oil, it was found that the yellowness (b*) of *Sample V30* was significantly lower than that of the control sample (*Sample C30*). The reduction in the yellowness without changes in the brightness and greenness of the sample could be due to the sole loss of carotenoid during the production. Carotenoid is one of the main antioxidants in olive oil (Velasco & Dobarganes 2002). With a lesser amount of yellow pigment carotenoid, it is likely that the antioxidant activity of this oil sample against oxidation is compromised. Oxidation induces changes to the structure of fatty acids in the oil and results in higher number of conjugated molecule structures (Morales & Przybylski 2000). Indeed, an increase in the level of conjugated diene (K232) is observed in *Sample V30*. Therefore, it is recommended that olive oil added with 0.15 g/mL Viscozymes during a 30 minute malaxation period (*Sample V30*) be stored appropriately to reduce the risk of oxidation and deterioration of its quality.

As shown in Table 4-8, addition of 0.15 g/mL Viscozymes to olive paste followed by a 30 minute malaxation period increased the yield of oil extraction and oil recovery of the extracted oil. Such improvement in the efficiency of olive oil extraction was observed in Gingin, Swan Valley and Margaret River (Appendix 11). Meanwhile, the increase in the peroxides value of the olive oil extracted with 0.15 g/mL Viscozymes is mostly due to the increase in the peroxides value in both of the extracted Gingin and Margaret River olive oil (Appendix 11). The olive oil extracted from Swan Valley olives subjected to addition of 0.15 g/mL Viscozymes did not have an elevated reading of peroxides value. The results indicate that the endogenous enzymes present in the Gingin and Margaret River olives were more responsive to the addition of 0.15 g/mL Viscozymes. Once activated, these endogenous enzymes tended to initiate the formation of hydroperoxides via the LOX pathway (Ranalli et al. 2003b) and led to an increase in the peroxides value in the extracted olive oil. It is likely that the addition of Viscozymes did not activate the endogenous enzymes present in Swan Valley olives. As a result, no hydroperoxides were found in the extracted Swan Valley olive oil. Therefore, no increment in the peroxides value in the extracted Swan Valley olive oil was observed.

As shown above, interaction between addition of Viscozymes and olive growing sites was significant on the quality of extracted olive oil. Indeed, the significant increase in the acidity level of olive oil extracted by 0.15 g/mL Viscozymes was observed in Swan Valley and Margaret River olive oil, but not in Gingin olive oil, when compared to their control samples (*Sample C30*). In particular, the acidity level of Margaret River reached the upper limit set by IOC for EVOO, recording a reading of 0.80 % (Appendix 11). It is anticipated that Viscozymes, in addition to its cell rupturing activity, was effective in hydrolyzing the triacylglycerols. As the triacylglycerols were broken down, there was an increase amount of free fatty acids, as reflected in the higher acidity level in the oil. The quality, especially, the storage ability of the extracted oil sample is therefore jeopardized by the addition of Viscozymes. Interestingly, as a higher concentration of Viscozymes was added, there was no significant increase in the acidity level, particularly in Swan Valley olive oil (*Sample V30[H]*) when compared to its control sample (Appendix 11). The results suggest that Viscozymes, when added at a higher concentration, could have exerted protective mechanism on the triacylglycerols present in Swan Valley olives.

#### 4.2.4.3 Addition of 0.15 g/mL Pectolyase

Enzyme preparation Pectolyase contains pectinase enzyme. It was added to break down the pectin in the cell structure. As shown in Table 4-8, addition of Pectolyase at 0.15 g/mL to the olive paste followed by a 30 minute malaxation period (*Sample P30*) did not significantly increase the yield of oil extraction, oil recovery, concentration of total phenolic compounds and antiradical activity of the extracted oil, when compared to the control sample (*Sample C30*). Such a finding may be attributable to the mono-pectinase activity of Pectolyase. As the structure of olive cells comprise of pectin as well as cellulose and hemicellulose (Najafian et al. 2009), enzymes with a single strain activity are not capable of breaking down the cell structure. Indeed, the pectinase activity exerted by Pectolyase is likely to be ineffective in breaking down the cellulose and hemicellulose of the cell structure. As a result, the cell components were not released into the oil phase, leading to no significant differences between the main quality parameters of *Sample P30* and the control sample (*Sample C30*). Indeed, as shown in Appendix 12, the yield of oil extraction, concentration of total phenolic

compounds and the antiradical activity of all *Sample P30*s extracted from olives harvested from Gingin, Swan Valley and Margaret River were not significantly different to those of the control sample (*Sample C30*). The result suggests that enzyme complex with arabanase, cellulase, β-glucanase, hemicellulase and xylanase, as illustrated by Viscozymes, can better target the layers of olive cell structure. Consequently, it is more effective in disrupting the cell structure and release more cell components when comparing to a sole pectinase enzymatic activity. Such assumption is verified by the poorer performance of mono-pectinase activity exerted by Pectolyase comparing to the enzyme complex Viscozymes, particularly on the oil recovery and concentration of total phenolic compouds in the extracted oil.

In addition to the yield of oil extraction, oil recovery and concentration of total phenolic compounds in the extracted oil, it is necessary to assess if the oil extracted by Pectolyase met the requirements set by IOC for EVOO. The peroxides value, acidity level, level of conjugated diene and triene in *Sample P30* were recorded as 19.98 mEq/kg, 0.42 % m/m oleic acid, 1.93 % and 0.14 %, respectively (Table 4-8). In this regard, the quality parameters of *Sample P30* have all met the IOC standards. Although the percentage of stearic acid in *Sample P30* is within the IOC standard, it was significantly increased to 2.97 %. The significant increment of saturated stearic acid in *Sample P30* could provide greater stability during the storage period of the oil. As a result, deterioration of quality of the olive oil is reduced. However, a systematic review of epidemiological studies also concluded that consumption of saturated fatty acids was directly linked to high incidence of cardiovascular diseases (Erkkilä et al. 2008; Hall 2009).

Meanwhile, the colour of *Sample P30* is significantly different to that of the control sample (*Sample C30*). The brightness (L*) of the oil was increased while the greenness (a*) and yellowness (b*) was reduced. Such findings are in agreement with the study conducted by Ranalli et al. (2005), who examined the effect of adding enzymes to Italian *Caroleo, Leccino* and *Dritta* olives. It was reported that, when treated with single strain enzymes, namely *Bioliva* or *Rapidase adex* D, the extracted olive oil had high brightness but low green and yellow intensities. It is possible that all of the single strain enzymes minimized the binding of oil droplets with polysaccharides complex, producing a clearer oil with greater brightness. However, the natural colourings entrapped in the hypoderm tissue were not released by the

mono-pectinase activity of Pectolyase (Ranalli et al. 2005). As a result, the greenness (a*) and yellowness (b*) in *Sample P30* were reduced. The reduction in the greenness (a*) and yellowness (b*) of the oil is attributable to the interaction between addition of Pectolyase and the olive growing sites. In particular, the olives grown at Margaret River were more responsive to addition of 0.15 g/mL Pectolyase. Ranalli et al. (2003b) highlighted that the intensity of greenness and yellowness of olive oil is associated with the amount of chlorophyll and carotenoids present in the extracted oil. Chlorophyll and carotenoid are effective antioxidants in olive oil that can improve the storage stability and health benefits of olive oil (Morales & Przybylski 2000). The reduction in the greenness and yellowness of extracted Margaret River's *Sample P30* suggests that these antioxidants present in Margaret River olives were oxidized during the olive oil extraction process. Indeed, Pectolyase could not improve the concentration of total phenolic compounds or the antiradical activity of the extracted oil (Table 4-8). It is likely that Pectolyase was unable to protect the antioxidants from being oxidized. As a result, the green chlorophyll and yellow carotenoid could have been oxidized during the extraction process when Pectolyase was added, resulting in a lower reading of greenness and yellowness in the extracted olive oil. The greenness and yellowness of *Sample P30* were also lower than those of the commercial EVOO samples (Appendix 10). In addition, the concentration of total phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition and level of conjugated triene were inferior to those of commercial EVOO samples.

In summary, the effect of adding 0.15 g/mL Pectolyase to the olive paste followed by a 30 minute malaxation period (*Sample P30*) was not as good as the addition of 0.15 g/mL citric acid or Viscozymes in improving the quality of the extracted olive oil. At the same concentration of 0.15 g/mL, citric acid and Viscozymes positively improved the efficiency of olive oil extraction. The yield of oil extraction and oil recovery of *Sample A30* and *Sample V30* were both significantly improved. However, such finding is not observed in the oil extracted with addition of 0.15 g/mL Pectolyase. In addition, the results indicate that none of the processing aids added were effective in improving the concentration of total phenolic compounds in the extracted oil despite the documented chelating ability of citric acid and its efficiency in preventing oxidation of phenolic compounds (Choe & Min 2006). With a higher concentration of

total phenolic compounds in the olive oil, it is anticipated that the oil would have a higher antiradical activity, greater sensory profile and longer shelf life (Gutierrez, Arnaud & Garrido 2001; Morales & Tsimidou 2000). The reader is reminded that the objective of this study was to improve the efficiency of oil extraction process without compromising the quality of the extracted oil. Although addition of 0.15 g/mL citric acid and addition of 0.15 g/mL Viscozymes had improved the olive oil extraction efficiency, the concentration of total phenolic compounds in these oil samples was not significantly improved. Therefore, it is worth exploring the effect of adding citric acid and Viscozymes at a higher concentration.

#### 4.2.4.4 Addition of citric acid and Viscozymes at higher concentration

With the aim of improving the concentration of phenolic compounds in olive oil, the concentration of citric acid and Viscozymes to be added to the olive paste was increased to 0.30 g/mL. The effect of adding 0.30 g/mL citric acid and 0.30 g/mL Viscozymes on the quality parameters of the extracted olive oil were shown in Table 4-8.

Comparing with the control sample (*Sample C30*), it was found that addition of 0.30 g/mL citric acid to the olive paste followed by a 30 minute malaxation period was effective in increasing the concentration of total phenolic compounds in the oil (*Sample A30[H]*), as illustrated in Figure 4-5. The increase in the concentration of total phenolic compounds to 266.32 mg/kg oil as observed in *Sample A30[H]* is attributed to the chelating effect of citric acid (Choe & Min 2006). The chelator effectively inactivated PPO, the enzyme which breaks down phenolic compounds. Indeed, Cerretani e al. (2008) stated that the reduction in the pH could partially inactivate PPO thus limiting the amount of phenolic compounds being oxidized. As a result, there is an increase in the concentration of total phenolic compounds. In addition, the increase in the concentration of total phenolic compounds could be due to the differences in the charges at the oil and water phase. As the water phase (where citric acid is dissolved in) is more acidic than the oil phase, the non-ionized phenolic compounds would be more inclined to migrate to the oil phase, resulting in an increase in the concentration of phenolic compounds in the extracted oil (Cerretani et

al. 2008). On the other hand, Aliakbarian et al. (2009) described the increase in the phenolic compounds as the result of greater degree of cell wall eradication. As lesser amount of phenolic compounds is bound by the polysaccharides, more of the phenolic compounds can be released into the oil phase. Consequently, the concentration of total phenolic compounds in the extracted oil is increased by the addition of citric acid.

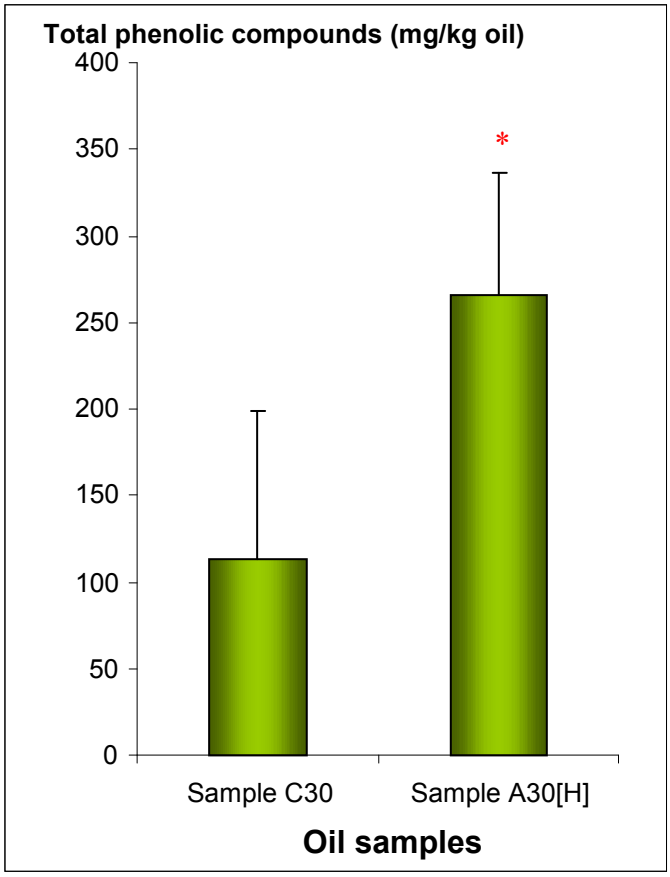



**Figure 4-5: Concentration of total phenolic compounds as affected by addition of 0.30 g/mL citric acid**

**The mean values of the concentrations of total phenolic compounds (mg/kg oil) are shown with standard deviation error bars. *Sample C30* is the control oil sample extracted by malaxation for 30 minutes with no addition of processing aids. *Sample A30[H]* is the oil sample extracted with addition of 0.30 g/mL citric acid. * indicates significant differences (P<0.05) between the concentrations of total phenolic compounds of the oil samples.**

Attributable to the higher concentration of total phenolic compounds retained in the olive oil, it is anticipated that the health benefits, sensory quality and storage stability of the oil can be improved. Indeed, the antiradical activity of the oil sample extracted with 0.30 g/mL citric acid (*Sample A30[H]*) was significantly increased from 32.49 to

47.61 % (Table 4-8). In addition, a lower peroxides value was found in *Sample A30[H]*, which indicates a lesser extent of oxidation in the oil. The peroxides value of *Sample A30[H]* was significantly reduced to 6.92 mEq/kg, more than half the value of *Sample C30*. The result is in agreement with the outcome reported by Cerretani et al. (2008). It is shown that citric acid, when added at 0.30 g/mL to the olive paste followed by a 30 minute malaxation period, is protective against oxidation of olive oil.

Meanwhile, the percentage of palmitic acid (C16:0) and linoleic acid (C18:2) of *Sample A30[H]* were significantly lower than those of *Sample C30*. Such observations are regarded as positive outcomes. The health benefits of *Sample A30[H]* is enhanced as suggested by the lower percentage of saturated palmitic acid. Furthermore, the lower percentage of linoleic acid gave rise to a greater ratio of MUFA:PUFA. The lower concentration of saturated fatty acid as well as the higher MUFA:PUFA ratio are associated with greater protective effect against cardiovascular diseases (Medeiros & Hampton 2007). To the best of the author's knowledge, no other research outcomes have been published on the effect of citric acid addition on the fatty acid composition, level of conjugated diene and triene as well as the colour of the extracted oil. Therefore, comparison and further elucidation of the finding collected from this study is not possible.

