**Author(s):** Bedgood, D.R. ; Bishop, A.G. ; Kalua, C.M. ; Prenzler, P.D.

**Title:** Flavour quality critical production steps from fruit to extra-virgin olive oil at consumption

**Journal:** Food Research International

**ISSN:** 0963-9969 **Year:** 2013 **Pages:** 2095 - 2103

**Volume:** 54 **Issue:** 2

**Abstract:** The production of olive oil comprises a number of production steps where the quality of the oil may be controlled through an understanding of how the production step influences key compounds such as volatiles and biophenols. In this study, critical production steps and significant inter-relationships between isolated production steps from fruit to oil-at-consumption were identified with the application of multivariate statistics. Having identified the key steps and relationships, sensory attribu ...

Elsevier Editorial System(tm) for Food Research International
Manuscript Draft

Manuscript Number: FOODRES-D-13-00398R1

Title: Flavour quality critical production steps from fruit to extra-virgin olive oil at consumption

Article Type: SI: Olive oil

Keywords: aroma; taste; pungency; stepwise linear discriminant analysis (SLDA); process control

Corresponding Author: Dr. Paul D. Prenzler,

Corresponding Author's Institution:

First Author: Curtis M Kalua

Order of Authors: Curtis M Kalua; Danny R Bedgood, Jr.; Andrea G Bishop; Paul D. Prenzler

Manuscript Region of Origin: AUSTRALIA

Abstract: The production of olive oil comprises a number of production steps where the quality of the oil may be controlled through an understanding of how the production step influences key compounds such as volatiles and biophenols. In this study, critical production steps and significant inter-relationships between isolated production steps from fruit to oil-at-consumption were identified with the application of multivariate statistics. Having identified the key steps and relationships, sensory attributes associated with volatile and compounds may be enhanced and maintained during production and through to consumption. Our study showed that flavour compounds (e.g. oleuropein and derivates) could be controlled through olive fruit properties whereas transfer of the best sensory attributes from the fruit (e.g. arising from C5 and C6 volatiles) was critically controlled during oil extraction. Once olive oil was produced, maintenance of quality was critically controlled through storage conditions. Consequently, quality attributes from phenolic and volatile compounds could be targeted for maximum transfer from the olive fruit to oil while taking into account the impact on aroma, bitterness, and pungency of fresh oils and the subsequent loss in flavour quality during storage and consumption.

11 April, 2013

Professor José Alberto Pereira
CIMO/Escola Superior Agrária, Instituto Politécnico de Bragança
Campus Sta Apolónia, Apt 1172, 5301-855 Bragança
Portugal.
Telf.: +351 273303277
Fax.: +351 273325405
E-mail: jpereira@ipb.pt

Dear José,
Please accept the revised manuscript "Flavour quality critical production steps from fruit to extra-virgin olive oil at consumption" by Kalua et al., for consideration in the Special Issue of Food Research International on Olive Oil.

We thank the reviewers for their helpful comments and have revised the manuscript accordingly. We trust that with these corrections the manuscript will be ready to be accepted for publication.

Kind regards,

Paul

Dr Paul D. Prenzler

**Response to Reviewers comments on "Flavour quality critical production steps from fruit to extra-virgin olive oil at consumption " by Curtis M. Kalua, Danny R. Bedgood, Jr., Andrea G. Bishop, and Paul D. Prenzler.**

**Reviewer #1:** The objective of this study, focused on the identification of critical production steps for virgin olive oil, by a multivariate statistical approach, that could be controlled to enhance and maintain flavour quality attributes associated with volatile compounds and phenolic compounds, is surely interesting and the obtained results could be important for the olive oil sector. However, I have a major criticism:

1. As specified in paragraph 2.1 and Table 1, authors elaborated the results obtained from their previous research articles (Kalua et al 2005; Kalua et al 2006a; Kalua et al 2006b; Kalua et al 2008), specifically four papers published from 2005 to 2008, focused on changes in phenolic and volatile compounds as a consequence of different critical steps (cultivar and maturity stage, fruit storage, malaxation time and temperature, storage). These manuscripts considered different olive cv (Frantoio, Corregiolo, Paragon), cultivated under different environmental conditions (different maturity indeces), harvested in different years (2003, 2004 and 2005 harvest season), processed after different fruit storage conditions and by different tecnological parameters (by Abencor system and by industrial mill).

   **Response:** All fruit was harvested in the 2005-06 season and used for results presented for critical control points in this manuscript. The work was just published in different years and the investigation on oil storage had to run for at least 12 months prior to collating the data and published. We have therefore added to the text (lines 71-73) the following to clarify the matter and avoid confusion brought about by the years the work was published.

   "Oil used to identify critical production steps was harvested in the same year (2005 -06 season) from olive fruits of similar maturity indices (**Table 1**) that were not significantly different and the fruit was similarly processed without fruit storage apart from the work on fruit storage."

   Additionally, further information has been provided in Table 1 on the samples, as suggested by Reviewer 2, to provide clarity on the conditions this study was undertaken.

   As for the different cultivars (Frantoio, Corregiolo, Paragon), we point out in lines 73-76 that these cultivars are reported to be similar (all from the Frantoio family), which was confirmed in our work on discrimination of olive oils (Kalua et al., 2005) as not being discriminated statistically with stepwise linear discriminant analysis (SLDA)

2. As authors know well, the sources of variability both natural and due to human choices are many and is very difficult to draw general conclusions using data coming from so different samples: I think that authors should confirm their suggestions through the develop of a new experimental plan that takes in consideration the suggested critical points and analyzes the markers (profiles in volatile and phenols) in well defined samples and applying a flow chart in which different conditions are applied to each critical point.

   As an alternative, they could arrange the present manuscript as a critical review instead of a research article.

   **Response:** As clarified above and in the text (lines 71-76), the samples were extracted from similar fruit and oil extracted using similar conditions. The work was just published in different years as we were collating our data and concluding on

some experiments e.g. the oil storage experiment. This ended up in the work being published in different years and different manuscripts

3. References: Please, check the ref. Carrasco-Pancorbo et al 2005, the two authors Lercker G. and Compagnone D. are lost (after Gallina-Toschi and before Fernandez-Gutierrez)

   **Response:** The deletion of authors would appear to be in line with the way Endnote applies the APA 6 referencing style. Additional authors have been added manually.

**Reviewer #2:** The work titled "Flavour quality critical production steps from fruit to extra-virgin olive oil at consumption" presented by Kalua, Bedgood, Bishop and Prenzler assessed and identified critical production steps from the production of olive oil to the final consumer, by determining volatile and phenolic composition of vegetable oils. The work is of high scientific interest, it is well written and well structured, well grounded, and has industrial application. The paper is inside the aims and scopes of Food Research International, and it fits the aims and scopes of the Special Issue. In my opinion this manuscript is possible to be published, however some points must be addressed before that.

4. Highlights: The highlights, in my opinion should be improved. I would add two new highlights: one highlighting the effect of fruits characteristics in biophenols; and other highlighting the extraction conditions and storage of olive oil in volatiles.

   **Response:** The new highlights are:

- New insights on the effect of fruit characteristics on biophenols
- New insights on the effect of extraction conditions and storage of olive oil on volatiles

**Material and Methods section:**

5. Section 2.1: In Table 1, in "Olive fruit colour" column, besides olives color authors must include the maturation index, if such data is available. In CP2 indicate the temperature used during storage. In CP4 and CP5, the temperature of storage was room temperature? Indicate it in Table 1.

   **Response:** Maturation index of the fruit is included and storage temperatures for CP2, CP4, & CP5 is also included in Table 1 as requested

6. Section 2.2: In phenolic compounds extraction 3×1mL is the correct extraction procedure or it was a mistake? How much time each extraction was conducted? Extraction conditions need to be better described.

   **Response:** The 3x1mL extraction is the correct extraction. We have added/modified the text (lines 99-101) for clarity on the extraction process as below:

"The hexane/methanolic mixture was vigorously shaken in an extraction flask (25mL) and the lower methanolic layer drained out after each extraction". Just after this sentence, we have replaced "The methanolic extracts" with "The combined methanolic extracts"

Time was not a big factor on the extraction efficiency but vigorous mixing through shaking and letting the mixture fully separate into two layers, the top hexane/oil phase and the lower methanol/water phase. Depending on the sample, phase separation was the factor determining how long each extraction takes but had minimal effect on extraction efficiency. A sample that formed emulsions generally took longer to separate (phase separation) and this was not typical for fresh samples but those of low quality, usually not meeting the extra-virgin quality status at the time of analysis.

7. Section 2.4: How many variables were considered for volatile compounds and phenolic compounds for variables selection in SLDA?

   **Response:** We have now provided this information in the text (lines 112-114) and the modified text reads as below:

   "Stepwise linear discriminant analysis (SLDA) was applied on nineteen volatile compounds and twenty-four phenolic compounds to select discriminating variables that characterised particular production steps (**Table 2**) ..."

8. Significance (p) must be presented always in italic. Correct it in the entire manuscript body.

   **Response:** Corrected in the entire manuscript

**Results and Discussion section:**

9. Section 3.1.1: Why hydroxytyrosol and hexanal are discussed in this section since they discriminate two critical production steps? Shouldn't they be discussed in section 3.1.2?