As previously shown, addition of 0.15 g/mL Viscozymes during the extraction of olive oil has significantly improved the oil recovery when compared to the control sample. It is anticipated that, by increasing the concentration of Viscozymes to 0.30 g/mL, the oil recovery can be further increased. Indeed, it was found that, when 0.30 g/mL Viscozymes was added to the olive paste during a 30 minute malaxation period (*Sample V30[H]*), the oil recovery of the oil was significantly improved from 54.08 to 73.32 % when compared to the control sample (*Sample C30*). In addition, the peroxides value of *Sample V30[H]* was significantly reduced to 6.61 mEq/kg, almost half of the amount recorded in *Sample C30*. The lower peroxides value suggests that this processing technique is capable of delaying the onset of oxidation in the oil. However, the concentration of total phenolic compounds in the oil was not significantly improved by the addition of 0.30 g/mL Viscozymes. The result suggests that enzyme complex could only enhance the release of oil droplets but not the phenolic compounds. Indeed, some protective mechanism against the oxidation of phenolic compounds needs to be applied to minimize the oxidation of phenolic

compounds during the oil extraction process. If the protective mechanism was not endorsed, the possible effect of adding 0.30 g/mL Viscozymes on increasing the concentration of total phenolic compounds in the extracted olive oil may be masked.

Italian and Spanish researchers appeared to be the only research groups that have been extensively working on the effect of enzymes on the quality of olive oil. All of the enzymes trialed, such as *Olivex*, *Glucanex*, *Rapidase adex* D, *Bioliva*, *Cytolase O*, were found to have positively affected the yield of oil extraction, phenolic compounds, antiradical activity and colour on the olive oil extracted from Italian *Caroleo*, *Leccino* and *Dritta* olives and Spanish *Arbequina* and *Picual* olives (García et al. 2001; Ranalli & De Mattia 1997; Ranalli et al. 2005; Ranalli et al. 2003a; Ranalli et al. 2003b; Ranalli, Sgaramella & Surricchio 1999; Vierhuis et al. 2001). The enzymes applied in their studies, although different to the Viscozymes added in this study, commonly contain pectolytic, cellulolytic and hemicellulolytic activity with glucosidase and endopolygalacturonase activities. Combination of these activities is effective in breaking down the cell structure of olive fruit and modifies the rheology of the olive paste (Chiacchierini et al. 2007). As a result, the cell components are greatly released into the oil matrix, resulting in a greater efficiency of oil extraction and higher quality olive oil. However, it should be noted that the processing techniques applied were different from that practiced in this study. The typical malaxation period performed by the other research was 60 minutes. In fact, it was found that the optimum malaxation period to which enzymes were added should be extended to 90 minutes for Italian *Coratina* olives (Aliakbarian et al. 2008). The extended malaxation period allows the enzymes to digest the olive paste over a prolonged period to release greater amount of phenolic compounds into the oil phase. Indeed, the concentration of total phenolic compounds was reported as significantly improved to the highest concentration of 844.30 µg/g oil. However, possible oxidation during the extended malaxation period as well as the deterioration of the quality of extracted olive oil needs to be prevented through proper extraction conditions. At the same time, the concentration of the enzymes may be increased during the malaxation period to ensure sufficient enzyme activity throughout the extended malaxation period.

The influences of olive cultivar and growing environment are significant on the quality of extracted olive oil. Indeed, the sensitivity of olives of different cultivars to enzymes was found to be different, as previously shown in the results reported by

Aliakbarian et al. (2008). In addition, the phenolic compounds in the oil extracted from Spanish *Arbequina* olives were enhanced by enzymes at a greater degree than the Spanish *Picual* olives (García et al. 2001). In this regard, it is not feasible to directly compare the results obtained from another olive cultivar with the quality of the *Frantoio* olive oil extracted in this study.

Recently, the effect of adding enzymes on the quality of Indian *Frantoio* olive oil was published (Sharma & Sharma 2007). The results concluded that adding 0.10 % of pectinase and cellulase enzymes followed by a 60 minute malaxation period was the most effective processing technique in significantly improve the yield of oil extraction to 9.89 %. However, such level of oil yield is still lower than that obtained in this study (Table 4-8). It is implied that the cell rupture activity exerted by pectinase and cellulase enzymes is not as effective as that of the enzyme complex Viscozymes. Viscozymes contain carbohydrases such as arabanase, cellulase, β-glucanase, hemicellulase and xylanase, which are more effective in rupturing the olive cell structure. The acidity level of the extracted Indian *Frantoio* olive oil was greater than the acceptable level set by IOC for EVOO. Although the peroxides value and fatty acid composition of the Indian *Frantoio* olive oil were similar to those observed in this study, the concentration of total phenolic compounds was significantly lower (0.165 mg/g) than that observed in this study (Table 4-8). The results suggest that the concentration of enzymes added in Sharma and Sharma's study (2007) was not sufficient to improve the efficiency of oil extraction process and the concentration of phenolic compounds in the extracted Indian *Frantoio* olive oil. Meanwhile, the variation between the reaction of Australian *Frantoio* olives and Indian *Frantoio* olives to enzymes could be attributable to the effect of olive growing sites. It appears that only the comparison of extracted oil from the same cultivar grown at the same growing site can provide meaningful conclusions.

The quality parameters of the oil extracted by the control processing technique (*Sample C30*) were significantly different to those of the oil extracted by addition of 0.30 g/mL citric acid (*Sample A30[H]*). In addition, the quality parameters of the oil extracted by the control processing technique (*Sample C30*) were significantly different from those of the oil extracted by addition of 0.30 g/mL Viscozymes (*Sample V30[H]*). However, the only significant differences between *Sample A30[H]* and *Sample V30[H]* are their antiradical activities. The antiradical activity of oil

extracted by addition of 0.30 g/mL citric acid (*Sample A30[H*) was significantly greater than that extracted by addition of 0.30 g/mL Viscozymes (*Sample V30[H]*). The differences may be due to different cell rupturing mechanism exerted by these processing aids on the quality of extracted olive oil. Therefore, it is better to compare the differences in the quality of extracted oil against addition of low and high concentration of individual processing aids. As shown in Table 4-8, addition of high concentration (0.30 g/mL) of citric acid is more effective than that of low concentration (0.15 g/mL) in improving the concentration of total phenolic compounds and the antiradical activity of the extracted oil. Another advantage of adding high concentration of citric acid is that it does not increase the acidity level of the oil as much. A lower acidity level is associated with a greater oxidative stability (Frega, Mozzon & Lercker 1999). Meanwhile, comparison between the oil quality subjected to addition of Viscozymes at low and high concentration identifies the sole difference as the acidity level between the extracted olive oil samples. The results suggest that addition of 0.30 g/mL Viscozymes to the olive paste is not necessary. Indeed, addition of 0.15 g/mL Viscozymes to the olive paste is a more economical approach to production of EVOO.

In general, addition of citric acid as well as addition of Viscozymes to the olive paste followed by a 30 minute malaxation period both has the potential to increase the yield of oil extraction, oil recovery and concentration of total phenolic compounds in the extracted oil. The extent of increment is further enhanced by addition of these processing aids at a higher concentration (0.30 g/mL), particularly in the case of citric acid. Meanwhile, these techniques did not significantly exert negative effects on the peroxides value, acidity level, fatty acid composition, oxidative stability and colour of the extracted olive oil. Indeed, the peroxides value, acidity level, level of oleic acid and linoleic acid, oxidative stability, greenness and yellowness of these oil samples were comparable to those of the commercial EVOO samples (Appendix 10).

In summary, the quality of olive oil extracted by malaxing the olive paste for 30 minutes with no addition of processing aids met the IOC standards set for EVOO. In addition, the quality is comparable to that of commercial EVOO samples. It is deduced that the quality of the *Frantoio* olives and the control processing techniques applied in this study are appropriate to produce EVOO. Generally, addition of processing aids to olive paste followed by a 30 minute malaxation period better enhanced the yield of oil extraction, oil recovery and the concentration of total phenolic compounds in the extracted olive oil when compared to extending the olive paste malaxation period to 60 minutes with no addition of processing aids. Meanwhile, the interactive effect between processing techniques and olive growing sites was significant in affecting the quality of extracted olive oil. Comparing to their control samples, addition of 0.30 g/mL citric acid to the olive paste followed by a 30 minute malaxation period improved the quality of olive oil extracted from Gingin and Swan Valley olives. Indeed, the oil recovery and concentration of total phenolic compounds in these oil samples were both significantly increased. In other words, this technique improves the efficiency of oil extraction process and minimizes the loss of bioactive phenolic compounds during the olive oil extraction process. It is anticipated that addition of 0.30 g/mL citric acid to the olive paste followed by a 30 minute malaxation period can enhance the health benefits of the extracted olive oil. In addition, the antiradical activity of Gingin olive oil and the yield of oil extraction of Swan Valley were improved when 0.30 g/mL citric acid was added to the olive paste. Meanwhile, addition of 0.30 g/mL Viscozymes to the olive paste followed by a 30 minute malaxation period also improves the yield of oil extraction and oil recovery of Gingin olive oil. On the other hand, while addition of 0.15 g/mL Viscozymes to the olive paste followed by a 30 minute malaxation period was the most effective technique in improving the oil recovery of Margaret River olive oil, the peroxides value and acidity level of the extracted oil reach the upper limit set by IOC for EVOO. In this regard, the significant interactive effect between the processing techniques and olive growing sites on the quality of extracted olive oil should be taken into consideration when adopting a new processing technique.

## 4.3 Optimum processing techniques in delivering designated quality parameters in the extracted olive oil

As previously shown, the quality of extracted olive oil is affected by various processing techniques. Nevertheless, it is necessary to identify the optimum processing technique(s) in delivering olive oil with designated quality that meets the IOC standards. As many of the quality parameters correlate with one another (Appendix 7), the correlated variables are grouped and discussed together as a subclass of quality. The effects of processing techniques were compared against each other and evaluated on the grouped quality parameters. The information can assist the identification of the most optimum processing techniques to produce good quality olive oil.

### 4.3.1 Yield of oil extraction and oil recovery

There is speculation that consumption of olive oil will continue to increase dramatically around the world (IOC 2008a). In addition, the shortfall of the estimated production volume was forecasted (AOA 2009; UNCTAD 2006). Therefore, it is important to rapidly identify a processing technique that can improve the yield of oil extraction and oil recovery during the production of olive oil.

In order to achieve the maximum level of yield of oil extraction, it is recommended to add citric acid at 0.15 g/mL and malax the olive paste for 30 minutes (Table 4-9). An alternative would be to add Viscozymes at 0.15 g/mL and malax the olive paste for 30 minutes. Both processing techniques were effective at significantly increasing the yield of oil extraction to around 12 %. They were also effective in improving the oil recovery to greater than 60 %. It is deduced that addition of 0.15 g/mL citric acid or Viscozymes to olive paste followed by a 30 minute malaxation period has promising potential to increase the production volume of olive oil in order to meet the increasing demand of olive oil around the world. However, it is important that the quality of olive oil is not compromised for higher production volume. In this regard, when adopting an ‘optimum’ processing technique, it is important to consider the effect of

processing techniques on other quality variables, such as the concentration of total phenolic compounds, antiradical activity, peroxides value, acidity level, fatty acid composition, oxidative stability and colour of the oil samples, as discussed below.

**Table 4-9: Yield of oil extraction and oil recovery (Mean ± S.D.) of the olive oil samples as extracted by various processing techniques**

| [#] Oil samples | Mean ± S.D. | |
|---|---|---|
| | Yield of oil extraction (%) | Oil recovery (%) |
| ***Sample C30*** | 11.24 ± 5.49 [a] | 54.08 ± 13.94 [a] |
| ***Sample C60*** | 10.01 ± 4.46 [a] | 57.15 ± 11.16 [a] |
| ***Sample A30*** | 12.06 ± 5.01 [b] | 62.16 ± 6.11 [b] |
| ***Sample V30*** | 12.61 ± 5.13 [b] | 64.79 ± 7.09 [b] |
| ***Sample P30*** | 10.54 ± 5.47 [a] | 54.47 ± 10.21 [a] |
| ***Sample A30[H]*** | 14.31 ± 2.12 [a] | 69.10 ± 2.35 [a] |
| ***Sample V30[H]*** | 14.94 ± 2.40 [a] | 73.32 ± 2.83 [a] |

[#] Descriptions of each sample are available in the abbreviation list
Different letters down the column indicate significant differences ($P<0.05$) on the quality parameter amongst the oil samples

## 4.3.2 Phenolic compounds and antiradical activity

The olive oil extraction technique currently applied in the olive oil industry results in a significant loss of phenolic compounds. The phenolic compounds are not retained in the extracted olive oil. Instead, approximately 90 % of the phenolic compounds present in the olive fruits ended up in the by product. The phenolic compounds are important contributing factor to the health benefits of olive oil, the stability of the oil during storage as well as the sensory profile of the oil (Bendini et al. 2007; Gutierrez, Arnaud & Garrido 2001; Morales & Tsimidou 2000; Servili et al. 2009). Loss of these bioactive phenolic compounds reduces the quality of olive oil. In addition, it poses disposal problems and is hazardous to our environment (Alburquerque et al. 2004; Alfano et al. 2008). It is therefore important to minimize the loss of phenolic compounds during the production of olive oil. In particular, as consumers become more aware of the health benefits contributed by the phenolic compounds present in olive oil, they demand olive oil with higher concentration of phenolic compounds.

It is found in this study that, addition of citric acid at 0.30 g/mL is the most effective processing technique to significantly increase the concentration of total phenolic compounds in the olive oil (Table 4-10). In addition, the antiradical activity of the oil sample is significantly improved by the addition of 0.30 g/mL citric acid to olive paste followed by a 30 minute malaxation period. Antiradical activity is important for both the health benefits and storage stability of olive oil (Del Carlo et al. 2004; Gutierrez, Arnaud & Garrido 2001; Lavelli 2002).