   **Response:** Discussion on hydroxytyrosol and hexanal has been moved to Section 3.1.2 and the modified text has been re-checked for flow of ideas. Thanks for your suggestion and picking this up

10. In this section I was expecting a more deep and scientific discussion. The entire section is well written and exposed, however I would like to see it more "chemically" discussed. For example, phenolic composition is drastically affected by ripening process. Oleuropein content in olives is drastically reduced from green (Maturation Index=0 or 1) to black olives (MI=5 to 7). At the same time oleuropein derivates, hydroxytyrosol and tyrosol contents arise in olive fruits. Fruit maturation is a critical step, since its influences phenolic composition, fatty acids profile as well, with that influencing olive oil quality, its oxidative stability (since many antioxidant compounds decrease their content, from biophenols to tocopherols passing through pigments, among others), and affecting bioactive properties as antioxidant and antimicrobial and antiproliferative capacities. Furthermore, besides aroma and flavor, olive oils appearance downgrades with maturation, changing from intense green to golden-yellow, a color less appreciated by consumers used to consume olive oil. The extraction process and its characteristics should be also considered within the results obtained by authors and LOX pathway (lipoxygenase) in the formation of GLV (green leaf volatiles) and other volatile compounds derived from unsaturated fatty acids. These aspects should be more addressed in a future revised version of the manuscript. With the

incorporation of such information readers that are not used to this kind of information can be further informed about what happens in both olive fruits and olive oils and correlate such data with the data obtained by authors, which is of major importance to olive oil academics, consumers and industrials.

**Response:** We initially thought that such a discussion may have been beyond the scope of this article. However, upon the suggestion of the reviewer, we have included a new section that covers the main points raised by the reviewer. We discuss the main chemical changes at each step, but we do not address colour as colour of the oil was not measured in our study. We also point readers to a number of reviews where the chemistry of the production steps is covered in much greater depth than is possible here. The revised text is (lines 142-190):

"***3.1 Overview of Chemical Changes in Olive Fruit and Oil During Olive Oil Production***

In order to understand the critical control points in olive oil production and what volatile and phenolic compounds may be associated with each step, we begin with a brief overview of the key steps in olive processing and what has been previously found regarding the chemistry occurring at each step. For a comprehensive overview of the chemistry of volatile and phenolic compounds associated with each production step the reader is directed to a review by Kalua et al. (2007), and a recent review on the malaxation step by Clodoveo (2012).

Figure 1 shows the production process for olive oil along with what can be measured at each step to provide some insight into quality. The process begins with olive fruit and the ripeness of the olive fruit is a key factor in olive oil quality since many chemical changes occur within the fruit during ripening. For example, oleuropein content decreases from unripe to ripe fruit and oleuropein derivatives (in oil) have been shown to discriminate maturity stage of the fruit used to produce the oil (Kalua, et al., 2005). Oleuropein derivatives are extremely important to oxidative stability and sensory properties of olive oil.

For volatile compounds in olive oil, the lipoxygenase (LOX) pathway is an important contributor to desirable C5 and C6 aroma compounds (also known as green leaf volatiles, GLV). The enzymes in the LOX pathway generally decrease in activity as the ripeness of the fruit increases, with many C6 aldehydes at maximum levels when the olive skin changes from green to purple (Angerosa & Basti, 2001). With the concentration of oleuropein and many volatiles decreasing with fruit ripening there is obviously a need to harvest olive fruit at the optimum time to maximise these positive compounds in the oil.

After ripening, the next key step in olive oil production is malaxation (**Figure 1**). This step is essential for maximising oil yield as the small oil droplets released from olive fruit at crushing, coalesce to form a continuous phase during the gentle mixing that occurs in malaxation. The changes to volatile and phenolic compounds during this step have been the subject of numerous studies (Kalua, Bedgood, et al., 2006a; Servili et al., 2012). Enzymatic reactions are important for both volatile production and the conversion of oleuropein to deglucosylated derivatives that are found in oil (oleuropein itself is only found in trace amounts in olive oil). These compounds, together with ligstroside derivatives, are key compounds for bitterness and pungency in extra virgin olive oil.

The final step in the production of olive oil is storage and in our previous work (Kalua, Bedgood, et al., 2006b) we were interested in both industrial storage and that which occurs during consumer use (Figure 1). In the latter, oxygen ingress is unavoidable and deterioration of the oil occurs quite rapidly. This leads to the loss of desirable volatiles and the production of undesirable ones. Moreover, as the oil is exposed to oxygen, antioxidant phenolic compounds also decrease with time. From a production perspective, it is important that desirable volatile and phenolic compounds are maximised in the oil prior to storage since inevitably they will decrease with time, and oil quality will suffer.

Having given this very brief overview of the effects of each production step on the chemistry of phenolic and volatile compounds, it is important to distinguish between studies that investigate this chemistry and the current study, which brings together previous work, but from a production and quality control viewpoint. As such, the intent of this work is not so much to understand the underlying chemistry during each production step – this is well known. Rather, we seek to apply process control theory to identify critical production steps and the volatile and phenolic compounds that are associated with these steps. By doing so critical production steps can be indentified and controlled to control quality at each step and therefore overall quality of olive oil. ”

**References section:**

11. In all references from Journal of the American Oil Chemists' Society, change "Journal of the American Oil Chemists Society" to "Journal of the American Oil Chemists' Society". You forget the apostrophe in "Chemists'"

    **Response:** References updated.

12. Page 19, lines 443-444: please provide the complete list of authors.

    **Response:** Corrected as recommended by Reviewer 1.

13. Page 19, lines 447-448: Provide the title in minor letters.

    **Response:** Corrected.

14. Page 19, line 452: Correct "Magalhaes" to "Magalhães" as well as in Page 3, line 20.

    **Response:** Corrected.

15. Page 19, lines 457-458: Put Olea europaea" in italic. Correct the reference, there are "." and "," in duplicate. Change "Advances in horticultural science" to "Advances in Horticultural Science"

    **Response:** Corrected.

16. Sometimes in the text references appear in with a final double bracket (example in page 3, line 21 and page 7, line 142). Look for possible further appearances an correct them.

    **Response:** Corrected.

17. Page 4, line 48: According to guidelines, reference should be Kalua et al., 2006b, since it has already been cited earlier (Page 3, line 23).

    **Response:** Despite several attempts at correcting this error, Endnote refuses to make the change, and I can't do it manually. Can this be corrected at the galley proof stage?

18. Page 5, lines 78-79: Change ". Kalua, Bedgood, et al., 2006b." to ". Kalua et al., 2006b."; equal at page 9, line 184; page 10, lines 204-205; page 15, lines 353-354;

    **Response:** See comment above.

    **Figures and Tables:**

19. Figure 1: "A typical flow diagram of the virgin olive oil production process showing possible changes in quality parameters". This caption is not in accordance with the diagram presented. Besides quality parameters (PV, FFA, specific coefficients of extinction at 232 and 270 nm, <DELTA>K and sensorial analysis) authors consider oil yield, ripening, moisture, volatile and phenolic profiles in the diagram. Oil yield, ripening, moisture, volatile and phenolic profiles besides influencing olive oils quality, they aren't quality parameters.

    **Response:** The caption of Figure 1 has been changed to:

    "A typical flow diagram of the virgin olive oil production process showing possible implications in oil quality, composition, physical/chemical aspects, and sensorial properties".

20. Change caption to something like this: "A typical flow diagram of the virgin olive oil production process showing possible implications in oils quality, composition and physical and chemical aspects".

    **Response:** Changed as suggested with minor modifications as shown in comment above.

21. Figure 3: Change "Ligstroside dialdehype" to "Ligstroside dialdehyde"

    **Response:** Changed.

## Highlights

- Identification of critical production steps for virgin olive oil
- Application of statistical process control and HACCP to olive oil production
- Description of how volatile and phenolic compounds are altered at critical points
- New insights on the effect of fruit characteristics on biophenols
- New insights on the effect of extraction conditions and storage of olive oil on volatiles
- Use of multivariate statistics to control compounds with aroma and taste

# Flavour quality critical production steps from fruit to extra-virgin olive oil at consumption

Curtis M. Kalua[a,b], Danny R. Bedgood, Jr.[a], Andrea G. Bishop[a], and Paul D. Prenzler*[a]

[a] School of Agricultural and Wine Sciences, E.H. Graham Centre for Agricultural Innovation, Locked Bag 588, Charles Sturt University, Wagga Wagga 2678, Australia.

[b] Present address: Kirkvue Road, Adelaide, SA 5076, Australia

Title Running Header: Critical production steps for extra-virgin olive oil.

* Corresponding author: Dr Paul D. Prenzler (pprenzler@csu.edu.au)

**Abstract**

The production of olive oil comprises a number of production steps where the quality of the oil may be controlled through an understanding of how the production step influences key compounds such as volatiles and biophenols. In this study, critical production steps and significant inter-relationships between isolated production steps from fruit to oil-at-consumption were identified with the application of multivariate statistics. Having identified the key steps and relationships, sensory attributes associated with volatile and compounds may be enhanced and maintained during production and through to consumption. Our study showed that flavour compounds (e.g. oleuropein and derivates) could be controlled through olive fruit properties whereas transfer of the best sensory attributes from the fruit (e.g. arising from C5 and C6 volatiles) was critically controlled during oil extraction. Once olive oil was produced, maintenance of quality was critically controlled through storage conditions. Consequently, quality attributes from phenolic and volatile compounds could be targeted for maximum transfer from the olive fruit to oil while taking into account the impact on aroma, bitterness, and pungency of fresh oils and the subsequent loss in flavour quality during storage and consumption.