**Table 4-10: Total phenolic compounds and antiradical activity (Mean ± S.D.) of the olive oil samples as extracted by various processing techniques**

| [#] Oil samples | Mean ± S.D. | |
|---|---|---|
| | Total Phenolic Compounds (mg/kg oil) | Antiradical activity (% inhibition of DPPH•) |
| ***Sample C30*** | 113.09 ± 86.02 [a] | 32.49 ± 5.92 [a] |
| ***Sample C60*** | 97.36 ± 71.12 [a] | 29.02 ± 4.80 [b] |
| ***Sample A30*** | 96.44 ± 91.62 [b] | 26.47 ± 8.69 [b] |
| ***Sample V30*** | 105.65 ± 95.71 [a] | 30.28 ± 8.94 [a] |
| ***Sample P30*** | 59.38 ± 17.88 [a] | 27.56 ± 8.26 [a] |
| ***Sample A30[H]*** | 266.32 ± 70.33 [b] | 47.61 ± 9.25 [b] |
| ***Sample V30[H]*** | 235.42 ± 73.59 [a] | 40.54 ± 5.61 [a] |

[#] Descriptions of each sample are available in the abbreviation list
Different letters down the column indicate significant differences ($P<0.05$) on the quality parameter amongst the oil samples

## 4.3.3 Fatty acid composition

Fatty acids are carboxylic acids with long hydrocarbon chains. Depending on the structure, the fatty acids can be grouped into saturated fatty acids and unsaturated fatty acids. The main fatty acids present in olive oil are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2). The fatty acid composition in olive oil serves as an indication of authenticity of the oil (Christopoulou et al. 2004; Stefanoudaki, Kotsifaki & Koutsaftakis 1999). Indeed, the IOC has established standards for the four main fatty acids in olive oil. The percentage of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) in EVOO should be between the range of 7.5-20.0, 0.5-5.0, 55.0-83.0 and 3.5-21.0 %, respectively. In order for an oil to be classified as EVOO, it is important that the fatty acid composition of the oil to lie within these ranges.

As shown in Table 4-11, extending the length of olive paste malaxation period to 60 minutes (*Sample C60*), addition of 0.15 g/mL citric acid (*Sample A30*), addition of 0.15 g/mL Viscozymes (*Sample V30*), addition of 0.15 g/mL Pectolyase (*Sample P30*) and addition of 0.30 g/mL citric acid (*Sample A30[H]*) affect the fatty acid composition of the extracted oil, when compared to the control oil sample (*Sample C30*). However, the fatty acid composition of olive oil extracted by 0.30 g/mL Viscozymes (*Sample V30[H]*) was not significantly different to that of the control sample. Despite the significant effect of the processing techniques on the fatty acid composition, the levels of palmitic acid (C16:0), stearic acid (C18:0) and linoleic acid (C18:2) in all of the oil samples extracted in the present study were within the IOC standard set for EVOO.

**Table 4-11: Fatty acid composition (Mean ± S.D.) of the olive oil samples as extracted by various processing techniques**

| [#] Oil samples | Mean ± S.D. | | | |
|---|---|---|---|---|
| | **Palmitic acid (C16:0)** | **Stearic acid (C18:0)** | **Oleic acid (C18:1)** | **Linoleic acid (C18:2)** |
| ***Sample C30*** | 14.48 ± 3.56 [a] | 1.93 ± 0.97 [a] | 80.40 ± 11.83 [a] | 15.30 ± 6.64 [a] |
| ***Sample C60*** | 15.43 ± 3.98 [a] | 2.18 ± 1.21 [b] | 81.31 ± 11.21 [a] | 17.78 ± 7.08 [a] |
| ***Sample A30*** | 17.15 ± 4.82 [b] | 2.36 ± 1.32 [b] | 86.71 ± 15.87 [b] | 18.23 ± 5.96 [b] |
| ***Sample V30*** | 16.48 ± 4.46 [b] | 2.19 ± 1.04 [a] | 83.73 ± 10.07 [a] | 17.76 ± 5.88 [a] |
| ***Sample P30*** | 17.00 ± 2.08 [a] | 2.97 ± 1.13 [b] | 84.85 ± 7.69 [a] | 18.01 ± 5.10 [a] |
| ***Sample A30[H]*** | 11.98 ± 1.31 [b] | 0.79 ± 0.18 [a] | 76.55 ± 3.53 [a] | 12.29 ± 2.64 [b] |
| ***Sample V30[H]*** | 11.11 ± 2.20 [a] | 0.74 ± 0.30 [a] | 72.06 ± 12.79 [a] | 11.08 ± 2.38 [a] |

[#] Descriptions of each sample are available in the abbreviation list
Different letters down the column indicate significant differences ($P<0.05$) on the quality parameter amongst the oil samples

It should be noted that the proportions of saturated stearic acid (C18:0) in the olive oil extracted by extended length of olive paste malaxation period (*Sample C60*) and the oil extracted by addition of 0.15 g/mL Pectolyase (*Sample P30*) were significantly higher than that of the control sample (*Sample C30*). Similar observation was recorded in olive oil extracted by addition of 0.15 g/mL Viscozymes (*Sample V30*),

where the proportion of saturated palmitic acid (C16:0) was significantly increased when compared to the control sample (*Sample C30*). The health concerns related to saturated fatty acid consumption should not be neglected. The results indicate that the practice of extending olive paste malaxation period to 60 minutes, addition of 0.15 g/mL Viscozymes as well as addition of 0.15 g/mL Pectolyase to olive paste followed by a 30 minute malaxation period should be avoided.

Similarly, addition of 0.15 g/mL citric acid to the olive paste followed by a 30 minute malaxation period also significantly increased the proportion of saturated palmitic acid (C16:0) in the extracted oil. In addition, there is a significant increase in the proportion of saturated stearic acid (C18:0). However, the addition of 0.15 g/mL citric acid also significantly improves the percentage of unsaturated oleic acid (C18:1) in the extracted oil. Oleic acid is the main monounsaturated fatty acid in olive oil. Increase in the percentage of oleic acid in the olive oil positively improves the MUFA:PUFA ratio in the oil. The high MUFA:PUFA ratio has been shown to have protective effect against cardiovascular diseases (Cicero et al. 2008; Medeiros & Hampton 2007). Therefore, addition of 0.15 g/mL citric acid to the olive paste is beneficial in improving the health benefits of the extracted oil. However, the percentage of oleic acid found in the oil sample extracted by addition of 0.15 g/mL citric acid (*Sample A30*) exceeded the IOC standard of 83 %. It is later found that addition of citric acid at 0.30 g/mL is the best applied technique that improves the fatty acid composition in the extracted olive oil. Citric acid, when added at 0.30g/mL, significantly lowers the percentage of saturated palmitic acid (C16:0). In addition, the MUFA:PUFA ratio of the oil extracted by 0.30 g/mL citric acid is significantly improved compared to its addition at a lower 0.15 g/mL. Furthermore, the percentage of oleic acid (C18:1) in the oil extracted by addition of 0.30 g/mL citric acid is within the acceptable range set by IOC. In summary, addition of citric acid at 0.30 g/mL produced olive oil that met the IOC standard for EVOO while providing potential health benefits to the olive oil consumers.

### 4.3.4 Oxidative stability

Oxidation greatly deteriorates the quality of olive oil. For examples, the concentration of total phenolic compounds in the oil is reduced; the sensory profile of olive oil becomes unacceptable due to oxidative rancidity; the acidity level and peroxides value in the oil are elevated as a result of oxidation. In addition, oxidation induces changes to the structure of the fatty acids. As a result, the level of conjugated diene and triene increases in the oil. Therefore, the IOC has established standards to determine the oxidative stability of olive oil. The oxidative stability is measured in terms of peroxides value, acidity level and the level of conjugated diene and conjugated triene present in the olive oil. The standards set by IOC for EVOO are $\leq$ 20 mEq/kg oil, $\leq$ 0.8 % m/m oleic acid, $\leq$ 2.5 % and $\leq$ 0.22 %, respectively.

All olive oil samples extracted by various processing techniques in this study met the IOC EVOO standards in terms of peroxides value, acidity level, level of conjugated diene and conjugated triene. Addition of processing aids significantly affected these quality parameters in the extracted oil samples (Table 4-12). In particular, addition of 0.15 g/mL citric acid significantly increases the peroxides value, acidity level, level of conjugated diene and conjugated triene in the extracted oil. Increase in the peroxides value indicates onset of oxidation (Kiritsakis & Christie 2000). Therefore, it is deduced that addition of 0.15 g/mL citric acid to olive paste followed by a 30 minute malaxation period exerted an adverse effect on the storage ability of the extracted oil. However, the problem can be rectified by increasing the concentration of citric acid to 0.30 g/mL. A similar phenomenon was observed for oil sample extracted with either 0.15 g/mL or 0.30 g/mL of Viscozymes. A general finding deduced from these results is that the addition of citric acid or Viscozymes at 0.30 g/mL is beneficial in protecting the extracted olive oil samples against oxidation.

**Table 4-12: Mean values and S.D. of some key quality parameters used for IOC classification of olive oil as extracted by various processing techniques**

| [#] Oil samples | Peroxides value (mEq/kg) | Acidity levels (% m/m oleic acid) | K232 (%) | K270 (%) |
|---|---|---|---|---|
| ***Sample C30*** | 14.24 ± 9.17 [a] | 0.24 ± 0.13 [a] | 1.84 ± 0.34 [a] | 0.12 ±0.10 [a] |
| ***Sample C60*** | 18.26 ± 8.10 [a] | 0.24 ± 0.14 [a] | 1.77 ± 0.33 [a] | 0.13 ± 0.09 [a] |
| ***Sample A30*** | 18.38 ± 8.40 [b] | 0.40 ± 0.33 [b] | 2.14 ± 0.24 [b] | 0.16 ± 0.12 [b] |
| ***Sample V30*** | 17.66 ± 8.15 [b] | 0.41 ± 0.34 [b] | 2.11 ± 0.22 [b] | 0.14 ± 0.11 [a] |
| ***Sample P30*** | 19.98 ± 5.84 [a] | 0.42 ± 0.26 [b] | 1.93 ± 0.33 [a] | 0.14 ± 0.07 [a] |
| ***Sample A30[H]*** | 6.92 ± 2.10 [b] | 0.16 ± 0.03 [a] | 1.82 ± 0.09 [a] | 0.04 ± 0.12 [a] |
| ***Sample V30[H]*** | 6.61 ±1.64 [b] | 0.18 ± 0.05 [a] | 1.80 ± 0.08 [a] | 0.03 ± 0.13 [a] |

[#] Descriptions of each sample are available in the abbreviation list
Different letters down the column indicate significant differences ($P<0.05$) on the quality parameter amongst the oil samples

## 4.3.5 Colour

In addition to the effectiveness of a processing technique in producing olive oil that meets the standards set by IOC for EVOO, the colour of the extracted oil should not be affected by the extraction technique. The reason is that colour affects the acceptability of an oil sample. Indeed, it is one of the main factors affecting consumers' buying behaviour (Criado et al. 2008; Romero et al. 2003). Colour is usually measured in terms of brightness (L*), greenness (a*) and yellowness (b*). Brightness of an oil sample does not reflect on the quality of an olive oil sample. However, it is often found inversely related to greenness and yellowness of the extracted oil (Ranalli et al. 2005). Therefore, measurement on the brightness of an oil sample indirectly provides a general idea on the effect of processing techniques on the greenness and yellowness of an oil sample. On the other hand, greenness and yellowness of olive oil give an indirect indication on the amount of chlorophyll and carotenoid present in the oil (Ranalli et al. 2003b). It is anticipated that the amount of these pigments is greater when the reading of greenness and yellowness in an oil sample is higher. As chlorophyll and carotenoid are effective antioxidant that can improve the storage stability and health benefits of olive oil (Morales & Przybylski 2000), it is essential to evaluate the effect of processing techniques on these colour parameters of the extracted oil.

The colour of the oil samples in terms of brightness (L*), greenness (a*) and yellowness (b*) was affected by the addition of processing aids applied in this study (Table 4-13). Both the addition of citric acid and Pectolyase at 0.15 g/mL reduced the greenness and yellowness of the extracted oil samples. Meanwhile, the increase in the brightness of these oil samples was expected as brightness is inversely proportional to the greenness and yellowness of the extracted oil (Ranalli et al. 2005). Addition of Viscozymes at 0.15 g/mL to the olive paste followed by a 30 minute malaxation period appears to have the least impact on the brightness and the greenness of the oil sample compared to the addition of the other two processing aids. However, when the concentration of these processing aids was increased to 0.30 g/mL, there were no significant changes on the colour of the extracted oil samples when compared to the control sample (*Sample C30*).

**Table 4-13: Appearance in terms of brightness, greenness and yellowness (Mean ± S.D.) of the olive oil samples as extracted by various processing techniques**

| [#] Oil samples | Mean ± S.D. | | |
|---|---|---|---|
| | **Brightness (L*)** | **Greenness (a*)** | **Yellowness (b*)** |
| ***Sample C30*** | 38.23 ± 0.56 [a] | -1.99 ± 0.81 [a] | 8.42 ± 3.57 [a] |
| ***Sample C60*** | 38.31 ± 0.59 [a] | -2.01 ± 0.68 [a] | 8.42 ± 3.06 [a] |
| ***Sample A30*** | 38.52 ± 0.47 [b] | -1.80 ± 0.78 [b] | 7.05 ± 2.96 [b] |
| ***Sample V30*** | 38.50 ± 0.37 [a] | -1.80 ± 0.80 [a] | 7.03 ± 2.94 [b] |
| ***Sample P30*** | 38.25 ± 0.47 [b] | -1.67 ± 0.78 [b] | 7.09 ± 3.67 [b] |
| ***Sample A30[H]*** | 39.03 ± 0.37 [a] | -2.25 ± 0.36 [a] | 9.11 ± 1.57 [a] |
| ***Sample V30[H]*** | 38.94 ± 0.44 [a] | -2.25 ± 0.46 [a] | 9.08 ± 2.06 [a] |

[#] Descriptions of each sample are available in the abbreviation list
Different letters down the column indicate significant differences ($P<0.05$) on the quality parameter amongst the oil samples

### 4.3.6 Sensory profile

The sensory profile of olive oil plays an important role in determining its quality. Good olive oil should have fresh, fruity, green note on the aroma with a balance of pungency, bitterness and fruitiness on the palate (Morales, Luna & Aparicio 2005). The sensations should all be intense and complex. Sensory profile of olive oil is contributed by a complex combination of different interactions between the compounds present in the oil sample (Angerosa et al. 2000; Gawel & Rogers 2009; Morales & Tsimidou 2000). In addition, the sensory attributes of olive oil correlate with each other (Appendix 13), giving rise to various unique sensory profiles. The sensory properties of olive oil are affected by processing techniques (Di Giovacchino, Sestili & Di Vincenzo 2002; Gomez-Rico et al. 2009). In this regard, the effects of various processing techniques applied in this study were evaluated on their impacts on the sensory profile of the extracted olive oil. In addition to comparison with the control oil sample, the aroma and palate attributes of the olive oil samples were also evaluated against those of the commercial EVOO samples (Table 4-14).

**Table 4-14: Sensory attributes (Mean ± S.D.) of the oil samples as extracted by various processing techniques**

| Samples | Mean ± S.D. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Aroma | | | Palate | | | | |
| | Absence of Faults | Complexity | Fruit Intensity | Absence of Faults | Balance | Complexity | Flavour Intensity | Total |
| **Sample C30** | 1.40 ± 0.83 $^{ac}$ | 0.97 ± 0.58 $^{a}$ | 1.50 ± 0.94 $^{a}$ | 1.37 ± 0.81 $^{a}$ | 1.17 ± 0.88 $^{a}$ | 1.10 ± 0.71 $^{ab}$ | 1.37 ± 0.85 $^{ad}$ | 8.87 ± 5.19 $^{abd}$ |
| **Sample C60** | 1.80 ± 0.42 $^{ad}$ | 1.15 ± 0.34 $^{a}$ | 1.70 ± 0.71 $^{a}$ | 1.80 ± 0.42 $^{a}$ | 1.80 ± 0.63 $^{a}$ | 1.40 ± 0.46 $^{ac}$ | 2.05 ± 0.55 $^{bc}$ | 11.70 ± 2.94 $^{ace}$ |
| **Sample A30** | 2.00 ± 0.00 $^{bd}$ | 1.55 ± 0.44 $^{a}$ | 2.20 ± 0.75 $^{a}$ | 1.75 ± 0.42 $^{a}$ | 1.60 ± 0.57 $^{a}$ | 1.75 ± 0.54 $^{cde}$ | 2.20 ± 0.48 $^{b}$ | 12.95 ± 2.51 $^{c}$ |
| **Sample V30** | 1.60 ± 0.70 $^{abc}$ | 1.15 ± 0.34 $^{a}$ | 1.90 ± 0.52 $^{a}$ | 1.50 ± 0.85 $^{a}$ | 1.60 ± 0.57 $^{a}$ | 1.30 ± 0.42 $^{ac}$ | 1.70 ± 0.48 $^{cd}$ | 10.75 ± 2.98 $^{abcd}$ |
| **Sample A30[H]** | 1.90 ± 0.32 $^{abd}$ | 1.40 ± 0.39 $^{a}$ | 1.85 ± 0.75 $^{a}$ | 1.70 ± 0.48 $^{a}$ | 1.70 ± 0.59 $^{a}$ | 1.90 ± 0.39 $^{d}$ | 2.25 ± 0.49 $^{b}$ | 12.65 ± 2.68 $^{cf}$ |
| **Sample V30[H]** | 1.80 ± 0.63 $^{abd}$ | 1.25 ± 0.68 $^{a}$ | 1.85 ± 0.94 $^{a}$ | 1.65 ± 0.67 $^{a}$ | 1.50 ± 0.75 $^{a}$ | 1.60 ± 0.88 $^{ade}$ | 1.70 ± 0.82 $^{abd}$ | 11.30 ± 4.79 $^{acd}$ |
| **Commercial EVOO samples** | 1.53 ± 0.75 $^{ac}$ | 1.25 ± 0.68 $^{a}$ | 1.95 ± 1.07 $^{a}$ | 1.28 ± 0.79 $^{a}$ | 1.30 ± 0.73 $^{a}$ | 1.24 ± 0.77 $^{ae}$ | 1.50 ± 0.86 $^{acd}$ | 9.98 ± 4.99 $^{def}$ |

[#] Descriptions of each sample are available in the abbreviation list

Different letters down the column indicate significant differences ($P<0.05$) on the quality parameter amongst the oil samples

As shown in Table 4-14, there were no significant differences between the control sample (*Sample C30*) and the commercial EVOO samples on all of the evaluated sensory attributes. In addition, there were no significant differences detected between the *complexity of aroma*, *fruit aroma intensity* and the *balance between bitterness, fruitiness and pungency on the palate* of all of the oil samples. The total score of *Sample C30* was also similar to that of the commercial EVOO samples. Such findings suggest that the control technique applied in this study could extract olive oil with comparable quality to the commercial oil samples.

In general, the various processing techniques applied in this study led to significant differences on the *absence of faults on aroma*, *absence of faults on the palate, complexity on the palate*, *flavour intensity on the palate* and the *total scores* between the extracted oil samples and the commercial EVOO samples. For examples, malaxing the olive paste over an extended malaxation period of 60 minutes with no addition of processing aids (*Sample C60*) improves the flavour intensity when compared to that of *Sample C30* and the commercial EVOO samples. Meanwhile, addition of 0.15 g/mL citric acid to the olive paste followed by a 30 minute malaxation period (*Sample A30*) significantly prevents faults on the aroma and the palate of the oil when compared to *Sample C30* and the commercial EVOO samples. *Sample A30* also has better complexity on the palate and greater intensity of flavour on the palate than *Sample C30* and the commercial EVOO samples. Due to its superior sensory profile, *Sample A30* has scored the highest total score amongst all the tasted olive oil samples. Therefore, addition of 0.15 g/mL citric acid to the olive paste followed by a 30 minute malaxation period can be adopted by the olive oil industry to produce olive oil with good sensory profile. It should be noted that increasing the concentration of citric acid to 0.30 g/mL (*Sample A30[H]*) also significantly improves the complexity of the sensory attributes on the palate and intensity of flavour on the palate. However, such practice is not as cost-efficient as the addition of 0.15 g/mL citric acid. Addition of 0.15 g/mL citric acid to the olive paste followed by a 30 minute malaxation period is sufficient to prevent faults on the aroma and palate of the extracted oil while improving the balance of fruitness, pungency and bitterness, the complexity and flavour intensity on the palate.

In summary, addition of 0.15 g/mL citric acid or Viscozymes to the olive paste followed by a 30 minute malaxation period is effective in improving the yield of oil extraction and oil recovery. However, these techniques are not effective in improving the concentration of total phenolic compounds in the extracted oil. In addition, they did not enhance the antiradical activity of the extracted oil. Instead, addition of 0.30 g/mL citric acid to the olive paste followed by a 30 minute malaxation period is more effective in increasing the concentration of total phenolic compounds and the antiradical activity of the extracted oil. In addition, the extracted oil has comparable oil extraction efficiency and lower peroxides value when compared to the control sample. Furthermore, addition of 0.30 g/mL citric acid to the olive paste followed by a 30 minute malaxation period did not negatively affect the fatty acid composition of the extracted oil. Instead, it has improved the MUFA:PUFA ratio of the oil. In addition, the colour parameters of the oil extracted with addition of 0.30 g/mL citric acid were not negatively affected. The sensory profile of the oil sample extracted by addition of 0.30 g/mL citric acid is also better than that of the control sample. Addition of 0.30 g/mL citric acid to the olive paste followed by a 30 minute malaxation period is deduced as the optimum processing technique for production of quality olive oil.

## 4.4 General discussion

In this study, a range of olive oil samples were extracted via different processing techniques from *Frantoio* olives harvested at three different maturity levels (MI1, MI4 and MI6) from three different olive growing sites (Gingin, Swan Valley and Margaret River) over a two year period. Maturity level of the harvested olives did not significantly affect the yield of oil extraction and the quality of the extracted oil. However, processing techniques and olive growing sites are two significant factors affecting the yield of oil extraction and the quality of the extracted oil.

In general, the olive oil extracted by malaxing the olive paste for 30 minutes with no addition of processing aids (*Sample C30*) has comparable quality to the commercial EVOO samples. It is therefore concluded that this control technique is appropriate in the production of EVOO. However, the quality of *Sample C30* can be enhanced by other processing techniques. In particular, extending the length of olive paste

malaxation period to 60 minutes increased the oil recovery of extracted olive oil. During an extended malaxation period, it is likely that greater coalescence of the oil droplets occurred. As bigger oil mass was formed, the oil mass could be easily separated from the solid matrix during the centrifuge phase. Consequently, there is an increase in the oil recovery when the olive paste malaxation period was extended to 60 minutes. However, such practice led to a reduction of antiradical activity in the extracted olive oil. It is anticipated that the antioxidant phenolic compounds and other components exerted protective mechanism on the oil against oxidation induced during the extended malaxation period. Consequently, these compounds were oxidized and were unable to act as antioxidants in the extracted oil. Therefore, there was a decrease in the antiradical activity when the oil extracted by extended malaxation period was assessed.

On the other hand, addition of citric acid, Viscozymes or Pectolyase at 0.15 g/mL to the olive paste followed by a 30 minute malaxation period produced olive oil with diverse quality. The differences are possibly due to the different mechanism by which these processing aids acted on the olive cell structure. In particular, citric acid is likely to induce changes on the pH of the system and affect the partition of phenolic compounds in the oil. On the other hand, enzyme preparations Viscozymes and Pectolyase are likely to have ruptured the cell wall and released the cell components (such as oil droplets and phenolic compounds) into the olive oil. The findings from the present study indicate that enzyme complex with arabanase, cellulase, β-glucanase, hemicellulase and xylanase activity is more effective than a singular pectinase activity in increasing the yield of oil extraction and oil recovery of the extracted oil. Indeed, Viscozymes is more effective in improving the efficiency of the olive oil extraction than Pectolyase. Such improvement can be further enhanced by adding the processing aids at a higher concentration. The oil recovery was significantly increased when 0.30 g/mL Viscozymes was added to the olive paste followed by a 30 minute malaxation period. In addition, the concentration of total phenolic compounds in the oil was significantly improved when 0.30 g/mL citric acid was added to the olive paste followed by a 30 minute malaxation period.

However, olives harvested from various growing sites reacted differently to the processing techniques. As a result, the quality of olive oil extracted was different. The results suggest that olive growing sites significantly affect the quality of olives and the

extracted oil. Indeed, when the olives harvested from Gingin, Swan Valley and Margaret River were crushed into olive paste and malaxed for 30 minutes with no addition of processing aids, the quality of these extracted oil samples varied. The reason is due to the variation in the quality of the olive fruits grown at different growing sites. In particular, the rainfall, temperature and latitude at a growing site as well as agronomic practices influence the development of the olive fruits. As a result, the physiology of the olive fruits is affected, which is directly influential on the quality of the extracted olive oil. Furthermore, it is thought that the amount of endogenous enzymes present in the olives at these growing sites is different. As a result, different biochemical pathways were activated during the extraction process, producing different intermediate products that affect the end quality of the extracted olive oil.

In addition to the significant effect of processing techniques and olive growing sites on the quality parameters of the extracted olive oil, the interaction between processing techniques and olive growing sites was also found significant in the present study. In particular, efficiency of oil extraction, concentration of total phenolic compounds, antiradical activity, oxidative stabilities, fatty acid composition and colour of the extracted oil samples were different. In Gingin, addition of 0.30 g/mL citric acid was found to improve the concentration of total phenolic compounds in the extracted oil. The high concentration of citric acid is likely to have chelated PPO, an enzyme which degrades phenolic compounds. In addition, the pH change induced by citric acid increased the partition of phenolic compounds in the extracted oil. Simultaneously, the antiradical activity of the oil was enhanced. Furthermore, the yield of oil extraction and oil recovery of Gingin olive oil were increased by addition of 0.30 g/mL Viscozymes. The enzyme preparations successfully ruptured the pectin, cellulose and hemicellulose of the olive fruits and increased the amount of oil droplets released into the oil phase. Fatty acid composition, peroxides value, acidity level, level of conjugated diene and triene as well as the colour of the extracted oil sample were not affected by these techniques. Similar effects on the extracted Swan Valley olive oil were observed when the oil sample was treated with either citric acid or Viscozymes. However, none of the processing techniques applied on Margaret River olives significantly improve the quality of the extracted olive oil. In fact, addition of citric acid negatively affected the quality of extracted Margaret River olive oil. The

yield of oil extraction, oil recovery and the concentration of total phenolic compounds were not improved by this technique. Furthermore, addition of citric acid reduced the antiradical activity of the Margaret River oil while significantly increasing the peroxides value, acidity level, percentage of saturated palmitic acid, level of conjugated diene and level of conjugated triene in the olive oil. Such observations are in contrast to the quality observed on Gingin and Swan Valley olive oil samples.

In summary, the effect of maturity level of the harvested olive fruits was not significant in affecting the quality parameters of the extracted olive oil. However, the quality parameters of extracted Western Australian olive oil samples were significantly affected by the effect of processing techniques, olive growing sites and the interaction between the processing techniques and olive growing sites. The possible mechanisms have been elucidated with the knowledge published in the literatures. The information is expected to assist the olive oil producers in understanding the mechanism of extracting high quality olive oil. They will be in a better position to accommodate the increased demand for high quality olive oil.

# Chapter 5 Conclusions

In this present study, the effects of olive growing sites, maturity level of the harvested olives, processing techniques applied during the extraction of olive oil and the interaction between these 3 independent variables on the quality parameters of the extracted olive oil were investigated. The results show that maturity level of the harvested olives is not a significant factor in affecting the quality of extracted olive oil. Processing techniques, olive growing sites and the interaction between processing techniques and olive growing sites were all significant in affecting the quality of the extracted olive oil.

Despite the significant effect of processing techniques on the quality of extracted olive oil, all of the quality parameters of the extracted olive oil met the IOC standards established for EVOO. Therefore, extending the length of olive paste malaxation period from 30 to 60 minutes, addition of 0.15 g/mL of either citric acid, Viscozymes or Pectolyase to the olive paste followed by a 30 minute malaxation period and addition of 0.30 g/mL citric acid or Viscozymes to the olive paste followed by a 30 minute malaxation period are appropriate techniques for the production of EVOO.

The quality of the extracted olive oil was compared with the commercial EVOO samples. Extending the length of malaxation period to 60 minutes produced olive oil with inferior quality compared to the commercial EVOO samples. Quality of the oil samples extracted with addition of either 0.15 g/mL citric acid, 0.15 g/mL Viscozymes or 0.15 g/mL Pectolyase was also inferior to that of the commercial EVOO samples. However, addition of 0.30 g/mL citric acid and Viscozymes produced olive oil with quality parameters comparable to those of the commercial EVOO samples.

Addition of 0.15 g/mL citric acid or Viscozymes followed by a 30 minute malaxation period was effective in improving the efficiency of oil extraction process. The yield of oil extraction of the extracted olive oil samples was significantly improved to 12.06 and 12.61 %, respectively. The oil recovery of the extracted olive oil samples was improved to 62.16 and 64.79 %, respectively. However, addition of 0.15 g/mL Pectolyase followed by a 30 minute malaxation period did not improve the yield of oil

extraction or oil recovery of the extracted olive oil sample. The results suggest that enzyme complex with arabanase, cellulase, β-glucanase, hemicellulase and xylanase activities are more effective in releasing the oil droplet entrapped in the layers of cells, when compared to enzyme with a single pectinase activity.