*Keywords*: aroma; taste; pungency; stepwise linear discriminant analysis (SLDA)

## 1. Introduction

The production of virgin olive oil commences in the grove where characteristics of the raw material, the olive fruit, determine the market value (Garcia, Magalhães, Fregapane, Salvador, & Paiva-Martins, 2012; Rotondi et al., 2004). Market value and classification of olive oil quality are dictated by flavour and its changes (Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2004; Kalua, Bedgood, Bishop, & Prenzler, 2006b). For instance, premium quality, fresh, extra-virgin virgin olive oil is characterised by a fruity aroma and a peppery finish. For such oil, it is common for consumers to pay high prices. In contrast, the lower grades of olive oil, which retail at low prices, are distinctly "flat" in flavour.

The distinctive flavours of premium quality virgin olive oil are due to volatile compounds while the pepperiness (more correctly pungency and bitterness) is attributable to phenolic compounds (Andrewes, Busch, de Joode, Groenewegen, & Alexandre, 2003; Dierkes et al., 2012; Servili et al., 2004). Interestingly, most flavour compounds are not present in significant quantities in fresh olives. They are formed during virgin olive oil production and might be altered by the time olive oil reaches the consumer. In particular, it is at the oil extraction step where most significant fruity aroma development occurs - to be lost thereafter during the distribution/retail supply chain and consumption.

Quality in the virgin olive oil industry is assessed with emphasis on the olive fruit and virgin olive oil in terms of “potential” and “real” quality, respectively (Pardo, Cuesta, & Alvarruiz, 2007). Real quality is that measured on olive oils sampled randomly from storage tanks (Pardo, et al., 2007) - common and standard for the olive oil industry. Evaluation of the sensory quality of virgin olive oils, usually assessed after oil extraction (real quality), involves perception of both favourable and unfavourable sensory attributes, with sensory defects used to classify oils into various grades (IOOC, 2003). Classification of olive oils based on potential quality is rare despite its importance in determining the real quality. Potential quality of olive oil is reached when healthy and clean olive fruits are selected at optimal maturity; processed at optimal conditions with quick separation of

residues and by-products. Once the potential and real quality are assessed, stability is evaluated to determine the commercial quality of the oils at the end of the maximum possible time of storage from bottling and distribution to supermarkets or retail outlets (Pardo, et al., 2007). Our previous experience (Kalua, Bedgood, et al., 2006b) has shown that virgin olive oil quality deteriorates rapidly once extracted and the oil further loses its flavour at supermarkets or retailers prior to consumption. This loss of flavour quality calls for a paradigm shift to consider the entire virgin olive oil production and supply chain to enhance and maintain quality during consumption.

The diversity and inter-relationships of factors affecting the status of virgin olive oil quality make it tremendously difficult to carry out a complete quality investigation along the entire virgin olive oil production and supply chain without multivariate statistical techniques (Aparicio & Luna, 2002). Through a proper statistical process control, critical production steps can be identified and flavour quality attributes associated with volatile compounds and phenolic compounds transferred from olive fruit and maintained in the oil until consumption. “Critical production steps” are processes or operations that must be maintained under strict control to ensure the production and maintenance of a premium quality product (FAO, 1990). At these critical production steps, olive oil flavour quality attributes – aroma, taste, and pungency – can be controlled. The objective of this study was to identify critical production steps for virgin olive oil that could be controlled to enhance and maintain flavour quality attributes associated with volatile compounds and phenolic compounds. The concept of critical production steps is novel to the olive oil industry although it is common in process engineering – statistical process control – and in food technology to ensure microbial safety with “Hazard Analysis and Critical Control Points (HACCP)”.

## 2. Materials and Methods

### *2.1 Selection of samples and virgin olive oil production steps*

Oil used to identify critical production steps was harvested in the same year (2005 -06 season) from olive fruits of similar maturity indices (**Table 1**) that were not significantly different and the fruit was similarly processed without fruit storage apart from the work on fruit storage. The olive fruits used were also from the same *Frantoio* family; *Corregiola* and *Paragon* (Kailis & Considine, 2002), which have been reported to have similar volatile and phenolic profiles (Kalua, Allen, Bedgood, Bishop, & Prenzler, 2005).

The optimum conditions (**Table 1**) for the production and maintenance of premium quality virgin olive oil as identified earlier (Kalua, et al., 2005; Kalua, Bedgood, Bishop, & Prenzler, 2006a; Kalua, Bedgood, et al., 2006b; Kalua, Bedgood, Bishop, & Prenzler, 2008), have been used to compare the relative changes in quality attributes from fruit to oil at consumer level. Although postharvest olive fruit storage is not a normal production operation, it was included in the process of identification of critical production steps (**Table 1**) as its likelihood to be part of the normal virgin olive oil production process increases when fruit supply exceeds processing capacity. The choice of fruit maturity and fruit storage represents changes in potential oil quality (Pardo, et al., 2007). Real quality (Pardo, et al., 2007) was represented through changes in quality just after oil extraction whereas changes during the distribution and supply chain were represented with oil storage in the absence of oxygen. Storage of oil in presence of oxygen simulates virgin olive oil changes that might occur during consumer use. Dark oil storage conditions (**Table 1**) were chosen since such conditions are recommended for the preservation of virgin olive oil quality (IOOC, 1990). This consideration therefore covers a likely oil production process from olive fruit to oil at consumer level.

### *2.2 Qualitative and quantitative analysis of phenolic compounds*

Qualitative and quantitative analysis of the phenolic compounds in **Table 2** was performed using liquid chromatography – electrospray ionization – mass spectrometry (LC-ESI-MS) and high-performance liquid chromatography – diode array detector (HPLC-DAD) respectively, as described in our earlier paper (Kalua, et al., 2005). Phenolic compounds were extracted with 50 + 50 (v/v)

methanol + water solutions (3 x 1 mL) from virgin olive oil (15 g) dissolved in hexane (15 mL). Gallic acid (0.5 mL, 100 μg/g) was added to the oil as an internal standard. The hexane/methanolic mixture was vigorously shaken in an extraction flask (25 mL) and the lower methanolic layer drained out after each extraction. The combined methanolic extracts, from the oil, were washed with hexane and filtered through 0.45 μm plastic non-sterile filter prior to qualitative and quantitative analysis.

### *2.3 Qualitative and quantitative analysis of volatile compounds*

Qualitative and quantitative analysis of the volatile compounds in **Table 2** was performed using solid phase microextraction - gas chromatography – mass spectrometry (SPME-GC-MS) and solid phase microextraction – gas chromatography – flame ionization detection (SPME-GC-FID) respectively, as described in earlier our papers (Kalua, Bedgood, & Prenzler, 2006) with a DVB-CAR-PDMS, 50/30 μm fibre.

### *2.4 Statistical data analysis*

Stepwise linear discriminant analysis (SLDA) was applied on nineteen volatile compounds and twenty-four phenolic compounds to select discriminating variables that characterised particular production steps (**Table 2**) as described earlier (Kalua et al., 2005). Volatile and phenolic compounds concentrations were of different magnitudes and to necessitate comparison on a similar reference scale, standardized normal variables (statistical z-scores) were generated for each production step (**Table 1**). A cumulative statistical z-score was re-calculated for the entire virgin olive oil production process (CP1 to CP5, **Table 1**). Cumulative z-scores were plotted (**Figures 2 – 4**) with Sigma Plot 10.0 (SPSS Inc., Chicago, USA) to compare the relative changes of volatile and phenolic compounds and identify critical production steps from olive fruit to extra-virgin olive oil at consumption. Significant differences ($p$<0.05) between production steps were determined using one-way ANOVA post hoc multiple comparison tests using Duncan's test with SPSS 15.0 (SPSS Inc., Chicago, USA).

### *2.5 Identification of critical production steps and their indicators*

Critical production steps were qualified with significantly different ($p$<0.05) statistical z-scores from zero, otherwise the production steps were under control for particular volatile and phenolic compounds. The different signs and magnitudes of z-scores (**Figure 2 – 4**) showed the different influences of virgin olive oil production steps (**Table 1**). Positive signs implied that optimising such steps maximise the compounds whereas negative z-scores indicate minimization of the compounds at particular production steps. Minimal changes in z-scores (**Figure 2 – 4**) showed controlled processes with minimal impact from a compound. The process of identifying either maximum or minimum changes in z-scores is akin to the concept of quality control charts in statistical process control.

The identification of indicators for critical production steps was achieved through simultaneous consideration of both discriminating variables and changes in virgin olive oil from fruit to oil during consumption. Volatile and phenolic compounds were identified as indicators when they discriminated a particular production step and showed a significantly different ($p$<0.05) statistical z-scores from zero. This compilation of indicators for critical production steps is analogous to the identification of hazards in HACCP.