In order to improve the oil quality, it is necessary to increase the concentration of the processing aids. Addition of 0.30 g/mL citric acid was the most effective processing technique to increase the concentration of total phenolic compounds in the extracted oil. The technique was also effective in improving the antiradical activity, lowering the percentage of saturated palmitic acid (C16:0) and improving the MUFA:PUFA ratio of the extracted oil. In addition, the oil extracted by addition of 0.30 g/mL citric acid was effectively protected against oxidation while the colour of the oil remained unchanged when compared to the control oil sample. The sensory profile of this oil was also better than other extracted oil samples.

The quality parameters of olive oil extracted from olives harvested from Gingin, Swan Valley and Margaret River were different. In general, extending the length of olive paste malaxation period to 60 minutes did not improve the efficiency of oil extraction process nor the quality of olive oil extracted from olives harvested from these three different olive growing sites. Addition of 0.30 g/mL citric acid and 0.30 g/mL Viscozymes significantly improved the quality of extracted Gingin and Swan Valley olive oil. However, none of the processing techniques could significantly improve the quality of Margaret River olive oil.

In summary, processing techniques, olive growing sites and the interaction between processing techniques and olive growing sites were significant in affecting the quality of extracted oil. However, maturity level of the harvested olive fruits did not affect any of the evaluated oil quality parameters. Extending the olive paste malaxation period to 60 minutes was not an effective processing technique in improving the efficiency of oil extraction or the quality of the extracted oil. Collectively, it was found that the efficiency of oil extraction and the quality of the oil can be improved by slight modification of the processing techniques. Addition of 0.30 g/mL citric acid during the production of olive oil improved the efficiency of oil extraction process, increased the concentration of phenolic compounds and antiradical activity in the oil as well as delivered olive oil with positive sensory attributes.

# Chapter 6 Future perspectives and recommendations

The most important finding from this study is that addition of 0.30 g/mL citric acid improves concentration of total phenolic compounds and antiradical activity of the extracted oil without reducing the yield of oil extraction and oil recovery. Therefore, addition of 0.30 g/mL citric acid has the potential to be applied during the production of olive oil. Application of this technique during the extraction of olive oil is expected to produce olive oil with enhanced health benefits. In particular, the high concentration of phenolic compounds in the extracted olive oil is protective against cardiovascular diseases and cancer.

In this preliminary study, only the quality of olive oil extracted from *Frantoio* olives was investigated. The results in the literature indicate that the composition of olives of different cultivar varies. In particular, the amount of endogenous enzymes present in the olives vary between cultivars, contributing to different fatty acid composition, phenolic compounds, volatile compounds and sensory profile in the extracted oil. Therefore, it would be valuable to determine the effect of the various processing techniques applied in this study on other olive cultivars.

The combined effect of processing aids and extended length of olive paste malaxation period on the quality of olive oil was not investigated in this study. However, in most of the studies reported in the literature, the olive paste was malaxed for 60 minutes after addition of enzyme preparations to the olive paste. The authors of these studies believe that it is appropriate to malax the olive paste for an extended period to enhance the effect of enzyme preparations on the quality of the extracted oil. It is speculated that the quality of the olive oil may be further improved by addition of processing aids followed by an extended olive paste malaxation period of 60 minutes. Further investigations of the combined effect of processing aids and extended malaxation period would be helpful to validate this speculation.

The results acquired from this study verified that the processing aids, namely citric acid and enzyme preparations, exert different effects on the olive cell structure. As a

result, the quality of the extracted oil varied when different processing aids were added. In particular, citric acid was more effective in improving the concentration of total phenolic compounds while Viscozymes was more effective in improving the oil recovery. It would be interesting to determine if there were synergistic effects between these processing aids on the quality of the extracted olive oil. Both citric acid and Viscozymes may be added at the same time to the olive paste during the extraction of olive oil. In addition, the effect of shorten and/or extended olive paste malaxation length on the oil extracted by using both citric acid and Viscozymes may be investigated.

The effect of olive growing sites on the quality of extracted olive oil was found significant in the present study. Therefore, more olive growing sites should be included in the future studies. With more results produced from these future studies, a database on the quality of olive oil can be established. Mapping of the quality of the olive oil processed by various processing techniques will be possible. A better understanding of the mechanism can thus be established from these data. In addition, the database can serve as a reference guide to the olive oil producers from different growing sites in adopting a suitable processing technique to improve the efficiency of olive oil extraction process and the quality of the extracted olive oil. It is anticipated that the efficiency of the production of quality olive oil can be increased. Consequently, the consumers would have sufficient supply of good quality olive oil.

# Chapter 7 References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# Appendices

## Appendix 1: Sensory profile evaluation sheet by IOC



**Figure 1**

**PROFILE SHEET FOR VIRGIN OLIVE OIL**

**INTENSITY OF PERCEPTION OF DEFECTS:**

Fusty/
muddy sediment ——————————➡

Musty-humid-
earthy ——————————➡

Winey-vinegary -
acid-sour ——————————➡

Metallic ——————————➡

Rancid ——————————➡

Others (specify) ——————————➡

**INTENSITY OF PERCEPTION OF POSITIVE ATTRIBUTES:**

Fruity ——————————➡

greenly □ ripely □

Bitter ——————————➡

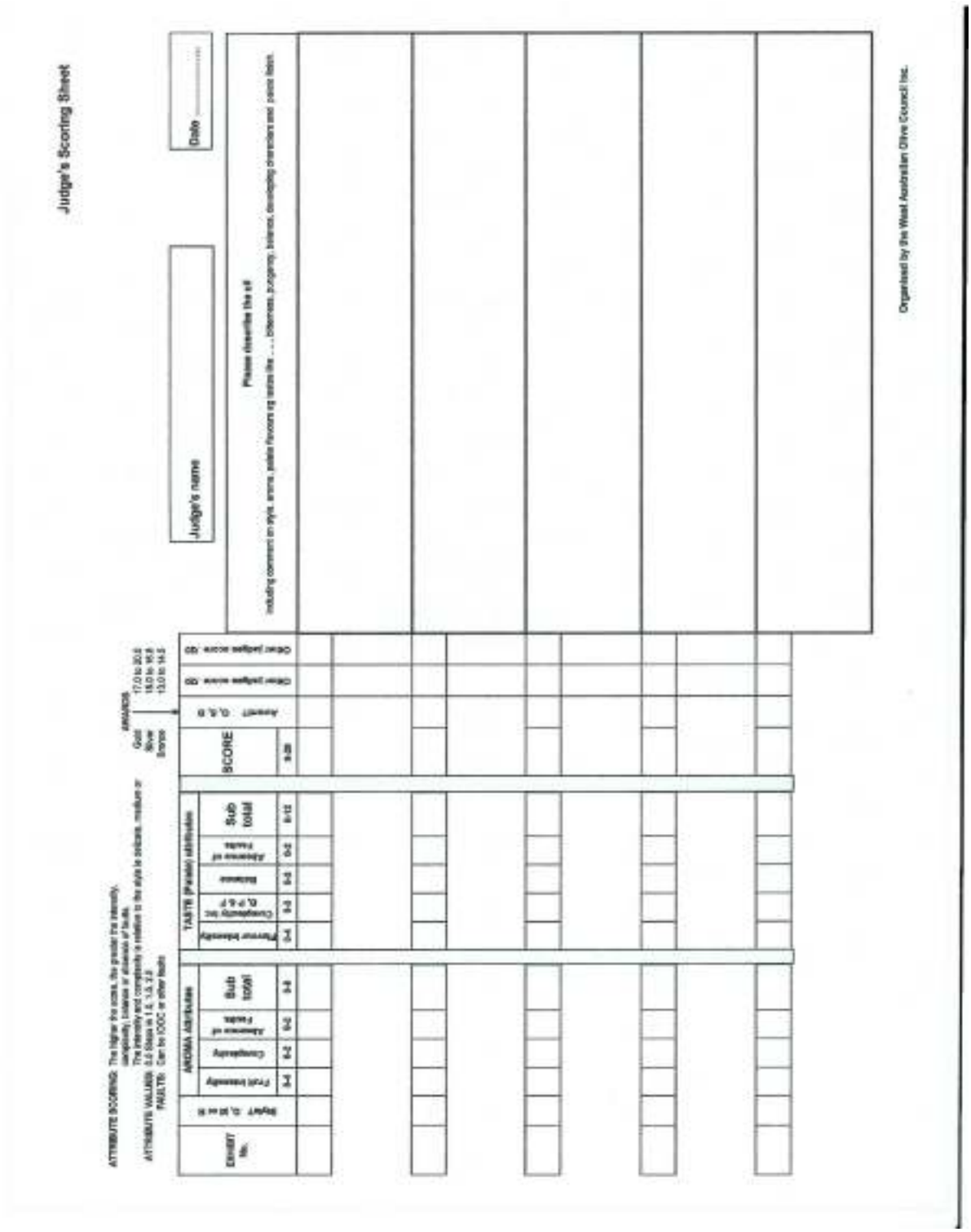
Pungent ——————————➡

**Name of taster**:

**Sample code**: **Date:**

**Comments:**

**Appendix 2: Sensory profile evaluation sheet developed by WAOC**



**Appendix 3: Screening form**

First name: _____ Family name: _____

Age group: <25 yo 25-35 yo 35-45 yo 45-55 yo 55-65 yo

Sex: _____ Ethnic/cultural background: _____

Contact number (office hours): _____

Email address: _____

1. Are you a smoker? Yes/No

2. Do you have any of the following?

   Dentures Yes/No

   Food allergies Yes/No

   Oral or gum diseases Yes/No

3. Are you on any medication? Yes/No

   If yes, please specify: _____

4. Do you have any restrictions on your diet? Yes/No

   If yes, please specify: _____

5. How frequently do you consume Mediterranean food?

   Never Yearly Monthly Weekly Daily

6. How frequently do you consume olive oil?

   Never Yearly Monthly Weekly Daily

7. What is your most frequent way of use of olive oil?

   Bread-dipping Salad dressing Cooking Baking

   Others, please specify: _____

8. Which types of olive oil do you normally consume?

   Extra Virgin Virgin Pure Light Pomace

9. Which types of olive oil do you like the best?

   Extra Virgin Virgin Pure Light Pomace

10. Are you available in June 2009? Yes/No

I, *(name stated as above)*, declared that the information given above is correct and true as on _____ (date).

_____( signature)

Adapted from Meilgaard, M. Civille, G.V. & Carr, B.T. 1991, Sensory Evaluation Techniques, 2nd edn, CRC Press, Inc., Boston.

**Appendix 4: Information sheet**

The study titled '*Yield and quality improvement of Western Australian olive oil by innovative biotechnology*' will be carried out in the Department of Food Science and Technology, School of Public Health in Curtin University of Technology's Bentley campus. The research is being conducted by Miss Hui Jun Chih and is supervised by Associate Professor Vijay Jayasena, Dr Tony James and Associate Professor Satvinder Dhaliwal. The project has received ethics approval from the Curtin University Human Research Ethics Committee. If needed, verification of approval (approval number: SPH-0045-2006) can be obtained by either writing to the Curtin University Human Research Ethics Committee, c/- Office of Research and Development, Curtin University of Technology, GPO Box U1987, Perth, 6845 or by telephoning (08) 9266 2784.

The aim of conducting sensory evaluation session is to understand and define consumers' expectations and acceptability of olive oil. Sensory panelists will be required to rank their preference of the olive oil samples based on the attributes listed on the sensory evaluation sheet. No personal harm or risk will be resulted from this study. The results are expected to provide valuable information to the Western Australian Olive Oil Industry in improving the yield and quality of Western Australian olive oil.

Involvement of the panelists in the sensory evaluation sessions is completely voluntary. Panelists are at liberty to withdraw from the study without prejudice or negative consequences. Panelists reserve all rights to question the researcher should there be any doubts about the process of sensory evaluation. All written personal information acquired from this study will remain confidential within a locked office in the School of Public Health. Access of the data is strictly available only to the researchers. Results of the study will be published as an average value of the group. Identification of the panelists will thus be impossible.

Should the participants require further information regarding this study, the researcher can be contacted on 9266 4465 or via email at huijun.chih@postgrad.curtin.edu.au

**Appendix 5: Consent form**

I have been clearly informed by the researcher of the purpose of this study "*Yield and quality improvement of Western Australian olive oil by innovative biotechnology*". I have read the information sheet and fully understood its content.

I understood that I have to evaluate the olive oil samples based on my acceptability and preference on the sensory evaluation form. To my understanding, all data collected from this study will remain confidential and my personal details will not be identified. I understood that I reserve the rights to attain satisfactory answers from the researchers regarding any doubts about this study in the future. I understood that my involvement with the project is purely voluntary and I can withdraw from the study without prejudice and negative consequences.

I hereby agree to volunteer and participate in this study conducted at School of Public Health, Curtin University of Technology in investigating the sensory attributes of Western Australian olive oil processed by different techniques. I am giving consent to the researcher in reporting my sensory evaluation data as an average result of the whole group.

Name: _____

Signature: _____

Date: _____

**Appendix 6: Quality parameters of commercial extra virgin olive oil (EVOO) samples in comparison with the olive oil extracted by malaxing the olive paste for 30 minutes with no addition of processing aids (*Sample C30*) and the olive oil extracted by malaxing the olive paste for 60 minutes with no addition of processing aids (*Sample C60*)**

| Quality parameters | Mean ± S.D. of quality variables | | |
|---|---|---|---|
| | ***Commercial EVOO samples*** | ***Sample C30*** | ***Sample C60*** |
| Total phenolic compounds (mg/kg oil) | 272.26 ± 97.91 | 113.09 ± 86.02 * | 97.36 ± 71.12 b |
| Antiradical activity (% inhibition of DPPH•) | 71.68 ± 19.31 | 32.49 ± 5.92 * | 29.02 ± 4.80 b |
| Peroxides value (mEq/kg) | 8.51 ± 3.12 | 14.24 ± 9.17 | 18.26 ± 8.10 a |
| Acidity level (% m/m oleic acid) | 0.23 ± 0.13 | 0.24 ± 0.13 * | 0.24 ± 0.14 b |
| C16:0 (%) | 7.25 ± 2.20 | 14.48 ± 3.56 * | 15.43 ± 3.98 b |
| C18:0 (%) | 0.82 ± 0.71 | 1.93 ± 0.97 * | 2.18 ± 1.21 b |
| C18:1 (%) | 74.50 ± 6.03 | 80.40 ± 11.83 | 81.31 ± 11.21 a |
| C18:2 (%) | 7.81 ± 4.07 | 15.30 ± 6.64 * | 17.78 ± 7.08 b |
| K232 (%) | 2.19 ± 0.34 | 1.84 ± 0.34 | 1.77 ± 0.33 b |
| K270 (%) | 0.04 ± 0.04 | 0.12 ± 0.10 * | 0.13 ± 0.09 b |
| ΔK | 0.00 ± 0.00 | 0.00 ± 0.02 * | 0.00 ± 0.03 b |
| L* | 37.77 ± 0.48 | 38.23 ± 0.56 | 38.31 ± 0.59 a |
| a* | -2.18 ± 0.08 | -1.99 ± 0.81 * | -2.01 ± 0.67 b |
| b* | 11.86 ± 0.74 | 8.42 ± 3.57 * | 8.42 ± 3.06 b |

* indicates significant differences ($P<0.05$) on the quality parameter between the oil samples

## Appendix 7: Correlation coefficient between the quality parameters of olive oil samples

| | Yield | Oil recovery | Phenolic compounds | Antiradical activity | Peroxides value | Acidity level | C16:0 | C18:0 | C18:1 | C18:2 | K232 | K270 | ΔK | L* | a* | b* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Yield** | 1.000 | 0.711** | 0.190* | 0.309** | -0.490** | 0.374** | -0.417** | -0.234** | 0.049 | -0.155 | -0.172 | -0.076 | -0.112 | 0.532** | 0.491** | -0.533** |
| **Oil recovery** | 0.711** | 1.000 | 0.416** | 0.493** | -0.626** | -0.103 | -0.535** | -0.383** | -0.036 | -0.413** | -0.118 | -0.303** | -0.134 | 0.507** | 0.046 | -0.134 |
| **Phenolic compounds** | 0.190* | 0.416** | 1.000 | 0.778** | -0.730** | -0.549** | -0.749** | -0.659** | -0.370** | -0.690** | -0.514** | -0.664** | -0.217* | 0.335** | -0.374** | 0.322** |
| **Antiradical activity** | 0.309** | 0.493** | 0.778** | 1.000 | -0.742** | -0.442** | -0.751** | -0.638** | -0.254** | -0.664** | -0.435** | -0.640** | -0.221* | 0.308** | -0.353** | 0.311** |
| **Peroxides Value** | -0.490** | -0.626** | -0.730** | -0.742** | 1.000 | 0.318** | 0.806** | 0.593** | 0.172 | 0.725** | 0.486** | 0.571** | 0.167 | -0.405** | 0.196* | -0.137 |
| **Acidity level** | 0.374** | -0.103 | -0.549** | -0.442** | 0.318** | 1.000 | 0.397** | 0.306** | 0.230* | 0.479** | 0.313** | 0.550** | 0.021 | -0.024 | 0.582** | -0.517** |
| **C16:0** | -0.417** | -0.535** | -0.749** | -0.751** | 0.806** | 0.397** | 1.000 | 0.695** | 0.537** | 0.823** | 0.401** | 0.539** | .0.178* | -0.398** | 0.197* | -0.165 |
| **C18:0** | -0.234** | -0.383** | -0.659** | -0.638** | 0.593** | 0.306** | 0.695** | 1.000 | 0.490** | 0.645** | 0.261** | 0.368** | 0.134 | -0.357** | 0.308** | -0.343** |
| **C18:1** | 0.049 | -0.036 | -0.370** | -0.254** | 0.172 | 0.230* | 0.537** | 0.490** | 1.000 | 0.385** | -0.028 | 0.049 | 0.172 | -0.284** | 0.079 | -0.106 |
| **C18:2** | -0.155 | -0.413** | -0.690** | -0.664** | 0.725** | .0.479** | 0.823** | 0.645** | 0.385** | 1.000 | 0.328** | 0.622** | 0.096 | -0.142 | 0.489** | -0.439** |
| **K232** | -0.172 | -0.118 | -0.514** | -0.435** | 0.486** | 0.313** | 0.401** | 0.261** | -0.028 | 0.328** | 1.000 | 0.444** | -0.016 | -0.141 | 0.106 | -0.066 |
| **K270** | -0.076 | -0.303** | -0.664** | -0.640** | 0.571** | 0.550** | 0.539** | 0.368** | 0.049 | 0.622** | 0.444** | 1.000 | 0.200* | -0.117 | 0.431** | -0.362** |
| **ΔK** | -0.112 | -0.134 | -0.217* | -0.221* | 0.167 | 0.021 | 0.178* | 0.134 | 0.172 | 0.096 | -0.016 | 0.200* | 1.000 | -0.101 | -0.017 | -0.005 |
| **L*** | 0.532** | 0.507** | 0.335** | 0.308** | -0.405** | -0.024 | -0.398** | -0.357** | -0.284** | -0.142 | -0.141 | -0.117 | -0.101 | 1.000 | 0.421** | -0.455** |
| **a*** | 0.491** | 0.046 | -0.374** | -0.353** | 0.196* | 0.582** | 0.197* | 0.308** | 0.079 | 0.489** | 0.106 | 0.431** | -0.017 | 0.421** | 1.000 | -0.967** |
| **b*** | -0.533** | -0.134 | 0.322** | 0.311** | -0.137 | -0.517** | -0.165 | -0.343** | -0.106 | -0.439** | -0.066 | -0.362** | -0.005 | -0.455** | -0.967** | 1.000 |

** Correlation is significant at the 0.01 level (2-tailed)

**Appendix 8: Quality variables of oil extracted from malaxing the olives harvested from each growing site for an extended period compared with the control sample**

| Quality parameters | # Samples | Mean ± S.D. of quality variables | | |
|---|---|---|---|---|
| | | Gingin | Swan Valley | Margaret River |
| Yield of oil extraction (%) | Sample C30 | 7.05 ± 3.27 $^{a,\alpha}$ | 9.56 ± 4.71 $^{a,\beta}$ | 16.84 ± 2.38 $^{a,\gamma}$ |
| | Sample C60 | 7.97 ± 2.97 $^{a,\alpha}$ | 10.57 ± 4.66 $^{a,\beta}$ | 16.95 ± 0.04 $^{a,\gamma}$ |
| Oil recovery (%) | Sample C30 | 50.59 ± 14.16 $^{a,\alpha}$ | 46.83 ± 13.98 $^{a,\alpha}$ | 63.61 ± 7.90 $^{a,\beta}$ |
| | Sample C60 | 60.15 ± 5.81 $^{b,\alpha}$ | 51.25 ± 14.19 $^{a,\beta}$ | 67.22 ± 0.11 $^{b,\gamma}$ |
| Total phenolic compounds (mg/kg oil) | Sample C30 | 125.92 ± 92.45 $^{a,\alpha}$ | 88.74 ± 50.59 $^{a,\beta}$ | 118.52 ± 102.07 $^{a,\alpha}$ |
| | Sample C60 | 117.66 ± 92.74 $^{b,\alpha}$ | 77.59 ± 45.33 $^{a,\beta}$ | 85.11 ± 26.97 $^{a,\alpha\beta}$ |
| Antiradical activity (% inhibition DPPH•) | Sample C30 | 31.99 ± 5.37 $^{a,\alpha}$ | 29.91 ± 6.90 $^{a,\beta}$ | 34.93 ± 5.12 $^{a,\gamma}$ |
| | Sample C60 | 29.74 ± 5.02 $^{a,\alpha}$ | 28.62 ± 4.97 $^{a,\alpha}$ | 27.38 ± 5.46 $^{a,\alpha}$ |
| Peroxides value (mEq/kg) | Sample C30 | 16.62 ± 9.05 $^{a,\alpha}$ | 20.06 ± 8.02 $^{a,\beta}$ | 7.02 ± 4.91 $^{a,\beta}$ |
| | Sample C60 | 18.51 ± 8.87 $^{b,\alpha}$ | 18.95 ± 8.41 $^{a,\beta}$ | 14.31 ± 4.44 $^{a,\gamma}$ |
| Acidity level (% m/m oleic acid) | Sample C30 | 0.15 ± 0.06 $^{a,\alpha}$ | 0.31 ± 0.11 $^{a,\beta}$ | 0.25 ± 0.14 $^{a,\beta}$ |
| | Sample C60 | 0.15 ± 0.07 $^{a,\alpha}$ | 0.27 ± 0.07 $^{a,\beta}$ | 0.54 ± 0.19 $^{b,\gamma}$ |
| C16:0 (%) | Sample C30 | 14.40 ± 3.77 $^{a,\alpha}$ | 16.47 ± 3.69 $^{a,\beta}$ | 12.90 ± 2.49 $^{a,\alpha}$ |
| | Sample C60 | 14.80 ± 3.20 $^{a,\alpha}$ | 16.47 ± 5.05 $^{a,\alpha}$ | 14.12 ± 2.90 $^{a,\alpha}$ |
| C18:0 (%) | Sample C30 | 2.20 ± 1.25 $^{a,\alpha}$ | 1.72 ± 0.78 $^{a,\beta\gamma}$ | 1.85 ± 0.79 $^{a,\alpha\gamma}$ |
| | Sample C60 | 2.26 ± 1.20 $^{a,\alpha}$ | 1.74 ± 1.05 $^{a,\alpha}$ | 3.54 ± 1.30 $^{b,\beta}$ |
| C18:1 (%) | Sample C30 | 78.02 ±12.68 $^{a,\alpha}$ | 75.04 ± 11.83 $^{a,\alpha}$ | 87.25 ± 7.81 $^{a,\beta}$ |
| | Sample C60 | 82.39 ± 9.00 $^{a,\alpha}$ | 78.17 ± 13.03 $^{a,\alpha}$ | 89.03 ± 14.74 $^{a,\alpha}$ |
| C18:2 (%) | Sample C30 | 13.85 ± 4.20 $^{a,\alpha}$ | 20.26 ± 8.37 $^{a,\beta}$ | 12.61 ± 4.95 $^{a,\alpha}$ |
| | Sample C60 | 14.41 ± 4.45 $^{a,\alpha}$ | 22.15 ± 8.20 $^{a,\beta}$ | 15.49 ± 2.96 $^{a,\alpha}$ |
| K270 (%) | Sample C30 | 0.09 ± 0.08 $^{a,\alpha}$ | 0.20 ± 0.12 $^{a,\beta}$ | 0.08 ± 0.08 $^{a,\alpha}$ |
| | Sample C60 | 0.09 ± 0.08 $^{a,\alpha}$ | 0.20 ± 0.08 $^{a,\beta}$ | 0.09 ± 0.01 $^{a,\alpha\beta}$ |
| L* | Sample C30 | 38.04 ±0.58 $^{a,\alpha}$ | 38.37 ± 0.72 $^{a,\alpha}$ | 38.30 ± 0.36 $^{a,\alpha}$ |
| | Sample C60 | 38.24 ± 0.70 $^{a,\alpha}$ | 38.39 ±0.56 $^{a,\alpha}$ | 38.32 ± 0.15 $^{a,\beta}$ |
| a* | Sample C30 | -2.36 ± 0.56 $^{a,\alpha}$ | -1.70 ± 0.69 $^{a,\beta}$ | -1.86 ± 1.01 $^{a,\beta}$ |
| | Sample C60 | -2.39 ± 0.60 $^{a,\alpha}$ | -1.71 ± 0.60 $^{a,\beta}$ | -1.46 ± 0.39 $^{a,\gamma}$ |
| b* | Sample C30 | 9.88 ±2.78 $^{a,\alpha}$ | 7.80 ± 3.84 $^{a,\alpha\beta}$ | 7.48 ± 3.84 $^{a,\beta}$ |
| | Sample C60 | 9.62 ±2.71 $^{a,\alpha}$ | 7.66 ± 3.33 $^{a,\beta}$ | 6.04 ± 1.56 $^{a,\gamma}$ |

*# Descriptions of the sample are available in the abbreviation list*

*Different letter down the column within a particular quality parameter indicates significant differences (P<0.05) between the quality parameter of oil samples with the control sample at the particular growing site*

*Different Greek letter across the row indicates significant differences (P<0.05) between the growing sites*

## Appendix 9: Quality variables of oil samples extracted from olives harvested from each growing site using citric acid compared with the control sample