## 3. Results and Discussion

### *3.1 Overview of Chemical Changes in Olive Fruit and Oil During Olive Oil Production*

In order to understand the critical control points in olive oil production and what volatile and phenolic compounds may be associated with each step, we begin with a brief overview of the key steps in olive processing and what has been previously found regarding the chemistry occurring at each step. For a comprehensive overview of the chemistry of volatile and phenolic compounds associated with each production step the reader is directed to a review by Kalua et al. (2007), and a recent review on the malaxation step by Clodoveo (2012).

Figure 1 shows the production process for olive oil along with what can be measured at each step to provide some insight into quality. The process begins with olive fruit and the ripeness of the olive

fruit is a key factor in olive oil quality since many chemical changes occur within the fruit during ripening. For example, oleuropein content decreases from unripe to ripe fruit and oleuropein derivatives (in oil) have been shown to discriminate maturity stage of the fruit used to produce the oil (Kalua, et al., 2005). Oleuropein derivatives are extremely important to oxidative stability and sensory properties of olive oil.

For volatile compounds in olive oil, the lipoxygenase (LOX) pathway is an important contributor to desirable C5 and C6 aroma compounds (also known as green leaf volatiles, GLV). The enzymes in the LOX pathway generally decrease in activity as the ripeness of the fruit increases, with many C6 aldehydes at maximum levels when the olive skin changes from green to purple (Angerosa & Basti, 2001). With the concentration of oleuropein and many volatiles decreasing with fruit ripening there is obviously a need to harvest olive fruit at the optimum time to maximise these positive compounds in the oil.

After ripening, the next key step in olive oil production is malaxation (**Figure 1**). This step is essential for maximising oil yield as the small oil droplets released from olive fruit at crushing, coalesce to form a continuous phase during the gentle mixing that occurs in malaxation. The changes to volatile and phenolic compounds during this step have been the subject of numerous studies (Kalua, Bedgood, et al., 2006a; Servili et al., 2012). Enzymatic reactions are important for both volatile production and the conversion of oleuropein to deglucosylated derivatives that are found in oil (oleuropein itself is only found in trace amounts in olive oil). These compounds, together with ligstroside derivatives, are key compounds for bitterness and pungency in extra virgin olive oil.

The final step in the production of olive oil is storage and in our previous work (Kalua, Bedgood, et al., 2006b) we were interested in both industrial storage and that which occurs during consumer use (Figure 1). In the latter, oxygen ingress is unavoidable and deterioration of the oil occurs quite rapidly. This leads to the loss of desirable volatiles and the production of undesirable ones. Moreover,

as the oil is exposed to oxygen, antioxidant phenolic compounds also decrease with time. From a production perspective, it is important that desirable volatile and phenolic compounds are maximised in the oil prior to storage since inevitably they will decrease with time, and oil quality will suffer.

Having given this very brief overview of the effects of each production step on the chemistry of phenolic and volatile compounds, it is important to distinguish between studies that investigate this chemistry and the current study, which brings together previous work, but from a production and quality control viewpoint. As such, the intent of this work is not so much to understand the underlying chemistry during each production step – this is well known. Rather, we seek to apply process control theory to identify critical production steps and the volatile and phenolic compounds that are associated with these steps. By doing so critical production steps can be indentified and controlled to control quality at each step and therefore overall quality of olive oil.

### *3.2 Discrimination of virgin olive oil production steps*

Many factors have been attributed to differences in virgin olive oil quality along the production line (**Figure 1**) and objective identification of critical steps and factors that enhance and maintain oil quality have been a challenge. Optionally, potential quality (Pardo, et al., 2007) could be assessed with a potential challenge of determining optimal fruit characteristics necessary to produce premium quality virgin olive oil. Once the oil is extracted, another challenge arises as to the optimum time to assess the real quality; is it when the oil is just transferred to the storage tanks or just before bottling? The emphasis on quality after bottling has often been on how a product will survive the distribution chain with little emphasis on how the oil will perform during consumption. With multiple steps along the virgin olive oil production line (**Figure 1**); what steps are critical to the production, maintenance and enhancement of premium quality oil? Applied in this study is a multivariate statistical approach of identifying discriminating variables during virgin olive oil production, followed by an exploration of relative concentration changes at particular production steps, which contributes to the understanding of flavour quality critical production steps.

Identification of variables that characterize steps along virgin olive oil production line (**Figure 1**) has shown to discriminate single or multiple production steps (**Table 2**). Changing variables that discriminate multiple production steps (Frequency > 1; **Table 2**) does not necessarily mean that the change is transferred to virgin olive oil at consumption. In cases where a variable is discriminated by multiple production steps, the impact of the other steps should be assessed. For instance, a volatile or phenolic compound might be enhanced at a certain production step but depleted at subsequent steps, an aspect that is explored later under identification of critical production steps. However, discrimination of a single production step simplifies the process of optimising the levels of certain quality attributes in virgin olive oil as changing the variable at a particular step (where it is discriminated) has a non-significant effect on the other production steps.

### *3.2.1 Variables that characterise single production steps*

Some of the volatile compounds and phenolic compounds discriminated single steps along the virgin olive oil production line (**Figure 1**). These discriminating variables were common in steps that involved the olive fruit. For instance, oleuropein hemiacetal and luteolin discriminated fruit maturity only (**Table 2**), which suggests that targeting these compounds at certain maturation stages could maximise or minimise concentrations in the fruit with negligible influence from subsequent processes along the production line. Vanillic acid, (+)-pinoresinol and 2-pentyl furan (**Table 2**) was another set of discriminating variables that was affected by olive fruit postharvest storage only. The emergence of volatile compounds and phenolic compounds that discriminate single production steps suggest that these compounds can be enhanced or minimised in virgin olive oil through control at such steps, as for fruit maturation and postharvest fruit storage described above. Solely discriminated production steps can be envisaged as critical control steps in the transferring of desirable or undesirable sensory attributes, imparted through the volatile or phenolic compounds (**Table 2**).

Additionally, some volatile compounds and phenolic compounds discriminate virgin olive oil in different matrices, such as olive fruit and bulk oil. Storage, whether in the fruit (CP2) or oil in absence of oxygen (CP5), was discriminated with ligstroside derivatives, (+)-acetoxypinoresinol, and (*E*)-2-hexen-1-ol (**Table 2**), which suggests that the occurrence of these phenolic compounds and volatile compounds is significantly influenced with storage. These compounds have been shown to decrease at optimum fruit and oil storage conditions (Kalua, Bedgood, et al., 2006b; Kalua, et al., 2008), which might entail a loss in quality as some of these compounds, such as ligstroside derivatives, are associated with the desirable pungency sensations (Dierkes, et al., 2012). In such cases where quality is lost, storage in whichever matrix should be discouraged, but possible simultaneous gains in quality with fruit storage based on other compounds, such as hydroxytyrosol and hexanal, should be considered and balanced if they contribute to maintenance of flavour quality at subsequent production steps as discussed in the next section.

### *3.2.2 Variables that characterise multiple production steps*

It is not always the case that compounds discriminate a single production process, but might be affected differently with similar operations - such as storage - depending on the matrix. For instance, hexanal and hydroxytyrosol discriminated both fruit storage and oil storage in the presence of oxygen (**Table 2**) with an enhancement during fruit storage (Kalua, et al., 2008) and a decrease in concentration during oil storage (Kalua, Bedgood, et al., 2006b). The different effects of fruit and oil storage on hydroxytyrosol and hexanal offers an opportunity of either maximising or minimising changes during virgin olive oil production at particular production steps. In addition to maximising hydroxytyrosol and hexanal during fruit storage, the same production step minimised the levels of vanillic acid, (+)-pinoresinol and 2-pentyl furan (Kalua, et al., 2008), which suggests that side effects should also be considered when maximising positive quality attributes. In the case of fruit storage, optimising storage time is not counter-productive as it maximises compounds associated with positive quality attributes, hydroxytyrosol and hexanal (Kiritsakis, 1998), and minimises compounds associated with poor quality virgin olive oils, such 2-pentyl furan (Vichi, Pizzale, Conte, Buxaderas,

& Lopez-Tamames, 2003) . On the other hand, the same fruit storage step minimises compounds associated with specific positive flavours, such as vanillic acid (Kiritsakis, 1998), and oil stability, (+)-pinoresinol (Owen et al., 2000), providing a chance of targeting certain quality attributes in virgin olive oil.

As such, virgin olive oil production steps should not be viewed in isolation – since other steps down the production line might be either counter-productive or complementary to each other. For instance, (*E*)-2-hexenal and tyrosol were discriminating variables along the entire production process from olive fruit to oil (**Table 2**). These compounds, (*E*)-2-hexenal and tyrosol, are closely associated with quality of virgin olive oil (Angerosa, Mostallino, Basti, & Vito, 2000; Kiritsakis, 1998) and affect both potential and real quality (Pardo, et al., 2007) differently along the virgin olive oil production line.

There were compounds that affected both potential and real quality shown through discrimination of production steps associated with both the olive fruit and oil, respectively. For instance, 1-penten-3-ol was a discriminating variable during both fruit maturity and oil extraction while hexanol discriminated fruit maturity, oil extraction and oil storage (**Table 2**). This observation of compounds discriminating multiple production steps that include the fruit imply that even when these compounds were maximised or minimised in the olive fruit, the compounds should still be controlled during and after oil extraction to maintain quality of virgin olive oil for the entire production process. Phenolic compounds, such as oleuropein aglycon and oleuropein derivatives, have shown a similar tendency of simultaneously discriminating fruit maturity, fruit storage, and oil extraction (**Table 2**) at which these compounds were transferred from olive fruit to oil.

On the other hand, there are some compounds more significantly associated with the fruit than oil thereby significantly affecting the potential quality of olive oil (Pardo, et al., 2007). For instance, (*Z*)-2-penten-1-ol (**Table 2**) discriminated production steps that changed with fruit characteristics only,

such as fruit maturity and fruit storage, but the volatile compound was not a discriminating variable with production steps after the oil was separated from the plant material, such as oil extraction and oil storage. This suggests that (*Z*)-2-penten-1-ol levels may be significantly altered with changes in fruit characteristics, with minimal effect from production conditions during and after oil extraction. The quality of the olive fruit might be critical in enhancing sensory attributes from volatile compounds, such as (*Z*)-2-penten-1-ol, and assure that the transferred attributes from olive fruit to oil were maximised and maintained.

Conversely, some compounds were more associated with the oil than olive fruit, significantly influencing the real quality of virgin olive oil (Pardo, et al., 2007). Acetic acid and octane were examples of such compounds that characterized oil extraction and oil storage without discriminating fruit maturity and postharvest fruit storage (**Table 2**). This observation implied that acetic acid and octane were significantly formed during oil extraction and oil storage with minimal influence from fruit characteristics at optimal conditions (**Table 1**). The production of these compounds is consistent with an earlier report (Vichi, et al., 2003) on the generation of these volatile compounds from oxidative quality deterioration during virgin olive oil storage. It can therefore be suggested that with proper control of oil extraction and oil storage conditions, octane and acetic acid might be minimised to enhance and maintain the quality of virgin olive oil, which is the current common practice where quality is controlled during and after oil extraction.

In general, most of the compounds that discriminated multiple virgin olive oil production steps were either C5 and C6 volatile compounds or oleuropein related compounds, which are associated with premium quality virgin olive oils (Angerosa, et al., 2000; Kiritsakis, 1998) . It is therefore not surprising when studies on virgin olive oil flavour quality enhancement focus on the transfer of positive quality attributes (often from C5 and C6 volatile compounds or oleuropein related compounds) from olive fruit to oil. Unfortunately, this quality enhancement drive does not consider

possible effects on flavour quality from different oil storage conditions and postharvest olive fruit handling, which can be maximised or minimised at critical production steps.

### *3.3 Identification of critical production steps for virgin olive oil flavour quality*

Identification of discriminating variables (**Table 2**) enables us to envisage compounds that can be minimised or maximised for an enhanced flavour, a combined sensation of smell/aroma and taste. Often, characterization of the virgin olive oil flavour quality is based on single production step (usually just after oil extraction) without inter-relating with other production steps. However, virgin olive oils may have different potential and real qualities (Pardo, et al., 2007) based on fruit characteristics and the extracted oil, respectively, which might have synergistic and antagonistic effects along the production line (**Figure 1**). Beyond potential and real quality, virgin olive oil continues to change through the distribution or supply chain and even during consumption when the oil is used. In this study, critical production steps were explored to understand how flavour quality is affected along the entire virgin olive oil production line (**Figure 1**).

#### *3.3.1 Identification of Critical Production Steps for aroma*

Virgin olive oil aroma is perceived when volatile compounds reach human olfactory sensors. More than 120 volatile compounds that contribute both positively and negatively to the sensory properties of olive oil have been identified (Aparicio & Luna, 2002). Although there are many volatile compounds that are odour active, it is not clear where these compounds can be controlled along the virgin olive oil production line (**Figure 1**).

Exploration of relative changes of volatile compounds from olive fruit to oil at consumption showed different changes at control points (**Figure 2**). Positive relative changes in virgin olive oil from fruit characteristics during both fruit maturity were observed for hexanal, (*Z*)-2-penten-1-ol and hexanol (**Figure 2**). Among these volatile compounds, (*Z*)-2-penten-1-ol and hexanol discriminated fruit maturity (**Table 2**). Fruit maturity can therefore be suggested as a critical production step for (*Z*)-2-

penten-1-ol and hexanol. The positive relative change during fruit maturity for hexanal did not discriminate the respective production step, but this compound was discriminated during fruit storage and oil storage with headspace (**Table 2**). Fruit storage (CP2) positively and significantly changed hexanal levels relative to oil storage with headspace (CP5) and during oil extraction (CP3), which significantly reduced hexanal (**Figure 2**) suggesting that fruit storage is critical for maximizing the levels of hexanal in virgin olive oil.

The switch from positive z-scores (during fruit maturity) to negative (during and after oil extraction) for hexanal and (*Z*)-2-penten-1-ol (**Figure 2**) implied that olive fruit quality was critical in maximising the levels of these volatile compounds in virgin olive oil. This is an illustration of aroma compounds capable of being promoted at a particular production step but lost along the production line if good manufacturing practices are violated.

Postharvest olive fruit handling steps (apart from oil storage without headspace) showed negative changes with hexanol and (*E*)-2-hexen-1-ol (**Figure 2**), indicating the importance of good manufacturing practices (IOOC, 1990) in maintaining aroma of virgin olive oil. The positive change during fruit maturity and negative changes thereafter implied that potential levels of hexanol might be maximised during fruit maturation but lost during subsequent production steps. Fruit maturity, oil extraction, and oil storage in the absence of oxygen were discriminated with hexanol (**Table 2**). Fruit maturity potentially maximised hexanol and the subsequent production steps minimised this volatile compound apart from oil storage without headspace (**Figure 2**). This illustrates the importance of fruit maturity in enhancing potential hexanol levels in virgin olive oil, which were later minimised during oil extraction and oil storage with headspace (**Figure 2**). The significantly higher hexanol change (**Figure 2**) with fruit maturity (CP1) and oil storage without headspace (CP4) than oil extraction (CP3) and oil storage with headspace (CP5) suggests that fruit maturity and oil storage without headspace were critical in controlling the levels of hexanol during virgin olive oil production. Unlike hexanol, pentanal (**Figure 2**) was minimised at optimum fruit maturity (CP1) and oil storage

without headspace (CP4) showing an opposite relative effect at similar production steps. This identifies fruit maturity and oil storage without headspace as the critical production steps for both pentanal and hexanol. The opposite effect at similar critical production steps for aroma compounds illustrate that olive oil production conditions can be purposely selected (or engineered) to impart the desired sensory attributes.

In general, volatile compounds have shown single critical production steps, mainly involving fruit characteristics. These single critical production steps dominated the determination of positive sensory properties of virgin olive oil. Positive z-score changes with fruit maturity and fruit storage were generally critical in maximising the sensory quality of virgin olive oil. Negative changes for most of the volatile compounds were observed after separating the oil from the fruit, which suggests that sensory quality of virgin olive oil declines after oil extraction. As the aroma of virgin olive oil was predominantly maximised through fruit characteristics by volatile compounds, it is interesting to investigate changes in the sensory quality of virgin olive oil linked to taste.

### *3.3.2 Identification of Critical Production Steps for taste*

Phenolic compounds have been associated with bitterness, pungency, and overall flavour quality of virgin olive oils (Dierkes, et al., 2012; Servili, et al., 2004). However, little is known on how these compounds can be either maximised or minimised along the virgin olive oil production line (**Figure 1**). In this study, we explore critical production steps associated with flavour of virgin olive oils from fruit to oil at consumption (**Figure 1**).

A way of maximising phenolic compounds in virgin olive oil during consumption is to optimise the initial concentrations of phenolic compounds through intervention at production steps that enhance positive changes. For instance, maximum positive changes in z-scores for oleuropein hemiacetal and ligstroside dialdehyde (**Figure 3**), were observed with fruit maturity (CP1), which suggests that secoiridoids can be maximised in virgin olive oil by harvesting olive fruits at a maturity stage when

these compounds are at peak concentration. However, to ensure that they are still available for the consumer, secoiridiod levels should be controlled down the production line.

While fruit maturity and fruit storage were critical in determining the concentrations of phenolic compounds in virgin olive oils, oil extraction may significantly change the levels of phenolic compounds with implications on sensory attributes. For instance, oleuropein hemiacetal had a positive change in z-scores with oil extraction (CP3, **Figure 3**), indicating that bitter oils (Mateos, Cert, Perez-Camino, & Garcia, 2004) were favoured by oil extraction, whereas ligstroside dialdehyde that account for the pungency (Andrewes, et al., 2003) had negative changes with oil extraction (CP3, **Figure 3**), indicating a loss of pungency. However, maximising sensory quality attributes after production steps that involve the olive fruit should be controlled carefully since concentrations of compounds such as oleuropein compounds depend on maturity and oil extraction parameters (Kalua, et al., 2005; Kalua, Bedgood, et al., 2006b).