| Quality parameters | [#] Samples | Mean ± S.D. of quality variables | | |
|---|---|---|---|---|
| | | Gingin | Swan Valley | Margaret River |
| **Yield of oil extraction (%)** | **Sample C30** | **7.05 ± 3.27** $^{a,\alpha}$ | **9.56 ± 4.71** $^{a,\beta}$ | **16.84 ± 2.38** $^{a,\gamma}$ |
| | **Sample A30** | 8.07 ±2.99 $^{a,\alpha}$ | 15.04 ± 1.53 $^{b,\beta}$ | 17.56 ± 4.14 $^{a,\gamma}$ |
| | **Sample A30[H]** | 12.51 ± 0.14 $^{a,\alpha}$ | 16.10 ± 1.23 $^{b,\beta}$ | |
| **Oil recovery (%)** | **Sample C30** | **50.59 ± 14.16** $^{a,\alpha}$ | **46.83 ± 13.98** $^{a,\alpha}$ | **63.61 ± 7.90** $^{a,\beta}$ |
| | **Sample A30** | 60.89 ± 5.04 $^{b,\alpha}$ | 62.28 ± 6.93 $^{b,\alpha}$ | 65.18 ± 7.90 $^{a,\beta}$ |
| | **Sample A30[H]** | 68.42 ± 0.58 $^{b,\alpha}$ | 69.79 ± 3.47 $^{b,\alpha}$ | |
| **Total phenolic compounds (mg/kg oil)** | **Sample C30** | **125.92 ± 92.45** $^{a,\alpha}$ | **88.74 ± 50.59** $^{a,\beta}$ | **118.52 ± 102.07** $^{a,\alpha}$ |
| | **Sample A30** | 118.06 ± 113.71 $^{a,\alpha}$ | 100.05 ± 69.57 $^{a,\beta}$ | 37.01 ± 2.51 $^{a,\alpha\beta}$ |
| | **Sample A30[H]** | 330.04 ± 11.68 $^{b,\alpha}$ | 202.60 ± 6.95 $^{b,\beta}$ | |
| **Antiradical activity (% inhibition DPPH•)** | **Sample C30** | **31.99 ± 5.37** $^{a,\alpha}$ | **29.91 ± 6.90** $^{ab,\beta}$ | **34.93 ± 5.12** $^{a,\gamma}$ |
| | **Sample A30** | 29.41 ± 7.27 $^{a,\alpha}$ | 25.43 ± 11.63 $^{a,\beta}$ | 20.68 ± 4.33 $^{b,\alpha\beta}$ |
| | **Sample A30[H]** | 54.98 ± 7.09 $^{b,\alpha}$ | 40.23 ± 0.65 $^{b,\beta}$ | |
| **Peroxides value (mEq/kg)** | **Sample C30** | **16.62 ± 9.05** $^{a,\alpha}$ | **20.06 ± 8.02** $^{a,\beta}$ | **7.02 ± 4.91** $^{a,\beta}$ |
| | **Sample A30** | 19.04 ± 8.55 $^{b,\alpha}$ | 16.34 ± 9.55 $^{a,\beta}$ | 19.79 ± 7.97 $^{b,\beta}$ |
| | **Sample A30[H]** | 5.97 ± 0.41 $^{ab,\alpha}$ | 7.86 ± 2.85 $^{a,\beta}$ | |
| **Acidity level (% m/m oleic acid)** | **Sample C30** | **0.15 ± 0.06** $^{a,\alpha}$ | **0.31 ± 0.11** $^{a,\beta}$ | **0.25 ± 0.14** $^{a,\beta}$ |
| | **Sample A30** | 0.18 ± 0.08 $^{a,\alpha}$ | 0.50 ± 0.35 $^{b,\beta}$ | 0.82 ± 0.14 $^{c,\beta}$ |
| | **Sample A30[H]** | 0.14 ± 0.01 $^{a,\alpha}$ | 0.19 ± 0.02 $^{ab,\beta}$ | |
| **C16:0 (%)** | **Sample C30** | **14.40 ± 3.77** $^{a,\alpha}$ | **16.47 ± 3.69** $^{a,\beta}$ | **12.90 ± 2.49** $^{a,\alpha}$ |
| | **Sample A30** | 17.42 ± 5.80 $^{b,\alpha}$ | 16.80 ± 5.23 $^{a,\beta}$ | 17.00 ± 0.52 $^{b,\alpha}$ |
| | **Sample A30[H]** | 11.39 ± 0.86 $^{ab,\alpha}$ | 12.58 ± 1.59 $^{a,\alpha}$ | |
| **C18:0 (%)** | **Sample C30** | **2.20 ± 1.25** $^{a,\alpha}$ | **1.72 ± 0.78** $^{a,\beta\gamma}$ | **1.85 ± 0.79** $^{a,\alpha\gamma}$ |
| | **Sample A30** | 2.41 ± 1.56 $^{a,\alpha}$ | 1.89 ± 1.20 $^{a,\alpha}$ | 2.92 ± 0.65 $^{a,\alpha}$ |
| | **Sample A30[H]** | 0.86 ± 0.11 $^{a,\alpha}$ | 0.72 ± 0.23 $^{a,\alpha}$ | |
| **C18:1 (%)** | **Sample C30** | **78.02 ± 12.68** $^{a,\alpha}$ | **75.04 ± 11.83** $^{a,\alpha}$ | **87.25 ± 7.81** $^{a,\beta}$ |
| | **Sample A30** | 88.17 ± 21.04 $^{b,\alpha}$ | 79.80 ± 6.80 $^{a,\alpha}$ | 93.40 ± 5.29 $^{a,\alpha}$ |
| | **Sample A30[H]** | 75.43 ± 4.27 $^{ab,\alpha}$ | 77.67 ± 3.04 $^{a,\alpha}$ | |
| **C18:2 (%)** | **Sample C30** | **13.85 ± 4.20** $^{a,\alpha}$ | **20.26 ± 8.37** $^{a,\beta}$ | **12.61 ± 4.95** $^{a,\alpha}$ |
| | **Sample A30** | 15.78 ± 4.55 $^{b,\alpha}$ | 21.69 ± 8.11 $^{a,\beta}$ | 19.18 ± 2.88 $^{a,\alpha}$ |
| | **Sample A30[H]** | 10.14 ± 0.78 $^{ab,\alpha}$ | 14.44 ± 1.72 $^{a,\beta}$ | |
| **K270 (%)** | **Sample C30** | **0.09 ± 0.08** $^{a,\alpha}$ | **0.20 ± 0.12** $^{a,\beta}$ | **0.08 ± 0.08** $^{a,\alpha}$ |
| | **Sample A30** | 0.11 ± 0.07 $^{a,\alpha}$ | 0.20 ± 0.19 $^{a,\beta}$ | 0.24 ± 0.02 $^{b,\alpha\beta}$ |
| | **Sample A30[H]** | 0.01 ± 0.03 $^{a,\alpha}$ | 0.09 ± 0.17 $^{a,\alpha}$ | |
| **L*** | **Sample C30** | **38.04 ±0.58** $^{a,\alpha}$ | **38.37 ± 0.72** $^{a,\alpha}$ | **38.30 ± 0.36** $^{a,\alpha}$ |
| | **Sample A30** | 38.30 ± 0.39 $^{a,\alpha}$ | 38.95 ± 0.44 $^{a,\beta}$ | 38.44 ± 0.30 $^{a,\alpha\beta}$ |
| | **Sample A30[H]** | 38.84 ± 0.37 $^{a,\alpha}$ | 39.22 ± 0.30 $^{a,\alpha}$ | |
| **a*** | **Sample C30** | **-2.36 ± 0.56** $^{a,\alpha}$ | **-1.70 ± 0.69** $^{a,\beta}$ | **-1.86 ± 1.01** $^{a,\beta}$ |
| | **Sample A30** | -2.45 ± 0.34 $^{a,\alpha}$ | -1.32 ± 0.55 $^{a,\beta}$ | -0.88 ± 0.19 $^{a,\beta}$ |
| | **Sample A30[H]** | -2.57 ± 0.13 $^{a,\alpha}$ | -1.94 ± 0.13 $^{a,\beta}$ | |
| **b*** | **Sample C30** | **9.88 ±2.78** $^{a,\alpha}$ | **7.80 ± 3.84** $^{a,\alpha\beta}$ | **7.48 ± 3.84** $^{a,\beta}$ |
| | **Sample A30** | 9.44 ± 1.63 $^{a,\alpha}$ | 5.29 ± 2.11 $^{a,\beta}$ | 3.73 ± 0.48 $^{a,\beta}$ |
| | **Sample A30[H]** | 10.44 ± 0.87 $^{a,\alpha}$ | 7.78 ± 0.28 $^{a,\beta}$ | |

[#] *Descriptions of the sample are available in the abbreviation list; Different letter down the column within a particular quality parameter indicates significant differences (P<0.05) between the quality parameter of oil samples with the control sample at the particular growing site; Different Greek letter across the row indicates significant differences (P<0.05) between the growing sites*

**Appendix 10: Quality parameters of commercial extra virgin olive oil (EVOO) samples in comparison with the olive oil extracted by addition of processing aids**

| Quality parameters of olive oil | [#] Samples (Mean ± S.D.) | | | | | |
|---|---|---|---|---|---|---|
| | ***Commercial EVOO samples*** | ***Sample A30*** | ***Sample V30*** | ***Sample P30*** | ***Sample A30[H]*** | ***Sample V30[H]*** |
| Total phenolic compounds (mg/kg oil) | 272.26 ± 97.91 [a] | 96.44 ± 91.62 [b] | 105.65 ± 95.71 [b] | 59.38 ± 17.88 [b] | 266.32 ± 70.33 [a] | 235.42 ± 73.59 [a] |
| Antiradical activity (% inhibition of DPPH•) | 71.68 ± 19.31 [a] | 26.47 ± 8.69 [b] | 30.28 ± 8.94 [b] | 27.56 ± 8.26 [b] | 47.61 ± 9.25 [ab] | 40.54 ± 5.61 [b] |
| Peroxides value (mEq/kg) | 8.51 ± 3.12 [a] | 18.38 ± 8.40 [b] | 17.65 ± 8.15 [a] | 19.98 ± 5.83 [b] | 6.92 ± 2.09 [a] | 6.61 ± 1.64 [a] |
| Acidity level (% m/m oleic acid) | 0.23 ± 0.13 [a] | 0.40 ± 0.32 [b] | 0.41 ± 0.34 [b] | 0.42 ± 0.26 [b] | 0.16 ± 0.03 [a] | 0.18 ± 0.05 [a] |
| C16:0 (%) | 7.25 ± 2.20 [a] | 17.15 ± 4.82 [b] | 16.48 ± 4.46 [b] | 17.00 ± 2.08 [b] | 11.98 ± 1.31 [b] | 11.11 ± 2.20 [b] |
| C18:0 (%) | 0.82 ± 0.71 [a] | 2.36 ± 1.32 [b] | 2.19 ± 1.04 [b] | 2.97 ± 1.13 [b] | 0.79 ± 0.18 [a] | 0.74 ± 0.30 [a] |
| C18:1 (%) | 74.50 ± 6.03 [a] | 86.70 ± 15.87 [a] | 83.73 ± 10.07 [a] | 84.85 ± 7.69 [b] | 76.55 ± 3.53 [a] | 72.06 ± 12.79 [a] |
| C18:2 (%) | 7.81 ± 4.07 [a] | 18.23 ± 5.96 [b] | 17.76 ± 5.88 [b] | 18.01 ± 5.10 [b] | 12.29 ± 2.64 [a] | 11.08 ± 2.38 [a] |
| K232 (%) | 2.19 ± 0.34 [ab] | 2.14 ± 0.24 [a] | 2.11 ± 0.22 [ab] | 1.93 ± 0.33 [b] | 1.82 ± 0.09 [ab] | 1.80 ± 0.08 [b] |
| K270 (%) | 0.04 ± 0.04 [a] | 0.16 ± 0.12 [a] | 0.14 ± 0.11 [a] | 0.14 ± 0.07 [b] | 0.04 ± 0.12 [ab] | 0.03 ± 0.13 [a] |
| ΔK | 0.00 ± 0.00 [a] | 0.01 ± 0.02 [b] | 0.00 ± 0.02 [b] | 0.00 ± 0.03 [b] | -0.01 ± 0.01 [a] | -0.01 ± 0.02 [a] |
| L* | 37.77 ± 0.48 [a] | 38.52 ± 0.47 [b] | 38.50 ± 0.37 [b] | 38.25 ± 0.47 [a] | 39.03 ± 0.36 [b] | 38.94 ± 0.44 [b] |
| a* | -2.18 ± 0.08 [a] | -1.80 ± 0.78 [b] | -1.80 ± 0.80 [b] | -1.67 ± 0.78 [b] | -2.25 ± 0.36 [a] | -2.25 ± 0.46 [a] |
| b* | 11.86 ± 0.74 [a] | 7.05 ± 2.96 [b] | 7.03 ± 2.94 [b] | 7.09 ± 3.67 [b] | 9.11 ± 1.57 [a] | 9.08 ± 2.06 [a] |

[#] Descriptions of each sample are available in the abbreviation list

Different letters across the row indicate significant differences ($P<0.05$) of the quality parameter amongst the oil samples

## Appendix 11: Quality variables of oil samples extracted from olives harvested from each growing site using Viscozymes compared with the control sample

| Quality parameters | # Samples | Mean ± S.D. of quality variables | | |
|---|---|---|---|---|
| | | Gingin | Swan Valley | Margaret River |
| **Yield of oil extraction (%)** | **Sample C30** | **7.05 ± 3.27** $^{a,\alpha}$ | **9.56 ± 4.71** $^{a,\beta}$ | **16.84 ± 2.38** $^{a,\gamma}$ |
| | **Sample V30** | 8.26 ± 3.15 $^{ab,\alpha}$ | 15.06 ± 0.93 $^{b,\beta}$ | 18.73 ± 3.38 $^{a,\gamma}$ |
| | **Sample V30[H]** | 12.98 ± 1.17 $^{b,\alpha}$ | 16.90 ± 1.26 $^{b,\beta}$ | |
| **Oil recovery (%)** | **Sample C30** | **50.59 ± 14.16** $^{a,\alpha}$ | **46.83 ± 13.98** $^{a,\alpha}$ | **63.61 ± 7.90** $^{a,\beta}$ |
| | **Sample V30** | 62.13 ± 7.16 $^{b,\alpha}$ | 65.50 ± 4.78 $^{b,\alpha\beta}$ | 69.71 ± 8.48 $^{b,\beta}$ |
| | **Sample V30[H]** | 73.23 ± 2.64 $^{b,\alpha}$ | 73.40 ± 3.60 $^{b,\alpha}$ | |
| **Total phenolic compounds (mg/kg oil)** | **Sample C30** | **125.92 ± 92.45** $^{a,\alpha}$ | **88.74 ± 50.59** $^{a,\beta}$ | **118.52 ± 102.07** $^{a,\alpha}$ |
| | **Sample V30** | 130.23 ± 118.98 $^{a,\alpha}$ | 105.16 ± 81.31 $^{a,\beta}$ | 51.07 ± 20.23 $^{a,\alpha\beta}$ |
| | **Sample V30[H]** | 300.94 ± 8.34 $^{a,\alpha}$ | 169.90 ± 24.28 $^{a,\beta}$ | |
| **Antiradical activity (% inhibition DPPH•)** | **Sample C30** | **31.99 ± 5.37** $^{a,\alpha}$ | **29.91 ± 6.90** $^{a,\beta}$ | **34.93 ± 5.12** $^{a,\gamma}$ |
| | **Sample V30** | 32.97 ± 7.57 $^{a,\alpha}$ | 26.76 ± 11.34 $^{a,\beta}$ | 29.51 ± 8.05 $^{a,\alpha}$ |
| | **Sample V30[H]** | 42.78 ± 6.55 $^{a,\alpha}$ | 38.30 ± 4.53 $^{a,\alpha}$ | |
| **Peroxides value (mEq/kg)** | **Sample C30** | **16.62 ± 9.05** $^{a,\alpha}$ | **20.06 ± 8.02** $^{a,\beta}$ | **7.02 ± 4.91** $^{a,\beta}$ |
| | **Sample V30** | 18.15 ± 9.00 $^{b,\alpha}$ | 16.22 ± 8.55 $^{a,\beta}$ | 18.68 ± 7.37 $^{b,\beta}$ |
| | **Sample V30[H]** | 5.83 ± 0.65 $^{ab,\alpha}$ | 7.39 ± 2.11 $^{a,\beta}$ | |
| **Acidity level (% m/m oleic acid)** | **Sample C30** | **0.15 ± 0.06** $^{a,\alpha}$ | **0.31 ± 0.11** $^{a,\beta}$ | **0.25 ± 0.14** $^{a,\beta}$ |
| | **Sample V30** | 0.16 ± 0.06 $^{a,\alpha}$ | 0.54 ± 0.38 $^{b,\beta}$ | 0.80 ± 0.19 $^{b,\beta}$ |
| | **Sample V30[H]** | 0.13 ± 0.02 $^{a,\alpha}$ | 0.22 ± 0.02 $^{ab,\beta}$ | |
| **C16:0 (%)** | **Sample C30** | **14.40 ± 3.77** $^{a,\alpha}$ | **16.47 ± 3.69** $^{a,\beta}$ | **12.90 ± 2.49** $^{a,\alpha}$ |
| | **Sample V30** | 17.03 ± 5.22 $^{b,\alpha}$ | 16.55 ± 4.86 $^{a,\alpha}$ | 15.11 ± 1.91 $^{a,\beta}$ |
| | **Sample V30[H]** | 11.41 ± 1.33 $^{ab,\alpha}$ | 10.81 ± 3.18 $^{a,\alpha}$ | |
| **C18:0 (%)** | **Sample C30** | **2.20 ± 1.25** $^{a,\alpha}$ | **1.72 ± 0.78** $^{a,\beta\gamma}$ | **1.85 ± 0.79** $^{a,\alpha\gamma}$ |
| | **Sample V30** | 2.20 ± 1.07 $^{a,\alpha}$ | 1.80 ± 1.18 $^{a,\alpha}$ | 2.76 ± 0.58 $^{a,\alpha}$ |
| | **Sample V30[H]** | 0.79 ± 0.12 $^{a,\alpha}$ | 0.70 ± 0.45 $^{a,\alpha}$ | |
| **C18:1 (%)** | **Sample C30** | **78.02 ± 12.68** $^{a,\alpha}$ | **75.04 ± 11.83** $^{a,\alpha}$ | **87.25 ± 7.81** $^{a,\beta}$ |
| | **Sample V30** | 86.39 ± 12.16 $^{b,\alpha}$ | 77.98 ± 5.13 $^{a,\alpha}$ | 86.39 ± 8.56 $^{a,\alpha}$ |
| | **Sample V30[H]** | 78.35 ± 6.08 $^{ab,\alpha}$ | 65.77 ± 15.92 $^{a,\alpha}$ | |
| **C18:2 (%)** | **Sample C30** | **13.85 ± 4.20** $^{a,\alpha}$ | **20.26 ± 8.37** $^{a,\beta}$ | **12.61 ± 4.95** $^{a,\alpha}$ |
| | **Sample V30** | 15.36 ± 4.32 $^{b,\alpha}$ | 21.26 ± 7.71 $^{a,\beta}$ | 17.91 ± 4.05 $^{a,\alpha}$ |
| | **Sample V30[H]** | 9.84 ± 0.85 $^{ab,\alpha}$ | 12.32 ± 2.97 $^{a,\alpha}$ | |
| **K270 (%)** | **Sample C30** | **0.09 ± 0.08** $^{a,\alpha}$ | **0.20 ± 0.12** $^{a,\beta}$ | **0.08 ± 0.08** $^{a,\alpha}$ |
| | **Sample V30** | 0.09 ± 0.07 $^{a,\alpha}$ | 0.20 ± 0.15 $^{a,\beta}$ | 0.17 ± 0.09 $^{a,\alpha\beta}$ |
| | **Sample V30[H]** | 0.01 ± 0.02 $^{a,\alpha}$ | 0.07 ± 0.19 $^{a,\alpha}$ | |
| **L*** | **Sample C30** | **38.04 ±0.58** $^{a,\alpha}$ | **38.37 ± 0.72** $^{a,\alpha}$ | **38.30 ± 0.36** $^{a,\alpha}$ |
| | **Sample V30** | 38.33 ±0.37 $^{a,\alpha}$ | 38.73 ±0.36 $^{a,\alpha}$ | 38.54 ± 0.12 $^{a,\alpha}$ |
| | **Sample V30[H]** | 38.64 ± 0.11 $^{a,\alpha}$ | 39.25 ± 0.44 $^{a,\alpha}$ | |
| **a*** | **Sample C30** | **-2.36 ± 0.56** $^{a,\alpha}$ | **-1.70 ± 0.69** $^{a,\beta}$ | **-1.86 ± 1.01** $^{a,\beta}$ |
| | **Sample V30** | -2.51 ± 0.34 $^{a,\alpha}$ | -1.23 ± 0.50 $^{a,\beta}$ | -1.03 ± 0.41 $^{a,\beta}$ |
| | **Sample V30[H]** | -2.62 ± 0.05 $^{a,\alpha}$ | -1.87 ± 0.31 $^{a,\beta}$ | |
| **b*** | **Sample C30** | **9.88 ±2.78** $^{a,\alpha}$ | **7.80 ± 3.84** $^{a,\alpha\beta}$ | **7.48 ± 3.84** $^{a,\beta}$ |
| | **Sample V30** | 9.63 ± 1.48 $^{a,\alpha}$ | 4.95 ± 1.80 $^{a,\beta}$ | 4.31 ± 1.37 $^{a,\beta}$ |
| | **Sample V30[H]** | 10.76 ± 0.36 $^{a,\alpha}$ | 7.40 ± 1.41 $^{a,\beta}$ | |