Conversely, some phenolic compounds were not controlled with fruit characteristics. For instance, (+)-pinoresinol was not promoted with fruit characteristics, but generated during oil extraction (CP3) and levels controlled with oil storage (CP4 and CP5, **Figure 4**). The depletion of (+)-pinoresinol during oil storage without headspace (CP4) and its enhancement during storage with headspace (CP5), presents this compound as a good candidate for an indicator of oxidative stress in olive oil. This observation was similar for 3,4-DHPEA-DEDA (**Figure 3**). Both (+)-pinoresinol and 3,4-DHPEA-DEDA can be good indicators of oxidative stress with critical production steps at oil extraction (CP3) and oil storage (CP4 and CP5).

Once the olive fruit is ready for harvest and fruit supply exceeds processing capacity, fruit storage prior to oil extraction is inevitable. During olive fruit storage (CP2), concentrations of most phenolic compounds was affected differently (**Figures 3** and **4**). For instance, fruit storage (CP2) had negative changes in z-scores for most phenolic compounds (**Figures 3** and **4**) apart from hydroxytyrosol.

Hydroxytyrosol showed a positive change in z-scores (**Figure 4**) during fruit maturity (CP1) and fruit storage (CP2), indicating that its changes in olive oil were significantly influenced by fruit characteristics. Phenolic compounds that can be maximised in the fruit, such as hydroxytyrosol (**Figure 4**), showed negative changes in z-scores when extracted from the olive fruit (CP3) and during oil storage (CP5). In such cases, it is critical to control the original concentrations of such phenolic compounds from the olive fruit and maintain their levels in the oil through proper oil storage - hence both fruit maturity (CP1) and oil storage (CP5) were critical in maintaining and enhancing virgin olive oil quality. The positive change for hydroxytyrosol during olive fruit storage (**Figure 4**), with mirror image changes to (+)-pinoresinol (**Figure 4**), might indicate the role of these two phenolic compounds in indicating the quality and stability of virgin olive oil.

Quality and stability of virgin olive oils are usually lost with storage in presence of oxygen (IOOC, 1990; Kiritsakis, 1998). A comparison of different virgin olive oil production steps showed that oil storage with headspace (CP5) had a negative change in z-scores for most phenolic compounds present in the olive fruit in contrast to the oil-derived compounds, 3,4-DHPEA-DEDA and (+)-pinoresinol (**Figures 3** and **4**). The decline of phenolic compounds during virgin olive oil storage is consistent with reports (Baldioli, Servili, Perretti, & Montedoro, 1996; Carrasco-Pancorbo et al., 2005) on the antioxidant activity of phenolic compounds. To preserve virgin olive oil quality, oil storage and exposure to oxygen should be discouraged, despite its inevitability during consumer use.

Generally, concentrations of phenolic compounds in virgin olive oils have been shown to depend upon multiple production steps. In cases where oxidative stability is of interest, it has been observed that phenolic compounds can be maximised in the fruit but these compounds were subsequently depleted during oil storage. Hence, phenolic compounds could be targeted for maximum transfer from the olive fruit to oil and maintain oil quality until consumption. On the other hand, oil extraction has shown to be critical for sensory qualities of fresh virgin olive oils - where bitter virgin olive oils were favoured, pungent oils were not.

## 4. Conclusions

Fruit characteristics were dominant in determining the flavour properties of virgin olive oils. Production steps involving olive fruits, such as fruit maturity and postharvest fruit handling, were exclusively critical for the development of volatile compounds in virgin olive oils, and as such were critical in transferring desirable sensory attributes from the fruit to oil and avoiding transfer of undesirable attributes. Fruit characteristics had more influence on volatile compounds than did oil extraction, suggesting the possibility of significant enhancement of sensory attributes during fruit maturity, which can be lost later during oil extraction. The changes in virgin olive oil sensory attributes during oil extraction emphasize the importance of fruit quality (potential quality) in the production and maintenance of premium quality virgin olive oil.

Changes in virgin olive oil during production from fruit to oil were inter-related and simultaneously affected at multiple production steps; defining the ultimate olive oil flavour quality at consumption. The dominant identification of critical changes in volatile compounds at fruit maturation, and phenolic compounds during oil storage and fruit maturation, illustrate the inter-relationships in virgin olive oil flavour attributes (aroma and taste) from fruit to oil during consumption. In general, quality attributes from phenolic compounds and volatile compounds can be targeted for maximum transfer from olive fruit to oil while considering the impact on aroma, bitterness, and pungency in fresh oils and the subsequent loss in quality during storage and consumption.

**Abbreviations used**

3,4-DHPEA-DEDA, 3,4 – dihydroxy phenyl ethyl alcohol – decarboxymethyl elenolic acid dialdehyde; IOOC, International Olive Oil Council; SPME-GC-MS, solid phase microextraction-gas chromatography – mass spectrometry; SPME-GC-FID, solid phase microextraction – gas chromatography – flame ionization detection; LC-ESI-MS, liquid chromatography – electrospray ionization – mass spectrometry; HPLC-DAD, high-performance liquid chromatography – diode array

detector; CP, control point; HACCP, hazard analysis and critical control points; SLDA, stepwise linear discriminant analysis; ANOVA, analysis of variance.

**Acknowledgements**

The authors gratefully acknowledge Vici Murdoch and Gerard Gaskin of Riverina Olive Grove for the assistance during the study. Financial support from Rural Industries Research & Development Corporation (RIRDC) and Charles Sturt University is gratefully acknowledged.

## References


Andrewes, P., Busch, J., de Joode, T., Groenewegen, A., & Alexandre, H. (2003). Sensory properties of virgin olive oil polyphenols: Identification of deacetoxy-ligstroside aglycon as a key contributor to pungency. *Journal of Agricultural and Food Chemistry, 51*(5), 1415-1420.

Angerosa, F., & Basti, C. (2001). Olive oil volatile compounds from the lipoxygenase pathway in relation to fruit ripeness. *Italian Journal of Food Science, 13*(4), 421-428.

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2000). Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chemistry, 68*(3), 283-287.

Aparicio, R., & Luna, G. (2002). Characterisation of monovarietal virgin olive oils. *European Journal of Lipid Science and Technology, 104*(9-10), 614-627.

Baldioli, M., Servili, M., Perretti, G., & Montedoro, G. F. (1996). Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *Journal of the American Oil Chemists' Society, 73*(11), 1589-1593.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Del Carlo, M., Gallina-Toschi, T., Lercker G., Compagnone D., Fernandez-Gutierrez, N. G. (2005). Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. *Journal of Agricultural and Food Chemistry, 53*(23), 8918-8925.

Cavalli, J. F., Fernandez, X., Lizzani-Cuvelier, L., & Loiseau, A. M. (2004). Characterization of volatile compounds of French and Spanish virgin olive oils by HS-SPME: Identification of quality-freshness markers. *Food Chemistry, 88*(1), 151-157.

Clodoveo, M. L. (2012). Malaxation: Influence on virgin olive oil quality. Past, present and future - An overview. *Trends in Food Science & Technology, 25*(1), 13-23.

Dierkes, G., Krieger, S., Duck, R., Bongartz, A., Schmitz, O. J., & Hayen, H. (2012). High-performance liquid chromatography-mass spectrometry profiling of phenolic compounds for evaluation of olive oil bitterness and pungency. *Journal of Agricultural and Food Chemistry, 60*(31), 7597-7606.

FAO. (1990). *5. Food inspection*. Rome: FAO.

Garcia, B., Magalhães, J., Fregapane, G., Salvador, M. D., & Paiva-Martins, F. (2012). Potential of selected Portuguese cultivars for the production of high quality monovarietal virgin olive oil. *European Journal of Lipid Science and Technology, 114*(9), 1070-1082.

IOOC. (1990). *Olive Oil Quality Improvement*. Madrid, Spain: International Olive Oil Council.

Trade Standard Applying to Olive Oil and Olive Pomace Oil. (2003).

Kailis, S. G., & Considine, J. A. (2002). The olive *Olea europaea* L. in Australia: 2000 onwards. *Advances in Horticultural Science, 16*(3-4), 299-306.

Kalua, C. M., Allen, M. S., Bedgood, D. R., Bishop, A. G., & Prenzler, P. D. (2005). Discrimination of olive oils and fruits into cultivars and maturity stages based on phenolic and volatile compounds. *Journal of Agricultural and Food Chemistry, 53*(20), 8054-8062.

Kalua, C. M., Allen, M. S., Bedgood, D. R., Bishop, A. G., Prenzler, P. D., & Robards, K. (2007). Olive oil volatile compounds, flavour development and quality: A critical review. *Food Chemistry, 100*(1), 273-286.

Kalua, C. M., Bedgood, D. R., Bishop, A. G., & Prenzler, P. D. (2006a). Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. *Journal of Agricultural and Food Chemistry, 54*(20), 7641-7651.

Kalua, C. M., Bedgood, D. R., Bishop, A. G., & Prenzler, P. D. (2006b). Discrimination of storage conditions and freshness in virgin olive oil. *Journal of Agricultural and Food Chemistry, 54*(19), 7144-7151.

Kalua, C. M., Bedgood, D. R., Bishop, A. G., & Prenzler, P. D. (2008). Changes in virgin olive oil quality during low-temperature fruit storage. *Journal of Agricultural and Food Chemistry, 56*(7), 2415-2422.

Kalua, C. M., Bedgood, D. R., & Prenzler, P. D. (2006). Development of a headspace solid phase microextraction-gas chromatography method for monitoring volatile compounds in extended time-course experiments of olive oil. *Analytica Chimica Acta, 556*(2), 407-414.

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

Mateos, R., Cert, A., Perez-Camino, M. C., & Garcia, J. M. (2004). Evaluation of virgin olive oil bitterness by quantification of secoiridoid derivatives. *Journal of the American Oil Chemists' Society, 81*(1), 71-75.

Owen, R. W., Mier, W., Giacosa, A., Hull, W. E., Spiegelhalder, B., & Bartsch, H. (2000). Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. *Food and Chemical Toxicology, 38*(8), 647-659.

Pardo, J. E., Cuesta, M. A., & Alvarruiz, A. (2007). Evaluation of potential and real quality of virgin olive oil from the designation of origin "Aceite Campo de Montiel" (Ciudad Real, Spain). *Food Chemistry, 100*(3), 977-984.

Rotondi, A., Bendini, A., Cerretani, L., Mari, M., Lercker, G., & Toschi, T. G. (2004). Effect of olive ripening degree on the oxidative stability and organoleptic properties of cv. Nostrana di Brisighella extra virgin olive oil. *Journal of Agricultural and Food Chemistry, 52*(11), 3649-3654.

Servili, M., Esposto, S., Taticchi, A., Urbani, S., Veneziani, G., Di Maio, I., Selvaggini, R., Gucci, R. (2012). From the Orchard to the Virgin Olive Oil Quality: a Critical Overview. In J. Tous, R. Gucci & P. Fevereiro (Eds.), *XXVIII International Horticultural Congress on Science and Horticulture for People* (Vol. 924, pp. 365-378).

Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G., & Morozzi, G. (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A, 1054*(1-2), 113-127.

Vichi, S., Pizzale, L., Conte, L. S., Buxaderas, S., & Lopez-Tamames, E. (2003). Solid-phase microextraction in the analysis of virgin olive oil volatile fraction: Modifications induced by oxidation and suitable markers of oxidative status. *Journal of Agricultural and Food Chemistry, 51*(22), 6564-6571.

**Figure Captions.**

**Figure 1.** A typical flow diagram of the virgin olive oil production process showing possible implications in oil quality, composition, physical/chemical aspects, and sensorial properties

**Figure 2**. Relative changes (z-scores) in volatile compounds at different control points (CP) from olive fruit to oil. CP1, fruit maturity; CP2, fruit storage; CP3, oil extraction; CP4, oil storage (without headspace); CP5, oil storage (with headspace). Different letters for a compound represent significantly ($p$<0.05) different z-scores for independent duplicate samples.

**Figure 3**. Relative changes (z-score) in phenolic compounds (secoiridoids and derivatives) at different control points (CP) from olive fruit to oil. CP1, fruit maturity; CP2, fruit storage; CP3, oil extraction; CP4, oil storage (without headspace); CP5, oil storage (with headspace); and 3,4-DHPEA-DEDA, 3,4 - dihydroxy phenyl ethyl alcohol – decarboxymethyl elenolic acid dialdehyde . Different letters for a compound represent significantly ($p$<0.05) different z-scores for independent duplicate samples.

**Figure 4**. Relative changes (z-score) in phenolic compounds (alcohols and lignans) at different control points (CP) from olive fruit to oil. CP1, fruit maturity; CP2, fruit storage; CP3, oil extraction; CP4, oil storage (without headspace); CP5, oil storage (with headspace). Different letters for a compound represent significantly ($p$<0.05) different z-scores for independent duplicate samples.

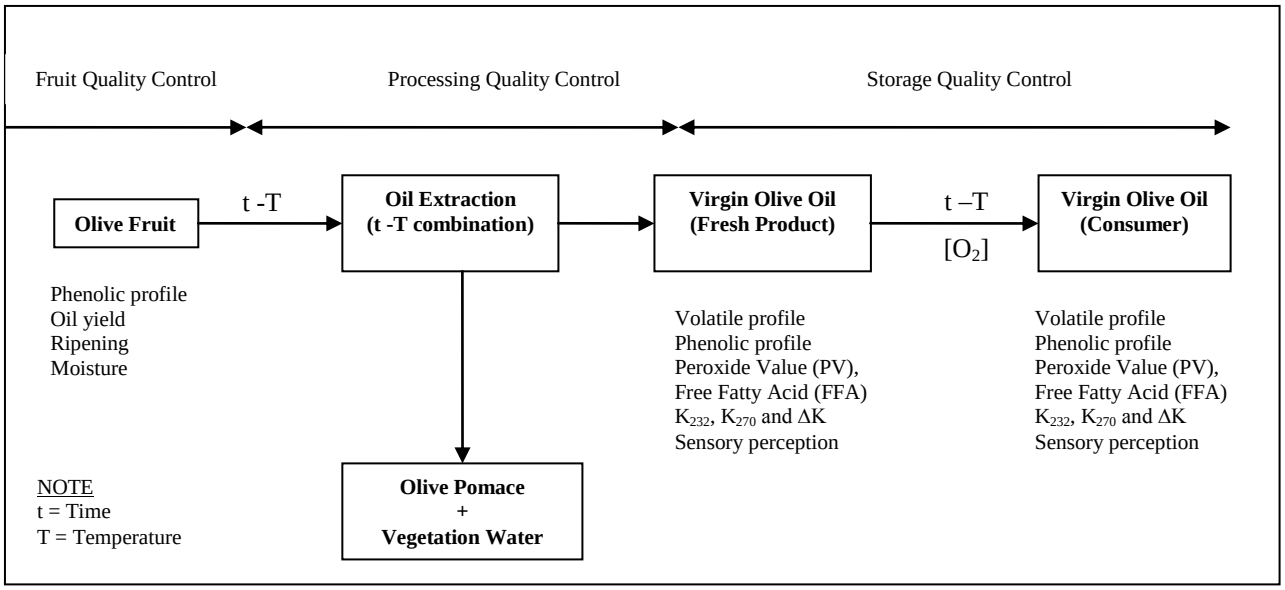
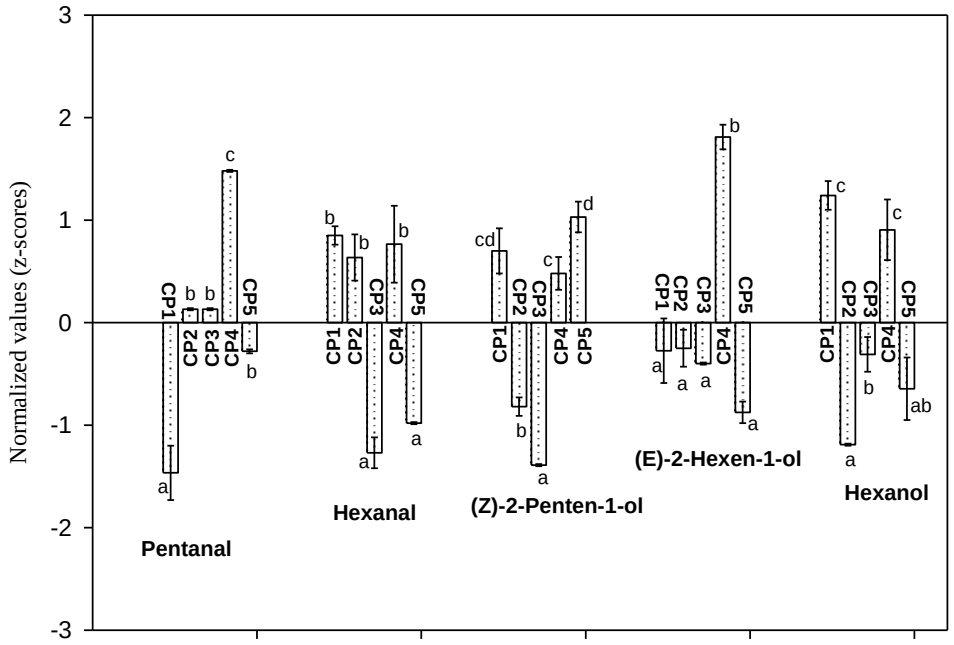

Fruit Quality Control
Processing Quality Control
Storage Quality Control
Olive Fruit
t -T
Oil Extraction
(t -T combination)
Virgin Olive Oil
(Fresh Product)
t –T
[O2]
Virgin Olive Oil
(Consumer)
Phenolic profile
Oil yield
Ripening
Moisture
Volatile profile
Phenolic profile
Peroxide Value (PV),
Free Fatty Acid (FFA)
K232, K270 and ΔK
Sensory perception
Volatile profile
Phenolic profile
Peroxide Value (PV),
Free Fatty Acid (FFA)
K232, K270 and ΔK
Sensory perception
NOTE
t = Time
T = Temperature
Olive Pomace
+
Vegetation Water

**Figure 1**




Volatile compounds (aldehydes and alcohols) relative changes during olive production