*# Descriptions of the sample are available in the abbreviation list; Different letter down the column within a particular quality parameter indicates significant differences (P<0.05) between the quality parameter of oil samples with the control sample at the particular growing site; Different Greek letter across the row indicates significant differences (P<0.05) between the growing sites*

## Appendix 12: Quality variables of oil sample extracted from olives harvested from each growing site using Pectolyase compared with the control sample

| Quality parameters | [#] Samples | Mean ± S.D. of quality variables | | |
|---|---|---|---|---|
| | | Gingin | Swan Valley | Margaret River |
| Yield of oil extraction (%) | Sample C30 | 7.05 ± 3.27 $^{a,\alpha}$ | 9.56 ± 4.71 $^{a,\beta}$ | 16.84 ± 2.38 $^{a,\gamma}$ |
| | Sample P30 | 6.27 ± 0.58 $^{a,\alpha}$ | 9.12 ± 4.95 $^{a,\alpha}$ | 16.44 ± 3.41 $^{a,\beta}$ |
| Oil recovery (%) | Sample C30 | 50.59 ± 14.16 $^{a,\alpha}$ | 46.83 ± 13.98 $^{a,\alpha}$ | 63.61 ± 7.90 $^{a,\beta}$ |
| | Sample P30 | 54.12 ± 8.51 $^{b,\alpha}$ | 45.97 ± 11.07 $^{a,\beta}$ | 60.95 ± 7.39 $^{a,\alpha}$ |
| Total phenolic compounds (mg/kg oil) | Sample C30 | 125.92 ± 92.45 $^{a,\alpha}$ | 88.74 ± 50.59 $^{a,\beta}$ | 118.52 ± 102.07 $^{a,\alpha}$ |
| | Sample P30 | 60.66 ± 11.38 $^{a,\alpha}$ | 54.30 ± 18.72 $^{a,\alpha}$ | 61.53 ± 24.47 $^{a,\alpha}$ |
| Antiradical activity (% inhibition DPPH•) | Sample C30 | 31.99 ± 5.37 $^{a,\alpha}$ | 29.91 ± 6.90 $^{a,\beta}$ | 34.93 ± 5.12 $^{a,\gamma}$ |
| | Sample P30 | 29.74 ± 3.81 $^{a,\alpha}$ | 23.06 ± 4.48 $^{a,\alpha}$ | 28.29 ± 12.73 $^{a,\alpha}$ |
| Peroxides value (mEq/kg) | Sample C30 | 16.62 ± 9.05 $^{a,\alpha}$ | 20.06 ± 8.02 $^{a,\beta}$ | 7.02 ± 4.91 $^{a,\beta}$ |
| | Sample P30 | 22.38 ± 1.18 $^{a,\alpha}$ | 24.49 ± 1.28 $^{a,\beta}$ | 14.02 ± 6.26 $^{b,\gamma}$ |
| Acidity level (% m/m oleic acid) | Sample C30 | 0.15 ± 0.06 $^{a,\alpha}$ | 0.31 ± 0.11 $^{a,\beta}$ | 0.25 ± 0.14 $^{a,\beta}$ |
| | Sample P30 | 0.19 ± 0.08 $^{a,\alpha}$ | 0.40 ± 0.05 $^{a,\beta}$ | 0.70 ± 0.19 $^{b,\gamma}$ |
| C16:0 (%) | Sample C30 | 14.40 ± 3.77 $^{a,\alpha}$ | 16.47 ± 3.69 $^{a,\beta}$ | 12.90 ± 2.49 $^{a,\alpha}$ |
| | Sample P30 | 16.99 ± 1.39 $^{a,\alpha\beta}$ | 19.09 ± 1.65 $^{a,\alpha}$ | 15.52 ± 1.86 $^{a,\beta}$ |
| C18:0 (%) | Sample C30 | 2.20 ± 1.25 $^{a,\alpha}$ | 1.72 ± 0.78 $^{a,\beta\gamma}$ | 1.85 ± 0.79 $^{a,\alpha\gamma}$ |
| | Sample P30 | 3.75 ± 1.21 $^{b,\alpha}$ | 2.38 ± 0.70 $^{a,\beta}$ | 2.50 ± 0.80 $^{a,\beta}$ |
| C18:1 (%) | Sample C30 | 78.02 ±12.68 $^{a,\alpha}$ | 75.04 ± 11.83 $^{a,\alpha}$ | 87.25 ± 7.81 $^{a,\beta}$ |
| | Sample P30 | 83.45 ± 5.51 $^{a,\alpha\beta}$ | 80.24 ± 3.07 $^{a,\beta}$ | 89.74 ± 9.86 $^{a,\alpha}$ |
| C18:2 (%) | Sample C30 | 13.85 ± 4.20 $^{a,\alpha}$ | 20.26 ± 8.37 $^{a,\beta}$ | 12.61 ± 4.95 $^{a,\alpha}$ |
| | Sample P30 | 15.94 ± 2.32 $^{a,\alpha}$ | 24.27 ± 4.95 $^{a,\beta}$ | 15.91 ± 4.04 $^{b,\alpha}$ |
| K270 (%) | Sample C30 | 0.09 ± 0.08 $^{a,\alpha}$ | 0.20 ± 0.12 $^{a,\beta}$ | 0.08 ± 0.08 $^{a,\alpha}$ |
| | Sample P30 | 0.11 ± 0.04 $^{a,\alpha}$ | 0.22 ± 0.05 $^{a,\beta}$ | 0.11± 0.07 $^{a,\alpha}$ |
| L* | Sample C30 | 38.04 ±0.58 $^{a,\alpha}$ | 38.37 ± 0.72 $^{a,\alpha}$ | 38.30 ± 0.36 $^{a,\alpha}$ |
| | Sample P30 | 37.94 ± 0.32 $^{a,\alpha}$ | 38.49 ±0.67 $^{a,\beta}$ | 38.43 ± 0.28 $^{a,\beta}$ |
| a* | Sample C30 | -2.36 ± 0.56 $^{a,\alpha}$ | -1.70 ± 0.69 $^{a,\beta}$ | -1.86 ± 1.01 $^{a,\beta}$ |
| | Sample P30 | -2.33 ± 0.55 $^{a,\alpha}$ | -1.56 ± 0.82 $^{a,\beta}$ | -1.00 ± 0.21 $^{b,\gamma}$ |
| b* | Sample C30 | 9.88 ±2.78 $^{a,\alpha}$ | 7.80 ± 3.84 $^{a,\alpha\beta}$ | 7.48 ± 3.84 $^{a,\beta}$ |
| | Sample P30 | 9.35 ±2.62 $^{a,\alpha}$ | 7.62 ± 5.02 $^{a,\alpha}$ | 4.13 ± 0.81 $^{b,\beta}$ |

[#] *Descriptions of the sample are available in the abbreviation list*

*Different letter down the column within a particular quality parameter indicates significant differences (P<0.05) between the quality parameter of oil samples with the control sample at the particular growing site*

*Different Greek letter across the row indicates significant differences (P<0.05) between the growing sites*

## Appendix 13: Correlation between the quality parameters and each sensory attribute

| Quality parameters | Aroma Absence of Faults | Aroma Complexity | Aroma Fruit Intensity | Palate Absence of Faults | Palate Balance | Palate Complexity | Palate Flavour Intensity | Total Sensory Score |
|---|---|---|---|---|---|---|---|---|
| **Yield** | -0.688** | -0.274 | -0.311 | -0.538 | -0.402 | -0.414 | -0.433 | -0.374 |
| **Total oil recovery** | -0.426 | -0.396 | -0.385 | -0.325 | -0.330 | -0.276 | -0.596* | -0.388 |
| **Phenolic compounds** | 0.294 | 0.036 | 0.041 | 0.102 | -0.108 | 0.243 | 0.008 | 0.096 |
| **Antiradical activity** | -0.262 | -0.379 | -0.402 | -0.447 | -0.694** | -0.238 | -0.394 | -0.410 |
| **Peroxides Value** | -0.212 | -0.053 | -0.184 | -0.219 | -0.133 | -0.193 | -0.133 | -0.204 |
| **Acidity level** | -0.178 | 0.255 | 0.134 | -0.104 | 0.028 | 0.095 | 0.121 | 0.070 |
| **C16:0** | 0.270 | 0.183 | 0.198 | 0.299 | 0.033 | 0.265 | 0.308 | 0.270 |
| **C18:0** | 0.006 | -0.163 | -0.110 | 0.003 | -0.222 | 0.000 | 0.003 | -0.044 |
| **C18:1** | -0.065 | -0.155 | -0.072 | 0.009 | -0.208 | -0.072 | 0.042 | 0.006 |
| **C18:2** | -0.106 | 0.205 | 0.173 | 0.017 | 0.058 | 0.166 | 0.128 | 0.080 |
| **K232** | -0.441 | -0.158 | -0.250 | -0.418 | -0.064 | -0.210 | -0.300 | -0.330 |
| **K270** | -0.223 | 0.108 | 0.041 | -0.185 | 0.155 | 0.050 | -0.125 | -0.110 |
| **ΔK** | 0.138 | -0.318 | -0.190 | -0.011 | -0.225 | -0.276 | -0.247 | -0.204 |
| **L*** | 0.159 | 0.307 | 0.440 | 0.262 | 0.422 | 0.155 | 0.358 | 0.333 |
| **a*** | -0.153 | 0.199 | 0.259 | 0.165 | 0.413 | 0.033 | 0.178 | 0.179 |
| **b*** | 0.194 | -0.116 | -0.193 | -0.088 | -0.366 | 0.077 | -0.122 | -0.069 |
| **Aroma Absence of Faults** | 1.000 | 0.737** | 0.656* | 0.865** | 0.481 | 0.810** | 0.781** | 0.818** |
| **Aroma Complexity** | 0.737** | 1.000 | 0.870** | 0.674* | 0.633* | 0.926** | 0.832** | 0.898** |
| **Aroma Fruit Intensity** | 0.656* | 0.870** | 1.000 | 0.724** | 0.722** | 0.744** | 0.735** | 0.869** |
| **Palate Absence of Faults** | 0.865** | 0.674* | 0.724** | 1.000 | 0.694** | 0.724** | 0.803** | 0.878** |
| **Palate Balance** | 0.481 | 0.633* | 0.722** | 0.694** | 1.000 | 0.561* | 0.742** | 0.754** |
| **Palate Complexity** | 0.810** | 0.926** | 0.744** | 0.724** | 0.561* | 1.000 | 0.842** | 0.888** |
| **Palate Flavour Intensity** | 0.781** | 0.832** | 0.735** | 0.803** | 0.742** | 0.842** | 1.000 | 0.931** |
| **Total Sensory Score** | 0.818** | 0.898** | 0.869** | 0.878** | 0.754** | 0.888** | 0.931** | 1.000 |

** Correlation is significant at the 0.01 level (2-tailed)