**Figure 2.**

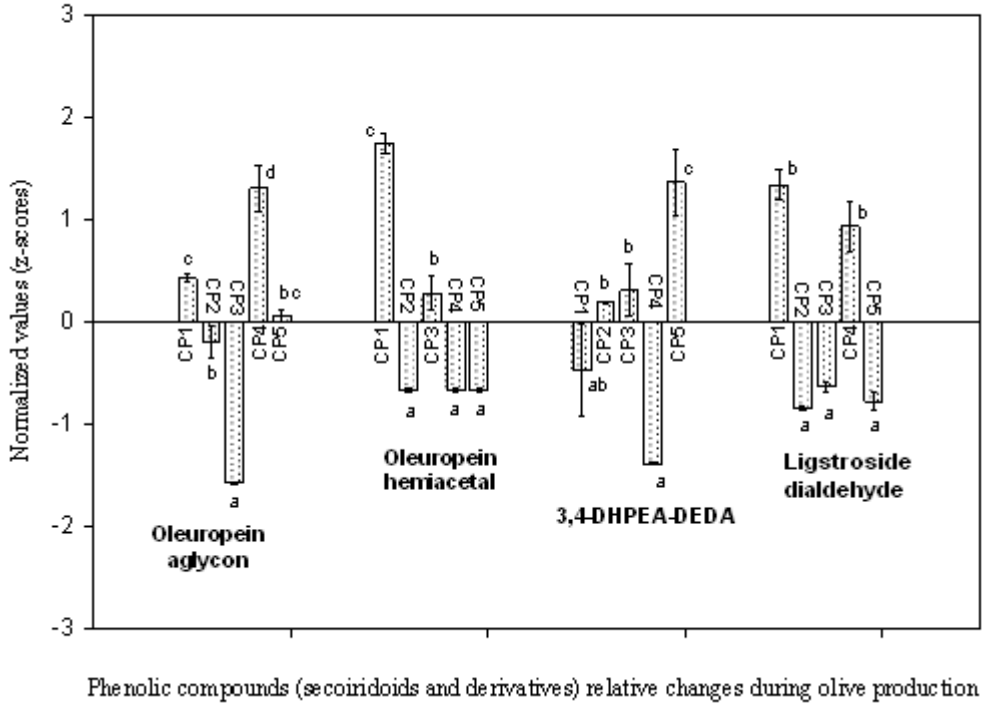
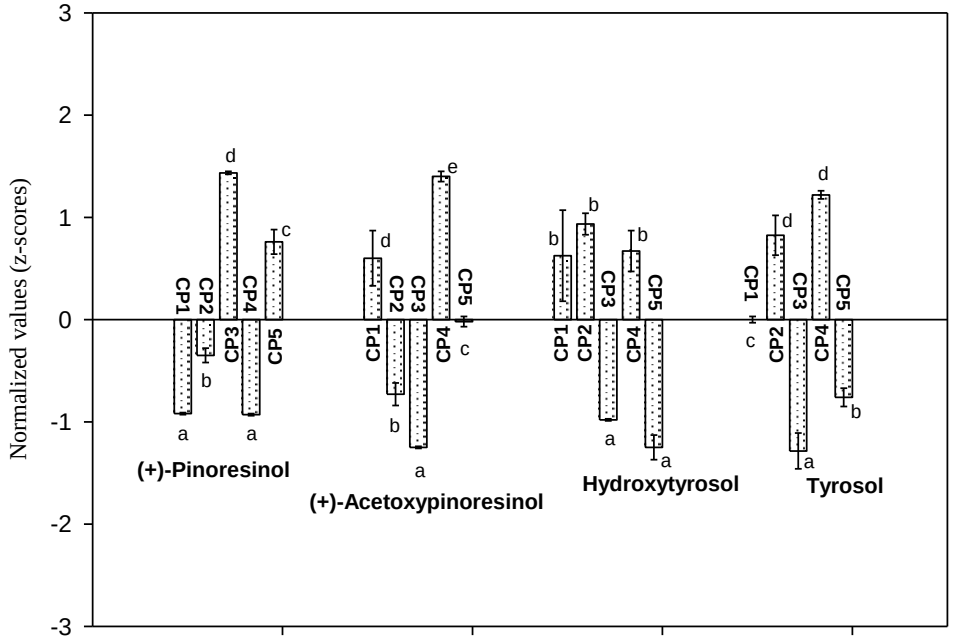

Normalized values (z-scores)
3
2
1
0
-1
-2
-3
CP1
CP2
CP3
CP4
CP5
Oleuropein aglycon
Oleuropein hemiacetal
3,4-DHPEA-DEDA
Ligstroside dialdehyde
Phenolic compounds (secoiridoids and derivatives) relative changes during olive production

**Figure 3.**




Phenolic compounds (lignans and alcohols) relative changes during olive production

**Figure 4.**

**Table 1.** Production conditions and samples used in the identification of critical production steps.

| Control Point (CP) | Production Steps | Optimum Conditions | Olive Cultivar | Olive Fruit Colour | Reference |
|---|---|---|---|---|---|
| CP1 | Fruit Maturity | Oil extracted from spotted olive fruits | *Corregiola* | Spotted (MI = 3.06 ± 0.68) | (Kalua, et al., 2005) |
| CP2 | Fruit Storage | Oil extracted from olive fruit stored at low temperatures (4 ± 2 °C) for 2 weeks | *Frantoio* | Spotted (MI = 2.92 ± 0.06) | (Kalua, et al., 2008) |
| CP3 | Oil Extraction | Malaxation at 30 °C for 60 min | *Corregiola* | Spotted and Green (MI = 2.4 ± 0.1) | (Kalua, Bedgood, et al., 2006a) |
| CP4 | Oil Storage (without headspace) | Oil stored at room temperature (24 ± 3 °C) in the dark for 4 months | *Paragon* | Spotted (MI = 3.06 ± 0.68) | (Kalua, Bedgood, et al., 2006b) |
| CP5 | Oil Storage (with headspace) | Oil stored at room temperature (24 ± 3 °C) in the dark for 2 months | *Paragon* | Spotted (MI = 3.06 ± 0.68) | (Kalua, Bedgood, et al., 2006b) |

"MI" represents maturity index of the olive fruit

**Table 2.** Discriminating variables for virgin olive oil production steps.

| Discriminating Variables | Flavour Attributes | Fruit Maturity | Fruit Storage | Oil Extraction | Oil Storage ($-O_2$) | Oil Storage ($+O_2$) | Freq [a] |
|---|---|---|---|---|---|---|---|
| **Oil Volatile Compounds** [b] | | | | | | | |
| **Aldehydes** | | | | | | | |
| Pentanal | Woody, bitter, oily aroma | × | × | × | × | ✓ | 1 |
| Hexanal | Green, grassy aroma | × | ✓ | × | × | ✓ | 2 |
| (*E*)-2-hexenal | Green aroma | ✓ | ✓ | ✓ | ✓ | ✓ | 5 |
| **Alcohols** | | | | | | | |
| (*Z*)-2-penten-1-ol | Green, plastic, rubber | ✓ | ✓ | × | × | × | 2 |
| 1-penten-3-ol | Green aroma | ✓ | × | ✓ | × | × | 2 |
| (*E*)-2-hexen-1-ol | Green, grassy aroma | × | ✓ | × | ✓ | × | 2 |
| Hexanol | Fruit, soft aroma | ✓ | × | ✓ | ✓ | × | 3 |
| **Miscellaneous** | | | | | | | |
| Acetic acid | Sour, vinegary flavour | × | × | ✓ | × | ✓ | 2 |
| Octane | Sweety, alcane | × | × | ✓ | ✓ | × | 2 |
| 2-pentyl furan | Associated with oil oxidation | × | ✓ | × | × | × | 1 |
| **Oil Phenolic Compounds** [b] | | | | | | | |
| **Secoiridoids** | | | | | | | |
| Oleuropein aglycon | Bitter taste | ✓ | ✓ | ✓ | × | × | 3 |
| Oleuropein derivatives | Bitter taste | ✓ | ✓ | ✓ | × | × | 3 |
| Oleuropein hemiacetal | Bitter taste | ✓ | × | × | × | × | 1 |
| 3,4-DHPEA-DEDA[c] | Bitter taste | × | × | ✓ | × | × | 1 |
| Ligstroside dialdehyde | Pungency sensation | × | ✓ | × | ✓ | × | 2 |
| Ligstroside derivatives | Pungency sensation | × | ✓ | × | ✓ | × | 2 |
| **Lignans** | | | | | | | |
| (+)-pinoresinol | No clear sensory attribute | × | ✓ | × | × | × | 1 |
| (+)-acetoxypinoresinol | No clear sensory attribute | × | ✓ | × | ✓ | × | 2 |
| **Flavonoid** | | | | | | | |
| Luteolin | No clear sensory attribute | ✓ | × | × | × | × | 1 |
| **Simple phenolic alcohols and acids** | | | | | | | |
| Hydroxytyrosol | Indirectly associated with premium olive oils | × | ✓ | × | × | ✓ | 2 |
| Tyrosol | Indirectly associated with poor quality olive oils | ✓ | ✓ | ✓ | ✓ | × | 4 |
| Vanillic acid | Associated with positive sensory quality | × | ✓ | × | × | × | 1 |

[a] Number of times (frequency) a variable discriminates a process along virgin olive oil production line;
[b] Concentrations (μg/g) of phenolic and volatile compounds;
[c] 3, 4 – dihydroxy phenyl ethyl alcohol – decarboxymethyl elenolic acid dialdehyde;